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The epidemiology of *Clostridium difficile* infection in Australia

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DECLARATION BY THE AUTHOR

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis. I have clearly stated the contribution of others to my thesis as a whole, including statistical assistance, survey design, data analysis, significant technical procedures, professional editorial advice, and any other original research work used or reported in my thesis. The content of my thesis is the result of work I have carried out since the commencement of my research higher degree candidature and does not include a substantial part of work that has been submitted to qualify for the award of any other degree or diploma in any university or other tertiary institution.

Luis Furuya Kanamori

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STATEMENT OF CONTRIBUTION TO JOINTLY AUTHORED WORKS CONTAINED IN THE THESIS

The following publications are included as part of this thesis:

1. **Furuya-Kanamori L**, Marquess J, Yakob L, Riley TV, Paterson DL, Foster NF, Huber CA, Clements AC. Asymptomatic *Clostridium difficile* colonization: epidemiology and clinical implications. *BMC Infect Dis* 2015;15:516 – Incorporated as a section in Chapter 2.

2. **Furuya-Kanamori L**, Stone JC, Clark J, McKenzie SJ, Yakob L, Paterson DL, Riley TV, Doi SA, Clements AC. Comorbidities, exposure to medications, and the risk of community-acquired *Clostridium difficile* infection: A systematic review and meta-analysis. *Infect Control Hosp Epidemiol* 2015;36:132-41 – Incorporated as a section in Chapter 2.

3. **Furuya-Kanamori L**, Clements AC, Foster NF, Huber CA, Hong S, Harris-Brown T, Yakob L, Paterson DL, Riley TV. Asymptomatic *Clostridium difficile* colonisation in two Australian tertiary hospitals, 2012–2014: A prospective, repeated cross-sectional study. *Clin Microbiol Infect* 2017;23:48.e1-7 – Incorporated as Chapter 3.

4. **Furuya-Kanamori L**, Riley TV, Paterson DL, Foster NF, Huber CA, Hong S, Harris-Brown T, Robson J, Clements AC. A comparison of *Clostridium difficile* ribotypes circulating in Australian hospitals and communities. *J Clin Microbiol* 2016;55:216-25 – Incorporated as Chapter 4.

5. **Furuya-Kanamori L**, Robson J, Soares Magalhaes RJ, Yakob L, McKenzie SJ, Paterson DL, Riley TV, Clements AC. A population-based spatio-temporal analysis of *Clostridium difficile* infection in Queensland, Australia over a 10-year period. *J Infect* 2014;69:447-55 – Incorporated as a section in Chapter 5.

6. **Furuya-Kanamori L**, Yakob L, Riley TV, Paterson DL, Baker P, McKenzie SJ, Robson J, Clements AC. Community-acquired *Clostridium difficile* infection in

Queensland, Australia. *Emerg Infect Dis* 2016;22:1659-61 – Incorporated as a section in Chapter 5.

7. **Furuya-Kanamori L**, McKenzie SJ, Yakob L, Clark J, Paterson DL, Riley TV, Clements AC. *Clostridium difficile* infection seasonality: patterns across hemispheres and continents - A systematic review. *PLoS One* 2015;10:e0120730 – Incorporated as a section in Chapter 5.

8. **Furuya-Kanamori L**, Doi SA, Paterson DL, Helms SK, Yakob L, McKenzie SJ, Garborg K, Emanuelsson F, Stollman N, Kronman MP, Clark J, Huber CA, Riley TV, Clements AC. Upper versus lower gastrointestinal delivery for transplantation of fecal microbiota in recurrent or refractory *Clostridium difficile* infection: A collaborative analysis of individual patient data from 14 studies. *J Clin Gastroenterol* 2017;51:145-50 – Incorporated as a section in Chapter 6.

9. **Furuya-Kanamori L**, Wangdi K, Yakob L, McKenzie SJ, Doi SA, Clark J, Paterson DL, Riley TV, Clements AC. 25-Hydroxyvitamin D concentrations and *Clostridium difficile* infection: A meta-analysis. *JPEN J Parenter Enteral Nutr* 2015 [Epub ahead of print] – Incorporated as a section in Chapter 6.

For each publication included in this thesis I was the lead author; however, given that each publication included contributions from several co-authors, the International Committee of Medical Journal Editors (ICMJE) authorship criteria recommendation was used to estimate my specific contribution to each paper based on the following four criteria: 1) Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; 2) Drafting the work or revising it critically for important intellectual content; 3) Final approval of the version to be published; 4) Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Chapter	Article title	Journal	Status	Number of co-authors	Student contribution (%)
2	Asymptomatic *Clostridium difficile* colonization: epidemiology and clinical implications	*BMC Infect Dis*	Published	7	70
2	Comorbidities, exposure to medications, and the risk of community-acquired *Clostridium difficile* infection: A systematic review and meta-analysis	*Infect Control Hosp Epidemiol*	Published	8	85
3	Asymptomatic *Clostridium difficile* colonisation in two Australian tertiary hospitals, 2012–2014: A prospective, repeated cross-sectional study	*Clin Microbiol Infect*	Published	8	75
4	A comparison of *Clostridium difficile* ribotypes circulating in Australian hospitals and communities	*J Clin Microbiol*	Published	8	75
5	A population-based spatio-temporal analysis of *Clostridium difficile* infection in Queensland, Australia over a 10-year period	*J Infect*	Published	7	80
5	Community-acquired *Clostridium difficile* infection in Queensland, Australia	*Emerg Infect Dis*	Published	7	80
5	*Clostridium difficile* infection seasonality: patterns across hemispheres and continents - A systematic review	*PLOS One*	Published	6	80
6	Upper versus lower gastrointestinal delivery for transplantation of fecal microbiota in recurrent or refractory *Clostridium difficile* infection: A collaborative analysis of individual patient data from 14 studies	*J Clin Gastroenterol*	Published	13	85
6	25-Hydroxyvitamin D concentrations and *Clostridium difficile* infection: A meta-analysis	*JPEN J Parenter Enteral Nutr*	Published	8	85

ADDITIONAL PUBLISHED WORKS BY THE AUTHOR

RELEVANT TO THE THESIS BUT NOT FORMING PART OF IT

1. Yakob L, Riley TV, Paterson DL, Marquess J, Magalhaes RJ, **Furuya-Kanamori L**, Clements AC. Mechanisms of hypervirulent *Clostridium difficile* ribotype 027 displacement of endemic strains: an epidemiological model. *Sci Rep* 2015;5:12666.

2. Sartelli M, Malangoni MA, Abu-Zidan FM, Griffiths EA, Di Bella S, McFarland LV, Eltringham I, Shelat VG, Velmahos GC, Kelly CP, Khanna S, Abdelsattar ZM, Alrahmani L, Ansaloni L, Augustin G, Bala M, Barbut F, Ben-Ishay O, Bhangu A, Biffl WL, Brecher SM, Camacho-Ortiz A, Cainzos MA, Canterbury LA, Catena F, Chan S, Cherry-Bukowiec JR, Clanton J, Coccolini F, Cocuz ME, Coimbra R, Cook CH, Cui Y, Czepiel J, Das K, Demetrashvili Z, Di Carlo I, Di Saverio S, Dumitru IM, Eckert C, Eckmann C, Eiland EH, Enani MA, Faro M, Ferrada P, Forrester JD, Fraga GP, Frossard JL, Galeiras R, Ghnnam W, Gomes CA, Gorrepati V, Ahmed MH, Herzog T, Humphrey F, Kim JI, Isik A, Ivatury R, Lee YY, Juang P, **Furuya-Kanamori L**, Karamarkovic A, Kim PK, Kluger Y, Ko WC, LaBarbera FD, Lee JG, Leppaniemi A, Lohsiriwat V, Marwah S, Mazuski JE, Metan G, Moore EE, Moore FA, Nord CE, Ordonez CA, Junior GA, Petrosillo N, Portela F, Puri BK, Ray A, Raza M, Rems M, Sakakushev BE, Sganga G, Spigaglia P, Stewart DB, Tattevin P, Timsit JF, To KB, Trana C, Uhl W, Urbanek L, van Goor H, Vassallo A, Zahar JR, Caproli E, Viale P. WSES guidelines for management of *Clostridium difficile* infection in surgical patients. *World J Emerg Surg* 2015;10:38.

Note: The two publications listed above are presented in Appendix 1.1 and 1.2.

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ABSTRACT

Background: *Clostridium difficile* was traditionally considered a nosocomial pathogen and research on *C. difficile* infection (CDI) has largely focused on symptomatic hospital-associated (HA)-CDI. Recent studies have pointed out the importance of asymptomatic *C. difficile* colonisation and community-associated (CA)-CDI in *C. difficile* epidemiology, yet our current understanding of these components is limited. Therefore, the objectives of my research were: 1) to identify risk factors associated with asymptomatic *C. difficile* colonisation; 2) to compare the predominant *C. difficile* ribotypes between symptomatic and asymptomatic patients; 3) to determine the spatio-temporal distribution of CA-CDI in Queensland and to examine its association with medication exposure at a population level; and 4) to investigate novel therapeutical options and risk factors for CDI.

Methods: I analysed datasets from different sources: 1) a prospective three-year repeated cross-sectional study in hospitalised patients; 2) a three-year longitudinal surveillance study of symptomatic CDI in the hospitals and the communities; 3) CDI data from Sullivan Nicolaides Pathology and quantities of medications prescribed in Queensland from the Pharmaceutical Benefits Scheme; and 4) published data. Depending on the nature of the data and the objectives, I analysed the data using multivariate regression models, regression models built in a Bayesian framework to incorporate spatially unstructured random effects, and meta-analytical models.

Results: Seven percent of admitted patients to two Australian tertiary hospitals were asymptomatically colonised by *C. difficile*. Toxigenic *C. difficile* (TCD)-colonisation was associated with gastro-oesophageal reflux disease, higher number of hospital admissions, and antimicrobial exposure; whereas, non-toxigenic *C. difficile* (NTCD)-colonisation was associated with chronic obstructive pulmonary disease and

chronic kidney failure. Asymptomatic *C. difficile* colonisation was seasonal with a higher prevalence in summer than winter. The predominant *C. difficile* ribotypes isolated in the hospital setting corresponded with those isolated in the community. Similarly, ribotypes isolated from symptomatic patients matched those isolated from asymptomatic patients in the hospitals. The proportion of positive CDI stool specimens increased 3-fold during the last decade in Queensland. The distribution of CDI had no evidence of spatial clustering at the postcode level and the observed increase of CA-CDI was not associated with variation in medication exposure at a population level. Faecal microbiota transplantation (FMT) was more effective for CDI recurrence/relapse when administered via colonoscopy/enema than gastroscopy/nasogastric tube. Lower levels of vitamin D were associated with CDI as well as severe forms of CDI.

Conclusions: I provided the first prevalence estimates of asymptomatic *C. difficile* colonisation in Australian hospitals. I also provided evidence that patient characteristics differed between asymptomatic NTCD- and TCD-colonisation. I found that the predominant ribotypes circulating in the communities concorded with those circulating in the hospitals. The findings suggested that asymptomatic colonised patients can act as a means of transmission between the hospital and community settings. I identified that over the past decade CDI has significantly increased in Queensland and antibiotic restriction policy in the community might have little effect on CA-CDI. I provided evidence of low levels of vitamin D is a risk factor for CDI and FMT for CDI recurrence/relapse should be preferably delivered via colonoscopy/enema.

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LIST OF ABBREVIATIONS

CA	Community-associated
CDI	*C. difficile* infection
CDT	*C. difficile* binary toxin
EIA	Enzyme immunoassay
FMT	Faecal microbiota transplantation
GHD	Glutamate dehydrogenase
HA	Hospital-associated
NTCD	Non-toxigenic *C. difficile*
PCR	Polymerase chain reaction
PMC	Pseudomembranous colitis
TCD	Toxigenic *C. difficile*

Chapter 1

Introduction

CHAPTER 1. Introduction

1.1. Background

Clostridium difficile infection (CDI) is the most common cause of infectious diarrhoea among inpatients worldwide. The incidence and severity of CDI have dramatically increased over the past three decades. The Centers for Disease Control and Prevention in the USA has catalogued *C. difficile* as "an immediate public health threat that requires urgent and aggressive action" [1]. It is estimated that *C. difficile* is responsible for over 400,000 infections and 29,000 deaths per year in the USA [2]. In recent years, *C. difficile* has surpassed methicillin-resistant *Staphylococcus aureus* as the most common bacterium causing healthcare-acquired infections in community hospitals [3] and it is estimated that CDI is responsible for over US$ 4.8 billion in excess healthcare costs per year in the USA [4].

1.1.1. History and evolution of *C. difficile*

C. difficile (or *Bacillus difficile* as it was initially called) is a Gram-positive, anaerobic, spore-forming bacterium first isolated in 1935 by Hall and O'Toole from newborn infants' meconium and stool specimens [5]; however, it was not until the late 1970s that the "*C. difficile* era" began [6]. Pseudomembranous colitis (PMC) was a common post-operative complication in the 1950-60s (at that time incorrectly attributed to *S. aureus*) among patients that received antibiotics [7-9]. In 1974, Tedesco and colleagues found that clindamycin was associated with PMC [10] and soon after that, in 1978 *C. difficile* toxin was identified as the aetiological agent of PMC [11-13]. Since then, studies using animal models have established the role of antibiotics and *C. difficile* toxins in the pathogenesis and virulence of the disease. Epidemiological studies conducted worldwide among inpatients have well documented the clinical presentation, the efficacy

of different treatment options, and the risk factors associated with healthcare-associated (HA)-CDI.

In the early 2000s, *C. difficile* once again attracted the attention of the research community, when a "new *C. difficile* era" began with two major epidemiological changes. Firstly, there was the emergence of hypervirulent strains of *C. difficile*. In North America, the *C. difficile* B1/NAP1/027 strain was responsible for a steep rise in CDI incidence (4-fold increase in Quebec, Canada from 1991 to 2003 [14, 15] and a 2.2-fold increase in the USA from 1996 to 2003 [16, 17]) and the majority of hospital outbreaks. This strain was associated with higher rates of recurrence [18] and more severe clinical presentations (i.e. toxic megacolon, septic shock) [14] with an unprecedented attributable mortality rate of 16.7% among elderly patients [19]. Moreover, *C. difficile* ribotype 027 has a distinct ability to spread between continents; by 2005-6 severe hospitals outbreaks due to *C. difficile* ribotype 027 were reported in seven European countries, by 2008-10 it reached Central America, Asia and Oceania [20], and by 2011-12 it reached South America [21] and Africa [22].

Secondly, *C. difficile* is a ubiquitous bacterium that can be found in the environment (i.e. soil and water), food, livestock and other domestic animals [23-25]; however, it was traditionally regarded as a nosocomial pathogen. Nowadays, *C. difficile* is no longer exclusively considered a nosocomial pathogen; in recent decades, a sharp increase in incidence of community-associated (CA)-CDI has been reported worldwide [26]. Furthermore, importation of CA-CDI cases into the hospital has been implicated in maintaining HA-CDI transmission [27]. The important role of CA-CDI in *C. difficile* epidemiology is increasingly being recognised, yet patient risk factors are less clearly defined for CA-CDI than for HA-CDI. In fact, patient risk profiles might significantly differ between CA- and HA-CDI since severe CDI cases have been reported in patients

from the community without the "traditional" CDI risk factors (i.e. age >65 years, exposure to antibiotics and hospital admissions) [28, 29].

1.1.2. *C. difficile* transmission

Some strains of *C. difficile* have the capacity to produce toxins (A, B and/or binary [CDT]). If a patient is exposed and ingests the spores of a toxigenic *C. difficile* (TCD) strain and these spores survive the gastric acid, they can germinate into vegetative cells and then penetrate the mucosa layer of the colon [30]. Disruption of the normal colonic biomass (e.g. through exposure to certain antibiotics) increases the host susceptibility and facilitates *C. difficile* colonisation [31]. After a patient is colonised, it is estimated that two-thirds would remain asymptomatic, while the remaining patients would developed symptoms of CDI [32]. However, currently it is unclear how long is the latent period, or the host (e.g. immune status, comorbidities) and pathogen (e.g. toxigenicity, ribotype) factors that determine whether a patient remains asymptomatic, or develops mild or severe symptoms.

The main modes of transmission of *C. difficile* are by direct contact (i.e. faecal-oral route) and indirect contact (i.e. contaminated fomites and surfaces) [33]. So far, airborne and food-borne routes have not been confirmed as being important for *C. difficile* transmission. Person-to-person transmission from symptomatic CDI patients, and environmental contamination, have been largely described in hospital wards [34, 35]; however, there is increasing evidence that "healthy" asymptomatic TCD-colonized patients have the potential to act as a reservoir and source of transmission in healthcare settings [34, 36]. Given that it is not cost-effective to screen for *C. difficile* all patients admitted to a hospital without diarrhoea, it might be beneficial to identify risk factors for asymptomatic TCD-colonisation, so that preventive and control measures can be targeted

at those patients with high risk of asymptomatic TCD-colonisation to reduce *C. difficile* transmission in hospitals.

Soon after TCD strains were identified as the aetiological agent of PMC, *C. difficile* strains without toxins were isolated from asymptomatic babies and adult patients [37]. The current knowledge on non-toxigenic *C. difficile* (NTCD) is scarce, given that the virulence of *C. difficile* is attributed to their toxins, and NTCD strains are considered non-pathogenic or benign. Some NTCD cases had been reported in the literature to cause symptomatic disease; however, currently it is accepted that mixed infections (TCD and NTCD strains) can be misclassified as exclusively NTCD strains if non-toxigenic strains were forming the predominant colonies [38]. Alternatively, clinical signs could be due to some other (infectious or non-infectious) aetiology, with NTCD isolated coincidentally.

It has been suggested that NTCD colonisation may provide some beneficial effect by providing a protective effect against TCD strains. A recent phase 2 randomised control trial has shown that administration of spores of NTCD strains significantly reduces CDI recurrence [39]. However, pathogenicity loci can be transferred from TCD to NTCD and the safety of administrating NTCD strains need to be carefully examined [40].

1.1.3. Diagnosis and treatment of *C. difficile*

There are different laboratory methods available that are routinely used to detect either *C. difficile* toxins (enzyme immunoassay [EIA] for toxin A/B, cell culture cytotoxicity neutralization assays, polymerase chain reaction [PCR] assays) or the bacterium (glutamate dehydrogenase [GHD] assays, anaerobic culture) in stool specimens. Current guidelines for diagnosis and treatment suggest using two-step algorithms to maximise cost-effectiveness, by first using a highly sensitive test (e.g. GHD assay) followed by a more specific test (e.g. PCR or culture) [41, 42]. Guidelines recommend that only patients with unformed stools should be tested as it is of great

importance that treatment targets the disease, not asymptomatic *C. difficile* colonized patients, given that CDI treatment involves antibiotics that can disrupt the normal colonic biomass of asymptomatic *C. difficile* colonized patients and might increase their risk of developing active forms of the disease.

Multiple international guidelines recommend metronidazole and vancomycin as first-line therapies for CDI [41, 42]. Although resistance to these antibiotics is not yet a problem for CDI, unparalleled high recurrence rates have been reported for both antibiotics recommended as first-line therapy (metronidazole [47.2%] and vancomycin [25.3%]) [18, 43]. A limited number of options is available for treatment of CDI recurrence (e.g. fidamoxicin, rifaximin or nitazoxanide [for children]). One treatment option that recent evidence suggests is highly effective (89% clinical resolution) is faecal microbiota transplantation (FMT) [44]. Nonetheless, it is still under debate whether FMT should be delivered via the upper (gastroscopy, nasogastric tube) or lower (colonoscopy, enema) gastrointestinal route.

1.1.4. Current status of *C. difficile* in Australia

In alignment with international findings, it has been identified that both CA-and HA-CDI cases increased in Queensland, Australia over the past decade [45] (Figure 1.1). These findings were confirmed by a national hospital surveillance study that reported a significant increase in CDI incidence across all Australian states/territories that could not be entirely explained by the adoption of more sensitive laboratory *C. difficile* detection methods [46]. In addition, CA-CDI incidence is rapidly increasing in Australia and recent studies have found that CA-CDI account for up to a third of CDI cases identified in Australian hospitals [47-50].

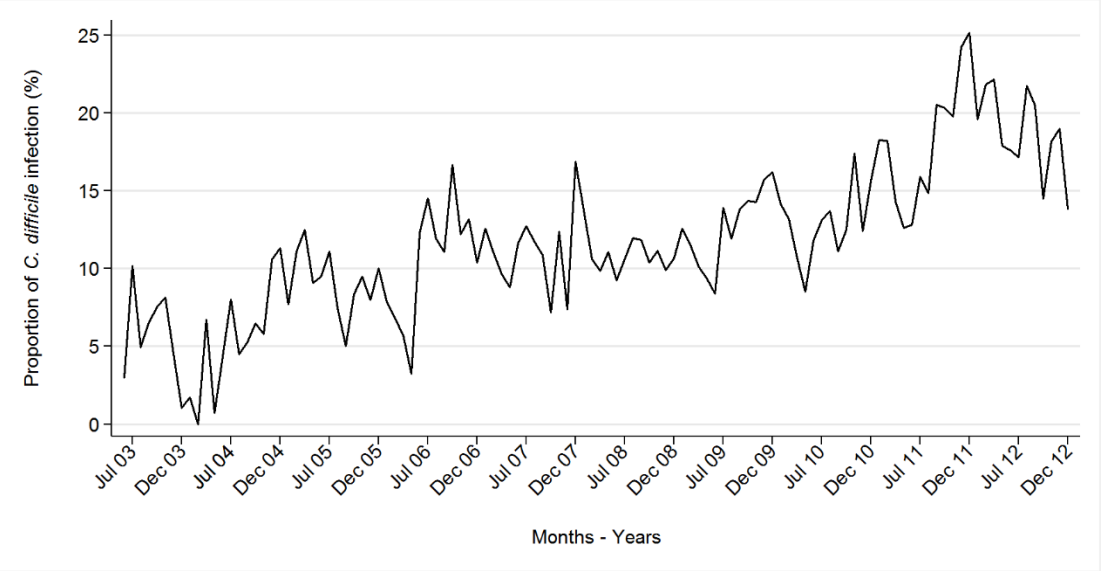

Figure 1.1. Proportion of submitted stool specimens that were positive *C. difficile* in Queensland between 2003 and 2012

In 2009, *C. difficile* ribotype 027 was first isolated in Western Australia from a patient returning from the USA [51] and in the subsequent year, the first autochthonous *C. difficile* ribotype 027 infection was reported in Victoria [52]. However, as opposed to North America and Europe, there is no evidence that *C. difficile* ribotype 027 has become established in Australia. Instead, *C. difficile* ribotype 244 has been reported to be the predominant binary toxin producing strain circulating in Australian hospitals [47, 48, 53]. Because CDI is not a notifiable disease in Australia [54], and few laboratories have the capability to perform *C. difficile* ribotyping, currently it is unknown which are the predominant ribotypes circulating in hospitals and communities and whether *C. difficile* ribotype 027 (or other hypervirulent strains) have been re-introduced. Little is currently known about asymptomatic *C. difficile* colonisation in Australia. This warrants further investigation particularly in the context of asymptomatic patients being a potential source of transmission.

1.2. Research objectives

The aim of this thesis is to gain insight into the components of *C. difficile* epidemiology that are poorly understood (asymptomatic colonisation, CA-CDI and the interaction between symptomatic and asymptomatic patients) and to explore new treatment options and novel risk factors in order to provide evidence that would assist in prevention, control and treatment of *C. difficile*. Using a simplified framework of the pathogenesis model of *C. difficile*, I depict the four main objectives of this thesis in Figure 1.2.

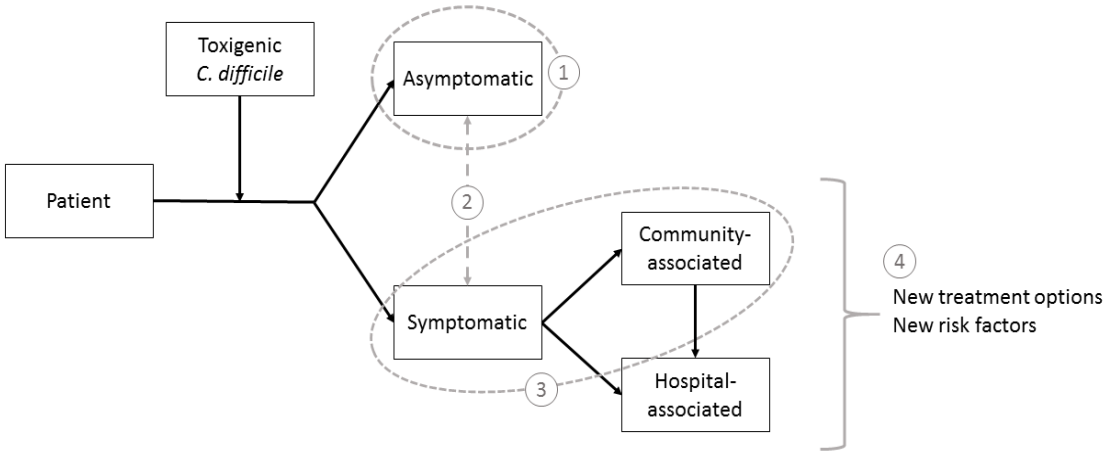

Figure 1.2. Framework of the pathogenesis model for *C. difficile* epidemiology and thesis objectives

[1] To estimate the prevalence of asymptomatic colonised patients in two Australian tertiary hospitals, identify risk factors associated with asymptomatic *C. difficile* colonisation and describe the predominant ribotypes isolated from asymptomatic patients.

[2] To compare *C. difficile* ribotypes from symptomatic HA-CDI, symptomatic CA-CDI and asymptomatic TCD-colonisation in two Australian states/territories and to identify risk factors associated with symptomatic forms of the infection.

[3] To determine the spatio-temporal distribution of CA- and HA-CDI in Queensland, Australia, examine the association between medication exposure at a population-level and CA-CDI cases, and describe the seasonal patterns of CDI worldwide.

[4] To investigate the efficacy of FMT for the treatment of recurrent/relapsing CDI and to quantify the relationship between vitamin D levels and the risk of CDI.

1.3. Thesis structure

This thesis consists of seven Chapters (Figure 1.3). The first Chapter includes a general introduction followed by a systematic review and a meta-analysis. The next four Chapters are a compilation of seven journal papers, and each of these Chapters addresses one of the four main objectives of the thesis. The last Chapter includes a discussion of the key findings.

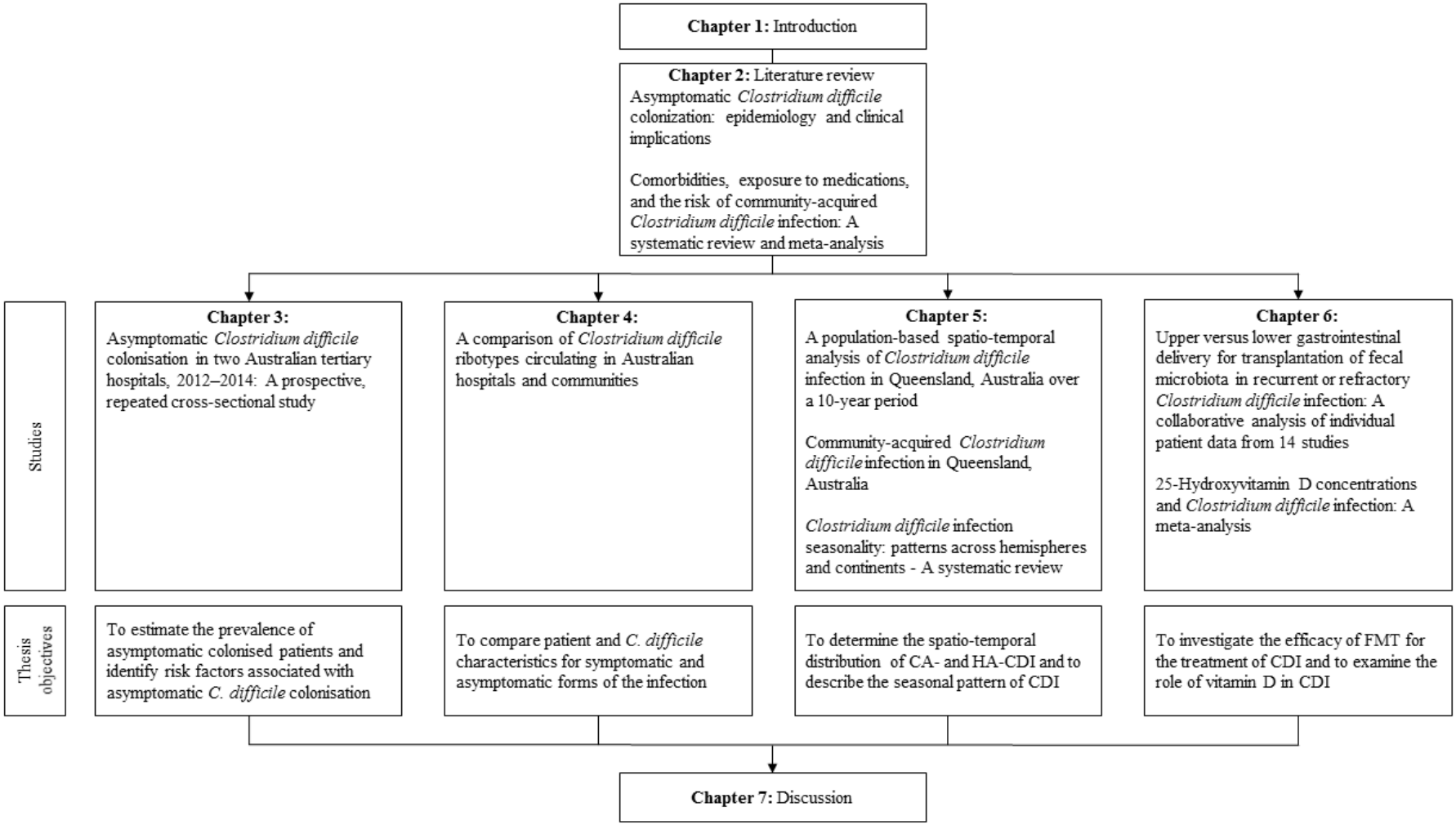

Figure 1.3. Thesis structure

Chapter 1 provides an introduction in which I described important changes in *C. difficile* epidemiology over the past decades. By describing the transmission mechanisms, diagnostic methods, treatment options, and the current status of *C. difficile* in Australia, I highlight which aspects of *C. difficile* epidemiology are currently not well studied and warrant further research. In addition, I provide the research objectives and the thesis structure.

Chapter 2 contains two papers. The first paper is a literature review in which I provided a critical appraisal of the case definitions used for asymptomatic *C. difficile* colonisation; the prevalence of asymptomatic *C. difficile* colonisation in different populations and settings; the role of asymptomatic *C. difficile* colonisation in *C. difficile* transmission; and the host and pathogen factors associated with asymptomatic *C. difficile* colonisation and progression to active disease. The second paper is a systematic literature review and meta-analysis in which I synthesised the current evidence using selected meta-analytical models for an association between commonly prescribed medications and comorbidities with CA-CDI.

Chapter 3 includes one paper that focusses on asymptomatic *C. difficile* colonisation. This paper includes an analysis of primary data from 1,380 asymptomatic patients prospectively enrolled in two Australian tertiary hospitals. In this study, I presented prevalence estimates of asymptomatic TCD- and NTCD-colonisation over three years and compare the variability in prevalence during summer and winter months and over a three-year period. I also reported the predominant toxin profiles and ribotypes isolated from asymptomatic patients and identified patient characteristics associated with TCD- and NTCD-colonisation.

Chapter 4 presents the results of a prospective observational study that examines the relationship between symptomatic patients (HA- and CA-CDI) and asymptomatic TCD colonised patients. In this study I analysed data from 324 patients that were

prospectively enrolled from two tertiary hospitals and two community-based laboratories in Australia over a three-year period. I compared patient characteristics for HA-, CA-CDI and asymptomatic TCD-colonisation and identified risk factors associated with symptomatic disease. I also described the changes and the predominant *C. difficile* ribotypes in the healthcare and community settings over the study period.

Chapter 5 consists of three papers that focus on symptomatic CDI, with an emphasis on CA-CDI and its spatio-temporal distribution. The first two papers are ecological studies in which I built regression models in a Bayesian framework to explore the spatio-temporal distribution of *C. difficile* in Queensland, Australia. First, I presented the spatio-temporal patterns and environmental factors (elevation, rainfall and land surface temperature) associated with HA- and CA-CDI. Then, I reported the association between medication exposure at a population-level and CA-CDI cases. In the third paper, I compiled data from 20 studies and described the global patterns of *C. difficile* seasonality.

Chapter 6 contains two clinical epidemiology papers in which I assessed treatment options and risk factors for CDI. The first paper is an individual patient data analysis of 14 published studies (305 patients) in which I evaluated the most effective delivery route for FMT for refractory and recurrent/relapsing CDI. In the second paper, I conducted a meta-analysis to investigate the association between levels of vitamin D and the risk of CDI.

Chapter 7 presents a general discussion of the main findings, in which I discussed the limitations of the studies, proposed potential future research ideas and provided conclusions and recommendations arising from the studies presented in Chapters 3-6.

1.4. References

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Chapter 2

Literature review

CHAPTER 2. Literature review

2.1. Context

Previous research has focused largely on symptomatic CDI cases occurring in the healthcare setting and often neglected the investigation of asymptomatic *C. difficile* colonisation as well as CA symptomatic cases. The main reasons for exploring these two aspects of *C. difficile* epidemiology (i.e. asymptomatic colonisation and CA-CDI) were that: 1) recent studies have suggested that patients who are asymptomatically colonised by *C. difficile* may have the potential to contribute to transmission of the bacterium in healthcare settings; and 2) there has been a steady increase in incidence of CA-CDI cases that has been reported worldwide in the past decades, including severe forms of the diseases being reported in the community among patients that were previously considered to be at low risk (i.e. young patients without prior exposure to antibiotics and/or healthcare facilities).

The literature review presented in this Chapter is divided into two parts. In the first part I systematically reviewed the literature regarding asymptomatic *C. difficile* colonisation. I described the heterogeneous case definitions used by different authors for asymptomatic colonisation/carriage with *C. difficile* and propose a new case definition. Then, I provided a summary of prevalence estimates of asymptomatic *C. difficile* colonisation in different populations and identified which population may be at higher risk of colonisation. I also summarised the potential role of transmission from asymptomatic colonised patients to uncolonised patients in the hospital. Finally, I compared the host and pathogen risk factors associated with asymptomatic *C. difficile* colonisation and active forms of the disease.

In the second part I meta-analysed the existing evidence of risk factors for CA-CDI. I used a novel meta-analytical method to pool the data from 12 case-control/nested

case-control studies (over 56 000 patients), and identified that exposure to antibiotics and corticosteroids and specific comorbidities (i.e. inflammatory bowel disease, renal failure, haematological cancer and diabetes) were associated with CA-CDI. Given that prescription of medications and comorbidities were not equally distributed across different age groups and geographical locations, I also presented the results of sensitivity analyses by continents and life stages.

2.2. Asymptomatic *C. difficile* colonisation

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Asymptomatic *Clostridium difficile* colonization: epidemiology and clinical implications

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Abstract

Background: The epidemiology of *Clostridium difficile* infection (CDI) has changed over the past decades with the emergence of highly virulent strains. The role of asymptomatic *C. difficile* colonization as part of the clinical spectrum of CDI is complex because many risk factors are common to both disease and asymptomatic states. In this article, we review the role of asymptomatic *C. difficile* colonization in the progression to symptomatic CDI, describe the epidemiology of asymptomatic *C. difficile* colonization, assess the effectiveness of screening and intensive infection control practices for patients at risk of asymptomatic *C. difficile* colonization, and discuss the implications for clinical practice.

Methods: A narrative review was performed in PubMed for articles published from January 1980 to February 2015 using search terms 'Clostridium difficile' and 'colonization' or 'colonisation' or 'carriage'.

Results: There is no clear definition for asymptomatic CDI and the terms carriage and colonization are often used interchangeably. The prevalence of asymptomatic *C. difficile* colonization varies depending on a number of host, pathogen, and environmental factors; current estimates of asymptomatic colonization may be underestimated as stool culture is not practical in a clinical setting.

Conclusions: Asymptomatic *C. difficile* colonization presents challenging concepts in the overall picture of this disease and its management. Individuals who are colonized by the organism may acquire protection from progression to disease, however they also have the potential to contribute to transmission in healthcare settings.

Keywords: *Clostridium difficile*, Carrier state, Asymptomatic, Infection

Background

Clostridium difficile is a Gram-positive, spore-forming, anaerobic bacillus that was first described in 1935 as part of the intestinal flora of newborn infants [1]. *C. difficile* is recognized as one of the most important pathogens in hospital and community healthcare settings, with a steadily rising global incidence of infection and concordant increase in mortality [2, 3]. The Centers for Disease Control and Prevention in the USA have assigned *C. difficile* as an urgent threat because of its association with antibiotic use and high mortality and morbidity [4].

The clinical spectrum of symptomatic *C. difficile* infection (CDI) ranges from mild diarrhea to severe complications such as pseudomembranous colitis, toxic megacolon, bowel perforation, sepsis, and death [5]. Symptomatic CDI is mediated through the production of toxins that are cytotoxic to epithelial cells of the colon, causing extensive inflammation and epithelial tissue damage to the host [6]. These toxins (toxins A and B) are implicated as the major virulence factors of *C. difficile*. An additional putative virulence factor, the binary toxin, is produced by some strains, particularly the more virulent epidemic strains such as BI/NAP1/027, and may also be present in the absence of toxin A or toxin B [7].

Asymptomatic *C. difficile* colonization is the condition where *C. difficile* is detected in the absence of symptoms

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of infection. It has been proposed that asymptomatic *C. difficile* colonized patients may be protected from progression to infection because they can mount a humoral immune response to clostridial toxins [8]. However, asymptomatic *C. difficile* colonized patients potentially act as an infection reservoir and may present a risk to others [9, 10]. The number of colonized patients is higher than symptomatic CDI cases among hospital patients, particularly when disease is endemic [11–13]. The prevalence of asymptomatic *C. difficile* colonization varies depending on a number of host, pathogen, and environmental factors. These features of asymptomatic *C. difficile* colonization are important to establish the contribution that asymptomatic *C. difficile* colonized patients make as potential vehicles of transmission of *C. difficile* in healthcare environments, particularly with the global spread of emergent hypervirulent toxigenic strains [14].

Few studies have synthesized evidence on the role and importance of asymptomatic *C. difficile* colonization in the progression to symptomatic CDI, the transmission of infection, or the challenges to CDI control. Therefore, we have reviewed published literature (Additional file 1) describing asymptomatic *C. difficile* colonization to better understand the prevalence, risk factors for colonization, mechanisms that may protect colonized patients from progression to symptomatic CDI or recurrent disease and the risk asymptomatic *C. difficile* colonized patients pose to non-colonized patients.

Definition of symptomatic *C. difficile* infection and asymptomatic *C. difficile* colonization

It is generally accepted that positive assays for *C. difficile* toxins are indicative of active disease and that the toxins are responsible for clinical symptoms [15, 16]. A validation study comparing reference tests for *C. difficile* (toxin assay positive versus cytotoxigenic *C. difficile* culture positive/toxin assay negative) showed that detection of toxins was associated with more severe CDI outcomes [17]. However, it has also been reported that patients with positive toxin assays can remain symptomless [8, 10, 18]. Therefore, the sole presence of *C. difficile* toxins is insufficient for a diagnosis of the disease. Consequently, symptomatic CDI has been defined as:

- The presence of diarrheal symptoms (three or more unformed stools in 24 or fewer consecutive hours) and either
  - ○ a stool test result positive for *C. difficile* toxins or
  - ○ detection of toxigenic *C. difficile*, or
  - ○ colonoscopic findings demonstrating pseudomembranous colitis [19].

To our knowledge there is no clear definition for asymptomatic CDI and the terms carriage and colonization are often used interchangeably. Table 1 provides case definitions for asymptomatic carriage and colonization identified in this review to illustrate the heterogeneity of the definitions used by different the authors and that both terms have been used without distinction. For the sake of clarity, while maintaining conventions of previous studies, we recommend the following definition for asymptomatic *C. difficile* colonization:

- The absence of diarrhea (or if present, attributable to a cause other than CDI) without colonoscopic or histopathologic findings consistent with pseudomembranous colitis, and either
  - ○ the detection of *C. difficile* or
  - ○ the presence of *C. difficile* toxins.

Table 1 A description of different case definitions for asymptomatic colonization and carriage with *C. difficile*

Term used	Case definition	Study reference
Colonization	Patients with symptomless colonization were defined as symptom-free, excluding patients recovering from *C. difficile* associated diarrhea.	Shim, 1998 [8]
	Asymptomatic *C. difficile* colonization was defined as a positive stool culture for *C. difficile* in the absence of diarrhea.	Loo, 2011 [13]
	A case of toxigenic *C. difficile* colonization was defined as an asymptomatic individual with *tcdB* gene detected in a fecal sample by real-time PCR	Hung, 2012 [109]
	Was not specifically defined and did not distinguish between colonization and infection. One colonized case was symptomatic at sampling time (personal communication).	Arvand, 2012 [30]
Carriage	Asymptomatic carriage was defined as a positive stool culture or cytotoxin test and the absence of diarrhea during hospitalization and during a 30-day period after discharge.	Kyne, 2000 [18]
	Asymptomatic carriage was considered when *C. difficile* or its cytotoxin was detected in stool from persons without gastrointestinal symptoms.	Simor, 1993 [67]
	Carriers were defined as positive for a toxigenic *C. difficile* screening test during the study period in the absence of a clinician ordered toxin screen determined by electronic medical record review. Carriers were categorized as persistent, transient, or indeterminate.	Curry, 2013 [75]

Novel to this definition of asymptomatic *C. difficile* colonization is the acknowledgment that symptoms associated with CDI can arise from alternative underlying conditions. Diarrhea commonly affects hospitalized patients and in the majority of the cases is attributable to non-infectious (e.g. medication side-effects, inflammatory bowel disease) and infectious causes other than CDI [20]. The proportion of cases of nosocomial diarrhea attributable to CDI may be within the range of 20 to 25 % [21, 22]. Identification of the etiology of diarrhea (or even to rule out *C. difficile*) could be challenging, particularly in critically ill patients. In cases where the underlying cause(s) of diarrhea cannot be identified (or CDI remains as a differential diagnosis), we suggest the use of algorithms such as the one proposed by Polage and colleagues [20]. They suggested that regardless of their antibiotic exposure status, CDI should be considered in all patients with clinically significant diarrhea. The evaluation of a patient should start by verifying the presence of diarrhea; the frequency, consistency, volume of stool, and duration of diarrhea should be taken into account along with associated symptoms/signs such as cramping, dehydration, fever, hypotension, or sepsis. If no clear infectious cause is identified, the medical history must be reviewed for non-infectious or iatrogenic (e.g. laxative overdose) causes.

There is no evidence that non-toxigenic *C. difficile* strains can cause disease [23]. In studies reporting CDI from patients harboring non-toxigenic strains, the cultured organisms were mixed with toxigenic stains and could not definitively be associated with disease [24, 25]. Hence, individuals with diarrhea who test positive only for non-toxigenic strains of *C. difficile* should be considered asymptomatically colonized unless there is supporting evidence of disease, such as endoscopic findings consistent with pseudomembranous colitis. In addition, colonization can be transient or long term often depending on the extent and frequency of exposure to *C. difficile.*

Epidemiology

Prevalence estimates of asymptomatic *C. difficile* colonization vary considerably between different patient groups (Table 2). Among healthy adults with no prior risk factors for CDI, asymptomatic *C. difficile* colonization prevalence varied between 0 and 15 % [15, 26–33]. The study reporting 15 % was a prospective cohort study carried out on seven groups of healthy individuals representing various occupations in Japan [32]. The range of asymptomatic *C. difficile* colonization prevalence among groups of study subjects was 4 to 15 %; the groups comprised university students, hospital workers, company employees, and defense force personnel. Among healthy newborns and infants, the prevalence of asymptomatic *C. difficile* colonization varied between 18 and 90 % [15, 34].

Few studies have examined asymptomatic *C. difficile* colonization in acute hospital care settings. In 1982, Gerding and colleagues detected 43/146 (29 %) patients colonized with non-toxigenic *C. difficile* strains [22]. Over the course of 10 years (1982–1991), Belmares and colleagues reported overall colonization with non-toxigenic strains in 10 % of the patients (ranged from 5 % in 1982 to 18 % in 1984) [35]. Most studies reporting asymptomatic *C. difficile* colonization have targeted elderly patients in dedicated long-term care facilities (LTCFs). Prevalence of asymptomatic *C. difficile* colonization among elderly residents ranged from 0 to 51 %, possibly because CDI is often endemic in units or institutions with elderly patients [9, 30, 36, 37].

Among adults, the highest prevalence of asymptomatic *C. difficile* colonization has been reported in patients with

Table 2 Prevalence of asymptomatic *C. difficile* colonization in different populations

Population type		Range of carriage rates	References
Healthy neonates and infants		18–90 %	[34, 110–113]
Healthy adults – general population		0–15 %	[15, 26–33]
Elderly in long-term care facilities, chronic care, or nursing homes		0–51 %	[9, 30, 37, 66, 67, 70, 114–116]
Hospital	*Elderly*	0.6–15 %	[26, 68, 69, 114, 117, 118]
	Inpatients (not specifically elderly)	4–29 %	[10, 13, 18, 22, 73, 79, 91, 105, 106, 109, 119–121]
	Rehabilitation (spinal)	11–50 %	[43, 45]
	HIV	4 %	[122]
	Healthcare workers	0–13 %	[26, 32, 123]
	Cystic fibrosis	18–47 %	[38–41]
	Hospital surgical patients on antibiotic prophylaxis	17 %	[124]
	Intensive care	7 %	[125]
	IBD (ulcerative colitis or Crohn's disease)	11 %	[95]
	Hematological malignancies	8 %	[94]

cystic fibrosis (CF) and in spinal/brain injury rehabilitation. Asymptomatic *C. difficile* colonization prevalence ranged from 18 to 47 % in studies among CF patients, substantially higher than other clinical subgroups (e.g. surgical patients) or general hospital inpatients [38–42]. In a case–control study, Bauer and colleagues found 26/55 (47 %) CF patients were asymptomatically colonized [38]. Yahav and colleagues reported 14 toxin-positive asymptomatic *C. difficile* colonized patients without evidence of diarrhea in a study of 30 CF patients compared to no toxin-positive individuals among non-CF patients [41]. Welkon and colleagues reported asymptomatic *C. difficile* colonization in 19/99 CF patients (19 %), with 12 strains being toxigenic [40]. Another study of CF patients reported asymptomatic *C. difficile* colonization in 12/37 (32 %) patients, rising to 43 % if patients were treated with antibiotics [39]. The heightened vulnerability of CF patients to asymptomatic *C. difficile* colonization rather than to disease has been attributed to an electrolyte transport defect in epithelial cells that may offer protection from the effects of clostridial toxins [41].

Rehabilitation patients also had higher asymptomatic *C. difficile* colonization prevalence than other groups. In one study, 11/22 (50 %) spinal cord rehabilitation patients were colonized and remained asymptomatic [43]. The asymptomatic *C. difficile* colonized patients in this study also had a significantly greater length of stay (median 57 days) compared to non-colonized patients (median 6 days). Stevens and colleagues found that for 7-day increments in length of stay, the risk of healthcare-associated CDI increased by 10 % [44]; this implies that on average, spinal cord rehabilitation asymptomatic *C. difficile* colonized patients will be at 52 % increased risk of developing CDI compared to non-colonized *C. difficile* patients. Another study of asymptomatic *C. difficile* colonization prevalence on admission to a rehabilitation ward reported that 9/54 (17 %) patients without prior colonization became colonized after admission [45]. Of these nine patients, six showed no symptoms of diarrhea. The increased colonization rate among this group of patients is thought to result from the rehabilitation therapy where group activities and socialization are encouraged, facilitating transmission.

Mechanism of colonization with C. difficile

The first stage in asymptomatic *C. difficile* colonization is the ingestion of *C. difficile* spores [46–48]. The spores survive the gastric acid and germinate into vegetative cells in the anaerobic environment of the colon. *C. difficile* has been isolated from samples of human jejunum, however the primary reservoir is the large intestine [49]. Vegetative *C. difficile* cells penetrate the mucus layer in the large intestine using flagella and enzymatic degradation of the colonic extracellular matrix [48]. Once the mucosal layer has been breached, in vitro assays have demonstrated that adhesion of *C. difficile* cells to intestinal epithelial cells is facilitated by bacterial surface layer proteins [50].

For colonization with vegetative *C. difficile* cells to occur, there must be a disruption of the normal intestinal microbiota which usually provides colonization resistance against *C. difficile* [51, 52]. The inhibitive effect of the natural gut microbiota may occur through competition for space and nutrients or the production of compounds that inhibit *C. difficile* proliferation [53]. The concept of colonization resistance is important to understand the mechanisms that result in the development of disease. Therefore, there is potential to introduce non-pathogenic organisms as probiotic agents or non-toxigenic *C. difficile* strains to compete with toxigenic *C. difficile* strains as novel prevention and treatment strategies [54, 55]. However, Brouwer and colleagues have challenged this concept as they found that transconjugation of the pathogenicity locus can occur from toxigenic to non-toxigenic *C. difficile* strains [56].

Toxin production and asymptomatic colonization

Secretion of toxins A and B usually occurs once *C. difficile* reaches the stationary phase. The first essential step for these toxins to exert their effects is binding to receptors on gut epithelial cells [6]. Disease symptoms commence with toxin catalysis in the cytosol. The catalyzed toxin products inactivate guanosine triphosphate binding Rho proteins [6]. The subsequent depolymerization of the actin cytoskeleton elicits a cellular response that includes neutrophil infiltration, resulting in inflammation, and the subsequent release of cytokines and interferon gamma [57, 58]. Cell death occurs by apoptosis following disaggregation of the actin cytoskeleton [59]. Consequently, extensive colonic inflammation and epithelial tissue damage occur, leading to rapid fluid loss into the large intestine, manifesting as acute diarrhea [6].

The role and importance of toxins A and B in progression to the disease state has been subject to debate. In early studies using hamster models, purified toxin A was shown to elicit symptoms consistent with disease, whereas toxin B would only elicit a response if co-administered with toxin A [60]. Consequently, it was suggested that toxin B exerted an effect following initial tissue damage by toxin A. The recovery of toxin A-negative, toxin B-positive strains from symptomatic patients has challenged the view that toxin A is the dominant virulence factor in CDI [61, 62]. Recent work with animal models using antibodies against toxins A and B showed that administration of anti-toxin B antibodies either alone or in combination with anti-toxin A was more effective at preventing the development of gastrointestinal symptoms consistent with CDI [63]. Lyras and colleagues constructed

mutant isogenic strains of *C. difficile* capable of producing either toxin A or toxin B. The toxin A producing strains lost their pathogenicity whereas the toxin B producing stains were as pathogenic in animal models as wild type strains [64]. However, another group using similar gene knockout methods to generate mutant strains produced conflicting findings with a role for both toxins A and B [65].

Toxigenic strains of *C. difficile* are the most prevalent among colonized patients; early studies cultured stool specimens and using enzyme immunoassay (EIA) or cell culture cytotoxicity neutralization assay reported the proportion of toxigenic strains among asymptomatic colonized patients was in excess of 50 % [31, 39, 40, 66–69]. These findings have been corroborated in later studies using real-time polymerase chain reaction (PCR) [27, 29, 30, 32, 70]. It is important to note that both EIA and PCR methods specifically target toxigenic *C. difficile* strains and could therefore bias results reporting a higher prevalence of these strains [71].

Duration of the colonized state

There is limited information about the duration over which individuals remain asymptomatic after coming in contact with *C. difficile* spores or the time taken to revert to a non-colonized state. In a randomized placebo-controlled trial, Johnson and colleagues compared the efficacy of vancomycin and metronidazole for eradication of *C. difficile* in asymptomatic colonized patients. Sixty, 80 and 100 % of the patients in the placebo group were negative for *C. difficile* after 40, 70 and >90 days follow-up, respectively [72]. In a prospective study, Samore and colleagues [73] compared the incidence of colonization in surgical, medical and intensive care wards. Thirty two colonized patients were followed on a weekly basis until they were discharged; 84 % of the colonized patients remained culture positive with median duration of colonization of 8.5 days (range 7–29 days). The study also showed that 3/20 (15 %) of the patients colonized with non-toxigenic strains, none of whom developed diarrhea, were positive for toxigenic strains at follow-up. Longer-term colonization and transmission was investigated among 1234 healthy Japanese volunteers, who included university students, hospital staff, and company employees [32]. Follow-up was performed on 38 asymptomatic patients between 5 and 7 months later. Of these 38 cases, *C. difficile* was re-isolated from 12 (32 %) individuals, half of whom yielded the same PCR ribotypes and pulsed-field gel electrophoresis types as previously. In a subsequent study by the same authors, a 6-month follow-up of 18 colonized healthy students found 10 (56 %) were no longer colonized and 8 (44 %) were colonized more than once, of whom 3 (38 %) harbored the same strain [27].

These findings suggest that there is marked variation in duration of the colonized state, however the role of repeated exposure from the environment or other colonized individuals was not investigated. Limited longitudinal data available about asymptomatic *C. difficile* colonization warrants further epidemiological studies to investigate the persistence of colonization and to understand the role of re-exposure to the organism over time.

Transmission from colonized patients

Person-to-person transmission in hospital wards, environmental contamination, and carriage of *C. difficile* on the hands of healthcare workers have been described extensively [74–77]. The main modes of transmission are by the fecal-oral route and direct contact with contaminated surfaces and fomites [78], although transmission between healthy individuals who are asymptomatically colonized has also been reported [32].

Spores from asymptomatically colonized patients are a potential source of CDI and may contribute to the transmission reservoir [9] and studies have clearly demonstrated that transmission from asymptomatically colonized patients can occur [75, 79]. Curry and colleagues investigated transmission potential of asymptomatic *C. difficile* colonized patients using multiple-locus variable number tandem repeat analysis. They found that 29 % of isolates from hospital-associated CDI cases were highly related to isolates from asymptomatic *C. difficile* colonized patients [75]. Clabots and colleagues reported that patients admitted from home without prior hospitalization in the previous month had the lowest prevalence of asymptomatic *C. difficile* colonization (6 %) but, because they represent the majority of admissions, they contributed the second-highest total number of *C. difficile* introductions to hospital, after patients readmitted to hospital within 30 days [79]. Similarly, the length of stay in hospital can also influence transmission. Fecal excretion of *C. difficile* spores occurs for up to 6 weeks following resolution of CDI symptoms [80, 81]. Furthermore, Riggs and colleagues demonstrated that even colonized patients who did not develop disease during a 6 months follow-up period were shedding spores into the environment [9]. The current CDI clinical practice guidelines from the Society of Healthcare Epidemiologists of America (SHEA) recommend maintaining contact precautions only until resolution of diarrhea. It has been suggested that contact precautions should be extended until time of discharge for patients recovering from CDI. However, there is no conclusive evidence to support extending contact precautions following CDI while patients remain asymptomatic during their hospital stay [81].

Asymptomatic *C. difficile* colonized patients in hospital have the potential to contaminate the environment and subsequently infect others [75]; however the transmission potential is lower in asymptomatic *C. difficile*

colonized patients than in those patients with active disease [10]. In one prospective study of acquisition rates in an endemic CDI setting, 38/128 (29 %) environmental samples from hospital rooms occupied by asymptomatic *C. difficile* colonized patients were contaminated compared to 90/128 (49 %) samples from rooms occupied by patients with disease. This corresponds with findings from another study of LTCF residents in which proportions of positive cultures from skin sites and environmental samples were highest among residents with disease, second highest among asymptomatic *C. difficile* colonized patients and lowest among non-colonized residents [9]. Moreover, Sethi and colleagues found that even 4 weeks after receiving therapy for CDI, the frequency of skin contamination (30/52; 58 %) and environmental shedding (26/52; 50 %) remained high in asymptomatic *C. difficile* colonized patients [81]. Samore and colleagues demonstrated that in an endemic situation carriage of *C. difficile* on the hands of healthcare workers was positively correlated with the extent of environmental contamination with *C. difficile* [82].

The spore-forming ability of *C. difficile* makes it distinct from other infectious organisms common to healthcare settings and introduces further challenges to reduce transmission. Spores can persist in the environment for long periods and require chlorine- [83] or peroxide-based [84] sporicidal agents or ultraviolet radiation devices [85] for environmental decontamination. Typically, hospital patients colonized with other multidrug-resistant organisms are isolated to prevent transmission, but this appears to be of limited value for asymptomatic *C. difficile* colonization. In an epidemiological model, Lanzas and colleagues demonstrated that transmission of *C. difficile* within a ward cannot be sustained unless new *C. difficile* colonized patients are introduced [86]. Therefore, the admission of asymptomatic *C. difficile* colonized patients plays an important role in sustaining *C. difficile* transmission within a ward [87]. A recent study, has demonstrated that nearly half of the *C. difficile* cases were genetically distinct from all previous cases, which suggests genetically diverse sources of infection [88]. Furthermore, Yakob and colleagues demonstrated, using a stochastic mathematical model, that screening for asymptomatic *C. difficile* colonization to segregate colonized patients from non-colonized patients had little impact on infection transmission because patients still in a latent period (exposed but not yet colonized) would not be detected [89].

Risk factors for asymptomatic *C. difficile* colonization and progression to active disease

Among inpatients with positive stool samples for *C. difficile*, McFarland and colleagues found that 52/83 (63 %) of the patients were asymptomatic and 31/83 (37 %) developed symptoms of CDI [10]. Currently, the time required to progress from asymptomatic *C. difficile* colonization to active CDI is unknown; however, epidemiological studies have identified risk factors associated with progression to disease. It is not surprising to find common risk factors for asymptomatic *C. difficile* colonization and disease because colonization with *C. difficile* is a necessary prerequisite of disease. The most significant epidemiological study to date to investigate risk factors for healthcare-associated asymptomatic *C. difficile* colonization identified that hospitalization within the last 12 months, exposure to corticosteroids, history of CDI and presence of antibody against toxin B were significantly associated with healthcare-associated asymptomatic *C. difficile* colonization [90]. Similar findings were described by Loo and colleagues in 2011, they identified chemotherapy, recent hospitalization, use of proton-pump inhibitors or histamine H2 antagonists, and presence of antibodies against toxin B were associated with healthcare-associated asymptomatic *C. difficile* colonization [13]. The study also found that antibiotic exposure (within 8 weeks of hospitalization) was as a risk factor for healthcare-associated CDI (OR 5.25, 95 % CI 2.15–12.82) but not for healthcare-associated asymptomatic *C. difficile* colonization (OR 1.04, 95 % CI 0.61–1.78). The apparent discrepancy between the results may indicate that disruption of the intrinsic intestinal microbiota due to antibiotic exposure is not a key feature for *C. difficile* colonization as it is for progression to disease. More recently, an investigation conducted in a tertiary care facility identified recent hospitalization, chronic dialysis, and corticosteroid use as independent risk factors for toxigenic asymptomatic *C. difficile* colonization on admission [91]. The eligible patients' first stool samples after admission were tested for toxigenic *C. difficile* by real-time PCR assay. While the study had limited generalizability, because the subjects who participated in the study were predominantly older (mean age 64 years), and due to the low proportion of enrolled subjects who provided samples (22 %), results were consistent with a previous study that reported renal disease, prior hospital admission, and prior CDI as risk factors for culture positivity on admission [73].

There are limited data about risk factors for asymptomatic *C. difficile* colonization among healthy populations. McNamara and colleagues investigated environmental factors associated with an increased risk of asymptomatic *C. difficile* colonization in a cohort of healthy farm workers. They found that reported weekly exposure to lake or pond swimming was associated with asymptomatic *C. difficile* colonization [29]; although, no biological plausible explanations were given for this finding by the authors. A number of factors act in concert before asymptomatic *C. difficile* colonization progresses to active disease. These factors can be categorized as host mediated or pathogen

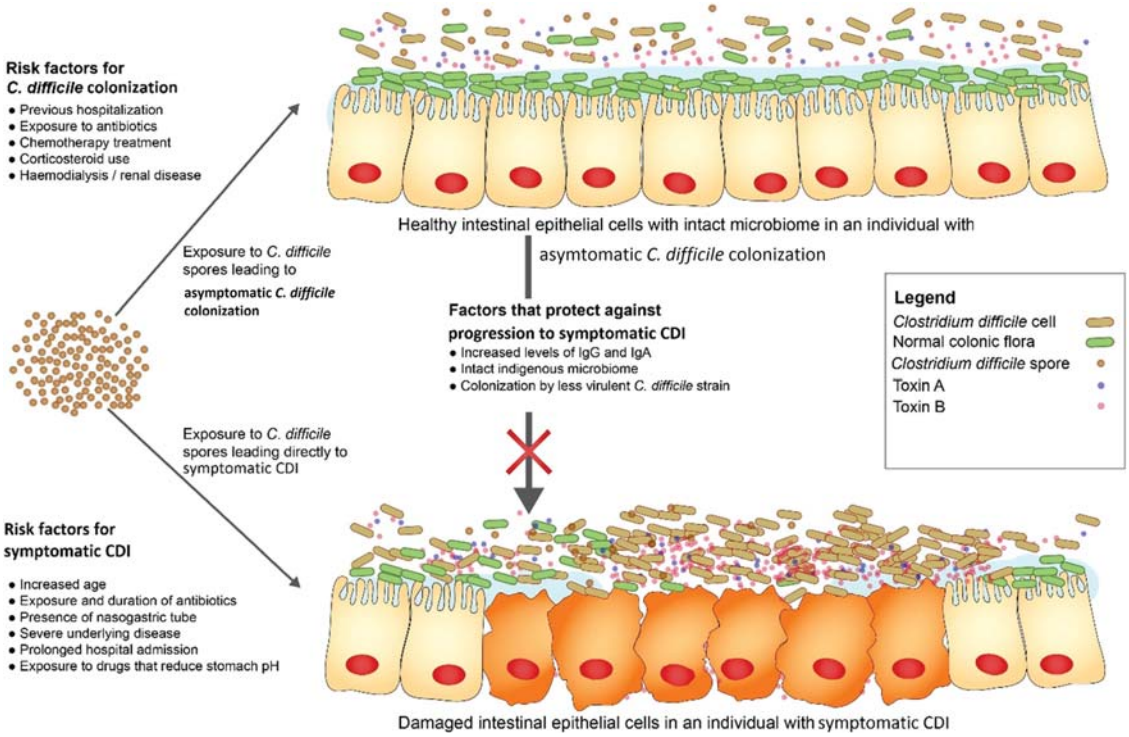

Fig. 1 Diagrammatic representation of asymptomatic *C. difficile* colonization and progression to symptomatic *C. difficile* infection (CDI). Following exposure to *C. difficile* spores, an individual may transiently or persistently harbor the organism without expressing typical CDI symptoms. In other situations ingestion of *C. difficile* spores or vegetative cells may lead directly to symptomatic CDI. Some individuals with asymptomatic *C. difficile* colonization may progress to symptomatic CDI, however a number of host and pathogen related factors can limit the progression of the disease

related. A diagrammatic representation of the mechanism of asymptomatic *C. difficile* colonization and progression to disease with risk factors is shown in Fig. 1.

Host-mediated factors

The most significant factor that leads to CDI is the disruption of intrinsic colonization resistance. This is a feature of the human intestine whereby indigenous microbiota, and the presence of compounds that inhibit bacterial germination and proliferation protect individuals against diseases caused by pathogenic organisms [54]. Factors that disrupt the intestinal microbiota thereby allowing *C. difficile* to flourish include treatment with antibiotics, proton-pump inhibitors, and chemotherapy agents in addition to physical effects of abdominal surgery and nasogastric tubes [13, 92].

Other host factors associated with an increased risk of CDI include advanced age, multiple comorbidities, suppressed immune system, inflammatory bowel disease and dense intestinal co-colonization with enterococci [27, 31, 69, 93–95]. It is worth pointing out that the observed association between advanced age and multiple comorbidities infection, and the increased risk of CDI, may be confounded by medication exposure given that polypharmacy is common among these groups of patients.

There is substantial evidence that asymptomatic *C. difficile* colonization has a protective effect against progression to disease through an immune-mediated response. In a prospective study of hospital patients showed that at the time of colonization, IgG levels were higher in asymptomatic *C. difficile* colonized patients compared to patients who subsequently developed diarrhea [18]. The same authors demonstrated that patients with a single episode of diarrhea had increased IgM levels against toxins A, B and non-toxin antigens compared to patients with recurrent disease, indicating that the presence of these antibodies conferred a protective effect [96]. Many healthy children and approximately 60 % of adults have detectable serum IgG and IgA antibodies to *C. difficile* toxins A and B, even when the organism is not detected [97, 98]. If antibodies are stimulated during infancy and through further exposure to *C. difficile* from the environment [99], it would suggest that protection against CDI is a dynamic host-mediated characteristic [18, 100]. The control of toxin-induced intestinal inflammation by

up-regulation of A_{2B} adenosine receptors in the intestinal epithelium can also reduce the progression of aggressive symptoms of disease [101]. In this study, an A_{2B} adenosine receptor antagonist did not reduce fecal toxin levels in animal models but conferred protection against progression of disease.

Pathogen factors

Colonization with non-toxigenic strains of *C. difficile* can offer protection against infection, suggesting a possible colonization resistance role through competition for nutrients or access to mucosal epithelial cells [55, 102]. Competition between clostridial strains may reduce the proliferation of pathogenic strains and the onset of disease symptoms [103]. Initial speculation was that toxigenic *C. difficile* strains may be in the minority among asymptomatic *C. difficile* colonized patients [104]; however, it has since been shown that the majority of strains are toxigenic.

Discussion and conclusion

Despite technological advances in *C. difficile* microbiology and epidemiology (e.g. genotyping), asymptomatic *C. difficile* colonization remains as a complex and challenging health problem as its epidemiological features vary considerably between study groups and settings. Several gaps in the current knowledge were identified in this review that should guide future research studies:

1. There is no consistent definition for asymptomatic *C. difficile* colonization; a standard definition across studies is urgently needed.
2. The time between acquisition of *C. difficile* and symptomatic disease is unknown but has been estimated to be between 1 and 2 weeks [8, 13, 105]. It has also been suggested that progression to disease happens within this short time after acquisition or does not occur at all [73].
3. Asymptomatic *C. difficile* colonized patients serve as a potential infection reservoir of horizontal transmission of *C. difficile* in a range of healthcare settings and the strain types isolated from patients with asymptomatic *C. difficile* colonization are predominantly toxigenic [9, 27, 30, 32, 40, 66, 70, 73, 91, 106]. However, whether the clinical outcomes differ in asymptomatic patients colonized with toxigenic *C. difficile* compared to non-toxigenic strains it is currently unknown; thus, we suggest that patients with diarrheal symptoms with non-toxigenic strains of *C. difficile* should be considered colonized unless there is definitive evidence of disease.
4. Estimates of asymptomatic colonization may be underestimated as stool culture is not practical in a clinical setting; however, this constitutes important future epidemiological study.

The current SHEA guidelines for CDI recommend that active screening for asymptomatic *C. difficile* colonization is not performed for infection control purposes [19]. Polage and colleagues retrospectively reviewed 6121 records of toxin negative patients and revealed that only one (0.02 %) had a laboratory confirmed complication of CDI. We emphasize that this recommendation for asymptomatic *C. difficile* colonization is still valid for the following important reasons: first, there are limited options to manage asymptomatic *C. difficile* colonized patients - they should not be treated because antimicrobial therapy does not eradicate spores [19, 72]; moreover treatment may render patients more susceptible to symptomatic CDI [107]; and second, asymptomatic *C. difficile* colonization might protect individuals from progressing to active diseases [8].

Given the transmission potential of asymptomatic *C. difficile* colonized patients, the increased prevalence among certain clinical groups, limited management options, and the limited utility of screening, we suggest a more pragmatic approach. Intensive infection control practices, normally reserved for diseased patients, should be targeted at individuals or clinical areas with higher risk of asymptomatic *C. difficile* colonization. For example, patient or unit-level risk assessments could target enhanced environmental cleaning and use of gloves for patient contact to limit the transmission of *C. difficile* from asymptomatic *C. difficile* colonized patients [108]. Empirical research should be conducted into the impact of targeted, risk-based, intensive infection control programs before changes to the current SHEA guidelines for asymptomatic *C. difficile* colonized patients are considered.

Additional file

Additional file 1: Search strategy and selection criteria.
(DOCX 100 kb)

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

The idea for this study was conceived by AC. The search was performed by LFK and JM. LFK and JM drafted the original manuscript. LY, TR, DP, NF, CH, AC revised the manuscript and provided input. LFK, JM, LY, TR, DP, NF, CH, AC read and approved the final manuscript.

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2.3. Risk factors for community-associated *C. difficile* infection

Furuya-Kanamori L, Stone JC, Clark J, McKenzie SJ, Yakob L, Paterson DL, Riley TV, Doi SA, Clements AC. Comorbidities, exposure to medications, and the risk of community-acquired *Clostridium difficile* infection: A systematic review and meta-analysis. *Infect Control Hosp Epidemiol* 2015;36:132-41.

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ORIGINAL ARTICLE

Comorbidities, Exposure to Medications, and the Risk of Community-Acquired *Clostridium difficile* Infection: A Systematic Review and Meta-analysis

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BACKGROUND. *Clostridium difficile* infection (CDI) has been extensively described in healthcare settings; however, risk factors associated with community-acquired (CA) CDI remain uncertain. This study aimed to synthesize the current evidence for an association between commonly prescribed medications and comorbidities with CA-CDI.

METHODS. A systematic search was conducted in 5 electronic databases for epidemiologic studies that examined the association between the presence of comorbidities and exposure to medications with the risk of CA-CDI. Pooled odds ratios were estimated using 3 meta-analytic methods. Subgroup analyses by location of studies and by life stages were conducted.

RESULTS. Twelve publications (n = 56,776 patients) met inclusion criteria. Antimicrobial (odds ratio, 6.18; 95% CI, 3.80–10.04) and corticosteroid (1.81; 1.15–2.84) exposure were associated with increased risk of CA-CDI. Among the comorbidities, inflammatory bowel disease (odds ratio, 3.72; 95% CI, 1.52–9.12), renal failure (2.64; 1.23–5.68), hematologic cancer (1.75; 1.02–5.68), and diabetes mellitus (1.15; 1.05–1.27) were associated with CA-CDI. By location, antimicrobial exposure was associated with a higher risk of CA-CDI in the United States, whereas proton-pump inhibitor exposure was associated with a higher risk in Europe. By life stages, the risk of CA-CDI associated with antimicrobial exposure greatly increased in adults older than 65 years.

CONCLUSIONS. Antimicrobial exposure was the strongest risk factor associated with CA-CDI. Further studies are required to investigate the risk of CA-CDI associated with medications commonly prescribed in the community. Patients with diarrhea who have inflammatory bowel disease, renal failure, hematologic cancer, or diabetes are appropriate populations for interventional studies of screening.

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INTRODUCTION

Although the previous literature has focused largely on healthcare-associated (HA) *Clostridium difficile* infection (CDI), the incidence, prevalence, and severity of community-acquired (CA) CDI has also increased.[1] Kuntz et al[2] reported similar incidence rates for CA-CDI (11.2 cases/100,000 person-years) and HA-CDI (12.1 cases/100,000 person-years) in the United States. Moreover, the emergence of "hypervirulent" strains of *C. difficile* in the community among patients previously considered to be at low risk of CDI (ie, young adults without antimicrobial exposure) clearly shows that the epidemiology of CDI is changing and that CDI is no longer exclusively a nosocomial infection, as it was previously considered.[1] It seems that the risk profile of patients from the community points more to increased numbers of younger patients without comorbidities, whereas in the hospital setting, elderly inpatients with multiple morbidities and exposed to polypharmacy remain most at risk.

Research, including through meta-analysis, has attempted to describe the risk of CDI specifically in the community setting and found that clindamycin, fluoroquinolones, cephalosporins, macrolides, penicillins, and sulphonamides/trimethoprim are associated with an increased CA-CDI risk.[3,4] The evidence, however, remains uncertain because these meta-analyses used the random effects (RE) model, which has been questioned for its overconfident results.[5] Exposure to gastric-acid suppressive drugs[6–11] and the presence of comorbidities[12–14] are associated with an increased risk of HA-CDI; but as with antimicrobials, the

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evidence remains inconclusive in the community setting. Therefore, the current meta-analysis was undertaken to pool the evidence from observational studies so that the magnitude and direction of the association between commonly prescribed medications and comorbidities with CA-CDI can be documented.

METHODS

Search Methodology

A systematic search was undertaken in 5 medical and life sciences databases (PubMed, Embase, Cochrane CENTRAL, Cumulative Index to Nursing and Allied Health Literature [CINAHL], and Scopus) from their inception to March 1, 2014 (Appendix 1). A related citation search was also performed; by combining the systematic search with the first 20 studies from the related citation search of selected articles in PubMed, a comprehensive evaluation of the published evidence can be achieved.[15]

Eligibility Criteria

The inclusion of studies was restricted to human studies, full-text articles written in English, studies reporting CA-CDI, and data presented in an extractable format. Conference presentations and abstracts, studies that exclusively compared CA-CDI with HA-CDI, and studies that presented data in a nonextractable format (ie, graphical representations) were excluded. Exclusions were also made for studies that investigated specific groups (ie, patients with human immunodeficiency virus or cirrhosis) because these were not considered representative of the general population.

Study Selection and Data Extraction

Two authors (L.F.-K. and J.C.S.) independently evaluated all the citations by titles and abstracts for studies that met the eligibility criteria. Full-text version articles of all potentially relevant studies were retrieved and independently assessed for eligibility. Data from the included studies were then independently extracted using a predefined tool (Appendix 2) and summarized in a spreadsheet by the same 2 authors. Extracted data were cross-checked by the 2 authors, and discrepancies during the selection of studies or data extraction were resolved through discussion and consensus following independent evaluation by another author (S.A.R.D.).

Quality Assessment

The quality of each study was assessed using a modified version of the Newcastle-Ottawa quality assessment scale for case-control studies. The modified scale assessed whether 7 safeguards against bias had been undertaken by the authors: (1) definition of cases and methods employed for *C. difficile* diagnosis, (2) selection of CA infection, (3) control definition and the method used to rule out *C. difficile*, (4) selection of controls from the community, (5) analysis adjusted for confounders, (6) method used for ascertainment of exposure, and (7) same method used to ascertain exposure for cases and controls. The quality criteria were combined into a univariate score as outlined in Table 1. The quality score was rescaled between zero and 1 (called *Qi*); this was done by summing the points of each component (maximum sum = 17) and dividing it by the highest sum obtained by a study within the meta-analysis, ensuring that the best quality study always had a *Qi* of 1.

Statistical Analyses

The outcome measure was the odds ratio (OR) for the association of CA-CDI with exposure to risk factors, such as antimicrobial drugs, gastric acid suppressant drugs (proton-pump inhibitors [PPI] and histamine-2-receptor antagonists), nonsteroidal anti-inflammatory drugs, aspirin, steroids, and the presence of comorbidities. The OR was pooled using 3 meta-analytic models. This was justified because some have expressed skepticism regarding the appropriateness of the conventional RE model[16] owing to its documented underestimation of the statistical error, which leads to overconfident results.[5,17–19] The other 2 models that were used were the quality effects (QE) model [20–21] and a novel method, the inverse variance heterogeneity (IVhet) model.[22] The QE model uses the *Qi* to redistribute the inverse variance weights in favor of the studies with higher methodologic quality and thus studies that provided higher quality of evidence contributed with a higher weighting towards the overall effect size.[21] This use of quality information via a univariate score does not imply that quality deficiencies can quantify bias. Rather, the quality score is used to rank studies by methodologic rigor and this rank is then linked with a synthetic bias variance that is added to the random error variance.[20] The other model used was the IVhet model that does not require input of quality information and so is less rigorous than the QE model.[22] Both of the latter models use a quasi-likelihood-based variance structure without distributional assumptions and thus have coverage probabilities for the confidence interval (CI) well above the nominal level.[22] The reported results are based on the IV het model; results using the QE and RE models have been presented for comparative purposes.

Statistically significant heterogeneity was defined as tau-squared statistic (τ^2) > 0, Cochran's Q test $P < .1$, or I^2 index >0%. A sensitivity analysis was conducted to determine the degree to which the findings vary depending on the geographic location where the studies were conducted (America or Europe) and life stages of the participants (children aged <2 years, children and adults, adults, or adults aged >65 years).

The *Doi* plots were used to evaluate the presence of publication bias, which plots the lnOR against the absolute value of the z-score for each study.[23] Funnel plots were not reported because the graphical assessment of publication bias requires at least 10 studies and even then can be difficult to interpret.[24]

The results of the analyses were considered statistically significant if the 95% CI did not include zero. Analyses were conducted using MetaXL, version 2.0 (EpiGear International).

TABLE 1. Modified Newcastle-Ottawa Quality Assessment Scale for Case-Control Studies Included in the Meta-analysis

Author, publication year	Definition of cases[a]	Case selection for community-acquired infection[b]	Definition of controls[c]	Control selection[d]	Analysis adjusted for confounders[e]	Ascertainment of exposure[f]	Method of ascertainment of exposure for cases and controls[g]	Total score (points)	*Qi* (total score/13)
Dial et al 2005[25]	1	1	1	2	2	3	1	11	0.85
Dial et al 2006[27]	0	1	0	2	2	3	1	9	0.69
Dial et al 2008[46]	1	1	1	1	3	3	1	11	0.85
Kuntz et al 2011[2]	1	2	1	2	3	3	1	13	1.00
Kutty et al 2010[30]	2	2	2	1	1	3	0	11	0.85
Lowe et al 2006[32]	1	2	0	1	2	3	1	10	0.77
Marwick et al 2013[31]	2	1	0	2	1	3	1	10	0.77
Naggie et al 2011[47]	2	2	2	1	2	1	1	11	0.85
Soes et al 2014[28]	3	2	3	2	0	1	1	12	0.92
Suissa et al 2012[48]	0	1	0	2	2	3	1	9	0.69
Vesteinsdottir et al 2012[44]	2	2	2	2	0	1	1	10	0.77
Wilcox et al 2008[49]	2	0	2	2	0	2	1	9	0.69

[a]Definition of cases: Method used for *Clostridium difficile* diagnosis: stool culture (3 points), toxin detection (2 points), clinical diagnosis or *International Classification of Diseases* (ICD) code (1 point), other or no description (0 points).
[b]Case selection for community-acquired infection: Patient not previously hospitalized and not a resident of a nursing home (2 points), patient not previously hospitalized or not a resident of a nursing home (1 point), no description (0 points).
[c]Definition of controls: Method used for exclusion (noninfection) of *C. difficile*: stool culture (3 points), toxin detection (2 points), clinical diagnosis or ICD code (1 point), other or no description (0 points).
[d]Control selection: Community (2 points), community and hospital (1 point), no description (0 points).
[e]Analysis adjusted for exposures other than the primary exposure of interest (sex, age, antimicrobial exposure, gastric acid–suppressive medication exposure or presence of comorbidities). Adjusted for 5 factors (3 points), 3–4 factors (2 points), 1–2 factors (1 point), or nonadjusted (0 points).
[f]Ascertainment of exposure: Objective methods, ie, charts or medical records (3 points), reported by the general practitioner (2 points), self-reported (1 point), no description (0 points).
[g]Method of ascertainment of exposure for cases and controls: Same (1 point), different (0 points).

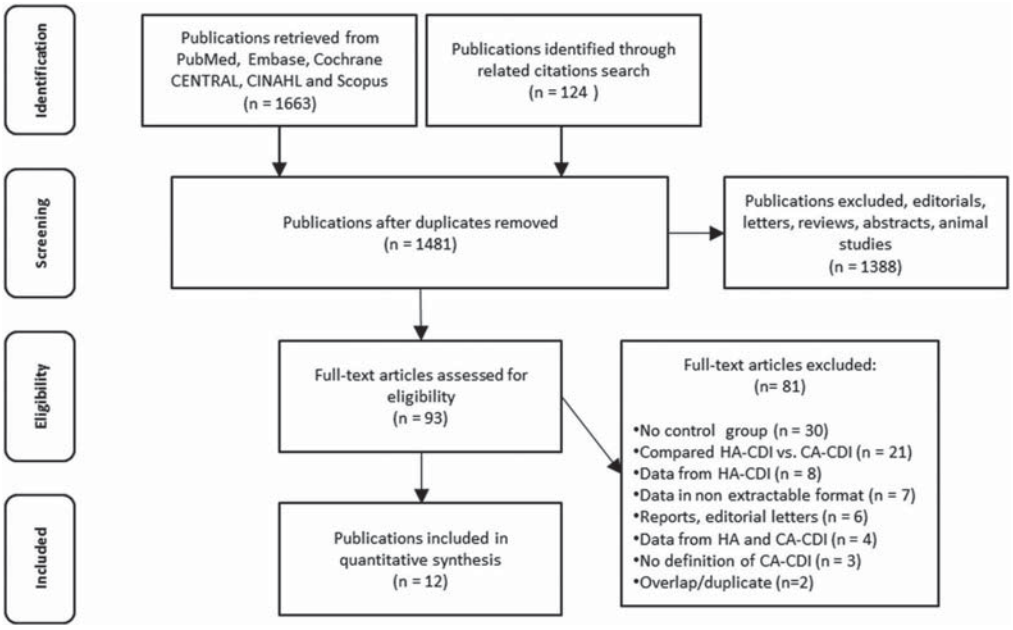

FIGURE 1. Preferred Reporting Items for Systematic Reviews and Meta-Analysis flowchart of the literature search conducted on March 1, 2014, for the meta-analysis.

RESULTS

Yield of Search Strategy

The initial search identified 1,663 publications. An additional 124 publications were retrieved throughout the related citations search. After excluding duplicate citations, 1,481 publications remained. After screening the publications by title and abstract, 1,388 were excluded. A full-text review of 93 publications was conducted, and 12 met the eligibility criteria and were selected for the meta-analysis (Figure 1).

There was overlap in subjects between 2 sets of publications. Two publications (Dial et al[25] and Delaney et al[26]) used data from the UK General Practice Research Database between 1994 and 2004 and a positive toxin test result for CDI as case definition to assess the risk of CA-CDI with antimicrobial exposure. Although Dial et al[27] also used data from the UK General Practice Research Database, the authors reported that there was no overlap between this and Dial et al[25] because they used different case definitions for CDI.[27] Additionally, 2 publications (Soes et al[28] and Soes et al[29]) reported results from the same Danish cohort. Therefore, Delaney et al[26] and Soes et al[29] were excluded from the analyses.

Characteristics of the Included Studies

Twelve publications were included in the meta-analysis. Two publications reported results divided into groups. Kutty et al[30] presented the results of 2 populations (Veterans Affairs and Durham County residents), whereas Soes et al[28,29] presented the results divided into 2 age groups (<2 years and ≥2 years). Among the included studies, 7 were case-control studies and 5 were nested case-control studies. The studies included covered more than 35 years of research and 56,776 patients in 6 different countries. The age of the participants ranged from 3 months to 101 years. Only one study[28,29] used exclusively positive *C. difficile* culture in the case definition and another study[31] used a combination of *C. difficile* culture or toxin test results in the case definition. All studies evaluated exposure to medication for at least 6 weeks and presence of comorbidities for at least 12 weeks prior to the index date, respectively (Table 2). The quality score of the studies ranged from 9 to 13 of 17 (Table 1).

Quantitative Synthesis

When examining the association between drug exposures and CA-CDI using the IVhet model, exposure to antimicrobials (OR, 6.18; 95% CI, 3.80–10.04) and corticosteroids (1.81; 1.15–2.84) were significantly associated with CA-CDI. Gastric acid–suppressing drugs were not associated with increased odds of CA-CDI (both PPIs and histamine-2-receptor antagonists: OR,1.58; 95% CI, 0.90–2.75; just PPIs: 1.61, 0.90–2.88; just histamine-2-receptor antagonists:1.24, 0.76–2.01). Statistically significant associations were found between CA-CDI and the presence of inflammatory bowel disease (OR, 3.72; 95% CI, 1.52–9.12), renal failure (2.64, 1.23–5.68), leukemia or

TABLE 2. Characteristics of the Studies Included in the Meta-analysis

Author, publication year	Data source	Study period	Study design	Study population	Age, mean (SD), years case/control	Male sex, % case/control	Community-acquired definition	Case definition	Control definition	Matching	Exposure to medication or presence of comorbidity, days prior to index date	N case/control
Dial et al 2005[25] & Delaney et al 2007[26]	GPRD, UK	1 Jan 1994–31 Dec 2004	Case-control	≥2 years registered in a general practice in the UK and ≥18 years old	71.0(16)/ 70.8(16)	35/42	Not hospitalized the year prior to the index date	Clinical diagnosis or positive toxin test results for CDI	No clinical diagnosis nor positive toxin test result for CDI	Practice location, age (±2 years)	Gastric acid suppressant, antimicrobials, NSAID, aspirin, 90 Comorbidity, 720	1,233/12,330
Dial et al 2006[27]	GPRD, UK	1 Jan 1994–31 Dec 2004	Case-control	Registered in the GPRD without clinical diagnosis or positive toxin test results for CDI 30 days to 1 year prior to the index date	65.0 (19.6)/ 64.9 (19.5)	36.6/41.5	Not hospitalized the year prior to the index date	Prescription of oral vancomycin therapy	No prescription for oral vancomycin	Practice location, age (±2 years)	Gastric acid suppressant, antimicrobials, 90 Comorbidity, 720	317/3,167
Dial et al 2008[46]	Régie de l'assurance maladie du Québec and MED-ECHO, Canada	1996–2004	Nested case-control	Hospitalized during the study period, ≥65 years old, and have not received metronidazole or oral vancomycin 90 days prior the index date	79.8 (6.8)/ 77.5 (6.3)	33.7/40.9	Not admitted to any type of institution in the 90-day period before the index date	First hospital admission with primary diagnosis of CDI (ICD-9 code 008.45)	No primary diagnosis of CDI during the first hospital admission	Unmatched Index date and date of first hospital admission	Antimicrobials, 45 Comorbidity, 720	836/8,360
Kuntz et al 2011[2]	University of Iowa Wellmark Data Repository, USA	1 Jan 2004–31 Dec 2007	Nested case-control	Patients with at least 1 year of health and pharmacy insurance	NR/NR	39.47/48.36	No history of long-term care facility 6 months or hospitalized 12 weeks before the index date	Primary or secondary diagnosis of CDI (ICD-9 code 008.45)	No diagnosis of CDI on or before the index date	Unmatched Index date	Gastric acid suppressant, antimicrobials, 180 Comorbidity,	304/3,040
Kutty et al 2010[30a]	VA infection control database and Surveillance database of the Duke University Hospital network, USA	Jan 2005–Dec 2005	Case-control	≥18 years old	VA: 62 (38–85)/64 (38–86)[c] Durham County: 61 (20–101)/55 (22–87)[c]	VA: 88/96 Durham County: 42/29	No history of healthcare exposure within 8 weeks of the index date	Nonformed stool specimen with positive toxin test results for CDI	Outpatients with no clinical diagnosis of diarrhea or positive toxin test results for CDI	Unmatched	Gastric acid suppressant, antimicrobials, NSAID, 90 Comorbidity, NR	VA: 36/108 Durham County: 73/48
Lowe et al 2006[32]	Ontario Drug Benefit Program, Canadian Institute for Health Information Discharge Abstract Database, Ontario Health Insurance Plan Database and Ontario Registered Persons Database, Canada	1 Apr 2002–31 Mar 2005	Nested case-control	≥66 years old, exposed to antimicrobials	78.7 (7.2)/78.0 (6.8)	59.8/60.5	Not hospitalized during the 90-day period prior to the index date nor patients from long-term care or nursing homes	Hospitalized with diagnosis of CDI (ICD-10 code A04.7)	Outpatient	Index date, sex, age (±1 years), antimicrobials prescribed	Gastric acid suppressant, 90 Antimicrobials, 60 Comorbidity, 180–720	1,389/12,303
Marwick et al 2013[31]	Health Information Center at the University of Dundee, Scotland	1 Nov 2008–31 Oct 2009	Nested case-control	≥65 year old	81 (8.9)/81 (8.9)	27.4/27.4	Not hospitalized during the 120-day period prior to the index	Diarrhea and a positive toxin test result for CDI or positive *C. difficile* culture and pseudomembranous colitis	NR	Sex, age (±1 years)	Gastric acid suppressant, antimicrobials, 180 Comorbidity, 360	62/620

Naggie et al 2011[47]	Duke University Medical Center, Durham Regional Hospital, Durham VA Medical Center, Salisbury VAMC and Asheville VAMC, USA	1 Oct 2006–31 Nov 2007	Case-control	≥18 years old	64 (50–73)/ 63 (52–74)[c]	44/45	Symptom onset in the community or within 72 hours of admission to a healthcare facility. Not hospitalized during the 12-week period prior to the index	Diarrhea and a positive toxin test results for CDI	Outpatient with no diagnosis of CDI	Unmatched Geographic location	Gastric acid suppressant, antimicrobials, NSAID, aspirin, 90 Comorbidity, 720	66/114
Soes et al 2014[28,29 b]	NR, Denmark	24 Aug 2009–28 Feb 2011	Nested case-control	Patients who had fecal sample submitted by their GP for microbiological testing due to diarrhea or other gastrointestinal symptoms	<2 years: 0.95 (0.30–1.98)/1.06 (0.25–1.98) ≥2 years: 50 (2–94)/ 50 (2–90) [c]	<2 years: 53/55 ≥2 years: 25/28	Not hospitalized during the 12-week period prior to the index or onset of symptoms within 48 hours of admission	Positive *C. difficile* culture	Negative *C. difficile* culture	Laboratory location, sex, age (±2 years if ≥5years; ±5 months if ≥6months and <4years; ±6 weeks if <6months)	Antimicrobials, 56 Gastric acid suppressant, NSAID, aspirin, 120 Comorbidity, 120	<2 years: 121/213 ≥2 years: 138/242
Suissa et al 2012[48]	GPRD, UK	1 Jan 1994–31 Dec 2005	Case-control	≥2 years registered in a general practice in the UK and ≥18 years old	NR/NR	NR/NR	Not hospitalized the year prior to the index date	First positive toxin test results for CDI, or first prescription of oral vancomycin	No clinical diagnosis, positive toxin test result for CDI or prescription of oral vancomycin	Practice location, age (±2 years)	Gastric acid suppressant, antimicrobials, NSAID, aspirin, 90 Comorbidity, 720	929/10,242
Vesteinsdottir et al 2012[44]	National University Hospital of Iceland, Iceland	1 Jul 2010–30 Jun 2011	Case-control	≥18 years old	65 (56–80)/ 65 (55–80)[c]	42.3/42.3	Not hospitalized during the 6-week period prior to the index or lived in a nursing facility and if hospitalized, diagnosed with CDI within the 72 hours of admission	Positive toxin test results for CDI	Negative toxin test results for CDI	Sex, age (±5 years)	Gastric acid suppressant, antimicrobials, 42 Comorbidity, 84	111/222
Wilcox et al 2008[49]	Cornwall and Leeds, UK	Jan 1999–Dec 1999	Case-control	Patients who had fecal sample submitted by their GP for microbiological testing	78 (4–100)/NR[c]	44/NR	Patients that attended the GP	Diarrhea and a positive toxin test results for CDI	Negative toxin test results for CDI	Sex, age categories	Antimicrobials, 180 Comorbidity, NR	40/112

NOTE. CDI, *Clostridium difficile* infection; GP, general practitioner; GPRD, General Practice Research Database; ICD, *International Classification of Disease*; index date, the date when the cases were identified; MED-ECHO, provincial hospital discharge summary; NR, not reported; NSAID, nonsteroidal anti-inflammatory drug; VA, Veterans Affairs.

[a]Presented in 2 groups: Patients from the VA and Durham County.

[b]Presented in 2 groups: Patients aged <2 years and ≥2 years.

[c]Age, median (range), years.

TABLE 3. Pooled Effect Size Using the IVhet Model, QE Model, and the RE Model

Exposure	IVhet model OR (95% CI)	QE model OR (95% CI)	RE model OR (95% CI)	Heterogeneity I^2 index %
Antimicrobials	**6.18 (3.80–10.04)**	**6.11 (3.92–9.55)**	**5.92 (4.21–8.32)**	87.90
Cephalosporins	1.80 (0.38–8.46)	2.09 (0.55–7.98)	**3.29 (1.20–9.05)**	98.39
Clindamycin	2.32 (0.14–37.99)	3.21 (0.30–34.55)	**8.35 (1.54–45.20)**	97.73
Fluoroquinolones	1.55 (0.32–7.57)	1.90 (0.51–7.05)	**3.59 (1.60–8.06)**	96.97
Macrolides	1.26 (0.49–3.24)	1.45 (0.64–3.28)	**2.15 (1.11–4.17)**	93.38
Penicillins	1.31 (0.57–3.01)	1.54 (0.75–3.16)	**2.40 (1.40–4.11)**	93.50
Tetracyclines	0.98 (0.68–1.41)	0.98 (0.67–1.41)	0.98 (0.68–1.41)[a]	0
TMP-SMX	1.26 (0.75–2.12)	1.30 (0.80–2.10)	1.37 (0.87–2.15)	77.37
Gastric acid suppressant	1.58 (0.90–2.75)	1.58 (0.95–2.63)	**1.58 (1.06–2.34)**	68.89
H2RA	1.24 (0.76–2.01)	1.24 (0.78–1.96)	1.37 (0.96–1.96)	73.95
PPI	1.61 (0.90–2.88)	1.63 (0.95–2.80)	**1.68 (1.11–2.55)**	92.23
Other medication				
Aspirin	0.97 (0.87–1.08)	0.96 (0.85–1.08)	0.97 (0.87–1.08)[a]	0
NSAIDs	1.14 (0.67–1.93)	1.04 (0.63–1.71)	0.83 (0.56–1.23)	90.42
Corticosteroids	**1.81 (1.15–2.84)**	**1.84 (1.22–2.77)**	**1.65 (1.14–2.38)**	34.79
Comorbidities				
Congestive heart disease	0.95 (0.45–2.01)	0.98 (0.46–2.06)	1.40 (0.77–2.54)	68.70
COPD	1.04 (0.93–1.16)	1.04 (0.93–1.16)	1.04 (0.93–1.16)[a]	0
Diabetes mellitus	**1.15 (1.05–1.27)**	**1.14 (1.04–1.26)**	**1.15 (1.05–1.27)[a]**	0
Diverticular disease	1.15 (0.98–1.36)	1.15 (0.98–1.35)	1.15 (0.98–1.36)[a]	0
GERD	1.02 (0.74–1.43)	1.03 (0.74–1.43)	1.07 (0.80–1.44)	45.53
Inflammatory bowel disease	**3.72 (1.52–9.12)**	**4.11 (1.78–9.49)**	**5.19 (2.49–10.83)**	89.39
Leukemia or lymphoma	**1.75 (1.02–3.03)**	**1.74 (1.01–3.01)**	**1.88 (1.09–3.21)**	38.95
Peptic ulcer	0.97 (0.60–1.57)	0.96 (0.59–1.56)	0.94 (0.58–1.51)	14.72
Renal failure	**2.64 (1.23–5.68)**	**2.59 (1.20–5.59)**	**3.02 (1.66–5.48)**	85.96
Solid cancer	1.34 (0.83–2.17)	1.35 (0.84–2.17)	**1.51 (1.01–2.27)**	81.64

NOTE. COPD, chronic obstructive pulmonary disease; GERD, gastroesophageal reflux disease; H2RA, histamine-2-receptor antagonists; IVhet, inverse variance heterogeneity; NSAIDs, nonsteroidal anti-inflammatory drugs; OR, odds ratio; PPI, proton-pump inhibitors; QE, quality effects; RE, random-effects; TMP-SMX, trimethoprim-sulfamethoxazole. Boldface type indicates statistically significant ORs.
[a]No heterogeneity, pooled estimated report using the inverse variance model.

lymphoma (1.75, 1.02–3.03), and diabetes mellitus (1.15, 1.05–1.27) (Table 3).

Visual inspection of the forest plots, Cochran's Q test (Appendix 3), τ^2 (results not shown), and I^2 index (Table 3 and Appendix 3) confirmed heterogeneity across studies, except for exposure to tetracyclines or aspirin and the presence of chronic obstructive pulmonary disease, diabetes, or diverticular disease.

Sensitivity Analysis

A sensitivity analysis was possible only for antimicrobial and PPI exposure because of the small number of studies in the other categories. When stratifying the studies by geographic location, the sensitivity analysis showed that antimicrobial exposure had a greater association with CA-CDI in the United States (OR, 9.16; 95% CI, 5.47–15.34) compared with European countries (4.54, 2.68–7.70; Appendix 4.1). Conversely, exposure to PPIs had a stronger association with CA-CDI in Europe (OR, 2.56; 95% CI, 1.40–4.71) compared with the United States (1.12, 0.64–1.95; Appendix 4.2).

The subgroup analysis by life stages showed that older adults (>65 years) had the highest risk (OR, 10.16; 95% CI, 5.56–18.58) of CA-CDI when exposed to antimicrobials, followed by children and adults (5.98, 4.67–7.67; Appendix 4.3). When exposed to PPIs, adults had the highest risk of CA-CDI (OR, 2.78; 95% CI, 2.02–3.81; Appendix 4.4).

Publication Bias

On visual inspection of the *Doi* plots, there was gross asymmetry for some exposures, suggesting publication bias in relation to cephalosporins, fluoroquinolones, macrolides, penicillin, presence of congestive heart failure, and presence of gastroesophageal reflux disease. The bias was toward selective publication that reported medication exposure and presence of comorbidities as risk factors for CA-CDI (Appendix 3).

DISCUSSION

Exposure to antimicrobials remained the strongest risk factor associated with CA-CDI. No statistical significance was observed in most analyses by antimicrobial class, likely because

the largest study (Lowe et al[32]) reported ORs close to the null value. However, point estimates confirmed a trend toward an association with CA-CDI regardless of antimicrobial class exposure. These observations corroborated previous findings published by Deshpande et al[3] and Brown et al[4] that suggested an increased risk of CA-CDI as a result of antimicrobial exposure.

Despite the growing evidence in the past decade with respect to increased risk of HA-CDI after exposure to PPIs[6,7,9–11] or histamine-2-receptor antagonists,[8,25] no significant association was observed in the community setting. The observed difference between the risk of CA-CDI and HA-CDI with gastric-acid suppressive medication can be explained by the overuse of these medications in healthcare facilities.[33] Exposure to corticosteroids was associated with CA-CDI. In contrast to antimicrobials that disrupt the normal gut microbiome, facilitating the proliferation of *C. difficile*,[34] and in contrast to gastric-acid suppressive medication that may allow survival of vegetative forms of *C. difficile*,[35] a plausible biological mechanism for the observed association could be the negative impact of corticosteroids on the gastrointestinal mucosal integrity.[36]

Previous studies found that gastrointestinal comorbidities such as inflammatory bowel disease[12] and cirrhosis[14] were associated with a worse prognosis in patients with CDI. Similarly, congestive heart disease, chronic pulmonary disease, renal failure, and malignant neoplasms were also associated with higher mortality rates among inpatients with CDI.[13] Among the comorbidities examined in this meta-analysis, inflammatory bowel disease was the strongest risk factor for CA-CDI, followed by renal failure and hematologic cancers. In patients with the described comorbidities, early identification and prompt treatment of CA-CDI may reduce mortality rates. The associations found between CA-CDI and comorbidities may be confounded by medication exposure, given that polypharmacy is common among patients with multiple comorbidities. Furthermore, the heterogeneous definition of CA-CDI across the studies (ie, not hospitalized the year prior to the index date versus not hospitalized 6 weeks prior to the index date) may also be a source of misclassification between CA- and HA-CDI, considering that patients with multiple comorbidities are more likely to be admitted to hospitals.

The sensitivity analyses suggested that risk of CA-CDI with exposure to antimicrobials and PPI differed between Europe and America. The observed difference might be due to the dissimilar prescription of antimicrobials[37] and/or the presence of different strains of *C. difficile* in Europe and America.[38] Similarly, the risk of CA-CDI with exposure to antimicrobials and PPI varied among the life stages. These findings were consistent with Sandora et al,[39] who reported a negative correlation between age and CA-CDI among pediatric populations, and with Lessa et al,[40] who reported a higher incidence of CDI among patients at both extremes of life (1–4 years of age and older than 65 years). In the past 2 decades, a 12-fold increased incidence of CA-CDI among the pediatric population[41] and numerous outbreaks in long-term care facilities[42] have been reported, indicating that infants, toddlers, and older adults should be considered at high risk of CA-CDI.

Although a comprehensive systematic search for studies was performed, publication bias could have resulted in additional positive associations being published, such as those between CA-CDI and exposure to cephalosporins, fluoroquinolones, macrolides, and penicillins and the presence of congestive heart disease and gastroesophageal reflux disease. The actual risks attributable to these risk factors could be less than what we have reported. Nevertheless, heterogeneity across studies could also result in effect size asymmetry, and this represents an alternative explanation to selective publication of positive results.

Recent meta-analyses have investigated the risk of CDI associated with exposure to antimicrobials[3,4,6] and gastric acid suppressant drugs[6–9] using the widely adopted RE model.[16] However, the coverage probability of the RE CI can be substantially below the nominal level of 95% and thus does not adequately reflect the statistical error, especially when there are few included studies.[5,22,43] By underestimating the statistical error, the RE model produces tight CIs that potentially cause overconfident results prone to type 1 error. Moreover, the assumption of normally distributed random effects is not easily verified.[43] The use of a moment-based common variance[16] within this model is in the redistribution of the weights from larger to smaller studies.[18] The QE and IVhet models have both been created to do away with the problems that affect the RE model and both have coverage of the CI at or above the nominal level.[22] As an example, with the clindamycin pooled estimates, the IVhet model distributed the weight (83.5%) toward the biggest study (Lowe et al[32]; n = 13,692). The QE model took into account the extra information regarding the quality of the studies and penalized the biggest study by reducing the assigned weight (from 83.5% to 69.0%) because it had the lowest quality score; whereas the RE model redistributed the weights by equalizing weights (by transferring from big to small studies) and thus, it gave a weight percentage to the biggest study (Lowe et al[32]; n = 13,692; weight 25.85%) that was similar to that of the smallest study (Vesteinsdottir et al[44]; n = 333; weight 23.98%). Moreover, the RE model produced a tighter CI (with a statistically significant result), but its coverage may have been under the nominal level and thus may not have captured the true value of the effect (Appendix 3.3).

Several limitations of the present meta-analysis were noted. Kuntz et al[2] and Marwick et al[31] reported a positive relationship between duration of exposure to antimicrobials and CA-CDI. However, the small number of studies precluded a subgroup analysis by duration of exposure to antimicrobials. All studies included in this meta-analysis were conducted in Northern Hemisphere countries. A recent study has described a different seasonal pattern of CDI in Australia that remains largely unexplained.[45] The epidemiologic patterns of *C. difficile* transmission and infection may differ between hemispheres and thus generalizability of the findings to Southern Hemisphere countries is limited.

In conclusion, while antimicrobial use remains the dominant risk factor for CA-CDI, corticosteroid use should also

be considered an important risk factor. Given these are commonly prescribed medications in the community, the attributable risk of CDI due to exposure may be high and thus further research is warranted. In addition, patients with inflammatory bowel disease, renal failure, and hematologic cancer are at higher risk of CA-CDI, making them appropriate populations for interventional studies of screening for *C. difficile*.

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SUPPLEMENTARY MATERIAL

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Chapter 3

Asymptomatic *C. difficile* colonisation

CHAPTER 3. Asymptomatic *C. difficile* colonisation

3.1. Context

Some strains of *C. difficile* have the capacity to produce toxins and these TCD strains are implicated in the development of CDI symptoms. However, not all patients that become colonised develop symptoms; in fact, it is estimated that up to two thirds of patients colonised with TCD strains remain asymptomatic. Although asymptomatic TCD-colonised patients do not require antibiotic therapy for *C. difficile*, recent studies have provided evidence that these patients have the potential to transmit the bacterium to uncolonised patients in the healthcare setting, who can then go on to have CDI. Furthermore, the implementation of preventive control measures (i.e. isolation precautions and environmental control) targeting asymptomatic TCD-colonised patients has shown to have an effect on reducing the incidence of symptomatic CDI cases.

Strains of *C. difficile* have been isolated that lack an intact pathogenicity locus or do not express bioactive toxins. These NTCD strains are considered to be benign and do not cause disease. Evidence from animal models suggests that colonisation with NTCD could protect against infection with TCD strains. Recently, results from a Phase II randomised controlled trial have showed that administration of the NTCD strain M3 to patients with recurrent CDI significantly reduces the recurrence of the disease.

It is clear that asymptomatic patients can be colonised by TCD or NTCD, yet these different types of strain seem to have antagonistic roles in *C. difficile* epidemiology. Therefore, in this Chapter I present the results of a large prospective study conducted in two Australian tertiary hospitals. In this study, 1380 asymptomatic patients (i.e. without diarrhoea) were enrolled during a three-year period with the following aims: 1) to estimate the prevalence of asymptomatic *C. difficile* colonisation; 2) to describe the seasonal variation of asymptomatic colonisation prevalence; and 3) to identify host factors

associated with TCD- and NTCD-colonisation. I presented the first prevalence estimates of asymptomatic *C. difficile* colonisation in Australia and compared them with those reported in North America and Europe. I reported that asymptomatic *C. difficile* colonisation prevalence varied seasonally. I also identified that patients colonised with TCD and NTCD do not share risk factors, which highlights the importance of considering separately asymptomatic TCD and NTCD-colonisation to better understand CDI epidemiology.

3.2. Risk factors for asymptomatic *C. difficile* colonisation

Furuya-Kanamori L, Clements AC, Foster NF, Huber CA, Hong S, Harris-Brown T, Yakob L, Paterson DL, Riley TV. Asymptomatic *Clostridium difficile* colonisation in two Australian tertiary hospitals, 2012–2014: A prospective, repeated cross-sectional study. *Clin Microbiol Infect* 2017;23:48.e1-7.

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Original article

Asymptomatic *Clostridium difficile* colonization in two Australian tertiary hospitals, 2012–2014: prospective, repeated cross-sectional study

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ABSTRACT

Objectives: To investigate the prevalence and risk factors for asymptomatic toxigenic (TCD) and non-toxigenic *Clostridium difficile* (NTCD) colonization in a broad cross section of the general hospital population over a 3-year period.
Methods: Patients without diarrhoea admitted to two Australian tertiary hospitals were randomly selected through six repeated cross-sectional surveys conducted between 2012 and 2014. Stool specimens were cultured under anaerobic conditions, and *C. difficile* isolates were tested for the presence of toxin genes and ribotyped. Patients were then grouped into noncolonized, TCD colonized or NTCD colonized for identifying risk factors using multinomial logistic regression models.
Results: A total of 1380 asymptomatic patients were enrolled; 76 patients (5.5%) were TCD colonized and 28 (2.0%) were NTCD colonized. There was a decreasing annual trend in TCD colonization, and asymptomatic colonization was more prevalent during the summer than winter months. TCD colonization was associated with gastro-oesophageal reflux disease (relative risk ratio (RRR) = 2.20; 95% confidence interval (CI) 1.17–4.14), higher number of admissions in the previous year (RRR = 1.24; 95% CI 1.10–1.39) and antimicrobial exposure during the current admission (RRR = 2.78; 95% CI 1.23–6.28). NTCD colonization was associated with chronic obstructive pulmonary disease (RRR = 3.88; 95% CI 1.66–9.07) and chronic kidney failure (RRR = 5.78; 95% CI 2.29–14.59). Forty-eight different ribotypes were identified, with 014/020 (n = 23), 018 (n = 10) and 056 (n = 6) being the most commonly isolated.
Conclusions: Risk factors differ between patients with asymptomatic colonization by toxigenic and non-toxigenic strains. Given that morbidity is largely driven by toxigenic strains, this novel finding has important implications for disease control and prevention. **L. Furuya-Kanamori, CMI 2017;23:48.e1–48.e7**

Introduction

Clostridium difficile infection (CDI) is the main cause of healthcare-associated diarrhoea. Toxigenic *C. difficile* (TCD) strains produce toxins A and B, and, increasingly, binary toxin (CDT), which are responsible for the clinical presentation of CDI, ranging from mild diarrhoea to severe life-threatening conditions such as pseudomembranous colitis [1]. It is estimated that up to two-thirds of patients who are exposed to *C. difficile* remain asymptomatic [2]. There is growing evidence that asymptomatic patients colonized

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with TCD can act as a source of *C. difficile* transmission and environmental contamination in hospitals [3,4]. However, not all *C. difficile* strains produce toxins, and it has been proposed that asymptomatic patients colonized with nontoxigenic *C. difficile* (NTCD) strains are protected from colonization by heterologous strains, including toxigenic strains, as a result of niche competition or stimulation of the mucosal immune response in the gastrointestinal tract [5].

Limited evidence indicates that asymptomatic colonized patients may potentially play a role in transmission [6]. The associated host risk factors (e.g. sex, age, comorbidities and medication exposure) and pathogen characteristics (e.g. toxigenic profile and predominant ribotypes) among this group are poorly understood [7]. Few studies have investigated the prevalence of asymptomatic TCD and NTCD colonization in a broad cross section of the general hospital patient population [8], nor have the between-season variability or temporal trends of prevalence been reported.

Therefore, a 3-year study with biannual surveys in adult patients was conducted in two Australian tertiary-care hospitals in different Australian states with the following aims: to estimate the prevalence of asymptomatic *C. difficile* colonization; to compare the prevalence during summer and winter months and over time; to describe the predominant toxin profiles and ribotypes isolated from asymptomatic patients; and to identify host factors associated with TCD and NTCD colonization.

Materials and Methods

Study setting and participants

The study was conducted in two tertiary hospitals in Australia, the Royal Brisbane & Women's Hospital (RBWH), with 929 beds in Brisbane, Queensland, and the Sir Charles Gairdner Hospital (SCGH), with 607 beds in Perth, Western Australia. The patients were prospectively recruited through six repeated cross-sectional surveys conducted between 2012 and 2014. Each year, two surveys were conducted, one starting in late summer (February–March) and the other in late winter (August–September).

On the morning of each survey day, a sampling frame of currently admitted patients in the wards (i.e. medical, surgical, intensive care units) to be surveyed was created in a spreadsheet, with each patient given a unique ID. Patient IDs were drawn at random from the spreadsheet (using a random number generator). If the patient's ID was randomly selected, was 18 years of age or older and did not present diarrhoea (i.e. 3 or more loose or liquid bowel motions per day), the research nurse approached the patient and invited him or her to participate in the study.

The study received the approval of the RBWH (HREC/11/QRBW/223), the Sir Charles Gairdner Group (2011-088), the University of Queensland (2011000898) and the University of Western Australia (RA/4/1/5186) human research ethics committees. All the participants (or a legal proxy) provided written informed consent for their inclusion in the study. In Western Australia, a waiver of consent was granted when a person was unable to provide consent but the person could be enrolled onto the study without any additional risk beyond their standard care.

Data collection

Patients were interviewed to obtain demographic data and information on known CDI risk factors (e.g. use of various medications before admission, history of CDI and hospital admissions). Patient medical records were reviewed to determine the date and the reason for the latest admission, recent history of diarrhoea, comorbid conditions, inpatient medication (e.g. antimicrobials, gastric acid suppressants, nonsteroidal anti-inflammatory drugs) and medical procedures (e.g. colonoscopy, surgery) during the admission.

If *C. difficile* was isolated from the patients' stool specimens, the patients were monitored while hospitalized and followed up after discharge on a monthly basis for 3 months. The follow-up interviews were used to determine the patients' clinical outcomes and whether they remained asymptomatic, were readmitted to a hospital, were diagnosed with CDI, developed colitis or died.

Specimen collection and processing

Specimens from the enrolled patients were obtained using a rectal swab from consenting patients. Stool specimens were obtained from patients who were enrolled and able to provide a stool specimen but who did not consent to provide a rectal swab.

Swabs were cultured for *C. difficile* within 30 minutes of collection and stool samples were cultured within 24 hours using our previously described methods [9], except that direct culture was performed on ChromID *C. difficile* agar (bioMérieux, Marcy l'Étoile, France) and plates were examined at 24 and 48 hours for characteristic growth. Broth enrichment in Robertson cooked meat medium containing 5 mg/L of gentamicin, 250 mg/L of cycloserine and 8 mg/L of cefoxitin was performed concurrently and ethanol shocked after 48 to 72 hours for subculture on ChromID agar if direct culture was negative. Putative *C. difficile* colonies were subcultured onto prereduced blood agar plates for identification by characteristic colony morphology and odour, chartreuse fluorescence under UV light and proline aminopeptidase production (Diatabs; Rosco Diagnostica, Taastrup, Denmark) at 48 hours. All agar plate incubations were performed at 35°C under anaerobic conditions.

C. difficile isolates were tested for the presence of toxin genes (*tcdA, tcdB* and *cdtA/cdtB*) and were polymerase chain reaction (PCR) ribotyped following previously described methods [9]. Strains that did not produce banding patterns matching an international ribotype in the reference collection were assigned local nomenclature (QX type).

Statistical analysis

All enrolled patients not experiencing diarrhoea who had *C. difficile* isolated from their stool were considered to have asymptomatic *C. difficile* colonization. If the strain isolated was positive for the presence of *tcdA, tcdB* or *cdtA/cdtB* genes, then the patient was considered asymptomatic TCD colonized; if the isolated strain was negative for all toxin genes, then the patient was considered asymptomatic NTCD colonized. Therefore, for the purpose of the analyses, patients were grouped into three categories according to their status with respect to *C. difficile* colonization at the time of enrolment: noncolonized, TCD colonized and NTCD colonized. The overall and specific survey prevalence of TCD and NTCD colonized patients were calculated.

Pearson's chi-square test and Fisher's exact test were used to compare categorical variables, and the Kruskal-Wallis *H* test was used to compare continuous variables across the three categories of *C. difficile* colonization. Univariate and multivariate multinomial logistic regression models were built with *C. difficile* colonization as the outcome and noncolonized patients as the reference category to identify predictors of TCD and NTCD colonized patients. After adjusting for age and sex of the patients, known risk factors for CDI (i.e. hospital admissions and exposure to antimicrobials), the inclusion of comorbidities and medication exposure during the current admission in the regression model were analysed through a stepwise forward selection with the Akaike information criterion as

the selection criterion. A significance level cutoff of p <0.05 was used for all analyses. All statistical analyses were conducted by Stata SE 14 (StataCorp, College Station, TX, USA).

Results

Prevalence of asymptomatic C. difficile colonization and seasonal variation

During the six surveys throughout the 3 years, 1380 patients were enrolled onto the study (595 and 785 patients from the RBWH and SCGH, respectively) (Supplementary Material 1). The median time between the patients being admitted to hospital and enrolment onto the study was 5 days (interquartile range 2–10 days), and 25% of the patients were enrolled within 48 hours of being admitted. There was no statistically significant difference in time between being admitted and enrolment for both hospitals and across the six surveys (Supplementary Material 2).

C. difficile was isolated from 104 patients (7.5%; 95% confidence interval (CI) 6.2–9.1). A higher prevalence of *C. difficile* colonization was observed at SCGH (9.8%; 95% CI 7.8–12.1) compared to RBWH (4.5%; 95% CI 3.0–6.5). Among the enrolled patients, 76 (5.5%; 95% CI 4.4–6.8) and 28 (2.0%; 95% CI 1.4–2.9) were colonized with TCD and NTCD strains, respectively. A higher prevalence of asymptomatic *C. difficile* colonization was observed during the summer surveys (8.8%; 95% CI 6.9–11.1) compared to the winter surveys (5.9%; 95% CI 4.2–8.1) (Fig. 1). The prevalence of asymptomatic *C. difficile* colonization was highest during the first survey (February–March 2012), when 33 out of 294 patients were colonized (11.2%; 95% CI 7.9–15.4), including 28 (9.5%; 95% CI 6.4–13.5) patients colonized with TCD strains. The lowest prevalence was observed during the fourth survey (August–September 2013); *C. difficile* was isolated from 14 (5.6%; 95% CI 3.1–9.2) patients among the 250 patients enrolled during that survey. The seasonal patterns were similar in both hospitals.

Characterization of C. difficile

Among the 104 *C. difficile* isolates, five toxin profiles were identified, with $A^+B^+CDT^-$ being the most common ($n = 71$, 68.3%). Three isolates (2.9%) were $A^+B^+CDT^+$, one (1.0%) was $A\text{-}B^+CDT^-$, one (1.0%) was $A^-B^-CDT^+$ and the remaining 28 isolates (26.9%) were $A^-B^-CDT^-$. Forty-eight different ribotypes were identified; the most common ribotype was the 014/020 group ($n = 23$, 22.1%), followed by 018 ($n = 10$, 9.6%), 056 ($n = 6$, 5.8%), 010 ($n = 5$, 4.8%) and 103 ($n = 5$, 4.8%). The four binary toxin–positive isolates were PCR ribotypes 063, 127, 251 and QX 220 (Fig. 2, Supplementary Material 3).

Predictors of toxigenic and nontoxigenic C. difficile colonization

The characteristics of patients enrolled onto the study are described in Table 1. There were no differences between non-colonized, TCD colonized and NTCD colonized patients in terms of sex proportion or mean age. Among the comorbidities, cancer prevalence was less common among NTCD colonized patients (7.1% vs. 34.7% (noncolonized) vs. 29.7% (TCD)). Gastro-oesophageal reflux disease and congestive heart failure were more prevalent among TCD colonized patients, while chronic obstructive pulmonary disease and chronic kidney disease were more prevalent among NTCD colonized patients. Five (0.4%), three (4.0%) and two (7.1%) noncolonized, TCD colonized and NTCD colonized patients, respectively, reported having a history of CDI. With regards to healthcare exposure, 64% of TCD and NTCD colonized patients had been admitted to hospital at least once in the previous year compared to 46% of noncolonized patients.

The reasons for the current admission did not significantly differ between the three *C. difficile* colonization categories (Table 2). Exposure to antimicrobials during the admission was common among all the patients; however, it was significantly higher in TCD (83.8%) and NTCD colonized patients (78.6%) compared to non-colonized patients (66.4%; p 0.004). There were no differences in other medication exposure (gastric acid suppressants, aperients, nonsteroidal anti-inflammatory drugs, glucocorticoids, chemotherapy or antidiarrhoeals) or medical procedures (insertion of orogastric tube, gastroscopy, colonoscopy or mechanical ventilation) during the admission across the colonization categories. In terms of surgical procedures, a significantly higher proportion of TCD colonized patients underwent orthopaedic (25.0%) and neurologic (14.5%) surgeries compared to noncolonized patients (12.9% orthopaedic and 5.6% neurologic) and NTCD colonized patients (10.7% orthopaedic and 3.6% neurologic) (p 0.016 and 0.013, respectively).

In the multivariate multinomial logistic regression model, factors associated with an increased relative risk ratio (RRR) of harbouring a TCD strain compared to noncolonized included having gastro-oesophageal reflux disease (RRR 2.20; 95% CI 1.17–4.14), number of hospital admissions in the previous year (RRR 1.24; 95% CI 1.10–1.39), exposure to antimicrobials during the period of

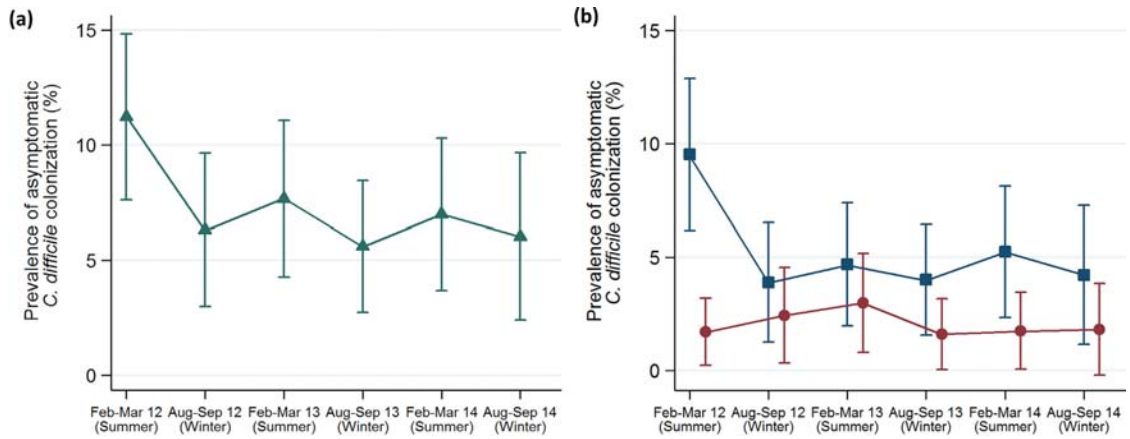

Fig. 1. (a) Seasonal variation of *Clostridium difficile* colonization prevalence and (b) variation by toxigenic profile. Green triangles, blue squares and red circles represent prevalence of overall, toxigenic and nontoxigenic *C. difficile* colonization, respectively. Vertical lines represent 95% confidence interval around prevalence estimates.

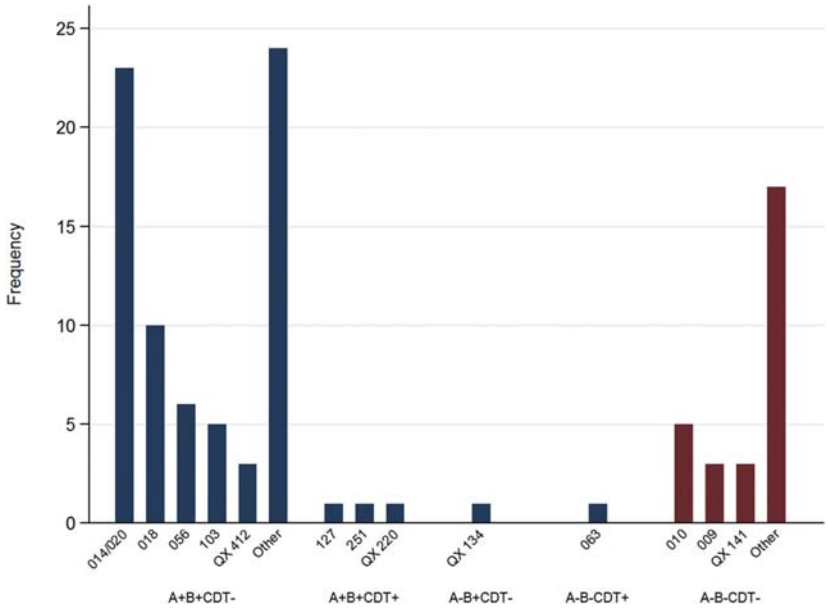

Fig. 2. Distribution of ribotypes among *Clostridium difficile* colonized patients. Blue and red bars represent frequency of toxigenic and nontoxigenic *C. difficile* strains isolated in study, respectively. Ribotypes with toxin profile of $A^{+}B^{+}CDT^{-}$ and $A^{-}B^{-}CDT^{-}$ and frequency of two or fewer were grouped into "other."

admission (RRR 2.78; 95% CI 1.23–6.28) and admission during the summer months (RRR 1.81; 95% CI 1.07–3.06) (Table 3). The regression model also revealed a decreasing annual trend in TCD colonization prevalence (RRR 0.68; 95% CI 0.47–0.97). For harbouring a NTCD strain relative to noncolonized, having chronic obstructive pulmonary disease (RRR 3.88; 95% CI 1.66–9.07) and chronic kidney failure (RRR 5.78; 95% CI 2.29–14.59) were associated with an increased RRR.

Over the 3-month follow-up, five colonized patients (4 (5.3%) TCD and 1 (3.6%) NTCD) reported developing CDI, and there were

Table 1
Patient characteristics

Characteristic	Noncolonized ($n = 1276$)	Toxigenic *Clostridium difficile* ($n = 76$)	Nontoxigenic *Clostridium difficile* ($n = 28$)	p[a]
Female sex	600 (47.0%)	40 (52.6%)	13 (46.4%)	0.633
Age, years, mean (SD)	61.8 (17.4)	64.1 (16.1)	64.3 (20.96)	0.414
Medical condition				
Cancer	441 (34.7%)	22 (29.7%)	2 (7.1%)	0.003
Diabetes mellitus	297 (23.4%)	18 (24.3%)	8 (28.6%)	0.806
Neurologic disorder	283 (22.3%)	23 (31.1%)	8 (28.6%)	0.165
GORD	256 (20.1%)	24 (32.4%)	7 (25.0%)	0.035
COPD	218 (17.2%)	17 (23.0%)	11 (38.3%)	0.005
Chronic kidney disease	107 (8.4%)	14 (18.9%)	9 (32.1%)	<0.001
Congestive heart failure	98 (7.7%)	12 (16.2%)	3 (10.7%)	0.029
Liver disease	90 (7.1%)	4 (5.4%)	2 (7.1%)	0.896
Inflammatory bowel disease	53 (4.2%)	3 (4.1%)	2 (7.1%)	0.578
Pregnancy	24 (1.9%)	0 (0.0%)	1 (3.6%)	0.384
Solid organ transplant	21 (1.7%)	1 (1.4%)	2 (7.1%)	0.118
HIV	5 (0.4%)	0 (0.0%)	0 (0.0%)	1.000
Smoking status				
Current	171 (13.4%)	7 (9.3%)	1 (3.6%)	0.218
Ever	754 (59.2%)	43 (58.1%)	13 (46.4%)	0.391
History of CDI (ever)	5 (0.4%)	3 (4.0%)	2 (7.1%)	<0.001
History of CDI in the last year	1 (0.1%)	0 (0.0%)	2 (7.1%)	0.002
Healthcare exposure 12 months before admission				
Admitted to hospital	584 (46.4%)	47 (64.4%)	18 (64.3%)	0.002
No. of admissions, median (IQR)	0 (0–2)	1 (0–3)	2 (0–3)	<0.001
LOS in the last admission, median (IQR)	4 (1–9)	6 (3–9)	8 (3–17)	0.997
Medication exposure 30 days before admission				
Antimicrobials	770 (63.4%)	51 (69.9%)	17 (60.7%)	0.506
Gastric acid suppressants	550 (44.5%)	29 (40.3%)	11 (39.3%)	0.685
Aperients	479 (43.4%)	29 (51.8%)	12 (46.2%)	0.453

CDI, *Clostridium difficile* infection; COPD, chronic obstructive pulmonary disease; GORD, gastro-oesophageal reflux disease; IQR, interquartile range; LOS, length of stay.
[a] p for comparison across noncolonized, toxigenic *C. difficile* and nontoxigenic *C. difficile*.

Table 2
Medication exposure and procedures during admission

Characteristic	Noncolonized ($n = 1276$)	Toxigenic *Clostridium difficile* ($n = 76$)	Nontoxigenic *Clostridium difficile* ($n = 28$)	p[a]
Reason for admission				
New medical/surgical problem	460 (36.8%)	35 (47.3%)	11 (39.3%)	0.175
Exacerbation of chronic condition	392 (31.4%)	19 (25.7%)	6 (21.4%)	
Infection	208 (16.7%)	12 (16.2%)	10 (35.7%)	
Elective surgery	171 (13.7%)	8 (10.8%)	1 (3.6%)	
Obstetric condition	18 (1.4%)	0 (0.0%)	0 (0.0%)	
Current length of stay, days, median (IQR)[b]	5 (2–10)	7 (4–17)	4 (2–8)	0.974
Medication exposure				
Any antimicrobial	836 (66.4%)	62 (83.8%)	22 (78.6%)	0.004
Cephalosporins	416 (32.6%)	34 (44.7%)	9 (32.1%)	0.092
Penicillins and β-lactamase inhibitors	377 (29.6%)	21 (27.6%)	13 (46.4%)	0.141
Penicillins	186 (14.6%)	12 (15.8%)	3 (10.7%)	0.866
Vancomycin	117 (9.2%)	12 (15.8%)	4 (14.3%)	0.095
Metronidazole	106 (8.3%)	16 (21.1%)	5 (17.9%)	<0.001
Macrolides	95 (7.5%)	3 (4.0%)	4 (14.3%)	0.174
Trimethoprim/sulfamethoxazole	75 (5.9%)	6 (7.9%)	3 (10.7%)	0.287
Ciprofloxacin	75 (5.9%)	5 (6.6%)	2 (7.1%)	0.770
Aminoglycosides	55 (4.3%)	5 (6.6%)	2 (7.1%)	0.340
Carbapenems	44 (3.5%)	6 (7.9%)	1 (3.6%)	0.114
Fluoroquinolones[c]	32 (2.5%)	3 (4.0%)	1 (3.6%)	0.448
Clindamycin	29 (2.3%)	4 (5.3%)	0 (0.0%)	0.233
Tetracyclines	22 (1.7%)	0 (0.0%)	2 (7.1%)	0.093
Other antimicrobials	33 (2.6%)	2 (2.6%)	0 (0.0%)	1.000
Gastric acid suppressants	686 (54.4%)	50 (67.6%)	16 (57.1%)	0.086
Proton pump inhibitors	643 (51.0%)	46 (62.2%)	16 (57.1%)	0.150
H2 blocker	75 (5.9%)	6 (7.9%)	1 (3.6%)	0.672
Aperients	590 (46.8%)	45 (60.8%)	15 (53.6%)	0.202
NSAIDs	382 (30.4%)	19 (26.0%)	12 (42.9%)	0.593
Glucocorticoids	331 (26.3%)	23 (31.1%)	7 (25.0%)	0.654
Chemotherapy	85 (6.8%)	2 (2.7%)	1 (3.6%)	0.406
Antidiarrhoeal	29 (2.3%)	3 (4.1%)	3 (10.7%)	0.080
Medical procedures				
Insertion of orogastric tubes	124 (9.8%)	8 (10.8%)	2 (7.1%)	0.885
Gastroscopy	81 (6.4%)	4 (5.4%)	2 (7.1%)	0.886
Colonoscopy	40 (3.2%)	1 (1.4%)	0 (0.0%)	0.780
Mechanical ventilation[d]	86 (6.8%)	10 (13.5%)	1 (3.6%)	0.158
Surgical procedures				
Orthopaedic	165 (12.9%)	19 (25.0%)	3 (10.7%)	0.016
Abdominal	137 (10.7%)	6 (7.9%)	1 (3.6%)	0.480
Cardiologic/thoracic	120 (9.4%)	4 (5.3%)	2 (7.1%)	0.499
Neurologic	72 (5.6%)	11 (14.5%)	1 (3.6%)	0.013
Oncologic	36 (2.8%)	0 (0.0%)	0 (0.0%)	0.381
Other surgical procedures	121 (9.5%)	5 (6.6%)	1 (3.6%)	0.571

IQR, interquartile range; NSAID, nonsteroidal anti-inflammatory drug.
[a] p for comparison across noncolonized, toxigenic *C. difficile* and nontoxigenic *C. difficile*.
[b] Time between admission and patient enrolment.
[c] Ciprofloxacin not included.
[d] Excludes mechanical ventilation during surgical procedures.

five deaths (4 (5.3%) TCD and 1 (3.6%) NTCD) not related to CDI. Given the small number of events, no statistical analysis was possible to compare the clinical outcomes of TCD and NTCD strains.

Discussion

The current study identified an asymptomatic *C. difficile* colonization prevalence of 7.5% across all hospital care wards, which was significantly lower than estimates recently reported in the United Kingdom (11%) [10] and the United States (21%) [11]. Likewise, the TCD colonization prevalence (5.5%) was lower compared to the pooled prevalence reported in a meta-analysis by Zacharioudakis *et al.* [8] (8.1% (95% CI 5.7–11.1) worldwide and 10.0% (95% CI 7.1–13.4) in North America). The prevalence of NTCD colonized patients in our study (2.0%) was significantly lower than that reported by Alasmari *et al.* (5.8%) [11], yet the ratios between nontoxigenic and toxigenic strains were similar in both studies (1:2.7). Inpatient hospital transfer has been identified as an important vehicle of *C. difficile* (symptomatic and asymptomatic) spread [12,13]. Given that hospital transfers in Australia mainly occur within a circumscribed health service area (http://www0.health.nsw.gov.au/policies/pd/2011/pdf/PD2011_031.pdf), the spread of any infectious disease may be limited and may contribute to the observed low prevalence of *C. difficile* colonization in our study. Australia's low population density might also contribute to less intense transmission in the community [14].

Notably, findings from this study conducted in two Australian cities located in a temperate climate zone suggest that asymptomatic *C. difficile* colonization has decreased from 2012 to 2014. In addition, it was noted that asymptomatic *C. difficile* colonization and symptomatic CDI displayed a synchronous seasonal trend, with higher prevalence during summer compared to winter months [15–19]. Understanding asymptomatic *C. difficile* seasonality is important because well-timed preventive and control measures targeting patients at high risk of asymptomatic colonization can be put in place to reduce transmission and emergence of new CDI cases.

Forty-eight different ribotypes were identified among the 104 asymptomatically colonized patients. Similar to the findings of

Table 3
Multinomial logistic regression models for predictors of toxigenic and nontoxigenic *Clostridium difficile* colonization

Characteristic	Toxigenic *C. difficile*		Nontoxigenic *C. difficile*	
	Univariate model, RRR (95% CI)	Multivariate model, RRR (95% CI)	Univariate model, RRR (95% CI)	Multivariate model, RRR (95% CI)
Female	1.25 (0.79–1.99)	1.33 (0.76–2.33)	0.98 (0.46–2.07)	0.82 (0.37–1.82)
Age (per decade)	1.08 (0.94–1.24)	1.07 (0.90–1.28)	1.09 (0.87–1.34)	0.99 (0.78–1.25)
Medical conditions				
Diabetes mellitus	1.06 (0.61–1.83)	1.26 (0.67–2.36)	1.31 (0.57–3.02)	0.96 (0.39–2.36)
Neurologic disorder	1.57 (0.95–2.62)	1.73 (0.94–3.17)	1.40 (0.61–3.20)	1.19 (0.49–2.87)
GORD	1.90 (1.15–3.16)	2.20 (1.17–4.14)	1.32 (0.56–3.14)	1.43 (0.54–3.73)
COPD	1.44 (0.82–2.52)	0.87 (0.42–1.80)	3.13 (1.44–6.77)	3.88 (1.66–9.07)
Chronic kidney disease	2.54 (1.37–4.69)	1.77 (0.83–3.75)	5.15 (2.28–11.67)	5.78 (2.29–14.59)
No. of admissions in year	1.25 (1.13–1.38)	1.24 (1.10–1.39)	1.24 (1.06–1.44)	1.14 (0.96–1.36)
Antimicrobial exposure 30 days before admission	1.34 (0.80–2.24)	0.95 (0.50–1.81)	0.90 (0.42–1.93)	0.60 (0.25–1.46)
Length of stay during current admission	0.99 (0.98–1.01)	1.00 (0.97–1.02)	0.99 (0.98–1.01)	1.00 (0.99–1.02)
Medications during admission				
Antimicrobials	2.62 (1.40–4.92)	2.78 (1.23–6.28)	1.86 (0.75–4.62)	2.40 (0.88–6.61)
Proton pump inhibitors	1.58 (0.97–2.55)	0.92 (0.50–1.72)	1.28 (0.60–2.73)	0.76 (0.31–1.82)
H2 blocker	1.39 (0.59–3.32)	1.14 (0.33–3.93)	0.58 (0.08–4.37)	0.70 (0.09–5.63)
Glucocorticoids	1.26 (0.76–2.10)	1.48 (0.82–2.66)	0.93 (0.39–2.22)	0.76 (0.30–1.93)
Year	0.78 (0.58–1.05)	0.68 (0.47–0.97)	0.94 (0.59–1.50)	0.84 (0.52–1.37)
Season—summer	1.73 (1.06–2.82)	1.81 (1.07–3.06)	1.13 (0.53–2.41)	1.25 (0.57–2.76)

CI, confidence interval; COPD, chronic obstructive pulmonary disease; GORD, gastro-oesophageal reflux disease; RRR, relative risk ratio.

Alasmari *et al.* [11] in the United States, our study found that the 014/020 group was the most common ribotype among asymptomatically colonized patients. However, none of the other ribotypes reported by Alasmari *et al.* (012, 053, 077 and 027) was identified among the colonized patients in Australia. The diversity of ribotypes identified in our study corresponds with surveillance studies among symptomatic CDI cases in hospitals in Queensland [20] and Western Australia [9]. Furthermore, the predominant ribotypes among symptomatic patients (014/020 group and 056) in the surveillance studies matches our findings in asymptomatic patients. These findings suggest that patients colonized with *C. difficile* may act as a source of transmission in the hospital for new CDI cases [3,4,21].

Our study corroborates data reporting that recent hospital admission increases the risk of TCD [2,4,8,22–24]. For each admission to a hospital in the previous 12 months, we found that the risk of TCD colonization increased by 24%. Gastro-oesophageal reflux disease was also associated with TCD; conversely, exposure to proton pump inhibitors (PPIs) during the admission was not a significant factor. Interestingly, medication exposure as a risk factor for TCD colonization remains uncertain. Our findings align with those reported by Kong *et al.* [23], who found no association between PPIs and TCD colonization; however, other studies have identified exposure to PPIs as a risk factor for asymptomatic *C. difficile* colonization [24,25]. Likewise, exposure to antimicrobials during the admission was associated with an increased risk of TCD, while previous studies found that TCD was instead associated with immunosuppressant use [23,24].

With regards to colonization by NTCD, a positive association was observed with chronic obstructive pulmonary disease (but not with smoking history). Chronic renal disease has been previously reported as a risk factor for TCD colonization [22,24], yet from our study findings, chronic renal disease was only associated with NTCD colonization. There is evidence that suggests that colonization with NTCD is protective against infection with TCD strains; hence, it is important to identify this group of patients and prevent the disruption of their "naturally" protected gut microbiome against TCD strains through the use of antimicrobials. Faecal microbiota transplantation has proven to be a highly effective therapeutic alternative for recurrent CDI; thus, future studies need to investigate the potential additional benefits of NTCD colonized donors compared to noncolonized donors.

Screening all inpatients without symptoms of diarrhoea for *C. difficile* will not be a cost-effective disease control measure; thus, by understanding the risk factors, resources could be allocated to those patients who are at high risk of being colonized by a TCD strain. Now that Longtin *et al.* [26] have reported that infection control measures (i.e. isolation precautions and environmental control) targeting asymptomatic TCD colonized patients significantly reduces the incidence of healthcare-associated CDI, identification of risk factors becomes crucial for screening patients at high risk of TCD colonization and allocating resources to reduce CDI transmission in the hospitals.

A striking finding of this study was that TCD and NTCD colonized patients did not share risk factors. This finding may suggest that colonization by TCD and NTCD strains are two different conditions. TCD colonization is likely more closely related to symptomatic CDI than NTCD colonization, given the fact that TCD colonized patients and CDI patients (and not NTCD colonized patients) share exposure to antimicrobials as their main risk factor.

We acknowledge that the study is limited by a number of factors. First, given the small number of events (new CDI cases and deaths) recorded during the follow-up period, it was not possible to elucidate patient and strain characteristics associated with clinical outcomes. Second, the majority of the specimens were collected using rectal swabs (84.3%). The positivity rate with rectal swabs was lower (6.79%) than with stool samples (11.52%), which could have influenced the low prevalence of asymptomatic *C. difficile* colonization identified in this study. However, collection of stool specimens was less convenient and less appealing to patients and would have negatively affected recruitment. Of further note is the fact that rectal swabs were guaranteed to be collected, as they were taken at the time of recruitment. Finally, the study was not designed to capture when a patient was exposed to *C. difficile;* thus, our study population may contain patients that acquired *C. difficile* in the community or in the hospital. Future studies need to investigate if community- and healthcare-associated asymptomatic colonized patients have different epidemiologic profiles as has been reported for symptomatic community- and healthcare-associated CDI cases.

One major strength of the current study was the number of enrolled patients. This is the first study with a sufficient sample size to determine independent (adjusted) risk factors separately for asymptomatic TCD and NTCD colonization. Additionally, given the long study period, ours is the first study to report seasonal differences in asymptomatic carriage over multiple years. Finally, it examined not only factors associated with asymptomatic colonization before hospital admission but also included factors to which patients were exposed during the admission, such as medical procedures (e.g. insertion of nasogastric tubes), surgical procedures and a detailed record of medication exposure, as well as after hospital discharge.

In conclusion, our study found a lower prevalence of asymptomatic TCD and NTCD colonized patients compared to previous studies elsewhere. It also found that risk factors for TCD and NTCD colonization were distinct from each other and that the prevalence of asymptomatic carriage was seasonal, indicating that carriage in the population is dynamic. Additional research is required to elucidate if current international guideline recommendations of not routinely screening and not providing treatment to asymptomatic colonized patients are still the best approach.

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Transparency Declaration

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.cmi.2016.08.030.

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Chapter 4

The relationship between symptomatic *C. difficile* infection and asymptomatic *C. difficile* colonisation

CHAPTER 4. The relationship between symptomatic *C. difficile* infection and asymptomatic *C. difficile* colonisation

4.1. Context

Mathematical modelling studies have estimated that the basic reproduction number (R_0) of *C. difficile* is less than one in hospital settings. Therefore, for CDI to be sustained, spread and cause epidemics in hospital settings, importation of new cases into hospitals is required. A contact-tracing study has determined that admitted cases from the community are the major source of new CDI cases that sustain transmission of *C. difficile* within hospital wards. Given that symptomatic CDI patients admitted to a hospital are typically isolated to prevent further transmission of the bacterium, the other plausible source of importation of *C. difficile* from the community into the wards is through asymptomatic *C. difficile* colonised patients. It is well documented that asymptomatic patients colonised with TCD strains shed spores into the environment, which can come into contact with uncolonised patients that may subsequently develop CDI symptoms.

Symptomatic cases of HA-, CA-CDI and asymptomatic TCD-colonised patients are interconnected, yet no study has investigated all these three components of *C. difficile* epidemiology as a whole in a non-endemic *C. difficile* 027 country. In this Chapter, I present the results of two prospective observational studies, conducted simultaneously, that examined the relationship between symptomatic patients (HA- and CA-CDI) and asymptomatic TCD-colonised patients. Three hundred and twenty-four patients with HA-CDI, CA-CDI or asymptomatic TCD-colonisation were enrolled from two tertiary hospitals and two community-based laboratories located in two different Australian states over a three-year period in order to: 1) compare patients' characteristics for HA-CDI, CA-CDI and asymptomatic TCD-colonisation; 2) identify risk factors associated with symptomatic forms of the disease relative to asymptomatic carriage; and 3) characterise

and compare the predominant *C. difficile* ribotypes circulating among symptomatic and asymptomatic patients. In this chapter, I found that in a non-endemic *C. difficile* ribotype 027 setting, patients' intrinsic characteristics were not associated with experiencing symptoms, rather the main factor that determined symptomatic CDI was antibiotic exposure. I identified that the same ribotypes, present in approximately the same proportions, were isolated from symptomatic patients in the hospital and the community, and asymptomatic patients. The fact that the same *C. difficile* ribotypes were circulating among symptomatic and asymptomatic patients reinforced the hypothesis that transmission between these two states of the disease occurs frequently, and that asymptomatic patients act as a vehicle for introduction of the pathogen from the community into hospitals.

4.2. *C. difficile* ribotypes circulating in Australian hospitals and communities

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Comparison of *Clostridium difficile* Ribotypes Circulating in Australian Hospitals and Communities

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ABSTRACT *Clostridium difficile* infection (CDI) is becoming less exclusively a health care-associated CDI (HA-CDI). The incidence of community-associated CDI (CA-CDI) has increased over the past few decades. It has been postulated that asymptomatic toxigenic *C. difficile* (TCD)-colonized patients may play a role in the transfer of *C. difficile* between the hospital setting and the community. Thus, to investigate the relatedness of *C. difficile* across the hospital and community settings, we compared the characteristics of symptomatic and asymptomatic host patients and the pathogens from these patients in these two settings over a 3-year period. Two studies were simultaneously conducted; the first study enrolled symptomatic CDI patients from two tertiary care hospitals and the community in two Australian states, while the second study enrolled asymptomatic TCD-colonized patients from the same tertiary care hospitals. A total of 324 patients (96 with HA-CDI, 152 with CA-CDI, and 76 colonized with TCD) were enrolled. The predominant *C. difficile* ribotypes isolated in the hospital setting corresponded with those isolated in the community, as it was found that for 79% of the *C. difficile* isolates from hospitals, an isolate with a matching ribotype was isolated in the community, suggesting that transmission between these two settings is occurring. The toxigenic *C. difficile* strains causing symptomatic infection were similar to those causing asymptomatic infection, and patients exposed to antimicrobials prior to admission were more likely to develop a symptomatic infection (odds ratio, 2.94; 95% confidence interval, 1.20 to 7.14). Our findings suggest that the development of CDI symptoms in a setting without establishment of hospital epidemics with binary toxin-producing *C. difficile* strains may be driven mainly by host susceptibility and exposure to antimicrobials, rather than by *C. difficile* strain characteristics.

KEYWORDS *Clostridium difficile*, asymptomatic, community-acquired infections, health care-acquired infection, ribotyping

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Over the past 3 decades, the epidemiology of *Clostridium difficile* infection (CDI) has markedly changed, and several countries have reported a significant increase in the incidence and severity of the disease as well as numerous hospital outbreaks. The changes have been partly attributed to the emergence of specific *C. difficile* strains (PCR ribotypes 001, 027, and 078) with increased toxin production and in some cases resistance to newer fluoroquinolones (1–3). CDI was previously exclusively considered a health care-associated CDI (HA-CDI) affecting elderly patients with multiple comorbidities and a recent history of antimicrobial exposure. However, patients in the

TABLE 1 Frequency distribution of *C. difficile* toxin profiles by source[a]

Toxin profile	No. (%) of patients		
	Symptomatic patients[b]		Asymptomatic patients with TCDc (n = 76)
	HA-CDI (n = 96)	CA-CDI (n = 152)	
A^+, B^+, CDT^+	4 (4.2)	7 (4.6)	3 (4.0)
A^+, B^+, CDT^-	83 (86.5)	139 (91.4)	71 (93.4)
A^-, B^+, CDT^+	1 (1.0)	2 (1.3)	0 (0.0)
A^-, B^+, CDT^-	1 (1.0)	1 (0.7)	1 (1.3)
A^-, B^-, CDT^+	0 (0.0)	0 (0.0)	1 (1.3)

[a]HA, health care associated; CA, community associated; CDI, *C. difficile* infection; TCDc, toxigenic *C. difficile* colonization.
[b]Nontoxigenic (A^-, B^-, CDT^-) *C. difficile* isolates were recovered from seven HA-CDI patients and three CA-CDI patients.

community are now also considered at risk of CDI, and *C. difficile* strains that are known to be highly pathogenic are now frequently isolated from patients with community-associated CDI (CA-CDI) (1). Severe cases of CA-CDI were reported among populations that were considered at low risk of CDI, including pregnant women and healthy young adults without antimicrobial exposure or contact with health care facilities (4, 5).

Symptoms of CDI can range from mild diarrhea to life-threatening conditions, such as pseudomembranous colitis, and are precipitated by the capacity of some *C. difficile* strains to produce toxins A and B and binary toxin (CDT). Similar to other infectious diseases, not all patients colonized with toxigenic *C. difficile* (TCD) strains become symptomatic. Loo et al. found that *C. difficile* ribotype 027 was the predominant strain isolated from symptomatic patients with HA-CDI, whereas asymptomatic patients were more likely to be colonized with other strains (6). However, it is unclear which host and pathogen features determine whether a patient colonized with *C. difficile* will remain asymptomatic or develop mild or severe forms of the disease in a setting where non-ribotype 027 strains are endemic. Although cases of *C. difficile* ribotype 027 infection have been reported in Australia (7, 8), *C. difficile* ribotype 027 has not yet become established, and the most common ribotypes circulating are 014/020, 056, and 002 (9, 10).

It has also been proposed that asymptomatic TCD-colonized patients act as a source of environmental contamination and may result in the emergence of new CDI cases, particularly in a hospital setting (11, 12). Furthermore, epidemiological studies and a mathematical modeling study have demonstrated that CA-CDI importation into the hospital may play a role in maintaining HA-CDI transmission (13–15).

Despite the growing evidence that HA-CDI, CA-CDI, and asymptomatic TCD colonization are interrelated and all three play a significant role in *C. difficile* epidemiology, no reported study has previously evaluated these three components of *C. difficile* at the same time. Therefore, the current study aimed to determine whether these three components are in fact interrelated by comparing the predominant *C. difficile* ribotypes and the characteristics of symptomatic and asymptomatic patients in the health care setting and in the community over a 3-year period.

RESULTS

Over the 3-year study period, 324 patients (96 with HA-CDI, 152 with CA-CDI, and 76 with asymptomatic TCD colonization) were enrolled. One hundred sixty-five patients (50.9%) were enrolled in Queensland, Australia, while 159 (49.1%) were enrolled in Western Australia.

Characteristics of *C. difficile* isolates. Five different toxin profiles were identified among the toxigenic *C. difficile* strains isolated (Table 1). The proportion of toxin profiles did not significantly differ between *C. difficile* categories (P = 0.816). The most common toxin profile was toxin A positive (A^+), toxin B positive (B^+), and CDT negative (CDT^-) (n = 293, 83.2%). Toxin A-negative (A^-), B^+, and CDT-positive (CDT^+) *C. difficile* isolates were recovered only from symptomatic patients (n = 3), while an A^-, toxin B-negative

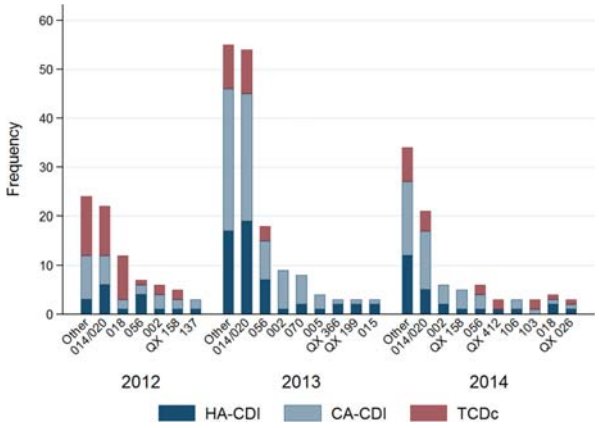

FIG 1 Distribution of ribotypes by year among symptomatic HA-CDI and CA-CDI patients and asymptomatic toxigenic *C. difficile*-colonized (TCDc) patients. Ribotypes found at a frequency of less than 3 isolates in a year were grouped into the other category.

(B^-), and CDT^+ isolate was recovered from only one asymptomatic patient. Nontoxigenic *C. difficile* strains were isolated from 10 symptomatic patients (7 with HA-CDI, 3 with CA-CDI), most likely due to coinfection with TCD strains that were not isolated.

Simpson's indices of diversity were 0.89, 0.89, and 0.88 for HA-CDI, CA-CDI, and asymptomatic TCD colonization, respectively. Although a high diversity of ribotypes (over 90) was identified during the study period, four *C. difficile* ribotypes (i.e., ribotypes 014/020, 056, 002, and 018) accounted for over 50% of the isolates. *C. difficile* ribotype 014/020 ($n = 97$, 29.9%) was the predominant ribotype throughout the 3-year study period among symptomatic patients (both patients with HA-CDI and patients with CA-CDI) and asymptomatic patients (Fig. 1 and the supplemental material). *C. difficile* ribotype 056 ($n = 31$, 9.6%) was the second most common ribotype isolated, followed by ribotype 002 ($n = 21$, 6.5%), which was predominantly found in CA-CDI patients, and ribotype 018 ($n = 18$, 4.9%), which was mainly found in asymptomatic TCD-colonized patients. Among all study patients, virulent ribotypes *C. difficile* 244, 078, 251, and 027 in particular were isolated from only four, two, one, and one CDI patients, respectively.

The predominant *C. difficile* ribotypes isolated from symptomatic HA-CDI patients were concordant with the ribotypes identified among asymptomatic TCD-colonized patients; for over 70% of the isolates from symptomatic patients, an isolate with a matching ribotype was isolated from an asymptomatic patient. Likewise, for 79% of the *C. difficile* isolates from the hospitals, an isolate with a matching ribotype was isolated from the community.

Patients' preadmission characteristics. The preadmission characteristics of the patients constituting the three *C. difficile* categories (HA-CDI, CA-CDI, and TCD colonization) are presented in Table 2. The proportion of females significantly differed between the three groups, with a higher proportion of females having CA-CDI (73.7%) than HA-CDI (52.1%) or asymptomatic TCD colonization (52.6%) ($P < 0.001$). Across the three groups, there was no statistically significant difference in health care exposure in the previous year. With regard to medication exposure in the month prior to enrollment, antimicrobials ($P = 0.031$) and gastric acid suppressants (P value < 0.001) were more often prescribed to patients that developed HA-CDI than to the other two groups, while laxatives ($P < 0.001$) were more often prescribed to patients that were asymptomatically colonized. The rates of household exposure to toddlers, elderly people, domestic animals, or livestock did not significantly differ between the groups. Ten percent of the symptomatic patients (HA-CDI patients [10.4%] and CA-CDI patients [10.0%]) reported having an episode of CDI in the past 12 months, whereas none of the asymptomatic TCD-colonized patients reported such an episode ($P < 0.001$).

TABLE 2 Patients' characteristics and health care, medication, and environmental exposure prior to enrollment[a]

Characteristic	Symptomatic patients		Asymptomatic patients with TCDc (n = 76)	*P* value		
	HA-CDI (n = 96)	CA-CDI (n = 152)		HA-CDI vs CA-CDI	HA-CDI vs TCDc	HA-CDI vs CA-CDI vs TCDc
No. (%) of female patients	50 (52.1)	112 (73.7)	40 (52.6)	<0.001	0.943	<0.001
Median (IQR) age (yr)	61.7 (49.2–75.0)	66.4 (49.1–75.4)	66.2 (54.8–76.8)	0.765	0.317	0.607
Health care exposure 12 mo prior to enrollment						
No. (%) of patients admitted to a hospital	62 (69.7)	105 (69.1)	47 (64.4)	0.924	0.476	0.729
Median (SD) no. of admissions	2.1 (2.2)	1.5 (1.6)	2.0 (2.6)	0.128	0.328	0.323
Median (IQR) LOS in the last admission	7 (4–16)	6 (3–10)	6 (3–9)	0.191	0.140	0.215
No. (%) of patients with medication exposure 30 days prior to enrollment						
Antimicrobials	83 (86.5)	117 (77.0)	51 (69.9)	0.066	0.008	0.031
Gastric acid suppressants	52 (54.7)	34 (22.4)	29 (40.3)	<0.001	0.64	<0.001
Laxatives	28 (29.2)	17 (14.2)	29 (51.8)	0.007	0.005	<0.001
No. (%) of patients with the following household exposure prior to enrollment:						
People <2 yr old	3 (3.1)	6 (4.0)	4 (5.3)	1.000	0.365	0.817
People >65 yr old	24 (25.3)	52 (34.2)	22 (29.3)	0.138	0.553	0.322
Cats	21 (21.9)	23 (15.1)	12 (15.8)	0.176	0.314	0.363
Dogs	30 (31.3)	63 (41.5)	28 (36.8)	0.106	0.441	0.269
Livestock	8 (8.3)	15 (9.9)	7 (9.2)	0.685	0.840	0.921
No. (%) of patients with the following smoking status:						
Current	8 (8.3)	10 (6.6)	7 (9.3)	0.604	0.819	0.740
Ever	52 (54.2)	61 (40.1)	43 (58.1)	0.031	0.608	0.016
No. (%) of patients with history of CDI in past year	10 (10.4)	15 (10.0)	0 (0.0)	0.916	0.003	<0.001

[a]HA, health care associated; CA, community associated; CDI, *C. difficile* infection; TCDc, toxigenic *C. difficile* colonization; IQR, interquartile range; LOS, length of stay.

Characteristics during admission and prior to specimen collection. The reason for admission and the procedures, comorbidities, and medication exposure that occurred during admission are described in Table 3. More patients with HA-CDI (11.5%) than asymptomatic TCD-colonized patients (1.4%) underwent a colonoscopy (P = 0.006); however, more asymptomatic TCD-colonized patients than HA-CDI patients required mechanical ventilation (P = 0.006) and underwent orthopedic (P < 0.001) and neurological (P < 0.001) interventions. Significantly lower proportions of patients with HA-CDI than asymptomatic TCD-colonized patients presented with chronic obstructive pulmonary disease (COPD) (P = 0.026) and neurological disorders (P = 0.042). Conversely, a higher proportion of patients with HA-CDI (16.7%) than asymptomatic colonized patients (4.1%) had inflammatory bowel disease (P = 0.008). In terms of medication exposure during the hospital admission, HA-CDI patients (74.0%) and TCD-colonized patients (77.6%) were equally exposed to antimicrobials (P = 0.578). However, penicillins and β-lactamase inhibitors (P = 0.010) were more often prescribed to patients who went on to develop HA-CDI than asymptomatic TCD-colonized patients. HA-CDI patients were more likely than asymptomatic TCD-colonized patients to have had chemotherapy (P = 0.019) and antidiarrheal medication (P = 0.019), while the latter group of patients was more commonly exposed to laxatives (P = 0.029).

Predictors of symptomatic and severe forms of the disease. The multivariate logistic regression model (Table 4) revealed that patients exposed to antimicrobials within 30 days prior to hospitalization were at a higher risk of developing symptoms (odds ratio [OR], 2.94; 95% confidence interval [CI], 1.20 to 7.14), whereas patients with COPD were at lower risk of developing symptoms of the infection (OR, 0.31; 95% CI, 0.12 to 0.83).

TABLE 3 Reason for admission and procedures, comorbidities, and medication exposure during admission but prior to specimen collection among patients with HA-CDI and asymptomatic toxigenic *C. difficile* colonization[a]

Characteristic	No. (%) of patients		*P* value
	Symptomatic patients with HA-CDI (*n* = 96)	Asymptomatic patients with TCDc (*n* = 76)	
Reason for admission			
New medical/surgical problem	25 (28.1)	35 (47.3)	0.022
Exacerbation of chronic condition	25 (28.1)	19 (25.7)	
Infection	31 (34.8)	12 (16.2)	
Elective surgery	8 (9.0)	8 (10.8)	
Medical procedures			
Insertion of orogastric tubes	8 (8.3)	8 (10.8)	0.680
Gastroscopy	13 (13.5)	4 (5.4)	0.049
Colonoscopy	11 (11.5)	1 (1.4)	0.006
Mechanical ventilation[b]	2 (2.1)	10 (13.5)	0.006
Surgical procedures			
Orthopedic	7 (7.3)	19 (25.0)	<0.001
Abdominal	12 (12.5)	6 (7.9)	0.327
Cardiological/thoracic	2 (2.1)	4 (5.3)	0.238
Neurological	0 (0.0)	11 (14.5)	<0.001
Oncological	5 (5.2)	0 (0.0)	0.052
Other surgical procedures	2 (2.1)	5 (6.6)	0.138
Medical conditions			
Cancer	42 (43.8)	22 (29.7)	0.061
Diabetes mellitus	21 (21.9)	18 (24.3)	0.706
Neurological disorder	17 (17.7)	23 (31.1)	0.042
Gastroesophageal reflux disease	26 (27.1)	24 (32.4)	0.448
Chronic obstructive pulmonary disease	10 (10.4)	17 (23.0)	0.026
Chronic kidney disease	22 (22.9)	14 (18.9)	0.527
Congestive heart failure	11 (11.5)	12 (16.2)	0.369
Liver disease	10 (10.4)	4 (5.4)	0.274
Inflammatory bowel disease	16 (16.7)	3 (4.1)	0.008
Diverticulosis	9 (9.4)	2 (2.7)	0.072
Solid organ transplant	7 (7.3)	1 (1.4)	0.069
Medication exposure			
Any antimicrobial[c]	71 (74.0)	59 (77.6)	0.578
Penicillins and β-lactamase inhibitors	45 (46.9)	21 (27.6)	0.010
Cephalosporins	29 (30.2)	34 (44.7)	0.050
Penicillins	11 (11.5)	12 (15.8)	0.407
Trimethoprim-sulfamethoxazole	11 (11.5)	6 (7.9)	0.437
Carbapenems	11 (11.5)	6 (7.9)	0.437
Ciprofloxacin	9 (9.4)	5 (6.6)	0.354
Aminoglycosides	8 (8.3)	5 (6.6)	0.448
Fluoroquinolones[d]	1 (1.0)	3 (4.0)	0.228
Clindamycin	1 (1.0)	4 (5.3)	0.120
Tetracyclines	1 (1.0)	0 (0.0)	0.442
Macrolides	0 (0.0)	3 (4.0)	0.084
Metronidazole	17 (17.7)	7 (9.2)	0.110
Vancomycin	7 (7.3)	6 (7.9)	0.882
Gastric acid-suppressive agents	59 (61.5)	41 (54.0)	0.321
Proton pump inhibitors	57 (59.4)	37 (48.7)	0.162
H2 blocker	4 (4.2)	5 (6.6)	0.480
Laxatives	28 (29.2)	34 (45.3)	0.029
Nonsteroidal anti-inflammatory drugs	18 (18.8)	13 (17.1)	0.780
Glucocorticoids	35 (36.5)	18 (23.7)	0.072
Chemotherapy	12 (12.5)	2 (2.6)	0.019
Antidiarrheal medication	12 (12.5)	2 (2.6)	0.019

[a]HA, health care associated; CDI, *C. difficile* infection; TCDc, toxigenic *C. difficile* colonization.
[b]Excludes mechanical ventilation during surgical procedures.
[c]Excludes metronidazole and vancomycin.
[d]Ciprofloxacin not included.

TABLE 4 Logistic regression for predictors of symptomatic HA-CDI compared to asymptomatic toxigenic *C. difficile* colonization

Characteristic	OR (95% CI)[a]	
	Univariate model	Multivariate model
Female	0.98 (0.53–1.79)	0.92 (0.45–1.85)
Age (per decade)	0.91 (0.76–1.09)	0.96 (0.78–1.19)
Admitted to a hospital in past 12 mo	1.27 (0.66–2.44)	1.05 (0.48–2.27)
Medication exposure 30 days prior to admission		
Antimicrobials	**2.78 (1.28–5.88)**	**2.94 (1.20–7.14)**
Gastric acid-suppressive agents	1.79 (0.96–3.33)	1.67 (0.76–3.57)
Medical conditions		
Cancer	1.85 (0.97–3.45)	1.15 (0.52–2.50)
Diabetes mellitus	0.87 (0.43–1.79)	0.72 (0.30–1.69)
Neurological disorder	**0.48 (0.23–0.98)**	0.50 (0.21–1.15)
Gastroesophageal reflux disease	0.78 (0.40–1.49)	0.74 (0.33–1.64)
Chronic obstructive pulmonary disease	**0.39 (0.17–0.91)**	**0.31 (0.12–0.83)**
Chronic kidney disease	1.27 (0.60–2.70)	1.16 (0.47–2.86)
Congestive heart failure	0.67 (0.28–1.61)	1.03 (0.35–3.03)

[a]OR odds ratio; CI, confidence interval. Boldface data indicate statistically significant results.

During the follow-up period, four TCD-colonized patients developed symptomatic CDI. Fifty-three and six patients with HA-CDI and CA-CDI, respectively, had recurrent CDI. Nine deaths were recorded, including three among participants with HA-CDI, two among participants with CA-CDI, and four among participants asymptomatically colonized with TCD. Three patients, all with HA-CDI, were admitted to an intensive care unit (ICU). No colectomies were recorded.

DISCUSSION

Previous studies that examined the relationship between *C. difficile* strains and the development of symptoms were conducted during an outbreak (16) or in settings where binary toxin-producing *C. difficile* strains were predominant (6); this is the first epidemiological study of *C. difficile* that was conducted simultaneously in a health care setting and a community setting and that examined symptomatic and asymptomatic patients in a setting without establishment of hospital epidemics with binary toxin-producing *C. difficile* strains. There was no difference in the ribotype diversity of the isolates across the HA-CDI, CA-CDI, and asymptomatic TCD-colonized patients, reflecting similar pathogen population structures. Furthermore, the most prevalent *C. difficile* ribotypes were similar across the HA-CDI, CA-CDI, and asymptomatic TCD-colonized patients, suggesting that transmission of *C. difficile* is occurring between the hospitals and the communities and that asymptomatic TCD-colonized individuals as well as symptomatic patients may be acting as a vehicle of transmission between these two settings.

The finding also suggests that *C. difficile* ribotypes may not be determinants of the development of symptomatic infection but, rather, that the development of symptoms may be mainly driven by host factors, such as immune state and disruption of the gut microbiome by exposure to antimicrobials or underlying conditions affecting the gastrointestinal tract (17–19). Our findings differ from those of a previous study in which a binary toxin-producing *C. difficile* strain (i.e., ribotype 027) was more likely than other strains to cause symptomatic disease (6). This difference could be explained by the very low prevalence of *C. difficile* ribotype 027 and other highly virulent binary toxin-producing strains in Australia, and therefore, our findings may be expected in other settings without hospital epidemics with binary toxin-producing *C. difficile* strains.

Several meta-analyses have described the risk factors for HA-CDI (20) and CA-CDI (21); however, female sex is not a well-documented risk factor for CA-CDI, and few studies have described this association (22–26). In our study, we found that nearly three-quarters of the CA-CDI cases occurred in women, whereas HA-CDI and asymp-

tomatic cases were equally distributed between the sexes. This observation may be mostly related to behavioral risk factors among women that occur in the community rather than physiological differences between the sexes. Among the behavioral factors occurring in the community that may put females at risk of CDI are higher rates of antimicrobial prescriptions (27, 28), vegetable consumption (29), and contact with children (30).

While there is no conclusive evidence that contaminated food leads to CDI in humans, studies have found that retail vegetables are contaminated with *C. difficile* strains similar to those affecting humans (31, 32). Likewise, the *C. difficile* ribotypes frequently isolated in the current study, such as 014/020 and 056, are common ribotypes found in piglets and veal calves, respectively, in Australia (33, 34). Therefore, the possibility of food being a vehicle of *C. difficile* transmission cannot be ruled out. Although our study did not find an association between the CDI category and contact with toddlers (30), this association needs to be assessed in the context of gender as an effect modifier. Due to the small number of participants that reported living with toddlers, this analysis was not possible.

Another interesting finding was that 10% of symptomatic patients in both settings (hospital and community) but none of the asymptomatic TCD-colonized patients reported having had a CDI in the previous year. While this may be explained by recall bias, given the greater awareness of the disease among the symptomatic patients, this finding may also reflect differences in immune system capacity, with previous infection not offering protection against further infection in these individuals. Those with some degree of immunosuppression might develop symptoms, and those with a fully functioning immune system might not develop symptoms irrespective of the toxigenicity of the *C. difficile* strains to which the patient had previously been exposed. This hypothesis warrants further investigation that would require measurement and comparison of the serum antibody, proinflammatory cytokine, and chemokine levels of noncolonized, asymptomatic *C. difficile*-colonized, and symptomatic CDI patients. However, indirect evidence from the current study supports our hypothesis, given that patients with some degree of immunosuppression (patients on chemotherapy) were more likely to develop symptoms.

This study supports reports elsewhere that inflammatory bowel disease is a risk factor for developing CDI (35); however, a finding that requires further investigation is that patients with COPD were less likely to develop symptoms. Wojciechowski and colleagues reported a reduced risk of CDI for patients with a COPD diagnosis and when systemic corticosteroids were used during antimicrobial treatment (36). This was corroborated by the findings of the present study, whereby COPD was statistically significantly associated with a reduced risk of CDI. Wojciechowski and colleagues argued that corticosteroids attenuate the host immune response to *C. difficile* toxins, thus reducing the toxin-induced cytokine release that is associated with systemic symptoms of CDI (36). Further studies are required to confirm the mechanism behind the association.

There are some limitations to this study. Although a large sample size ($n = 342$) of patients was enrolled, the small number of significant health outcomes (i.e., deaths, ICU admission) recorded during the follow-up period precluded statistical analyses to elucidate whether HA-CDI was associated with more severe outcomes than CA-CDI. In addition, more discriminatory strain typing methods (e.g., multilocus variable-number tandem-repeat analysis and whole-genome sequencing) are required to conclusively determine specific transmission events between community and hospital CDI cases as well as the role of asymptomatic colonized patients.

In summary, similar *C. difficile* ribotypes were circulating in the community and hospitals in this study of two Australian states, suggesting the carryover of strains between settings. Furthermore, asymptomatic and symptomatic patients were colonized with similar *C. difficile* ribotypes, suggesting that in a setting without establishment of hospital epidemics with binary toxin-producing *C. difficile* strains, the development of symptoms may be primarily driven by host characteristics rather than *C. difficile* toxigenicity or ribotype. Future epidemiological studies in settings without

hospital epidemics with binary toxin-producing *C. difficile* strains are needed to confirm our findings and determine the role of patient-, antibiotic-, and *C. difficile* strain-related factors in the development of symptoms.

MATERIALS AND METHODS

Study setting. Two studies were simultaneously conducted over a 3-year period (2012 to 2014) in two Australian states. The first study examined symptomatic patients with HA-CDI and CA-CDI, whereas the second study examined asymptomatic *C. difficile*-colonized patients in a health care setting.

The first study enrolled patients in two tertiary care hospitals, The Royal Brisbane and Women's Hospital (RBWH) with 929 beds in Brisbane, Queensland, Australia, and The Sir Charles Gairdner Hospital (SCGH) with 607 beds in Perth, Western Australia. Patients in the community who submitted specimens through their general practitioner (GP) to coordinating laboratories (Sullivan Nicolaides Pathology in Brisbane, Queensland, Australia, and PathWest Laboratory Medicine, Clinipath Laboratories, and Western Diagnostic Pathology in Perth, Western Australia, Australia) were also enrolled. This study used a census design, in which all the stool specimens submitted during the study period to the hospitals and the laboratories by patients 18 year of age or older and experiencing diarrhea were screened for *C. difficile*. If the specimen was positive for the *C. difficile* toxin A or B gene, the patient was invited to participate in the study. HA-CDI was defined as health care facility-onset, health care facility-associated CDI constituting the onset of diarrhea 48 h or more after admission to a hospital and as community-onset, health care facility-associated disease constituting the onset of symptoms in a patient who had been discharged from a health care facility within the previous 4 weeks. CA-CDI was defined as community-onset CDI in a patient who had not been admitted to a health care facility in the previous 12 weeks or as health care facility-onset CDI within 48 h or less of admission to the hospital (37).

The second study has been previously described elsewhere (38). In brief, six cross-sectional surveys (two per year) were conducted at RBWH and SCGH. Randomly selected hospitalized patients aged 18 years or older without diarrhea were approached and invited to participate in the study. Patients who were not experiencing diarrhea and who had a toxigenic *C. difficile* strain (positive for the presence of *tcdA*, *tcdB*, and/or the *cdtA* and *cdtB* genes) isolated from their stool were considered to have asymptomatic TCD colonization and were included in the current analysis.

The studies received the approval of RBWH (approval no. HREC/11/QRBW/223), the Sir Charles Gairdner Group (approval no. 2011-088), The University of Queensland (approval no. 2011000898), and The University of Western Australia (approval no. RA/4/1/5186) Human Research Ethics Committees. All the participants (or a legal proxy) provided written informed consent for their inclusion in the study. In Western Australia, a waiver of consent was granted when a person was unable to provide consent but the person could be enrolled in the study without any additional risk beyond that associated with their standard care.

Data collection. A questionnaire with questions regarding the patient's age, sex, occupation, previous hospital admissions, and use of medications in the previous 30 days and his or her cohabitants' ages was administered to all patients from both studies. For hospitalized patients at RBWH and SCGH, medical records were accessed to obtain additional information and to determine the date and the reason for the current admission, comorbidities, as well as the inpatient medications provided and procedures performed prior to specimen collection. Each participant was followed up on a monthly basis for 3 months by examination of the patient's records and a short interview for hospital patients and by a telephone interview for discharged or CA-CDI cases. The follow-up interviews were used to determine the clinical outcomes of the patients (whether they developed symptoms, had a recurrence of CDI, underwent a colectomy, were admitted to an ICU, or died).

Stool specimen collection and processing. As previously described (38), direct stool specimen culture was performed on ChromID *C. difficile* agar (bioMérieux). Broth enrichment in Robertson's cooked meat medium followed by ethanol shock and subculture on ChromID *C. difficile* agar at 48 to 72 h was performed if the direct culture result was negative. Putative *C. difficile* colonies were subcultured onto prereduced blood agar plates under anaerobic conditions. *C. difficile* isolates were tested for the presence of toxin genes and were ribotyped by PCR as previously described (39). Strains that did not produce banding patterns matching the pattern for an international ribotype in the reference collection were assigned a local nomenclature (QX type).

Statistical analysis. The frequency of *C. difficile* ribotypes was tabulated by year and *C. difficile* category (HA-CDI, CA-CDI, and asymptomatic TCD colonization) to identify the predominant ribotypes circulating in each category and to examine the changes in ribotype profile over the study period. Simpson's index of diversity was calculated for each category to compare the diversity of ribotypes isolated across the three categories.

Pearson's chi-square test and Fisher's exact test were used to compare categorical variables, whereas the Wilcox-Mann-Whitney U test and Kruskal-Wallis H test were used to compare continuous variables between participant groups. Multivariate logistic regression models were built to identify predictors of symptomatic disease. After adjustment for the age and sex of the patients and known risk factors for CDI (i.e., prior hospital admissions and exposure to antimicrobials and gastric acid-suppressive agents), the inclusion of comorbidities in the regression model was done through a stepwise forward selection by use of the Akaike information criterion as the selection criterion. A significance level cutoff of a *P* value of 0.05 was used for all analyses. All statistical analyses were conducted using Stata SE, version 14 (Stata Corporation, College Station, TX).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/JCM.01779-16.

TEXT S1, PDF file, 0.05 MB.

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Chapter 5

Community-associated *C. difficile* infection

CHAPTER 5. Community-associated *C. difficile* infection

5.1. Context

Understanding the spatio-temporal distribution of infectious diseases, including identification of clusters and environmental drivers that are associated with an increase in transmission of the disease, can provide insights for planning preventive and control programs in the community. CDI was historically considered primarily a nosocomial infection, thus our current knowledge is very poor in terms of its spatio-temporal distribution and environmental drivers in the community. In this Chapter, I present three ecological studies that focussed on symptomatic CDI with an emphasis on CA-CDI and its spatio-temporal distribution.

In the first study I analysed *C. difficile* data collected at Sullivan Nicolaides Pathology across Queensland over 10 years and environmental data from the WorldClim project in order to determine the spatio-temporal distribution of CA- and HA-CDI and to identify patient and environmental variables associated with CDI. I built a logistic regression model in a Bayesian framework with proportion of submitted stool samples that were positive for *C. difficile* as the outcome and fixed effects for sex, age, source of the sample (healthcare-facility or community), elevation, rainfall, temperature, seasons of the year, time in months and spatially unstructured random effects at the postcode level. I reported an increasing annual trend in HA- as well as in CA-CDI over the study period and a strong seasonality with a higher proportion of positive samples submitted during the summer months.

Given that the steady increase in CA-CDI cases in Queensland could not be explained by patient or environmental factors, in the second study presented in this

Chapter, I explored whether an increase in medication exposure at a population-level is driving the increase in CA-CDI. For this analysis, I used the same dataset from Sullivan Nicolaides Pathology but restricted the analysis to CA-CDI cases and incorporated data from the Pharmaceutical Benefits Scheme for 11 commonly prescribed drugs. A logistic regression model was built, in which I incorporate fixed effects for sex, age, drug prescribed, year and spatially unstructured random effects at the statistical area 4 level as predictors for CA-CDI. I reported that exposure to different medications (including antibiotics) at a population-level was not associated with CA-CDI; therefore, a more holistic investigation is required to identify alternative factors (e.g. transmission of the pathogen from food/animals or from the hospitals into the community) to determine what is driving the increase in CA-CDI in the wider population.

In the first study presented in this Chapter I found that a higher proportion of submitted stool samples were positive for *C. difficile* during the summer months. This finding contradicts a reported hypothesis that CDI peaks in winter months because of the higher incidence of respiratory infections, which leads to an increase in antibiotic prescriptions during winter months, which is known to be the strongest risk factor for CDI. Therefore, I compiled data from all published studies and described the global patterns of *C. difficile* seasonality. I found that CDI had a similar seasonal pattern in the Northern and Southern Hemispheres, characterized by a peak in spring and lower incidence during summer/autumn, which was consistent with the first study in this Chapter.

5.2. Spatio-temporal analysis of *C. difficile* infection in Queensland, Australia

Furuya-Kanamori L, Robson J, Soares Magalhaes RJ, Yakob L, McKenzie SJ, Paterson DL, Riley TV, Clements AC. A population-based spatio-temporal analysis of *Clostridium difficile* infection in Queensland, Australia over a 10-year period. *J Infect* 2014;69:447-55.

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A population-based spatio-temporal analysis of *Clostridium difficile* infection in Queensland, Australia over a 10-year period

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Summary *Objectives:* To identify the spatio-temporal patterns and environmental factors associated with *Clostridium difficile* infection (CDI) in Queensland, Australia.
Methods: Data from patients tested for CDI were collected from 392 postcodes across Queensland between May 2003 and December 2012. A binomial logistic regression model, with CDI status as the outcome, was built in a Bayesian framework, incorporating fixed effects for sex, age, source of the sample (healthcare facility or community), elevation, rainfall, land surface temperature, seasons of the year, time in months and spatially unstructured random effects at the postcode level.
Results: C. difficile was identified in 13.1% of the samples, the proportion significantly increased over the study period from 5.9% in 2003 to 18.8% in 2012. CDI peaked in summer (14.6%) and was at its lowest in autumn (10.1%). Other factors significantly associated with CDI included female sex (OR: 1.08; 95%CI: 1.01–1.14), community source samples (OR: 1.12; 95%CI: 1.05–1.20), and higher rainfall (OR: 1.09; 95%CI: 1.02–1.17). There was no significant spatial variation in CDI after accounting for the fixed effects in the model.

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Conclusions: There was an increasing annual trend in CDI in Queensland from 2003 to 2012. Peaks of CDI were found in summer (December—February), which is at odds with the current epidemiological pattern described for northern hemisphere countries. Epidemiologically plausible explanations for this disparity require further investigation.

Introduction

Clostridium difficile is a Gram-positive, toxin-producing anaerobic bacterium. Worldwide, *C. difficile* infection (CDI) is a major cause of antibiotic-associated diarrhea in hospitalized patients.[1] Infection and progression to disease are facilitated by exposure to antibiotics, which disrupt the normal gut microbiome and permit proliferation of *C. difficile*.[1,2] The spectrum of disease caused by *C. difficile* ranges from asymptomatic infection or uncomplicated diarrhea to severe conditions, such as pseudomembranous colitis and toxic megacolon, which may progress to colonic perforation, peritonitis, shock and death.[1]

Over the past two decades, the incidence and severity of CDI have increased globally.[3—14] While most studies have focused on the escalating rates in industrialized countries in the northern hemisphere,[3—9] it is increasingly being recognized that CDI is a major public health threat in less developed countries; for example, studies in Argentina,[10] Chile,[11] India,[12] Iran,[13] and Peru[14] have shown a consistently high prevalence of CDI (6—17%) among hospital inpatient cases. The worldwide increase in incidence in the last decades has resulted in massive economic losses.[15] It is estimated that CDI costs US healthcare facilities approximately US $800 million annually.[16]

Until the mid-2000s, severe cases of CDI were typically only reported for high-risk individuals such as elderly patients with comorbidities.[17] However, recent studies have also reported severe cases of CDI among groups that were previously considered to be at low risk, such as healthy people in the community without exposure to antibiotics,[18] children,[19] and peripartum women.[20] Of additional concern are so-called "hypervirulent" strains of *C. difficile*, such as PCR ribotype 027/North American pulsefield type 1, that have recently emerged. A fluoroquinolone-resistant PCR ribotype 027 strain has caused hospital outbreaks of severe CDI with high mortality rates in Canada,[21] the USA,[22,23] and Europe[24] since the mid-2000s. In 2009, the first case of *C. difficile* PCR ribotype 027 infection was identified in Australia.[25] Subsequently, two outbreaks of hypervirulent strains of *C. difficile* have been reported in hospitals[26,27] and one in the community[28] in Australia.

Several studies conducted in the northern hemisphere have consistently described a seasonal pattern in which the incidence of CDI increases during winter, which has been assumed to be associated with higher antibiotic consumption as a result of increased respiratory infections.[29—31] However, little is known about the seasonal pattern and the environmental conditions associated with CDI in southern hemisphere countries. Furthermore, current evidence suggests a potential role of the community in the epidemiology of CDI that has not been sufficiently assessed because previously, CDI was considered primarily a nosocomial infection.[32—34]

The aims of the current study were: (1) to determine the spatio-temporal distribution of laboratory-confirmed CDI from the community and healthcare facility specimens in the state of Queensland, Australia over a 10-year period, and (2) to determine patient and area-level variables associated with CDI.

Methods

Ethics statement

The study was approved by The University of Queensland Medical Research Ethics Committee (2013000812). Since the study consisted entirely of secondary analysis of patient de-identified data, the requirement for informed consent was waived.

Study area

The state of Queensland occupies the north-eastern portion of Australia and it is situated between the 10° and 29° south latitude and between 138° and 154° east longitude. Queensland has an approximate area of 1.73 million square kilometers,[35] making it the sixth-largest administrative subdivision of a country in the world, and is divided into 73 local government areas and 424 postcodes. Although highly variable due to the large size of the state, the climate can be described as mild and dry in winter and warm and humid in the summer, with peaks in rainfall and temperature between December and February.[36] The population of Queensland recently exceeded 4.5 million people, with most people concentrated in the southeast of the state, where the state capital Brisbane is located, and in large coastal settlements such as Townsville and Cairns.[37]

C. difficile data

Data were obtained from Sullivan Nicolaides Pathology, which has 267 collection centres located throughout the state as well as specimen collection at most private hospitals and nursing homes. Stool samples submitted for *C. difficile* toxin gene detection from May 2003 to December 2012 were included in the study. For the entire period of the study, laboratory confirmation of CDI was based on PCR detection of gene targets in the pathogenicity locus. From 2003 to August 2010, the molecular target was *tcdE* and the assay was agarose gel based. From August 2010 onwards, the detection method changed to real time PCR with dual targets *tcdE* and *tcdB*. The age and sex of the person from whom the specimen was obtained (referred to

hereafter as the patient), the source of the specimen (whether it was submitted by a patient from the community or a healthcare facility), the date the specimen was collected and the postcode where the patient lived were provided by the laboratory. The case definition of community-associated CDI used for the study was based on a positive *C. difficile* toxin gene detection from a specimen submitted by a patient from the community. Likewise, the case definition of healthcare facility-associated CDI was based on a positive *C. difficile* toxin gene detection from a specimen submitted by a patient from either a hospital or a nursing home.

Recurrence of *C. difficile* associated-diarrhea is a common event, occurring in approximately 25% of cases,[38,39] with an average relapse time of 4–28 days.[40–42] Thus, only the first positive specimen from a patient within 30 days was included in the study as a new CDI case to avoid the inclusion of recurrent cases as primary events. If another specimen from the same patient was positive by PCR for *C. difficile* toxin genes after 30 days, it was considered a new infection and included in the study as a different CDI event. In case a patient submitted more than one specimen within a 30-day period and all the specimens were negative by *C. difficile* toxin genes, only the first specimen was included in the study. The initial data set included 36,607 specimens. After excluding the repeat *C. difficile* cases within one month, the final data set contained data from 24,496 specimens collected from 392 postcodes in Queensland. The data were aggregated by month and postcode. The total number of specimens from which *C. difficile* toxin gene was identified as the numerator and the number of specimens examined as the denominator.

Environmental variables tested for association with CDI

High-resolution (1 square kilometer) raster maps of interpolated long-term average monthly rainfall (in millimeters), minimum, maximum and mean temperatures (in degrees Celsius) and elevation (in meters above mean sea level) were obtained from the WorldClim project (www.worldclim.org) website.

Rainfall, elevation and temperature maps were imported into a geographical information system (GIS; Quantum GIS version 1.8.0-Lisboa, QGIS Development Team) and linked spatially to a digitized boundary map of the 424 postcodes across Queensland. The postcode mean values of elevation, rainfall and temperature were extracted in the GIS. The values of rainfall, elevation and temperature were then standardized to have mean $= 0$ and standard deviation $= 1$ to improve identifiability in the subsequent regression models. Minimum, maximum and mean temperature showed high correlation ($r > 0.90$, $p < 0.001$), thus only mean temperature was selected and included in the subsequent regression models.

Statistical analysis

To identify whether the *C. difficile* cases with community origin were leading or lagging the healthcare facilities' *C. difficile* cases, cross-correlation coefficients (CCF) were used to examine the correlation and lag value (in months) between CDI in the community and from healthcare facilities using the temporal trend data from 2003 to 2012.

A Bayesian spatio-temporal model was constructed using WinBUGS version 1.4.3 (Medical Research Council Biostatistics Unit, Cambridge, UK). A binomial logistic regression model was developed with the following parameters: fixed effects for age, sex, source of the sample, elevation, long-term average monthly rainfall and mean temperature, time in months from May 2003 to December 2012, season (summer, December–February; autumn, March–May; winter, June–August; and spring, September–November) and spatially unstructured random effects at the postcode level.

The individual data were aggregated by postcode, month, sex and source of the sample (community or healthcare facilities). The model was of the form:

$$Y_{i,j} \sim \text{Binomial}\left(n_{i,j,k,l}, p_{i,j,k,l}\right)$$

$$\text{logit}\left(p_{i,j,k,l}\right) = \alpha + \sum_{n=1}^{p} \beta_n \times \mathbf{x}_{i,j,k,l} + u_i$$

where $Y_{i,j,k,l}$ is the number of samples with CDI in postcode i, month j, sex k and source l; $n_{i,j,k,l}$ is the number of stool samples examined in postcode i, month j, sex k and source l; $p_{i,j,k,l}$ is the probability of CDI for the stool samples examined in postcode i, month j, sex k and source l; α is the intercept; $\sum_{n=1}^{p} \beta_n \times \mathbf{x}_{i,j,k,l}$ is the matrix of covariates (month, sex, source, average age for the postcode–month–sex–source group, elevation, mean temperature, mean rainfall, and season). In addition, an unstructured postcode-level random effect (u_i) was included in the model. Non-informative prior distributions were used for α (uniform prior with bounds $-\infty$ and ∞) and the covariates (normal prior with mean $= 0$ and precision $= 1 \times 10^{-5}$). The random effects were assumed to follow a normal distribution, with a mean of zero and a variance of $1/\tau$, where the precision (τ) was given a gamma prior distribution with shape and scale parameters $= 0.5$.

Moran's *I* statistic was used to assess spatial autocorrelation in CDI cases at a postcode level.[43] Moran's *I* statistic (-0.002; 95%CI: -0.005 to 0.001) indicated spatial randomness (i.e. no autocorrelation, or clustering), which provided the basis for not including a spatially structured random effect in the model.

After an initial burn-in of 1000 iterations, the parameters of each model were monitored for the subsequent 10,000 iterations. Convergence was assessed by visual inspection of history and density plots. Convergence occurred within the first 1000 iterations for each model. Ten thousand values from the posterior distribution of each parameter were stored and summarized using descriptive statistics (posterior mean, 95% posterior credible interval). Choropleth maps of the proportion positive to *C. difficile* and model random effects were created in the GIS.

Results

Descriptive analysis

Among the 24,496 specimens in the data set, *C. difficile* toxin genes were identified in 3203 (13.1%) specimens. Fifty-six percent of the patients who submitted a stool sample were female. The patients' age range was 0—104 years (median 61.91 years, IQR 39—77 years), age presented a bimodal distribution peaking at 0—2 years and 78—82 years (Table 1). The percentage of CDI-positives increased over the study period, with a yearly average of 5.9% and 5.5% in 2003 and 2004, respectively, to 18.8% in 2012 (Fig. 1A). A clear seasonal pattern was evident, with the average monthly percentage varying from 10.1% in April to 14.6% in December (Fig. 1B). The map of the proportion of CDI did not show a clear spatial pattern (Fig. 2). The cross-correlation coefficient (CCF = 0.65) indicated that community-associated *C. difficile* cases and the healthcare facilities-associated *C. difficile* cases are significantly positively correlated at lag = 0 months (−1 month to 3 months).

Spatio-temporal model of CDI

Female sex (OR 1.08; 95%CI 1.01—1.14) and stool sample from a community source (OR 1.12; 95%CI 1.05—1.20) were significant predictors of CDI. There was a significant, positive relationship between CDI and rainfall (OR 1.09; 95%CI 1.02—1.17), indicating a 9% increase in odds per 100 mm of monthly rainfall. Additionally, there was a positive temporal trend of CDI (effect of an annual change, adjusted for other factors, including seasonality: OR 1.12; 95%CI 1.02—1.13). The odds of CDI decreased by 16% (OR 0.84; 95%CI 0.75—0.93) during the autumn months (March—May) when compared with the summer months (December—February), indicating a significant seasonal pattern of CDI. No statistically significant associations were found between CDI and mean age, elevation or mean monthly temperature (Table 2). The map of the random effects of CDI for the regression model showed no obvious spatial patterns or evidence of clustering of high-risk postcodes.

Discussion

The study provided insight into the spatial-temporal patterns of CDI in Queensland. Consistent with other studies conducted in industrialized countries in the northern hemisphere,[3,5—8] where the incidence of CDI has increased over the past two decades, the current study found a significant, increasing trend in the proportion of CDI for the last decade in Queensland, Australia. Identifying the causative factors of the increasing trend should be the focus of ongoing research. One explanation might be the increased use of broad spectrum antibiotics[44,45] which is a risk factor for hospital-acquired[46] and community-acquired[47] CDI. Another factor could be the rising rate of proton pump inhibitors prescribed in Australia,[48] which has also been demonstrated to be associated with an increased risk of CDI.[49] Additionally, the emergence of new strains of *C.*

Table 1 Demographic characteristics of the patients as per *C. difficile* infection.

Variable	*C. difficile* infection	
	Positive	Negative
	N (%)	*N* (%)
Stool samples	3203 (13.08)	21,293 (86.92)
Sex		
Male	1261 (11.94)	9304 (88.06)
Female	1942 (13.94)	11989 (86.06)
Age		
0—9 years	213 (13.47)	1368 (86.53)
10—19 years	86 (13.52)	550 (86.48)
0—29 years	216 (12.68)	1487 (87.32)
30—39 years	263 (12.44)	1851 (87.56)
40—49 years	289 (12.12)	2095 (87.88)
50—59 years	274 (9.67)	2560 (90.33)
60—69 years	454 (11.77)	3402 (88.23)
70—79 years	656 (14.90)	3748 (85.12)
80—89 years	607 (14.84)	3483 (85.16)
90—99 years	141 (16.00)	740 (84.00)
≥100 years	4 (30.77)	9 (69.23)
Source		
Healthcare facilities	1410 (13.06)	9387 (86.94)
Community	1792 (13.08)	11907 (86.92)

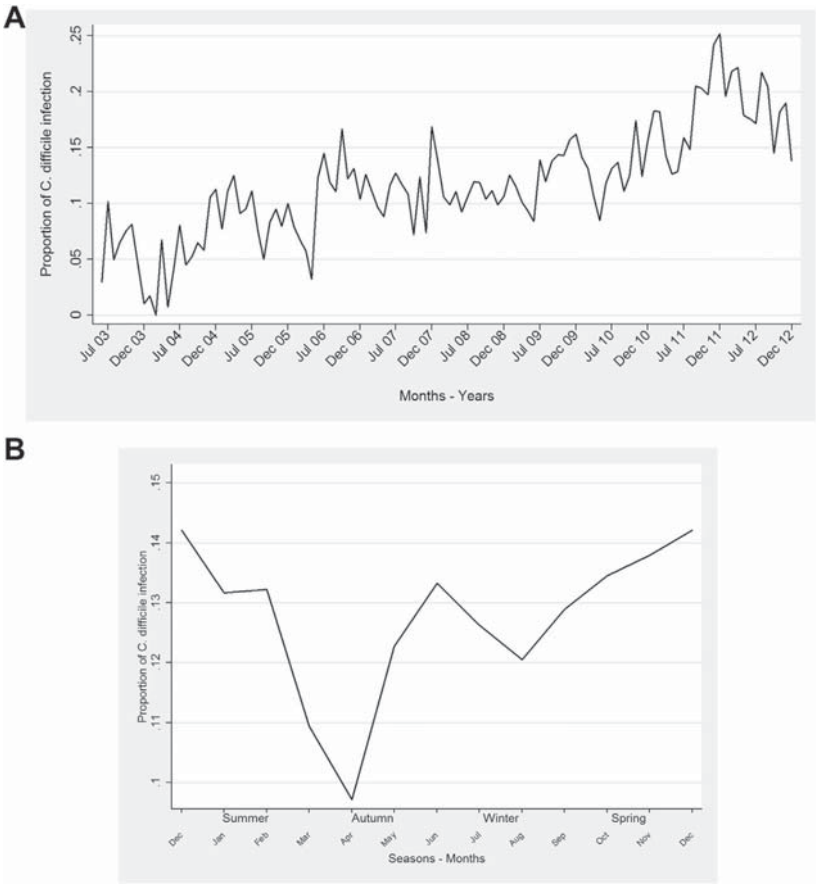

Figure 1 Variation of the proportion of *C. difficile* infection in Queensland, Australia by (A) temporal pattern between 2003 and 2012 and (B) seasons — months.

difficile that are more readily transmitted should be considered.[25–27]

There are several potential confounders that might affect temporal trends in laboratory detection of CDI such as the increase in submissions in response to increasing *C. difficile* awareness among physicians over time.[50,51] However, the current study investigated the proportion of specimens that were *C. difficile* toxin gene positive, which should not have been sensitive to more physicians submitting specimens; if anything, a decreasing trend would likely result due to a lower threshold of suspicion for samples to be submitted. Whilst molecular techniques were utilized for the entire period of the study there was a change to a more sensitive and specific multiplex PCR[52,53] in August 2010, the annual change in CDI, adjusted for other factors, before August 2010 (OR 1.11; 95%CI 1.01–1.14) and after August 2010 (OR 1.13; 95%CI 1.02–1.17) followed a similar trend. Therefore, the statistically significant increase in CDI found in the past decade was not due to the variation of the diagnostic method used by the laboratory.

Seasonal patterns of CDI with respiratory co-infections have been described in the USA[29,30] and Canada.[31] Although the mechanism is currently unknown, CDI occurrence has been associated with incidence peaks of pneumonia,[29] influenza[29–31] and respiratory syncytial virus[31] in winter and with the increased rates of antibiotics prescribed during this season.[31] Conflicting with the increased incidence of *C. difficile* cases in the USA and Canada during the northern hemisphere winter (December–February) but consistent with a recent study conducted in Australia,[54] the current study found peaks of CDI during the southern hemisphere summer (December–February) and a lower proportion of CDI during the Australian autumn (March–May). One explanation could be the difference in antibiotic prescribing practices compared to northern hemisphere countries: in Australia, the use of broad spectrum antibiotics is restricted, potentially attenuating any peaks in CDI incidence associated with respiratory infections in the winter. After a reduction in the prescription of third-generation cephalosporins in Western Australia in the late 1990s, the incidence of CDI significantly decreased by 50%.[55,56] An alternative or additional explanation could be the movement of people or food commodities into Australia from endemic countries, which increase during the summer months.[57] Asymptomatic carriers or imported foods from northern hemisphere countries may act as a vehicle for the pathogens during the southern hemisphere summer.

This study confirms a statistically significant association between rainfall and CDI, which raises another possible explanation: that the disparity between the proportion of CDI in Queensland and CDI rates in North America could be partly a result of physical environmental factors such as

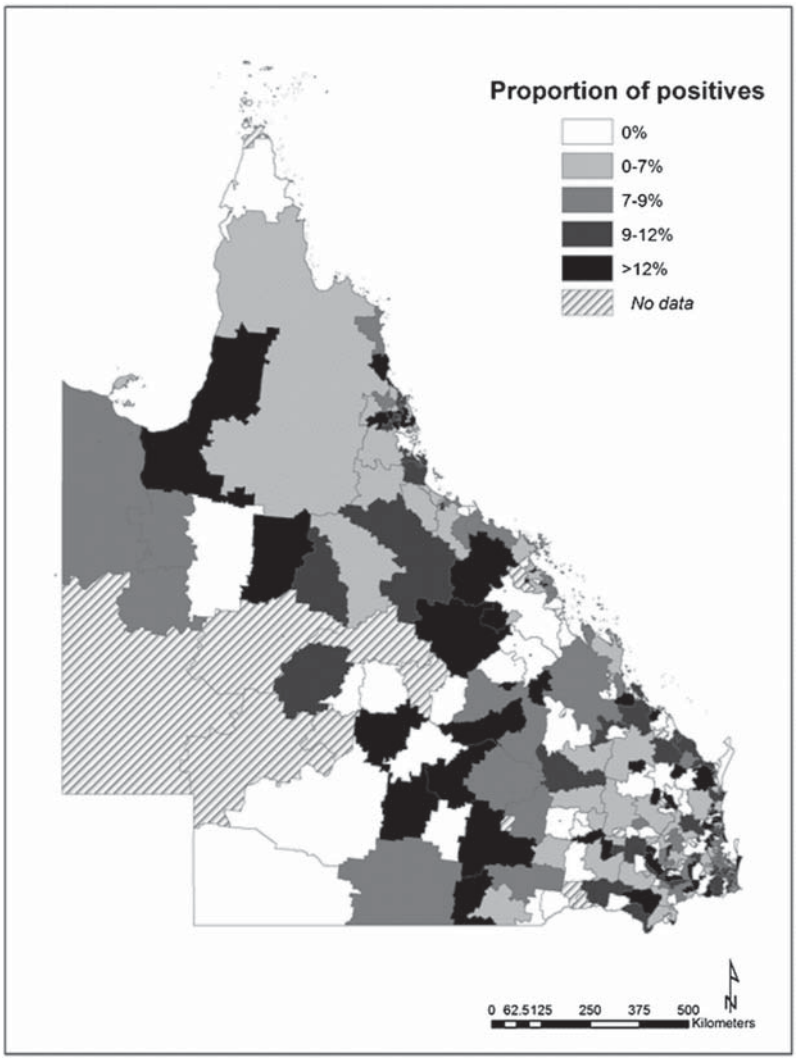

Figure 2 Proportion of *C. difficile* infection by postcodes in Queensland, Australia between 2003 and 2012 by quintiles.

seasonal rainfall patterns, which peak during the summer in Queensland.

Although, CDI has been considered predominantly a healthcare-associated infection, similar proportions of CDI were found from the community and healthcare facilities. Little is known about asymptomatic carriage in the community or community-acquired CDI in Queensland. Importantly, recent studies conducted in the USA and Canada have found that community-acquired CDI increased in incidence and severity particularly in elderly women.[33,34] The findings of the current study indicate that healthcare facilities-associated CDI and community-associated CDI cases are significantly positively correlated, raising the possibility that CDI rates in the healthcare facilities might be an important driver of CDI transmission in the community and vice versa. A contact tracing study conducted by Walker et al.[58] and mathematical modeling studies of Yakob et al.[32,59] demonstrated that carrier importation into the hospital from the community plays a significant role in the epidemiology of *C. difficile*, but the flow of CDI from the hospital back into the community requires further investigation.

There were several limitations in our study. Perhaps the most important limitation was the fact that Sullivan Nicolaides Pathology does not receive all *C. difficile* stool specimens in Queensland. Public hospital laboratories receive specimens collected in public hospitals in the state, and there is another large private pathology provider with a similar market share to Sullivan Nicolaides Pathology that receives samples from the community, nursing homes and private hospitals. This prevented the use of the population at risk as the denominator. However, an advantage of the current study is that the denominator used (total number of submissions) was likely to have been robust to the effects of differential geographical and temporal coverage of Sullivan Nicolaides Pathology services. A second important limitation is that the classification of the source was based entirely on the origin of the stool specimen at the collection point. Unfortunately, data regarding whether a stool specimen from the community came from a patient that was previously hospitalized or whether a patient developed diarrhea within 48 h of admission to a hospital were not available. Therefore, the classification of

Table 2 Regression model for the association of demographic, environmental characteristics, temporal pattern and spatially unstructured random effects at the postcode level with *C. difficile* infection.

Variable	OR	95%CI limits	
		Lower	Upper
Source	**1.12**	**1.05**	**1.20**
Sex	**1.08**	**1.01**	**1.14**
Age (per decades)	1.01	0.99	1.02
Elevation (in meter ×100)	1.02	0.99	1.04
Temperature (in °C ×10)	1.00	0.99	1.02
Rainfall (in mm ×100)	**1.09**	**1.02**	**1.17**
Temporal pattern (in months ×12)	**1.12**	**1.02**	**1.13**
Season			
Autumn	**0.84**	**0.75**	**0.93**
Winter	1.07	0.90	1.26
Spring	1.04	0.93	1.16
Variance (unstructured)	64.17	30.55	126.10

OR, odds ratio; CI, confidence interval.
Reference value for sex is male, for source is healthcare facility, for season is summer.
Statistically significant ORs are emboldened.

community and hospital sources might not have provided a highly accurate indication of the location of exposure of the patient. Finally, the inability to match the antibiotics received prior to the patient submitting stool samples with the result of the *C. difficile* toxin genes result precluded adjusting the regression model results for antibiotics exposure. Further investigation, including incorporating data regarding prescribed antibiotics in Australia, is required to understand the observed seasonal pattern and establish whether, and why, it differs from the one described in northern hemisphere countries.

The current study provides new evidence for a significant increase in the prevalence of CDI in Queensland, Australia, over the past 10 years. In addition, it also provides evidence of a seasonal pattern of CDI that does not appear to coincide with the seasonal patterns described in the northern hemisphere. Future CDI risk assessment should assess drivers of both of these temporal aspects of *C. difficile* epidemiology. Additionally, the finding of a lack of spatial heterogeneity in CDI in Queensland during the last decade argues against a geographically targeted approach surveillance.

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5.3. Community-acquired *C. difficile* infection and medication exposure in Queensland, Australia

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Community-Acquired *Clostridium difficile* Infection, Queensland, Australia

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To the Editor: In Queensland, Australia, a steady increase in community-acquired (CA) *Clostridium difficile* infections (CDI) during 2003–2012 could not be explained by patients' demographic characteristics or environmental

factors (*1*). Several risk factors have been implicated in the increased rates of CA-CDI, primarily exposure to antimicrobial drugs, gastric acid–suppression drugs, and corticosteroids (*2*). Given the recent rise in prescription of corticosteroids and proton pump inhibitors in Australia, we hypothesized that the observed increase in CA-CDI was associated with increased drug prescriptions.

To test our hypothesis, we analyzed a subset of data used in a previous study (*1*), which included fecal samples from patients seen by general practitioners in the community from January 2008 through December 2012. The samples were submitted to Sullivan Nicolaides Pathology (Taringa, Queensland, Australia) for *C. difficile* toxin gene detection. After samples submitted from healthcare facilities and nursing homes were excluded, the final dataset contained data from 14,330 fecal samples. We aggregated the data by patient sex, age categories, year, and statistical area level 4 (SA4). For each sex-age-year-SA4 group, we used as numerators the numbers of CA-CDI cases identified and as denominators the numbers of samples submitted for microbiological testing.

The Australian Department of Human Services provided data from the Pharmaceutical Benefits Scheme. The quantities of 11 anatomic therapeutic chemical drugs were accessed by patient sex, age group, year, and SA4. Corresponding with the CA-CDI data, medication data to be analyzed were then aggregated by sex, age group, year, and SA4.

For each medication, we built binomial logistic regression models, using CA-CDI status as the outcome, in a Bayesian framework, incorporating fixed effects for sex, age group, quantity of drug prescribed, year (2008–2012), and spatially unstructured random effects at the SA4 level. After performing an initial burn-in, we stored and summarized 1,000 values from the posterior distribution of each parameter by using descriptive statistics (posterior mean, 95% posterior credible interval [95% CrI], and p value). We examined multiple pairwise comparisons of CA-CDI and medication exposure; thus, we used the Holm adjustment for p values to avoid inflation and to control the familywise error rate.

Of the 14,330 fecal samples tested, 1,430 (10%) were positive for *C. difficile*. The proportion of positive fecal samples increased over the 5-year period, from 7.10% in 2008 to 12.72% in 2011 and 11.48% in 2012 ($p<0.001$). After adjusting the regression models for sex, age group, temporal pattern, and spatial distribution, we found that exposure to antimycobacterial drugs (odds ratio [OR] 1.09; 95% CrI 1.02–1.16) and anthelmintic drugs (OR 1.07; 95% CrI 1.01–1.13) were associated with increased odds of CA-CDI. After post hoc Holm adjustments, no statistically significant association between medication exposure and CA-CDI was observed (Table).

Our findings suggest that the increase in CA-CDI proportion was not associated with population-level medication exposure in Queensland during 2008–2012. CA-CDI epidemiology in Queensland might be driven by a group of factors other than medication exposure, such as transmission of the pathogen from food, animals, or hospitals into the community. Studies have confirmed the risk for foodborne and animalborne spread of *C. difficile* into the community (*3*). In Australia and New Zealand, importation of onions and garlic from the United States and Mexico might be responsible for increased CDI cases during Southern Hemisphere summers (*4*), and high prevalence of *C. difficile* colonization in piglets has been identified (*5*). However, the role of these factors in leading to CA-CDI cases remains unknown.

A recent contact tracing study in the United Kingdom demonstrated that a considerable proportion of CDIs among patients in healthcare settings originated from the community (*6*); this finding was supported by another study, which showed that in Queensland, more than two thirds of patients with CA-CDI required hospitalization (*7*). Currently, there is no evidence of a reverse-infection route (healthcare-acquired CDI being transmitted to persons in the community). However, Sethi et al. documented environmental shedding of *C. difficile* by inpatients for several weeks after resolution of symptoms (*8*). Therefore, the possibility that asymptomatic patients might be a source of transmission after hospital discharge needs to be examined. In recent years, epidemiologic models

Table. Binomial logistic regression models for medication exposure adjusted for sex, age group, temporal pattern, and spatial distribution among patients with community-acquired *Clostridium difficile* infection, Queensland, Australia, 2003–2012

Medication exposure	Odds ratio (95% credible interval)	p value	Holm-adjusted p value
Drugs for acid-related disorders	1.052 (0.943–1.163)	0.348	0.819
Drugs for constipation	1.056 (0.963–1.151)	0.235	0.781
Antidiarrheal drugs	1.106 (0.994–1.218)	0.051	0.379
Antithrombotic drugs	1.073 (0.955–1.197)	0.224	0.781
Corticosteroids for systemic use	1.043 (0.952–1.133)	0.348	0.819
Antibacterial drugs for systemic use	1.083 (0.990–1.174)	0.067	0.425
Antimycotic drugs for systemic use	1.035 (0.944–1.126)	0.454	0.819
Antimicrobial drugs for mycobacterial infections	1.089 (1.023–1.155)	0.006	0.063
Anti-inflammatory drugs	1.070 (0.970–1.170)	0.158	0.700
Antiprotozoal drugs	1.037 (0.953–1.123)	0.394	0.819
Anthelminthic drugs	1.068 (1.008–1.127)	0.021	0.189

exploring the role of CDI coming from the community into the hospital have become increasingly popular (*9*); however, to the best of our knowledge, only 1 modeling study described CDI dynamics within the wider community (*10*). Although this approach is innovative, we acknowledge some limitations. Medication exposure was used as a proxy, based on the average prescription in the community, and it cannot be applied to the individual patient. In addition, we were unable to adjust the regression model for the presence of concurrent medical conditions and other unmeasured confounders.

Exposure to medications, particularly antimicrobial drugs, probably influences CA-CDI pathogenesis (*2*). However, our community-based assessment indicates that a more holistic exploration is needed to identify alternative factors driving increases in CA-CDI cases in the wider population.

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5.4. Seasonality of *C. difficile* infection

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RESEARCH ARTICLE

Clostridium difficile Infection Seasonality: Patterns across Hemispheres and Continents – A Systematic Review

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Abstract

Background

Studies have demonstrated seasonal variability in rates of *Clostridium difficile* infection (CDI). Synthesising all available information on seasonality is a necessary step in identifying large-scale epidemiological patterns and elucidating underlying causes.

Methods

Three medical and life sciences publication databases were searched from inception to October 2014 for longitudinal epidemiological studies written in English, Spanish or Portuguese that reported the incidence of CDI. The monthly frequency of CDI were extracted, standardized and weighted according to the number of follow-up months. Cross correlation coefficients (XCORR) were calculated to examine the correlation and lag between the year-month frequencies of reported CDI across hemispheres and continents.

Results

The search identified 13, 5 and 2 studies from North America, Europe, and Oceania, respectively that met the inclusion criteria. CDI had a similar seasonal pattern in the Northern and Southern Hemisphere characterized by a peak in spring and lower frequencies of CDI in summer/autumn with a lag of 8 months (XCORR = 0.60) between hemispheres. There was no difference between the seasonal patterns across European and North American countries.

Conclusion

CDI demonstrates a distinct seasonal pattern that is consistent across North America, Europe and Oceania. Further studies are required to identify the driving factors of the observed seasonality.

Introduction

Clostridium difficile is the most common cause of antibiotic-associated diarrhea among hospital inpatients [1]. The incidence and severity of *C. difficile* infection (CDI) have increased worldwide in the last two decades [2].

Understanding the seasonal patterns of infectious diseases is crucial to identify factors associated with an increased risk of infection and to implement control measures during the time of year when interventions are likely to have the greatest impact [3]. Epidemiological studies have documented a seasonal variation in the frequency of CDI, yet the mechanisms responsible for its variability remain poorly understood. Specifically, in the USA and Canada, the incidence of CDI has been reported to increase during boreal winter months (February–March) [4–6]. Antibiotic exposure is strongly associated with CDI [7–10]; consequently, it has been proposed that the observed CDI seasonality in the Northern Hemisphere is associated with the higher incidence of respiratory infections, which leads to an increase in antibiotic prescriptions during winter months [11,12].

In Australia, even though antibiotic consumption also peaks during winter (August) [13]; recent epidemiological studies have found that the seasonal pattern of *C. difficile* is not characterized by an increased number of CDI during winter months [14,15]. This indicates that CDI in Australia may not conform to currently proposed mechanisms of *C. difficile* seasonality, suggesting that factors in addition to antibiotic exposure might be driving the seasonality. Therefore, the aim of the current review was to pool the existing evidence to describe the global patterns of CDI seasonality and to facilitate improved understanding of underlying mechanisms.

Methods

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines were followed in this systematic review [16]. A systematic search was undertaken in three medical and life sciences databases (PubMed, Embase and Latin American and Caribbean Health Sciences Literature [LILACS]) from their inception to October 1st 2014 for longitudinal epidemiological studies that reported the incidence of CDI. Search terms included were "*Clostridium difficile*" and "season", the specific keywords and connectors used in the systematic search strategy for each database are listed in S1.A Search strategy.

The inclusion of studies was restricted to human studies, full-text articles or abstracts written in English, Spanish or Portuguese. Studies with at least 12 months follow-up that reported the incidence of CDI or the proportion of stool specimens examined in which *C. difficile* was detected, per month or per season, were included. CDI intervention studies were excluded from the review because of the interference that interventions might have on transmission dynamics. Exclusions were also made for studies that reported the number of positive samples detected for *C. difficile* without reporting the total number of samples that were tested; unless the authors stated that the number of stool samples examined per month was constant across the

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follow-up period. Corresponding authors were contacted for further information regarding the total number of samples examined per month/season. The characteristics of the excluded studies are listed in S1 Table.

Two authors (LFK and LY) independently examined all the citations by title and abstracts for studies that met the inclusion criteria. Full-text version articles of all potentially relevant studies were retrieved and independently extracted. Data presented in a graphical format were extracted using Plot Digitizer version 2.6.6 (http://plotdigitizer.sourceforge.net/). Data from all the included studies were extracted and summarized in a spreadsheet. The extracted data were cross-checked by the two authors, discrepancies during the selection of studies or data extraction were resolved through discussion and consensus. The quality of the selected studies was assessed independently by the same two authors using the Newcastle-Ottawa scale (NOS) [17].

The extracted data (incidence of CDI or proportion of positive stool specimens for *C. difficile*) were standardized to have a mean = 0, a minimum value = −1, and a maximum value = 1 for comparison across studies. A weight between zero and 1 was assigned to each study proportional to the number of months of follow-up. The number of months of follow-up for each study were divided by the number of months of follow-up of the study with the longest follow-up period; this ensured that the study with the longest follow-up period received a weight of 1.

The weighted average of the standardized monthly incidence were then plotted by hemispheres and continents to compare the seasonal patterns of CDI in each setting. An additional plot in which weighted average of the standardized CDI data from the Southern Hemisphere was shifted 6 months to align the meteorological seasons between hemispheres was created for ease of comparison.

Cross correlation coefficients (XCORR) were used to examine the correlation and lag value (in months) between the weighted average of standardized monthly incidence of CDI across hemispheres and continents using the extracted temporal data.

Results

The search identified 244 publications; after screening the publications by title and abstract, 171 publications were excluded. After a full-text review of 41 publications was conducted, 20 studies met the inclusion criteria and were selected for the review (Fig. 1). Of the 20 studies, 18 were conducted in Northern Hemisphere countries and only 2 in the Southern Hemisphere. Among the studies from the Northern Hemisphere, 13 were from North America and 5 from Europe. The 2 studies from the Southern Hemisphere were from Oceania (Australia; Tables 1 and 2). No studies from South America, Africa or Asia were identified despite additional efforts to target these regions in our search strategy (S1.B Search strategy). Using the NOS, all the studies but two were identified as high quality (≥80% NOS score; Table 1 and S2 Table).

A similar seasonal pattern was observed between the Northern and Southern Hemisphere. In the Northern Hemisphere, CDI rates peaked during March – April (early boreal spring) and were at their lowest during the second half of the year. CDI increased in the Southern Hemisphere during the second half of the year and peaked in the last trimester of the year (October – November, the mid austral spring – Fig. 2A and 2B). The XCORR peaked (0.60) at lags = 8, indicating that the rise in the weighted average of the standardized monthly incidence of CDI in the Southern Hemisphere lagged the Northern Hemisphere by 8 months (i.e. it occurred two months later relative to the onset of spring in the Southern Hemisphere as compared to the Northern Hemisphere). The lowest value was identified (–0.76) at lag = 1, which indicates that at lag = 1 month the weighted average of the standardized monthly incidence of CDI in the Northern Hemisphere decreases while it increases in the Southern Hemisphere.

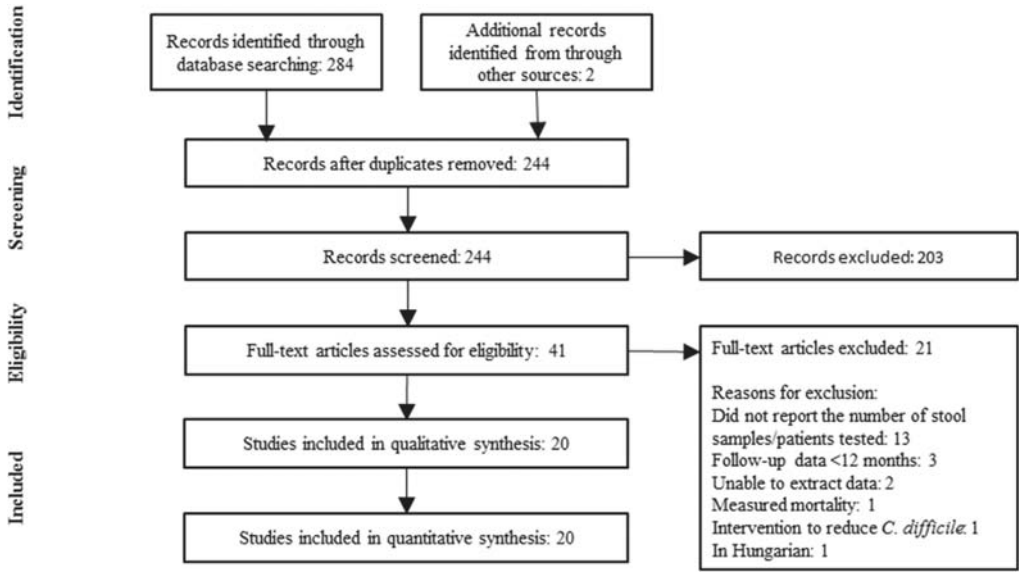

Fig 1. PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) flowchart of the literature search conducted on the 1st October 2014.

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When the studies were grouped by continents, a similar trend was observed in the Northern Hemisphere between North American and European countries. This observation was confirmed by the peak of XCORR = 0.69 at lag 0 months. Both presented a higher frequency of CDI during the first half of the year, with peaks of CDI in March and April in Europe and North America, respectively (Fig. 3).

Discussion

The findings of the current systematic review suggested that the Northern and Southern Hemisphere countries exhibit similar seasonal patterns characterized by CDI peaking in spring and being at their lowest during summer/autumn months. Antibiotic consumption in the community also follows a seasonal pattern. In North American and European countries the consumption of antibiotics mainly peaked in January-February, whereas in Australia antibiotic consumption peaked in August [13]. Hensgens *et al.* found that after cessation of antibiotic therapy, patients remain at higher risk of CDI for up to 3 months [18]. Therefore, the observed seasonality may indicate a lag of 2–3 months between antibiotic exposure and CDI. It is not surprising that several studies have found co-seasonality of CDI and respiratory tract infection [11,12,19]. In these studies, the respiratory infections often lead CDI by 1 month which could be explained by the corresponding incidence of respiratory tract infection and antibiotic prescription in the community [20].

Risk factors in addition to antibiotic exposure such as environmental variables (temperature, precipitation, altitude, etc.) could also be involved in the observed seasonality as they have also been demonstrate to affect the dynamics of numerous infectious diseases [3,21]. In a previous study we found that the odds of CDI infection increased by 9% (OR: 1.09; 95%CI: 1.02 to 1.17) per 100 mm increase in monthly rainfall in Queensland, Australia [14]. Respiratory tract infection transmission dynamics are highly dependent on environmental factors [21];

Table 1. Characteristics of included studies.

	Location	Data source	Start	Finish	Follow-up (months)	NOS scores
Archibald *et al*., 2004 [4]	All USA	National Nosocomial Infections Surveillance System	Jan 1987	Dec 2001	180	3/5
Brown *et al*., 2013 [11]	All USA	U.S. National Hospital Discharge Survey	Jan 1993	Dec 2008	192	8/9
Burckhardt *et al*., 2008 [40]	Saxony, Germany	State of Saxony Surveillance	Jan 2002	Dec 2006	60	4/5
Camacho-Ortiz *et al*., 2009 [41]	Mexico City, Mexico	Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubiran	Jan 2003	Dec 2007	60	4/5
Damani *et al*., 2011 [42]	Armagh, Northern Ireland	Craigavon Area Hospital	Jan 2007	May 2010	41	3/5
Deorari *et al*., 1999 [34]	Alberta, Canada	Alberta Children's Hospital	Apr 1993	Mar 1995	24	9/9
Dubberke *et al*., 2009 [43]	MO, MA, OH, IL, UT (USA)	Five hospitals	Jul 2000	Jun 2006	72	5/5
Faires *et al*., 2014 [44]	Ontario, Canada	A community hospital in Southern Ontario	Aug 2006	Feb 2011	55	5/5
Furuya-Kanamori *et al*., 2014 [14]	Queensland, Australia	Sullivan Nicolaides Pathology	May 2003	Dec 2012	117	4/5
Gilca *et al*., 2010 [5]	Quebec, Canada	MED-ECHO and Quebec's provincial surveillance	Apr 1998	Mar 2006	97	4/5
Gilca *et al*., 2012 [12]	Quebec, Canada	Quebec's provincial surveillance	Jan 2005	Dec 2008	48	8/9
Jagai and Naumova, 2009 [6]	All USA	Centers for Medicare and Medicaid Services	Jan 1993	Dec 2004	144	4/5
MacDonald *et al*., 1993 [45]	Manitoba, Canada	A tertiary care referral hospital	May 1990	May 1992	23*	5/5
McFarland *et al*., 2007 [46]	Washington, USA	Veterans Administration Puget Sound Health Care System	Jan 2004	Dec 2004	12	8/9
Reil *et al*., 2012 [47]	Northern Bavaria, Germany	Synlab Medical Care Service Centre Wieden	Jan 2000	Dec 2009	120	4/5
Reveles *et al*., 2014 [48]	All USA	U.S. National Hospital Discharge Survey	Jan 2001	Dec 2010	120	4/5
Slimming *et al*., 2014 [15]	All Australia (except Northern Territory)	450 public hospitals	Jan 2011	Dec 2012	24	5/5
Sonnenberg, 2009 [49]	All England	Hospital Episode Statistics	Apr 1995	Mar 2006	132	4/5
von Muller *et al*., 2011 [50]	Saarland, Germany	The University of Saarland Hospital	Apr 2008	Jun 2010	27	4/5
Wong-McClure *et al*., 2012 [30]	NR, Costa Rica	A tertiary care hospital	Jan 2009	Jun 2011	30	4/5

NOS: Newcastle-Ottawa Scale, *NR*: Not reported, *MO*: Missouri, *MA*: Massachusetts, *OH*: Ohio, *IL*: Illinois, *UT*: Utah

* January 1991 not included, a nursing strike made data unretrievable.

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therefore, caution is advised for future studies drawing an association between CDI and environmental factors because of the possible confounder of co-seasonality in CDI and respiratory infections. Because CDI was traditionally viewed as a nosocomial disease, studies that assess the relationship between environmental factors and CDI are scant and this is a research gap that requires substantial development. The observed difference of two-month lag between the Southern and Northern Hemisphere (relative to the onset of spring) may be explained by the

Table 2. Measures of monthly *C. difficile* infection incidence.

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Archibald *et al*., 2004 [4] (Cases/10,000 patient-days)	4.25	4.46	4.69	3.21	3.93	2.53	2.28	1.97	1.01	2.60	1.19	2.15
Brown *et al*., 2013 [11] (Cases/1,000 discharges)	8.36	8.42	8.67	8.75	8.65	8.35	8.19	8.22	8.27	7.97	7.71	7.90
Burckhardt *et al*., 2008 [40] (Cases/100,000 persons)	6.15	6.15	6.67	6.67	6.67	5.60	5.60	5.60	6.28	6.28	6.28	6.15
Camacho-Ortiz *et al*., 2009 [41] (Cases/1,000 discharges)	7.97	9.63	7.08	10.55	8.85	11.06	9.92	14.37	16.44	3.97	5.06	5.46
Damani *et al*., 2011 [42] (Percentage of positive samples)	10.48	10.36	10.16	6.58	7.53	9.30	11.17	13.43	12.93	11.96	15.40	12.98
Deorari *et al*., 1999 [34] (Percentage of positive samples)	22.64	14.64	9.36	19.73	0.09	33.18	41.91	35.36	44.27	49.91	42.27	25.55
Dubberke *et al*., 2009 [43] (Cases/10,000 patient-days)	9.02	9.30	9.39	10.03	9.17	9.13	8.28	9.32	9.32	9.43	9.36	9.28
Faires *et al*., 2014 [44] (Cases/10,000 patient-days)	0.18	0.79	0.90	1.66	1.59	1.50	1.45	1.18	1.49	0.70	1.50	1.11
Furuya-Kanamori *et al*., 2014 [14] (Percentage of positive samples)	13.42	13.39	12.55	10.07	11.24	13.39	13.08	13.33	12.84	14.22	14.09	14.67
Gilca *et al*., 2010 [5] (Cases/1,000 discharges)	11.29	10.10	9.08	8.29	7.83	6.92	7.31	8.02	8.63	10.05	10.43	11.50
Gilca *et al*., 2012 [12] (Cases/1,000 discharges)	12.76	11.82	11.85	11.04	10.09	8.99	8.70	7.65	7.30	7.70	8.12	8.40
Jagai and Naumova, 2009 [6] (Cases/10,000 persons)	0.53	0.48	0.49	0.51	0.50	0.46	0.50	0.50	0.49	0.54	0.47	0.54
MacDonald *et al*., 1993 [45] (Cases/100,000 patient-days)	2.97	5.49	2.39	5.49	4.33	5.92	8.97	3.98	4.96	4.93	9.50	2.94
McFarland *et al*., 2007 [46] (Cases/10,000 patient-days)	21.90	40.51	42.70	28.47	36.86	15.33	11.31	23.36	24.09	34.67	34.67	31.02
Reil *et al*., 2012 [47] (Percentage of positive samples)	10.99	10.99	10.96	10.96	10.96	11.44	11.44	11.44	10.94	10.94	10.94	10.99
Reveles *et al*., 2014 [48] (Cases/1,000 discharge)	6.60	7.00	7.60	6.70	7.30	7.00	6.80	7.00	6.70	7.10	6.00	6.90
Slimming *et al*., 2014 [15] (Cases/10,000 patient-days)	3.32	3.32	3.80	3.80	3.80	3.53	3.53	3.53	4.27	4.27	4.27	3.32
Sonnenberg, 2009 [49] (Cases/1,000 admissions)	0.80	0.92	1.03	1.03	0.92	0.76	0.68	0.60	0.69	0.58	0.69	0.66
von Muller *et al*., 2011 [50] (Percentage of positive samples)	7.35	7.06	8.42	9.12	6.51	9.26	5.11	7.91	10.34	6.36	11.29	10.74
Wong-McClure *et al*., 2012 [30] (Cases/10,000 patient-days)	10.68	15.19	6.69	12.62	7.53	8.02	8.67	4.13	7.41	5.14	9.74	8.50

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climatic zones where the studies included in the review are located. Australia, which is located in tropical and sub-tropical zones was the only country included in the review from the Southern Hemisphere; whereas the Northern Hemisphere countries included were mainly located in a temperate zone (USA, Canada, Germany, Ireland, and England). Von Boeckel *et al*. found that countries further from the equator (temperate zone) have a prominent seasonal pattern in antibiotic consumption characterized by peaks during winter, whereas antibiotic consumption is fairly constant across the months in countries located in tropical and sub-tropical zones [13]. Furthermore, Tamerius *et al*. described a similar one-month lag between the start of influenza epidemic in temperate Northern Hemisphere countries (November, end of boreal autumn) and the start of influenza epidemic in Australia (June, start of austral winter)[22]. In both cases, the influenza epidemic starts 3–4 months before the peak of CDI (March – April in Northern Hemisphere and October – November in Southern Hemisphere).

Despite contrasting antibiotic prescribing practices in outpatients between North America and Europe, the results indicate a similar seasonal pattern between European and North American countries. Patrick *et al*. found that the antibiotic consumption in the community was higher in British Columbia, Canada, than in Sweden, Germany, United Kingdom, Denmark and The Netherlands [23]. Of particular interest is the high consumption rate found in Canada compared to Denmark for some antibiotic classes such as fluoroquinolones (1.44 versus 0.15

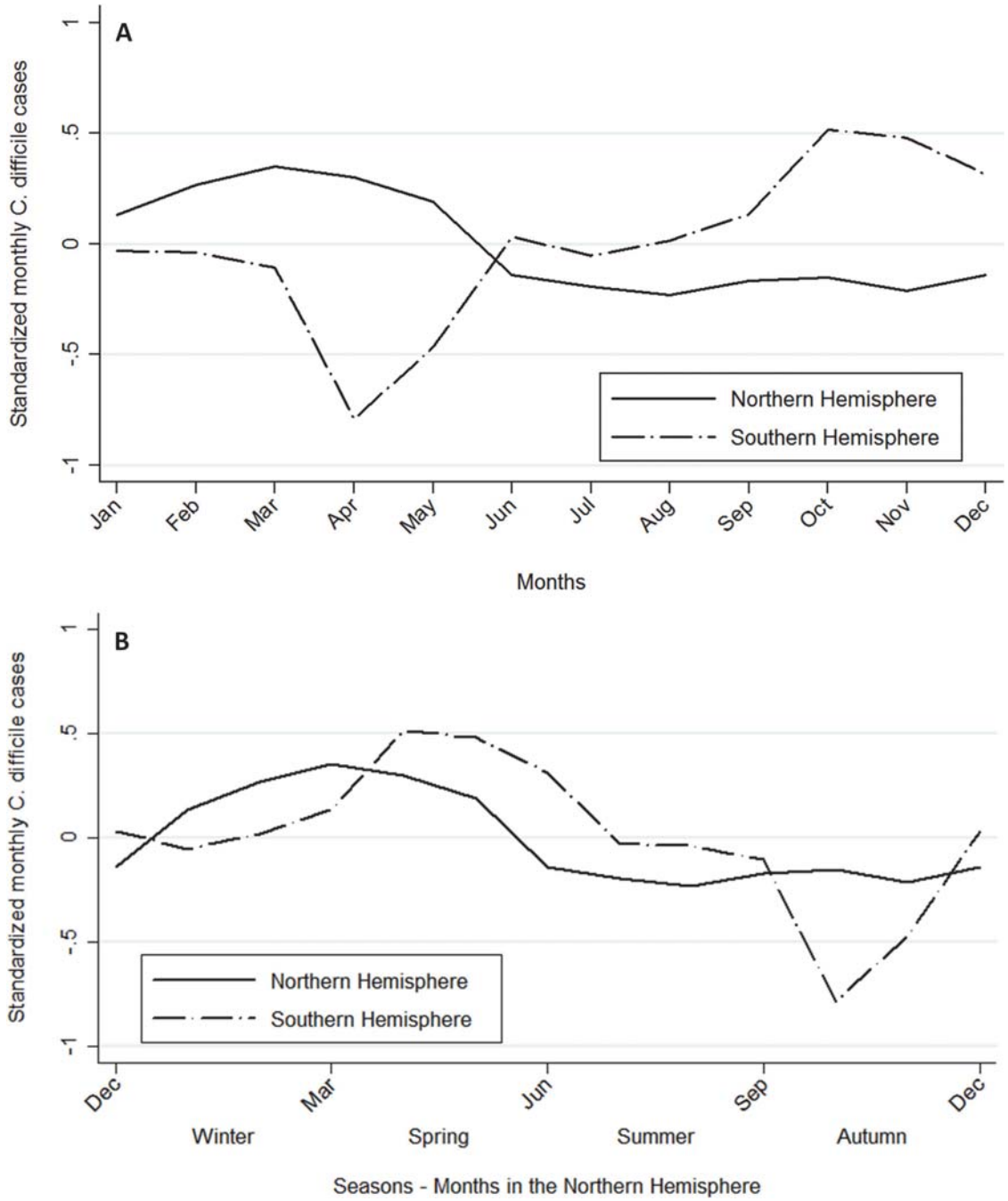

Fig 2. (a)Weighted average of the standardized monthly incidence of *C. difficile* infection by hemisphere. (b)Weighted average of the standardized monthly incidence of *C. difficile* infection by hemispheres. For ease of comparison, the Southern Hemisphere plot was moved 6 months (in the x-axis) thus the meteorological seasons align between hemispheres.

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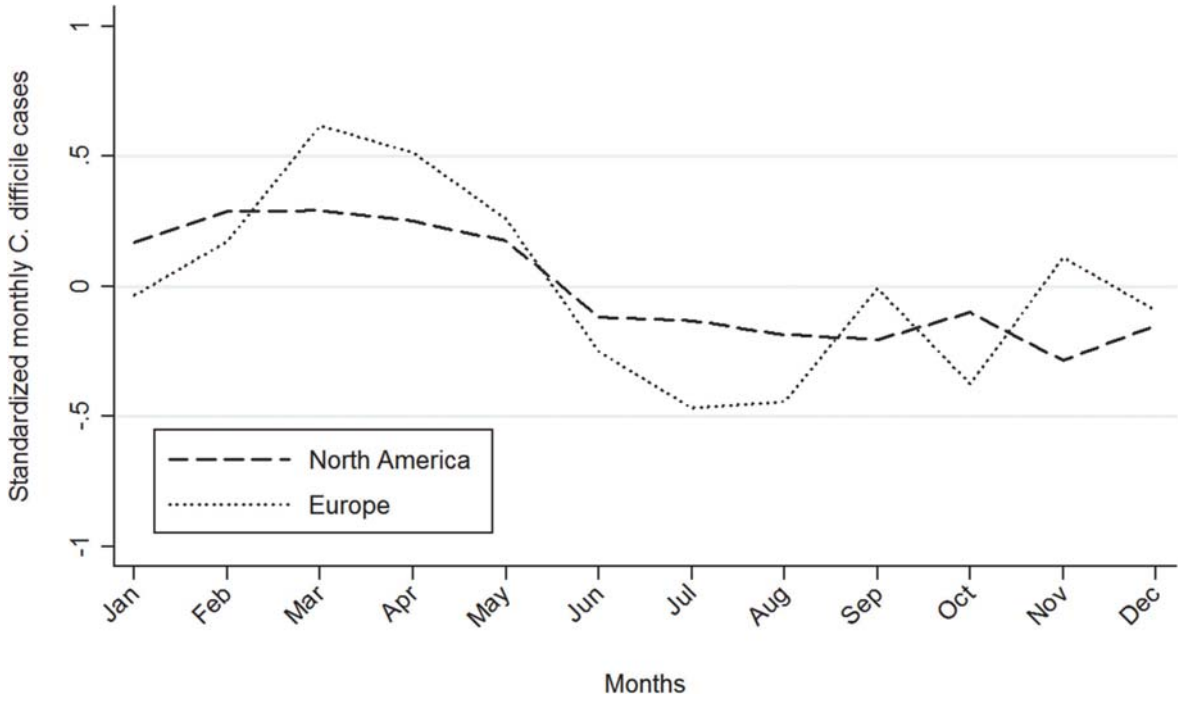

Fig 3. Weighted average of the standardized monthly incidence of *C. difficile* infection by Northern Hemisphere continents.

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defined daily doses [DDDs]/1000 inhabitant-days), macrolides (1.59 versus 0.92 DDDs/1000 inhabitant-days), and cephalosporins (1.86 versus 0.02 DDDs/1000 inhabitant-days) as these antibiotic classes have been associated with an increased risk of community-acquired CDI [7,8]. A similar trend in antibiotic prescribing was observed in children; higher rates of use of cephalosporins (89.1 versus 0.2 prescriptions/1000 children), lincosamides (2.3 versus 0.1 prescriptions/1000 children), macrolides (148.0 versus 42.6 prescriptions/1000 children), and fluoroquinolones (1.4 versus 0.5 prescriptions/1000 children) were reported in Canada compared to Denmark [24]. This finding supports the need to investigate additional factors (other than antibiotic exposure [11,12]) that would contribute towards a broader understanding of CDI seasonality.

Exposure to proton pump inhibitor (PPI) [25] and glucocorticoid [26] has been associated with an increased risk of CDI, however no study has yet examined the temporal relationship between monthly PPIs or glucocorticoids prescription rates and CDI seasonality. Additional factors such as the introduction of new strains of the pathogen via trade in livestock, commodities and/or movement of people (asymptomatic colonized patients such as tourists or business travellers, or hospital transfers) across boundaries should be evaluated when assessing possible factors associated with the seasonality of CDI [27]. Rodriguez-Palacios *et al.* reported a possible seasonality in contamination of retail meat in Canada with higher prevalence of *C. difficile* in January – February (11.5%) compared to other months of the study (4.0%) [28]. Riley has implicated the importation of onions and garlic from USA and Mexico into Australia in the increase in CDI during October – December in Western Australia [29].

Although a comprehensive review was carried out, several limitations were noted. First, only two studies were identified that reported the seasonality of CDI in Southern Hemisphere countries [14,15]. Furthermore, both studies were conducted in Australia. This may limit the generalizability of the findings for Southern Hemisphere countries only to Australia. However, the identified gap in information should encourage further investigation particularly in countries in South America, Africa and Asia. Second, there was a small number of studies from countries located between the Tropic of Cancer and the Tropic of Capricorn. The study conducted by Wong-McClure *et al.* [30] in Costa Rica was the only study from the Northern Hemisphere located in a tropical zone, precluding the comparison between the seasonality of CDI in temperate and sub-tropical/tropical climates. Despite the documented changes in CDI epidemiology [2], the increase in community-acquired CDI [31], and the different risk profiles between community- and hospital-acquired CDI patients [32], our study was also limited by the inability to compare the community- and hospital-acquired CDI seasonal patterns. Despite the increasing incidence of CDI among the paediatric population [33] only one study (Deodari *et al.* [34]) was identified that described the CDI seasonality in children; therefore, generalizability of the findings may be limited among this population. Potential factors that may contribute to differences in monthly CDI incidence that could not be accounted for in this review, such as hospital characteristics (e.g. staffing, overcrowding), CDI diagnosis ascertainment, severity of underlying illness, infection control practices, and CDI strain need to be assessed in future studies.

As the studies included in the review reported the measures of monthly CDI using different units, the values were standardized to compare the monthly CDI incidence across the studies. By doing so, the magnitude of the seasonality measured by the amplitude between the peak and the trough was lost. Although, the magnitude of the seasonality could be masked, the observed patterns should not be affected by the standardization. Finally, the weight allocated to each study was based on the number of follow-up months and not on the sample size as the number of participants or stool samples examined during the study period was not available for all the studies included in this review.

Understanding the seasonality of an infectious disease and the driving factors are of utmost importance for planning prevention and control strategies [21,35]. Recently, several epidemiological models of CDI have been constructed to inform control strategies for this disease of increasing incidence and severity [36–39]. However, none has yet incorporated the effects of seasonality and this will be difficult to achieve without better understanding of the underlying mechanisms. The current review provided evidence of a similar CDI seasonal pattern across hemispheres which differs from the seasonality that was previously proposed. Further studies are required to identify exposure to medications and environmental factors associated with the observed seasonality.

Supporting Information

S1 PRISMA Checklist.
(DOC)

S1 Table. Excluded studies.
(DOCX)

S2 Table. Study quality assessment.
(DOCX)

S1 Text. Search Strategy.
(DOCX)

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Author Contributions

Conceived and designed the experiments: LY. Analyzed the data: LFK SM AC. Wrote the paper: LFK SM LY JC DP TR AC. Conducted the systematic review: JC.

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Chapter 6

New therapeutical options and risk factors for *C. difficile* infection

CHAPTER 6. New therapeutical options and risk factors for *C. difficile* infection

6.1. Context

During the past three decades a steady increase in CDI has been reported worldwide. The Centers for Disease Control and Prevention in the USA catalogued *C. difficile* as "an immediate public health threat that requires urgent and aggressive action" in 2013. In some regions in the USA, *C. difficile* has now surpassed methicillin-resistant *Staphylococcus aureus* as the main cause of hospital-acquired infection. In this Chapter, I present two clinical epidemiological studies that provide evidence to help treat and prevent CDI. These studies include an analysis of a therapeutical option for CDI recurrence and a novel risk factor that will aid the recognition of patients at risk of developing CDI.

First, given the high recurrence rates that have been reported for patients treated with first-line antibiotics recommended by clinical guidelines (metronidazole [47.2%] and vancomycin [25.3%]) and the limited antibiotic therapeutical options available for CDI recurrence (i.e. fidaxomicin and rifaximin), I investigated FMT, a non-pharmacological therapeutical option for CDI treatment. In this study, I pooled individual-patient data from 14 studies (305 patients) to examine the efficacy of FMT in terms of risk of recurrence/relapse of CDI, and compared the upper (i.e. gastroscopy or nasogastric tube) and the lower (i.e. colonoscopy or enema) gastrointestinal routes for the delivery of FMT. I reported that the clinical resolution with FMT was much higher than reported for vancomycin and fidaxomicin. In addition, I found that the risk of clinical failure significantly increased after 30 days if FMT was delivered via the upper gastrointestinal route compared to the lower gastrointestinal route.

Early recognition of new CDI cases is crucial to reduce the transmission of the pathogen. Current guidelines recommend that CDI should be suspected in all hospitalised patients with diarrhoea and all patients with a history of exposure to antibiotics, gastric acid suppression and/or chemotherapy. However, as *C. difficile* epidemiology has dramatically changed since the earlies 2000s, risk factors may also have changed. Thus, we should actively seek to identify patients at risk beyond those with the "traditional" CDI risk factors. In view of vitamin D being implicated in multiple immune response processes, in the second study presented in this Chapter, I investigated the effects of vitamin D concentration on CDI risk. I systematically searched for epidemiological studies that examined the association between vitamin D concentration and CDI; meta-analysed data from 8 studies (4,479 patients) and reported that lower concentrations of vitamin D were associated with CDI as well as presentation of more severe forms of CDI.

6.2. Faecal microbiota transplantation for *C. difficile* infection

Furuya-Kanamori L, Doi SA, Paterson DL, Helms SK, Yakob L, McKenzie SJ, Garborg K, Emanuelsson F, Stollman N, Kronman MP, Clark J, Huber CA, Riley TV, Clements AC. Upper versus lower gastrointestinal delivery for transplantation of fecal microbiota in recurrent or refractory *Clostridium difficile* infection: A collaborative analysis of individual patient data from 14 studies. *J Clin Gastroenterol* 2016 [Epub ahead of print].

Upper Versus Lower Gastrointestinal Delivery for Transplantation of Fecal Microbiota in Recurrent or Refractory *Clostridium difficile* Infection

A Collaborative Analysis of Individual Patient Data From 14 Studies

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Goals: The aim of this study was to compare upper gastrointestinal (UGI) versus lower gastrointestinal (LGI) delivery routes of fecal microbiota transplantation (FMT) for refractory or recurrent/relapsing *Clostridium difficile* infection (CDI).

Background: FMT has been proven to be a safe and highly effective therapeutic option for CDI. Delivery, however, could be via the UGI or LGI routes, and it is unclear as to which route provides better clinical outcome.

Study: A systematic search for studies that reported the use of FMT for CDI treatment was conducted. Individual patient data that included demographic (age and sex) and clinical (route of FMT delivery, CDI outcome after FMT, and follow-up time) information were obtained. Kaplan-Meier cumulative hazard curves and Cox proportional hazard models were used to assess clinical failure after FMT by the route of delivery.

Results: Data from 305 patients treated with FMT (208 via LGI route and 97 via UGI route) for CDI were analyzed. At 30 and 90 days, the risk of clinical failure was 5.6% and 17.9% in the UGI group compared with 4.9% and 8.5% in the LGI delivery route group, respectively. A time-varying analysis suggested a 3-fold increase in hazard of clinical failure for UGI delivery (hazard ratio, 3.43; 95% confidence interval, 1.32-8.93) in the period after 30 days.

Conclusions: FMT delivered via the LGI seems to be the most effective route for the prevention of recurrence/relapse of CDI. A randomized controlled trial is necessary to confirm whether FMT delivered via the LGI is indeed superior to that delivered via the UGI route.

Key Words: *Clostridium difficile*, infection, fecal transplantation

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For 25 years, the first-line therapies recommended by the Society of Healthcare Epidemiology of America and Infectious Diseases Society of America for *Clostridium difficile* infection (CDI) have been metronidazole and vancomycin.[1] However, unacceptably high rates of CDI recurrences are reported for both metronidazole (47.2%)[2] and vancomycin (25.3%).[3] Given the poor clinical outcomes exhibited by the main antimicrobial agents in patients with recurrent CDI, alternative therapies were investigated and the use of fecal microbiota transplantation (FMT) was found to be effective. The latter consists of the instillation of normal microbiota via donor faces to correct the imbalance of the colonic microbiota caused by factors such as antimicrobials or gastric acid suppressants that disrupt the normal balance of the microbiome and permit the proliferation of pathogenic *C. difficile* strains.[4]

FMT was first used in humans to treat pseudomembranous colitis >60 years ago,[5] and the first case of confirmed CDI successfully treated with FMT dates back to 1983.[6] Four systematic reviews[7–10] and a meta-analyses (89% clinical resolution)[11] have described FMT as a highly effective and safe therapy for recurrent CDI. A randomized controlled trial (RCT) by van Nood et al[12] confirmed that

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clinical resolution was significantly higher in patients assigned to receive FMT (81%) compared with those patients who received vancomycin (31%) for the treatment of recurrent CDI. Similarly, another recent double-blind RCT among patients with recurrent CDI has shown high rates of clinical cure (91%) in patients who received FMT with donor stool.[13]

As evidence for its efficacy accumulated, controversy around the most appropriate delivery route for FMT began to emerge. There is evidence that upper gastrointestinal (UGI) delivery by gastroscope, nasojejunal, or nasogastric tube (NGT) may be less effective than lower gastrointestinal (LGI) (eg, enema or colonoscopy) delivery,[8] but other reports do not confirm this finding.[11,14] We therefore undertook this project to shed more definitive light on this issue through a collaborative analysis of individual patient data reported in the literature.

MATERIALS AND METHODS

Search Strategy, Eligibility Criteria, and Study Selection

A systematic search was conducted through 3 medical and life sciences databases (PubMed, Embase, and Cochrane CENTRAL). These databases were searched from their inception to August 2015 without language restriction for publications that reported the instillation of stool from a healthy human donor into a patient via any delivery modality (NGT, enema, or colonoscopy) for the treatment of recurrent or refractory CDI. Search terms included were "*Clostridium difficile*," "infection," "fecal," "intestinal," "microbiota," "transplantation," and "donor"; the specific key words and connectors used in the systematic search for each database are listed in the supplementary material S1 (Supplemental Digital Content 1, http://links.lww.com/JCG/A230).

The inclusion of studies was restricted to human studies; full-text peer-reviewed articles; studies with laboratory-confirmed CDI (enzyme immunoassay or PCR for the presence of toxins and/or genes) or endoscopic evidence of pseudomembranous colitis; and studies that clearly reported the follow-up period and the clinical outcome for each patient. Series of cases with 5 or more patients were included to reduce positive outcome bias associated with case reports. Studies in which FMT was used to treat other conditions such as inflammatory bowel disease (ulcerative colitis or Crohn's disease) or irritable bowel syndrome were excluded. Exclusions were also made for studies that reported the use of a culture mixture of enteric bacteria (bacteriotherapy) instead of a healthy donor's stool.

If a study reported aggregated patient data, the corresponding authors were invited to participate in this collaborative analysis by providing individual patient data on a set of core clinical variables consisting of demographics (age and sex), *C. difficile* outcomes in follow-up (recurrence, relapse, refractory, cured), and follow-up time. Data from each study were recorded in a uniform format after resolution of data queries and checked against the associated publications for accuracy.

Statistical Methods

The primary outcome measure of this study was time to clinical failure, defined as recurrence or relapse from the FMT intervention, and the principal research question was whether this time-to-event after FMT was modified by the route of delivery after adjusting for patient demographic characteristics.

Data from all of the 14 studies were pooled for descriptive analyses, and Kaplan-Meier cumulative hazard curves were created. Given that rapid recrudescence after FMT is highly undesirable and therefore ought to be given more weight, the Gehan-Breslow-Wilcoxon test was used to compare the cumulative hazard between the delivery routes, as it is more sensitive to early differences between events.

Cox proportional hazards models were also used to adjust for the effect of age and sex of the patients stratified by the type of study contributing the data (inpatient studies, outpatient studies, or mixed studies). It would have been ideal to stratify by study unit per se, but that was not possible given the size of the data set. The proportional hazards assumption was checked by both the visual appearance of the stratified Kaplan-Meier plots and by testing for a nonzero slope in a generalized linear regression of the scaled Schoenfeld residuals on functions of time. On the basis of these analyses, it was clear that the hazard ratios for delivery route varied over the early (up to 30 d) and late period (beyond 30 d); therefore, a binary time-dependent term (period up to 30 d and period beyond 30 d) was created and the interaction between the latter and delivery route modeled to allow a different hazard ratio for the early ($\leq$30 d) and late (> 30 d) follow-up periods.[15,16] The significance level was set at $P < 0.05$, and all statistical analyses were conducted using Stata IC, version 12.1 (Stata Corporation; College Station, TX).

RESULTS

Search Results

The initial search identified 1428 records. One thousand eleven publications remained after excluding duplicate citations. After screening the publications by title and abstract, 855 were excluded. Full-text reviews of the remaining 156 publications were conducted: 13 met the eligibility criteria and were selected for the analysis. No additional publications were identified through other sources.

Nine studies[17–25] that did not meet our eligibility criteria as aggregated patients' data were reported, and thus corresponding authors were contacted to provide individual patient data to include their studies in the analysis. Only 1 author[25] shared their patients' data; therefore, data from 14 studies were included in the current study (S2).

Patient Characteristics

Among the 14 studies,[25–38] the majority (n = 8) included both inpatients and outpatients.[25–28,31–33,36] Eight studies included patients with recurrent CDI,[25,26,29–31,34–36] 5 included patients with either recurrent or refractory CDI,[27,28,32,33,38] and only 1 focused exclusively on refractory cases.[37] Ten studies used patient-selected donors (spouse or relatives),[25,27,29–31,33–37] only 1 study used unrelated donors,[28] and the remaining studies used a combination of selected and unrelated donors (Table 1).[26,32,38]

The 14 studies yielded a total of 305 patients; 65% of the patients were female, 6% of the patients were below 18 years old, and ages ranged from 1 to 94 years [median, 72 y; interquartile range (IQR), 54 to 81 y]. FMT was delivered via the LGI route in 208 patients and via the UGI route in 97 patients. The overall median follow-up time was 175 days (IQR, 80 to 354 d), with longer follow-up periods

TABLE 1. Characteristics of the Included Studies

References	Data Source	Study Period	Male (%)	Mean Age (Range) (y)	Patient Type (Inpatient, Outpatient, Mixed)	CDI Type (Recurrent, Refractory, Both)	Donor (Patient Selected, Anonymous, Both)	Delivery Modality	Stool Sample Preparation/ Dose	Median Follow-up Period (Range) (d)	Successful Treatment/ Patients Treated
Aas et al[26]	SMDC; Duluth, MN	June 1994-August 2002	28	73 (51-88)	Mixed	Recurrent (≥ 2 laboratory-confirmed CDI relapses)	Both	Nasogastric tube	30 g stool/ 50-70 mL sterile NaCl	90 (3-90)	17/18
Emanuelsson et al[27]	Skaraborgs Hospital Skovde, Sweden	1994-2011	38	67 (25-93)	Mixed	Both (severe relapsing and therapy-resistant CDI)	Patient selected	Enema	50 g stool/ 500 mL sterile NaCl	546 (0-4071)	14/24*
Garborg et al[25]	Sorlandet Hospital, Kristiansand, Norway	1994-2008	48	75 (53-94)	Mixed	Recurrent	Patient selected	Gastroscopy or colonoscopy	50-100 stool/ 250 mL sterile NaCl	80 (21-80)	29/40
Kassam et al[28]	NR	NR	52	69 (26-87)	Mixed	Both	Anonymous	Enema	150 g stool/ 300 mL sterile water	390 (0-695)	25/27
Kelly et al[29]	Women and Infants Hospital, Providence, RI	NR	8	59 (19-86)	Outpatient	Recurrent (≥ 3 CDI recurrences)	Patient selected	Colonoscopy	6-8 tbs stool/ 1 L sterile water or NaCl	280 (0-885)	24/26
Kronman et al[30]	Seattle Children's Hospital, Seattle, WA	August 2011-May 2014	30	7 (1-14)	Outpatient	Recurrent (≥ 3 CDI recurrences)	Patient selected	Nasogastric tube	30 g stool/ 100 mL NaCl	44 (13-700)	9/10
MacConnachie et al[31]	NR	September 2003-NR	7	82 (68-95)†	Mixed	Recurrent	Patient selected	Nasogastric tube	30 g stool/ 150 mL NaCl	70 (0-168)	11/15
Mattila et al[32]	Helsinki UCH, Turku UCH, Satakunta CH, Turku MH, Helsinki MH, Finland	November 2007-February 2010	40	73 (22-90)	Mixed	Both	Both	Ileocolonoscopy	20-30 mL stool/ 100-200 mL water	354 (89-354)	62/70
Mellow and Kanatzar[33]	INTEGRIS Digestive Health Center, Oklahoma City, OK	July 2009-April 2010	54	67 (32-87)	Mixed	Both	Patient selected	Colonoscopy	Stool amount NR/NaCl amount NR	147 (30-295)	11/13
Pathak et al[34]	NR/Community Hospital	NR (3 y)	33	72 (37-90)	Inpatient	Recurrent	Patient selected	Colonoscopy	6-8 tbs stool/ 1 L tap water	266 (59-856)	11/12
Rohlke et al[35]	NCGC, Oakland, CA and University of Washington Medical Center, Seattle, WA	September 2004-July 2009	11	49 (29-82)	Outpatient	Recurrent	Patient selected	Colonoscopy	Stool amount NR/NaCl amount NR	738 (177-1918)	15/19

TABLE 1. (*continued*)

References	Data Source	Study Period	Male (%)	Mean Age (Range) (y)	Patient Type (Inpatient, Outpatient, Mixed)	CDI Type (Recurrent, Refractory, Both)	Donor (Patient Selected, Anonymous, Both)	Delivery Modality	Stool Sample Preparation/ Dose	Median Follow-up Period (Range) (d)	Successful Treatment/ Patients Treated
Russell et al[36]	MassGeneral Hospital for Children, Boston, MA	2009-2013	60	8 (1-19)	Mixed	Recurrent	Patient selected	Nasogastric tube or colonoscopy	30-40 g stool/ 250 mL NaCl	368 (59-1564)	9/10
Silverman et al[37]	Patients' home, Toronto, ON, Canada	NR	57	65 (30-88)	Outpatient	Refractory	Patient selected	Enema	50 mL stool/ 200 mL NaCl	207 (118-413)	7/7
Zainah et al[38]	Henry Ford Hospital, Detroit, MI	May 2010-June 2012	36	73 (52-92)	Inpatient	Both	Both	Nasogastric tube	30-50 g stool/ Tap water amount NR	100	14/14

*Patients treated with rectal bacteriotherapy were not included.
†Median age reported.
CH indicates central hospital; CDI, *Clostridium difficile* infection; MH, municipal hospital; NaCl, 0.9% sodium chloride; NR, not reported; SMDC, St. Mary's/Duluth Clinic Health System; tbs, tablespoons; UCH, University Central Hospital.

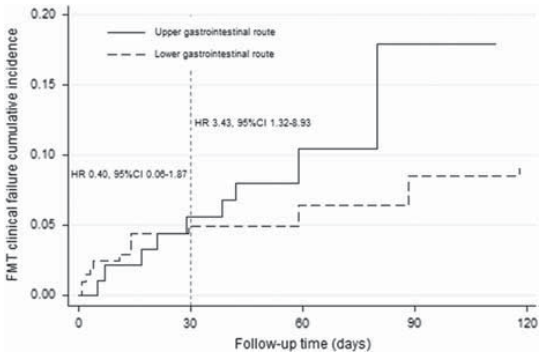

FIGURE 1. Kaplan-Meier cumulative hazard curves by delivery route. CI indicates confidence interval; FMT, fecal microbiota transplantation; HR, hazard ratio.

(median, 354 d; IQR, 118 to 413 d) recorded in studies that used the LGI route compared with those that used the UGI route (median, 80 d; IQR, 80 to 90 d).

Recurrence/Relapse After FMT

At 30 days of follow-up, the cumulative clinical failure rates for FMT were similar for both routes of FMT delivery [UGI, 5.60%; 95% confidence interval (CI), 2.37%-12.94% vs. LGI, 4.93%; 95% CI, 2.68%-8.96%]. However, at 90 days of follow-up, the cumulative clinical failure rates for FMT via the UGI route (17.91%; 95% CI, 11.19%-27.89%) was double that seen with the LGI route (8.53%; 95% CI, 5.39%-13.37%). When the Kaplan-Meier cumulative hazard curves by delivery route were compared, there was a statistically significant difference ($\chi^2_1 = 4.85$, $P = 0.028$) between them favoring the LGI route (Fig. 1).

When the delivery route was modeled as a time-varying covariate, the unadjusted Cox regression model showed that the hazard of FMT clinical failure after 30 days was 3-fold higher [hazard ratio (HR), 3.65; 95% CI, 1.47-9.05] when delivered via the UGI route compared with the LGI route. There was no effect seen of route of delivery in the early period. The late period route effect remained (HR, 3.43; 95% CI, 1.32-8.93) when adjusted for age and sex of the patient (Table 2).

DISCUSSION

The overall (84.6%) rate of clinical resolution for CDI and by delivery routes (UGI route 83.5% and LGI route

TABLE 2. Cox Proportional Hazards Regression With Consideration of Route of Delivery as a Time-varying Covariate

Delivery Route (Reference Category: Lower)	Unadjusted Hazard Ratio (95% CI)	Adjusted Hazard Ratio (95% CI)*
Interaction with time period		
Upper: up to 30 d	1.03 (0.35-3.02)	0.40 (0.09-1.87)
Upper: after 30 d	**3.65 (1.47-9.05)**	**3.43 (1.32-8.93)**

Main effect of time-period exhibited collinearity (ie, was nonvariant within failure event risk sets) and omitted from the table. Statistically significant hazard ratios and CI values are emboldened.
*Adjusted for age and sex.
CI indicates confidence interval.

85.1%) were comparable to the ones previously reported in a meta-analysis by Kassam et al.[11] These results are largely superior to those reported for metronidazole and vancomycin when used to treat recurrent/relapsing CDI.[39] A RCT that compared FMT, vancomycin, and vancomycin plus bowel lavage was stopped on ethical grounds after the interim analysis because of the greater effectiveness of FMT compared with vancomycin with or without bowel lavage for the treatment of recurrent CDI.[12] More recently, another RCT has reported a significantly higher clinical cure rate of recurrent CDI among the patients in the FMT arm compared with those in the placebo arm.[13]

After adjusting for patients' demographic characteristics, patients treated via the UGI route had 3-fold the hazard of clinical failure compared with those treated via the LGI route in the period commencing 30 days post-intervention (HR, 3.43; 95% CI, 1.32-8.93). This is the first study that has assessed the delivery route with a sample size big enough to identify a difference during the follow-up period. Previously, Youngster et al[14] reported results from a randomized, open-label, controlled pilot study to compare the outcome of FMT delivered via colonoscopy and NGT; however, because of the small sample size (n = 10 patients in each arm), no statistically significant difference was observed between the routes of delivery in the follow-up period (70% and 60% cured in the colonoscopy and NGT group, respectively; $P = 0.6$).

Although a fatal complication (aspiration pneumonia) associated with the FMT procedure has been reported in the literature,[40] no major procedural adverse effects, such as bowel perforation or death related to the procedure, were reported among the 305 patients included in this study. Furthermore, in 3 studies (n = 55)[29,30,35] the treatment was provided exclusively to outpatients and in 1 study (n = 7)[37] FMT was self-administered at the patients' home.

Despite the clear findings, we acknowledge some limitations in the current study. First, data from 8 studies[17–24] were unable to be accessed after unsuccessful contact with the corresponding authors. Second, adjustments in the regression model for other confounders such as comorbidities (inflammatory bowel disease, malignancies), exposure to antimicrobial (pre-FMT and post-FMT), or gastric acid suppressant drugs were not possible. Third, the data we include is observational, and we could not stratify by study unit (though we did by study type), as numbers contributed were small and thus there is a possibility that these results could have been influenced by some bias. Finally, the small number of children (n = 19) limits the generalizability of the results in this subpopulation.

Several other questions still remain unanswered. The ideal protocol for FMT is currently unknown. Questions around optimal pre-FMT and post-FMT patient care, patient selection criteria, donor screening, and stool preparation need to be carefully assessed. In addition, a new technique to deliver FMT (frozen encapsulated inoculum) has provided initial promising results.[41] A RCT with sufficient sample size is required to answer whether this is indeed a better delivery modality or not. A definitive RCT should also include a cost-effectiveness analysis and a measure of patient well-being. Until such a study is undertaken, this collaborative analysis of individual patient data, despite the heterogeneity across studies, suggests that the current evidence is in favor of the LGI route for FMT delivery (unless contraindications to colonoscopy or enema are present).

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6.3. Low concentration of vitamin D and the risk of *C. difficile* infection

Furuya-Kanamori L, Wangdi K, Yakob L, McKenzie SJ, Doi SA, Clark J, Paterson DL, Riley TV, Clements AC. 25-Hydroxyvitamin D concentrations and *Clostridium difficile* infection: A meta-analysis. *JPEN J Parenter Enteral Nutr* 2015 [Epub ahead of print].

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25-Hydroxyvitamin D Concentrations and *Clostridium difficile* Infection: A Meta-Analysis

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Abstract

Background: Well-known risk factors for *Clostridium difficile* infection (CDI) are exposure to antibiotics and gastric acid suppressants. Recent studies have provided some evidence of an association between hypovitaminosis D and the risk of CDI. Therefore, this meta-analysis aimed to pool all the existing evidence to investigate the association between 25-hydroxyvitamin D (25[OH]D) and CDI. *Methods*: A systematic search was conducted in 3 databases (PubMed, Embase, and Web of Sciences) for epidemiological studies that examined the association between mean 25(OH)D concentrations and CDI as well as between 25(OH)D status and CDI severity or recurrence. 25(OH)D status was defined as "lower" or "higher" at a threshold concentration of <20 or ≥20 ng/mL, respectively. Pooled effect sizes were computed using the inverse variance heterogeneity model of meta-analysis. *Results*: Eight publications (n = 4479 patients) were included in the meta-analysis. The mean concentration of 25(OH)D in patients with CDI was 3.54 ng/mL (95% confidence interval [CI], 0.39–6.89 ng/mL) lower than in patients without CDI. Patients with lower 25(OH)D status had a higher odds (odds ratio [OR], 1.61; 95% CI, 1.02–2.53) of developing severe CDI compared with those with a higher 25(OH)D status. No significant association was found between 25(OH)D status and CDI recurrence. *Conclusion*: The results of this meta-analysis suggest that lower mean concentrations of 25(OH)D were associated with CDI. A lower 25(OH)D status increased the odds of severe CDI but not of CDI recurrence. (*JPEN J Parenter Enteral Nutr*. XXXX;xx:xx-xx)

Keywords

Clostridium difficile; infection; recurrence; severity; vitamin D; 25-hydroxyvitamin D

Clinical Relevancy Statement

Clostridium difficile infection (CDI) is the leading cause of antibiotic-associated nosocomial diarrhea. Recent studies have reported contradictory evidence of hypovitaminosis D as a novel risk factor for CDI; therefore, the current meta-analysis was conducted to examine the association between 25-hydroxyvitamin D (25[OH]D) concentrations and CDI. The results of the pooled estimates reveal a lower mean concentration of 25(OH)D in patients with CDI and an increased odds of severe CDI in patients with a lower 25(OH)D status.

Introduction

Clostridium difficile is a Gram-positive, spore-forming anaerobic bacillus, and worldwide it is the main cause of infectious diarrhea in hospitalized patients. The incidence and severity of *C difficile* infection (CDI) has increased in the past decades mainly due to the emergence of hypervirulent strains.[1] It is estimated that the additional CDI attributable length of stay in acute care facilities ranges from 2.8–6.4 days with an estimated cost per CDI case of up to US$15,397.[2] The economic burden to the US healthcare system attributable to CDI in 2008 was estimated at US$4.8 billion.[3]

Traditionally, CDI has been associated with exposure to antimicrobials and gastric acid suppressant medications; however, a recent study has reported an association between higher concentrations of 25-hydroxyvitamin D (25[OH]D) and reduction in risk of CDI in patients with inflammatory bowel disease.[4] Furthermore, Abdelfatah et al[5] found a protective effect against severe cases of CDI in patients with concentrations of 25(OH)D >20 ng/mL. In contrast, van der Wilden et al[6] did not find an association between 25(OH)D concentrations and CDI severity.

Given the heavy burden on health systems imposed by CDI and the current contradictory evidence around 25(OH)D and CDI, a meta-analysis was conducted to assess the impact of 25(OH)D status on CDI.

Methods

A systematic review/meta-analysis (secondary analysis of de-identified published data) was conducted, and thus ethics committee approval and informed consent were not required.

Search Strategy and Eligibility Criteria

A systematic search with no language restrictions was undertaken in 3 medical and life sciences databases (PubMed, Embase, and Web of Sciences) from their inception to August 2015. Search terms included were *Clostridium difficile* and *vitamin D;* the specific keywords and connectors for each database are listed in the supplementary material.

The inclusion of studies was restricted to published (full-text or conference abstracts) epidemiological studies in humans that reported concentrations of 25(OH)D in an extractable format. The studies were included if they reported mean 25(OH)D concentrations or data around the CDI-related outcomes of severity or recurrence. Studies that reported findings in animal models were excluded. No exclusion criteria were considered for indirect methods to detect CDI cases such as *International Classification of Diseases* (*ICD*) codes, as these have proven to be highly specific for CDI.[7] Similarly, no restrictions about CDI severity scores were considered as most score indices have a good sensitivity and specificity.[8]

Study Selection and Data Extraction

Two researchers (L.F.-K. and K.W.) independently assessed all the citations by titles and abstracts followed by a full-text review of all potentially relevant studies. Data from the included studies were then independently extracted in a spreadsheet by the same 2 researchers. The recorded fields included study identifiers (authors, publication year), study characteristics (design, setting, inclusion criteria, sample size), and mean 25(OH)D concentrations and outcome measurement (CDI, CDI severity, CDI recurrence). 25(OH)D status of "lower" or "higher" was defined based on concentrations <20 ng/mL and ≥20ng/mL.[9] The extracted data were then cross-checked by the 2 researchers, and any discrepancies during the selection of studies or data extraction were resolved through discussion and consensus.

Statistical Analyses

The effect sizes for the difference in mean 25(OH)D concentrations across CDI diagnosis status and the odds ratios (ORs) for the association between 25(OH)D status and CDI severity or CDI recurrence were pooled using the inverse variance heterogeneity (IVhet) model.[10] Statistical heterogeneity among studies was assessed by both the Cochran's Q and I^2 index; heterogeneity was defined as low (I^2 < 25%), moderate (I^2 = 26%–50%), and high (I^2 > 50%). While I^2 is the percentage of variability that is due to between-study heterogeneity, $1 - I^2$ is the percentage of variability that is due to sampling error. The latter is affected by study size; thus, when the studies become very large, the sampling error tends to 0 and I^2 tends to 1.[11] Such heterogeneity may not be clinically relevant, and studies with relatively large I^2 in this situation may still be usefully pooled if other measures such as Q or τ^2 remain relatively small and clinically relevant heterogeneity is unlikely to be present. In addition, the model used to pool effect sizes (IVhet model) takes account of the uncertainty due to heterogeneity and adjusts the confidence interval (CI) adequately,[10] which does not happen with the random-effects model, thus again justifying pooling in the face of heterogeneity documented using the I^2 index.

The meta-analyses were conducted using MetaXL v3.0 (EpiGear International, Sunrise Beach, Australia).

Results

Yield of Search Strategy

The search strategy identified 147 records in the 3 databases after removal of duplicate records. Of these, 121 studies were excluded based on a review of title and abstract. Full texts of the remaining 26 studies were reviewed, and 8 articles were selected and included in the final analyses (see Figure 1).

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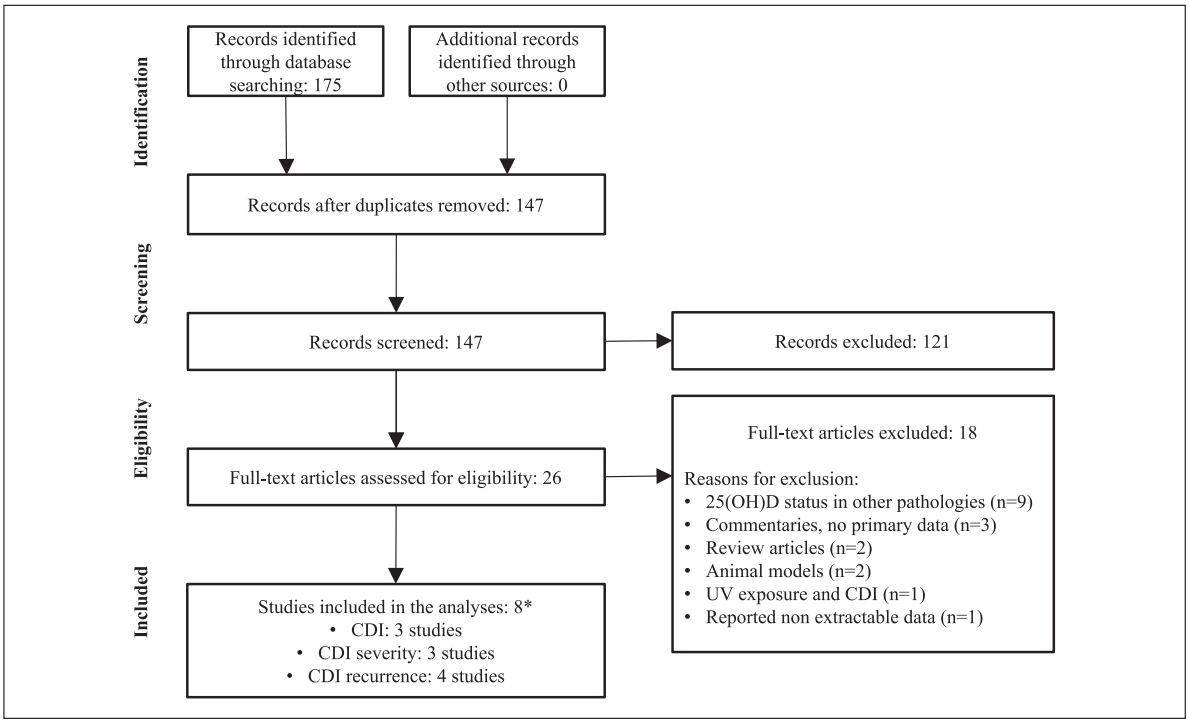

Figure 1. Preferred reporting items for systematic reviews and meta-analyses (PRISMA) flow diagram. CDI, *Clostridium difficile* infection; 25(OH)D, 25-hydroxyvitamin D; UV, ultraviolet.

Characteristics of Included Studies

All the studies were conducted in healthcare settings in the United States. Half of the studies were conducted prospectively,[4,6,12,13] one of which only enrolled patients with inflammatory bowel disease.[4] Among the included studies, 3 reported 25(OH)D concentrations by *C difficile* diagnosis outcome (infected vs noninfected).[4,14,15] Three studies[5,6,16] assessed the association between 25(OH)D status (<20 ng/mL vs ≥20 ng/mL) and CDI severity (mild vs severe). Finally, 4 studies[5,12,13,16] examined the association between 25(OH)D status (<20 ng/mL vs ≥20 ng/mL) and CDI recurrence (see Table 1).

Quantitative Synthesis

The pooled mean difference in 25(OH)D concentrations between patients with and without CDI was −3.54 ng/mL (95% CI, −6.89 to −0.39 ng/mL), and thus mean 25(OH)D was lower in patients with CDI. Patients with lower 25(OH)D status were at higher odds of developing severe CDI compared with those with higher 25(OH)D status (OR, 1.61; 95% CI, 1.02 to 2.53). No significant difference was found between patients with lower vs higher 25(OH)D status in terms of CDI recurrence (OR, 1.26; 95% CI, 0.56 to 2.83; see Figure 2). Moderate (I^2 = 48%) and high (I^2 = 63%) heterogeneity was observed for the mean difference in 25(OH)D concentrations across CDI status group and the OR for 25(OH)D status and CDI recurrence, respectively. No heterogeneity (I^2 = 1%) more than expected due to chance was observed for the OR for 25(OH)D status and CDI severity. Despite the different degrees of heterogeneity, the CIs under the IVhet model adequately account for the uncertainty due to heterogeneity and retain nominal coverage. Due to the limited number of studies included in each meta-analysis, visual inspection of the funnel plots was not possible to assess the presence of publication bias.

Discussion

Our findings provide some evidence that lower mean concentrations of 25(OH)D were present in patients diagnosed with CDI and that CDI severity was associated with a lower 25(OH) status. Paradoxically, pooled estimates did not reveal an association between 25(OH)D concentrations and CDI recurrence. One possible explanation for this finding may be differences in the duration of follow-up time used by the researchers. For instance, when CDI recurrence was defined as "within 30 days," Arramraju et al[12] and Wang et al[13] found a significant association between lower 25(OH)D status and recurrence; however, when CDI recurrence was evaluated in a longer follow-up period of 56 and 90 days, Abdelfatah et al[5] and Wong

Table 1. Characteristics of the Included Studies.

Authors	Setting	Study Design	Inclusion Criteria	Patients' Age, Mean (SD), y	Sample Size (25(OH)D <20/≥20 ng/mL)	Outcome Measured
Abdelfatah et al, 2015[5]	Akron General Medical Center, Akron, Ohio	Case-control study (2007–2013)	Hospitalized patients with positive *Clostridium difficile* toxin assay and recorded 25(OH)D concentration	68.7 (16.7)	271 (133/138)	Severity of CDI and recurrence of CDI associated with 25(OH) D status
Ananthakrishnan et al, 2014[4]	Massachusetts General Hospital and Brigham and Women's Hospital, Boston, Massachusetts	Cohort study	Patients with inflammatory bowel disease and recorded plasma 25(OH) D concentration	60.5 (16.9)/ 48.7 (18.0)[a]	3188 (20.4 [12.8] /27.1 [12.7])[b]	Development of CDI associated with 25(OH)D concentrations
Arramraju et al, 2010[12]	New York Hospital Queens, Flushing, New York	Cohort study (2008–2009)	Admitted patients with positive *C difficile* toxin assay	NR	62 (34/28)	Resolution of CDI (no recurrence) associated with 25(OH)D status
Quraishi et al, 2015[14]	Massachusetts General Hospital and Brigham and Women's Hospital, Boston, Massachusetts	Retrospective cohort study (1993–2006)	Patients aged ≥18 years with documented 25(OH) D concentration prior to admission. Patients without vitamin D supplementation or prior CDI.	63 (18)	568 (17 [10]/ 19 [12])[b]	Development of hospital-acquired CDI associated with 25(OH)D concentrations
Sahay and Ananthakrishnan, 2014[15]	Massachusetts General Hospital, Boston, Massachusetts	Case-control study (2010–2013)	Patients with positive *C. difficile* toxin assay and recorded 25(OH)D concentration	62 (19)	116 (28.5 [15.4]/ 33.8 [12.8])[b]	Community-acquired CDI associated with 25(OH)D concentrations
van der Wilden et al, 2015[6]	Massachusetts General Hospital, Boston, Massachusetts	Cohort study (2011–2013)	Admitted patients with confirmed CDI	62 (19)	100 (43/57)	Severity of CDI associated with 25(OH)D status
Wang et al, 2014[13]	New York Hospital Queens, Flushing, New York	Cohort study (2008–2009)	Hospitalized patients with positive *C difficile* toxin assay	75 (17)	62 (38/24)	Mortality and CDI recurrence associated with 25(OH)D status
Wong et al, 2015[16]	Akron General Medical Center, Akron, Ohio	Case-control study (2007–2012)	Hospitalized patients diagnosed with CDI and recorded 25(OH) D concentration within 3 months of CDI	68 (15.7)/ 71 (4.4)[c]	112 (56/56)	Severity of CDI and recurrence of CDI associated with 25(OH) D status

CDI, *Clostridium difficile* infection; NR, not reported; SD, standard deviation; 25(OH)D, 25-hydroxyvitamin D.
[a]Mean (SD) age for patients with CDI/mean (SD) age for patients without CDI.
[b]Mean (SD) of the patients with CDI/mean (SD) of the patients without CDI.
[c]Mean (SD) age for patients with 25(OH)D <20 ng/mL/mean (standard deviation) age for patients with 25(OH)D ≥20 ng/mL.

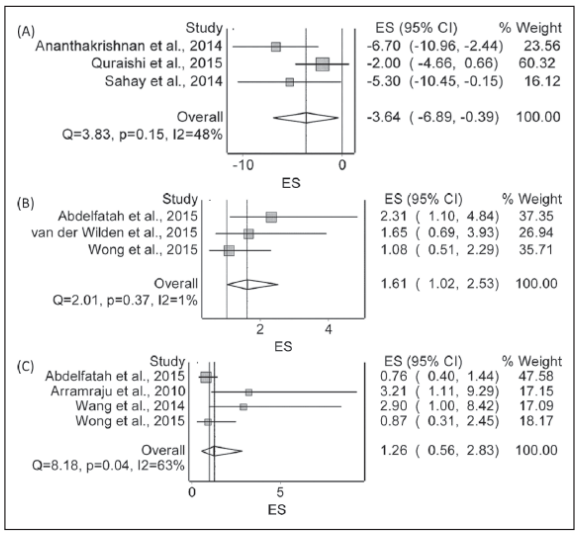

Figure 2. Forest plots depicting (A) the weighted mean difference in 25(OH)D concentrations across CDI status groups, (B) the odds ratio for 25(OH)D status and CDI severity, and (C) the odds ratios for 25(OH)D status and CDI recurrence. CDI, *Clostridium difficile* infection; ES, effect size; 25(OH)D, 25-hydroxyvitamin D.

et al[16] did not find an association. We must point out, however, that study considerations may have had a role in this discrepancy. For example, Wang et al[13] categorized patients who died as a "nonresolution" of CDI, which may have led to an overestimation of failure to resolve *C difficile*, since the exact cause of mortality in each patient was unknown. In addition, other factors such as exposure to certain antibiotics (cephalosporins, aminopenicillins, and clindamycin), proton pump inhibitor use, increased patient age, and number of previous admissions[17] may have affected the CDI recurrence pooled estimate since controlling for these covariates was not possible.

The findings align with those reported by Youssef and colleagues,[18] who described an association between 25(OH)D deficiency (<20 ng/mL) and other hospital-associated infections such as bacterial sepsis and methicillin-resistant *Staphylococcus aureus* colonization. The findings are also in line with those hypothesized beneficial effects of vitamin D supplementation on the reduction of surgical site infections as well as catheter-associated urinary tract infections.[18] In addition, ecological studies have reported an inverse relationship between ultraviolet B ray exposure (a major promotor of vitamin D synthesis) and CDI mortality[19] or influenza cases complicated by pneumonia.[20] Our findings therefore add to the growing body of evidence identifying a potential role of lower 25(OH)D status in infectious disease susceptibility.

Although the mechanisms by which 25(OH)D may act as an immunomodulator for CDI are not fully understood, possible explanations are available. Vitamin D plays a vital role in innate (nonspecific) immune response through the stimulation of nitric oxide,[21] cathelicidins,[22] and β-defensin 2[23] production in macrophage lysosomes and epithelial cells. Furthermore, vitamin D also modulates cell-mediated immunity via the differentiation of naive T cells into regulatory $CD4^+$ T lymphocytes.[24] The immunomodulatory activity of vitamin D has also been described in patients with autoimmune disease (multiple sclerosis, systemic lupus erythematosus, and rheumatoid arthritis) in which supplementation of vitamin D resulted in a reduction in disease severity.[25–27]

This is the first meta-analysis that examines a potentially new risk factor for CDI; however, several limitations were noted that warrant future research. First, the strains of *C difficile* ribotypes infecting individuals differ by country/region, and certain *C difficile* ribotypes are associated with different outcomes (eg, recurrence, severity, mortality). The studies included here were all conducted in the Northeast or Midwest regions of the United States, and *C difficile* ribotypes were not taken into account. Second, due to the limited number of studies identified, subgroup analysis by the source of CDI (healthcare vs community acquired) was not possible. Finally, no studies were identified that examined the effect of 25(OH)D in asymptomatic *C difficile* colonized individuals. Given that this group of people are a potential source of CDI and may contribute to the transmission of the pathogen, further epidemiological studies are required to investigate the role of 25(OH)D in this particular group of people. In view of the safety of vitamin D supplements and their potential to favorably influence the outcome or burden of CDI, we recommend the implementation of randomized controlled trials to examine the effect of vitamin D supplementation in the reduction of CDI occurrence and CDI severity.

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Statement of Authorship

L. Furuya-Kanamori, D. L. Paterson, T. V. Riley, and A. C. A. Clements contributed to conception/design of the research; L. Furuya-Kanamori, K. Wangdi, L. Yakob, S. J. McKenzie, S. A. R. Doi, and J. Clark contributed to acquisition, analysis, or interpretation of the data; L. Furuya-Kanamori and K. Wangdi drafted the manuscript; and L. Yakob, S. J. McKenzie, S. A. R. Doi, J. Clark, D. L. Paterson, T. V. Riley, and A. C. A. Clements critically revised the manuscript. All authors read and approved the final manuscript and agree to be fully accountable for ensuring the integrity and accuracy of the work.

Supplementary Material

Supplementary material for this article is available online at http://pen.sagepub.com/supplemental.

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Chapter 7

Discussion

CHAPTER 7. Discussion

7.1. Introduction

The body of research of CDI has largely focused on symptomatic HA-CDI. The current evidence, however, appears insufficient to develop interventions that successfully control the spread of *C. difficile* and prevent severe outbreaks, such as those reported in the early 2000s in North America and Europe. Therefore, in this thesis, I have presented studies that investigated the role of asymptomatic *C. difficile* colonisation and symptomatic CA-CDI in the epidemiology of *C. difficile*, as well as investigating novel therapeutical alternatives and risk factors for CDI.

7.2. Key research findings

The role of asymptomatic colonisation in the epidemiology of CDI remains poorly understood. Despite the growing evidence that asymptomatic colonisation with TCD and NTCD may play different roles, no study with sufficient sample size or defined pathogen characteristics has independently examined host risk factors for asymptomatic TCD- and NTCD-colonisation. The study detailed in Chapter 3 identified that asymptomatic TCD- and NTCD-colonised patients do not share similar risk factors; thus, they should be considered separately to better understand CDI epidemiology. Additionally, given that morbidity is largely driven by TCD strains, this novel finding has important implications for disease control and prevention, because it may assist the identification of patients at high risk of TCD colonisation. A study recently reported that screening all admitted patients to healthcare facilities for *C. difficile* and placing isolation precautions on those who were asymptomatically TCD colonised significantly reduced the incidence of HA-CDI [1]. Our finding suggested that such screening programmes can be more efficiently targeted at specific high-risk groups.

This thesis has also provided the first estimates of asymptomatic *C. difficile* colonisation prevalence in Australian hospitals. Although the overall prevalence was lower than reported in North America and Europe [2], it is of great concern that 1 in 13 admitted patients to Australian hospitals were colonised with *C. difficile* (and 1 in 18 were colonised with TCD strains) and can contaminate wards, yet no interventions are in placed to prevent or reduce the risk of transmission from these admitted patients. Highly discriminatory molecular typing methods (e.g. multilocus variable-number tandem repeat analysis or whole-genome sequencing) are needed to decisively conclude that HA-CDI cases are acquired from asymptomatic TCD-colonised patients in hospital wards. However, the research detailed in Chapters 3 and 4 provides compelling evidence that transmission of the pathogen from asymptomatic TCD-colonised patients to uncolonised patients -that would subsequently become symptomatic HA-CDI- is occurring in Australian hospital wards. The studies found that the predominant *C. difficile* ribotypes isolated from symptomatic HA-CDI patients were concordant with the ribotypes identified among asymptomatic TCD-colonised patients, in fact over 70% of the isolates from symptomatic patients had a matching ribotype isolated from an asymptomatic patient.

In order to sustain *C. difficile* transmission in hospitals, new cases of CDI need to be admitted to the wards, because the basic reproduction number (R_0) of *C. difficile* in hospitals is less than one [3]. The study presented in Chapter 4 found that *C. difficile* ribotypes circulating in two Australian hospitals corresponded with *C. difficile* ribotypes isolated from CA-CDI cases from the hospital service areas. These findings provide further evidence that importation of new cases from the community into the hospital is a plausible source of new cases to maintain transmission of *C. difficile* in hospitals settings. Symptomatic CDI cases admitted to a hospital will rapidly be detected and placed under isolation and contact precautions for the duration of diarrhoea, to reduce their

transmission potential [4]. This study also found that predominant *C. difficile* ribotypes among asymptomatic TCD-colonised patients were similar to those isolated among symptomatic CA-CDI patients. Therefore, a possible source of introduction of CDI cases into the hospitals is through asymptomatic TCD-colonised patients. These patients have the potential to transmit the disease, yet could go unnoticed during their hospitalisation because the current practice is not to screen asymptomatic (non-diarrheic) patients.

C. difficile ribotyping is not routinely done in Australia; thus, this study also provided a useful insight into the predominant ribotypes circulating in two major Australian cities. Although a wide variety of ribotypes (over 90) were identified during the study period, it was clear that particular *C. difficile* ribotypes were predominant and well established in Australia; *C. difficile* ribotypes 014/020, 056, 002 and 018 accounted for over 50% of the isolates among asymptomatic and symptomatic in the hospital and the community settings. The diversity of ribotypes identified in this prospective three-year study corresponded with surveillance snapshot studies among symptomatic CDI cases in hospitals in Queensland [5] and Western Australia [6]. This study also revealed that *C. difficile* ribotype 027 has not established as a predominant ribotype in Australia. The restricted use of fluoroquinolones in Australia may have prevented the establishment of *C. difficile* ribotype 027 and the emergence of other fluoroquinolone-resistant *C. difficile* ribotypes. *C. difficile* ribotype 244 was the most commonly binary toxin producing ribotype isolated in the study. Ribotype 244 belongs to the same *C. difficile* clade as the epidemic ribotype 027 and it was responsible of a community outbreak in October 2011 in Western Australia [7]. The fact that highly virulent *C. difficile* ribotypes such as 027, 078, 244, and 251 were found in the study to be circulating in Australian hospitals and communities imposes a latent risk of an outbreak. The findings presented in Chapter 4 also suggest that *C. difficile* ribotypes may not be determinants of the development of symptomatic infection, but rather development of symptoms is mainly

driven by host factors such as immune state and disruption of the gut microbiome by exposure to antimicrobials or underlying conditions affecting the gastrointestinal tract.

The results of the research presented in Chapter 5 have significant public health implications for CDI preventive and control programmes. No identified severe outbreaks of *C. difficile* have been reported in Queensland during the past decade; however, it is alarming that the proportion of CDI-positives stool samples detected in hospitals and communities has risen 3-fold over the last decade in Queensland. Moreover, the biggest burden of CDI in Queensland (and probably in the whole of Australia) might be occurring in communities [5]. Similar trends in CDI incidence have been documented in other states and territories in Australia [8], yet it is unclear the reasons for this steep increase in CDI cases and currently it is uncertain what is the best approach to control and reduce CDI incidence.

While third-generation cephalosporin restriction policies have been shown to have an effect in reducing the incidence of CDI in hospitals [9], the research presented in this thesis found that a similar policy may have a small or null effect in reducing the incidence of CA-CDI given that medication exposure (e.g. antibiotics, gastric acid suppressive agent and corticosteroids) was not associated at a population level with CA-CDI. Despite at an individual level antibiotic exposure is well known to be the greatest risk factor for CDI; our finding at a population level indicated that medication (including antibiotics) exposure was not associated with CA-CDI. This can be explained by the heterogeneous distribution of health determinants and risks factors in the population.

In communities, identification of clusters could make it possible to identify health risks and prepare strategies to contain them [10]. However, by mapping and analysing the distribution of CDI in Queensland, it was clear that the implementation of control measures for CA-CDI will need to target the wider population in order to be successful as CDI had no clear spatial patterns or identifiable ‘hotspots’.

The seasonality of asymptomatic *C. difficile* colonisation (Chapter 3) and symptomatic CDI (Chapter 5) in Australia did not follow the assumed seasonal pattern of respiratory infections, which in other parts of the world have been associated with higher antibiotics prescriptions rates and therefore higher incidence of CDI in winter months. Another group recently analysed data on symptomatic CDI from Victoria, Australia (located in different climate zone to Queensland), and found that indeed, CDI peaked at the end of summer in that state, and not during winter as reported in the Northern Hemisphere countries [11]. These consistent findings from two geographically separate Australian states reinforces the need for a more holistic approach – not focused solely on antibiotic exposure – to identify the drivers associated with the seasonal dynamics of CDI, as well as asymptomatic colonisation, in Australia. Sources of transmission other than human-to-human need to be investigated, including food or livestock, which have been reported to be contaminated with similar *C. difficile* ribotypes to those affecting humans [12, 13].

Resistance to antibiotics used to treat CDI is not yet a problem; however, treatment of recurrent and refractory CDI remain problematic. Therapeutical options recommended by international clinical guidelines for CDI recurrence or refractory disease are limited. The latest drug specifically developed and approved by the US Food and Drug Administration (FDA) for CDI was Fidaxomicin (Dificid or Difiklir). This drug is reserved for the 2nd or subsequent recurrence or as 2nd line therapy for refractory disease; however, its high price tag precludes its widespread use. Another drug specifically designed for *C. difficile* recurrence is Bezlotoxumab, a human monoclonal antibody against *C. difficile* toxin B, which has completed Phase 3 studies and currently is awaiting FDA approval. The development of a new drug is a lengthy and costly process; thus, clinicians and researchers are constantly investigating new non-pharmacological therapeutical options or alternative usages for existing drugs.

Among the non-pharmacological options, FMT is a promising therapeutical alternative for recurrent and relapse CDI due to its efficacy and safety. In fact, The Australasian Society of Infectious Diseases updated guidelines for the management of CDI recommends the use of FMT for the 2nd or subsequent recurrences or as the 2nd line therapy for refractory cases [14]. However, the current level of evidence for FMT is weak and the recommendations are mainly derived from series of cases. Limited comparative effectiveness research has been conducted in terms of the most effective delivery route for FMT. Therefore, by compiling data from multiples studies, the research detailed in Chapter 6 provides a strong level of evidence that will guide clinical practice. This is the first study that compared the effectiveness of upper (i.e. gastroscopy or nasogastric tube) versus lower (i.e. colonoscopy or enema) gastrointestinal routes for the delivery of FMT for recurrence or relapse of CDI as a function of time. In the study, both FMT delivery routes were found to be superior to vancomycin for treating CDI recurrence or relapse. In addition, the study revealed that after 30 days post-FMT via the upper gastrointestinal route, patients had a 3-fold increased risk of clinical failure compared to those that received FMT via the lower gastrointestinal route. Therefore, current clinical guidelines should favour the lower gastrointestinal route for FMT delivery, unless contraindications to colonoscopy or enema are present.

Vitamin D is a key requirement of both the innate and adaptive immune response. It enhances antimicrobial effects of macrophages and monocytes by stimulating the chemotactic and phagocytic capabilities of immune cells [15]. Lower levels of vitamin D have been associated with bacterial sepsis and methicillin-resistant *S. aureus* colonization [16]. Furthermore, astute clinicians and researchers have noticed that supplementation of vitamin D can have a beneficial effect on reducing surgical site infections as well as catheter-associated urinary tract infections [16]. With that in mind, the research presented in Chapter 6 aimed to investigate if vitamin D levels were associated with the occurrence

of CDI, severity of the disease and recurrence of CDI. The systematic review found the evidence around vitamin D and CDI to be contradictory [17, 18], prompting a meta-analysis of all published studies around this topic. The meta-analytic results revealed that serum levels of vitamin D were significantly lower in patients with CDI than patients without the infection in hospital and community settings. Additionally, it was found that low levels of vitamin D were associated with severe forms of the infection and poor clinical outcomes (i.e. mortality) in hospitalised patients. More studies are required, including randomised controlled trials that examine the effect of vitamin D supplementation on CDI. However, given the safety and low cost of vitamin D supplements and the results found in the meta-analysis, vitamin D supplementation has the potential to be included in future clinical guidelines as a complementary treatment to the standard therapies for CDI.

7.3. Limitations

While the evidence presented in this thesis included results from multiple studies, all conducted using robust statistical methods, there were some limitations in relation to the data that may have affected the findings. For the studies presented in Chapters 3 and 4, the enrolment rate ranged from 30-50% depending on the site and the months when the patients were approached. It is important to assess whether refusal to participate was occurring randomly or non-randomly (i.e. whether certain groups of patients were more or less likely to participate). With that in mind I analysed the limited data available from patients that refused to enrol and compared them with those that agreed to participate in the study. There were no differences in term of sex or age between participants and non-participants; however, patients that were admitted to medical wards were less likely to participate compared to patients in surgical wards and intensive care units. Given the refusal to participate, the interviewers could not enquire about the reason(s) for their

decision nor review their medical records to obtain further information. Therefore, it is uncertain if selection bias was introduced due to refusal to participate in the study and how this may affect the generalisability of the results.

The first two studies presented in Chapter 5 analysed data collected from Sullivan Nicolaides Pathology. One of the main limitations was the accuracy of the ascertainment as to whether a positive sample was a HA- or a CA-CDI case, given that hospital length of stay and prior hospital admission information were not available. Second, Sullivan Nicolaides Pathology does not process all the stool samples for *C. difficile* in Queensland, and it has a similar market share to another large private laboratory, from which data were not available. Regional differences for selecting one of the providers may be present due to market penetration and access to these laboratories particularly in rural and remote areas, which may have introduced sampling bias. This also meant the denominator for the study was number of stool submissions rather than the population at risk. This could have introduced bias through different patterns of behaviour among submitting physicians in space and time.

For the second study presented in Chapter 5, it was not possible to investigate if CDI seasonality was associated with certain medication exposures (in particular antibiotics). The number of prescriptions for different classes of medications increased in November and December and sharply decreased in January and February for every year of the study period. The reason for the temporal pattern in medication prescription was not related to actual medication use; instead, it was due to the Medicare Safety Net, which provides a higher Medicare benefit once a threshold is reached for the rest of the calendar year. This prompts patients to stockpile medications towards the end of the year, resulting in the temporal pattern of prescriptions.

Individual patient data (as opposed to aggregated data, which are commonly used for meta-analyses) allowed examination of the effect of the route of FMT administration

as a function of time (Chapter 6), but comorbidities and medication exposures that are known to influence the outcome of CDI were not accounted for in the multivariate analysis because the data were not available or could not be shared by the researchers due to strict policies about disclosing patient information. However, the model used in the study adjusted for age, sex and in or out-patient status as a proxy for severity of the disease had a good data fit and predictive ability.

7.4. Potential future research

This thesis addressed some of the gaps in *C. difficile* epidemiological knowledge, particularly with regards to in asymptomatic colonisation and CA-CDI that were identified in the literature reviews. However, there are several potential areas for further research that would assist in improving CDI preventive, control and treatment strategies (Figure 6.1), and these include:

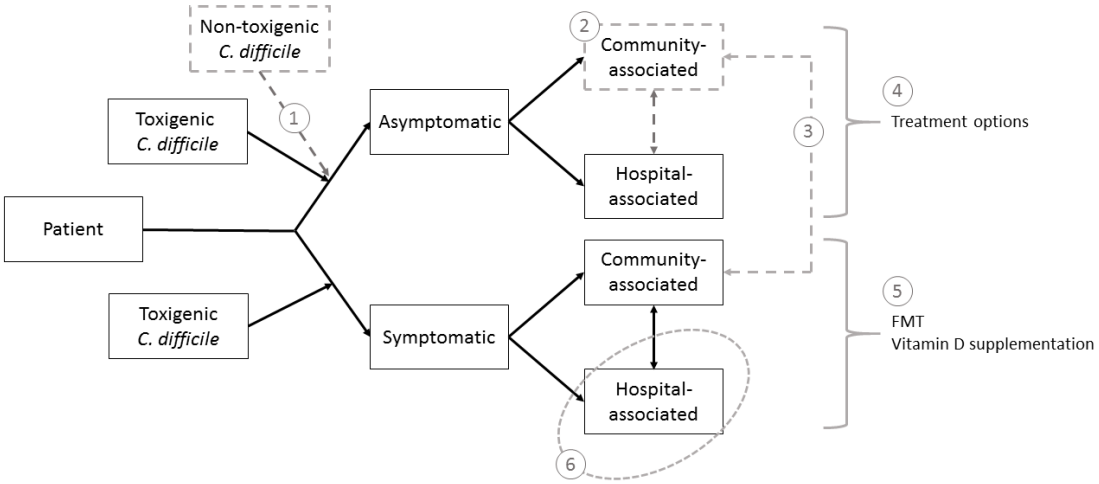

Figure 6.1 Areas for potential future research in *C. difficile*

[1] Asymptomatic NTCD colonisation has shown a protective effect in animal models. Administration of NTCD spores as a potential preventive measure for healthy (uncolonised) individuals at high risk of CDI needs to be assessed.

[2] The biggest reservoir of asymptomatic *C. difficile* colonised patients may be in the community, yet there are no recent estimates of asymptomatic *C. difficile*

colonisation prevalence outside of healthcare facilities nor a clear understanding of which patients are at high risk of being colonised in the community.

[3] Asymptomatic colonised patients in hospitals can transmit the pathogen. It is uncertain the extent to which this occurs in the community; if such transmission is widespread, it is crucial to determine the extent to which asymptomatic colonised patients in the community are responsible for new CA-CDI cases.

[4] It is unknown how to manage an asymptomatic colonised patient to prevent further transmission or development of CDI symptoms. It is necessary to evaluate if certain classes of antibiotics or other therapeutical approaches can be used to treat *C. difficile* in this group of patients without disrupting the gut microbiome and precipitating disease.

[5] In order to adjust for possible confounders and provide higher level of evidence, randomised controlled trials are needed to evaluate the best route of administration of FMT and the effect of vitamin D supplementation on CDI.

[6] An updated evaluation of the economic burden attributable to CDI in Australian hospitals is urgently required, the last study conducted in Australia dates back to 1996 [19].

7.5. Conclusions

The research findings from this thesis have important implications for control and prevention of CDI. Firstly, I provided the first prevalence estimates of asymptomatic *C. difficile* colonisation in Australian hospitals and showed that asymptomatic colonisation has a seasonal pattern. I also provided evidence that patients' characteristics are different between asymptomatic NTCD- and TCD-colonised patients, and it is crucial to make this distinction given that only the latter group is implicated in the transmission of the disease, whereas the former group might be protected. Secondly, I described the most common *C.*

difficile ribotypes isolated in two Australian tertiary hospitals and surrounding communities. I found that the predominant *C. difficile* ribotypes circulating in the communities match those isolated in the healthcare setting, suggesting that asymptomatic colonised patients can act as a means of transmission between the community and hospital settings. Thirdly, I demonstrated that development of CDI symptoms is mainly driven by patient's characteristics and exposure to antibiotics rather than C. difficile strains in a non-endemic 027 setting. Fourthly, I showed that the proportion of submitted stools positive for *C. difficile* has significantly increased in Queensland over the past decade and antibiotic restriction policy at a community-level might have little effect on CA-CDI incidence. Finally, I provided evidence of low concentrations of vitamin D were associated with CDI, as well as recommendations for clinical guidelines for the most appropriate delivery route of FMT for CDI recurrence/relapse.

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Appendix 1.1

Mechanism of hypervirulent *Clostridium difficile* ribotype 027 displacement of endemic strains: an epidemiological model

SCIENTIFIC REPORTS

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Mechanisms of hypervirulent *Clostridium difficile* ribotype 027 displacement of endemic strains: an epidemiological model

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Following rapid, global clonal dominance of hypervirulent ribotypes, *Clostridium difficile* now constitutes the primary infectious cause of nosocomial diarrhoea. Evidence indicates at least three possible mechanisms of hypervirulence that facilitates the successful invasion of these atypical strains: 1) increased infectiousness relative to endemic strains; 2) increased symptomatic disease rate relative to endemic strains; and 3) an ability to outcompete endemic strains in the host's gut. Stochastic simulations of an infection transmission model demonstrate clear differences between the invasion potentials of *C. difficile* strains utilising the alternative hypervirulence mechanisms, and provide new evidence that favours certain mechanisms (1 and 2) more than others (3). Additionally, simulations illustrate that direct competition between strains (inside the host's gut) is not a prerequisite for the sudden switching that has been observed in prevailing ribotypes; previously dominant *C. difficile* strains can be excluded by hypervirulent ribotypes through indirect (exploitative) competition.

Clostridium difficile is a globally significant enteric pathogen with rapid emergence in the Americas, Asia, Oceania and Europe[1]. It is reported to be the leading cause of infectious diarrhoea in healthcare facilities of developed nations[2], and the burden of disease caused by this pathogen is receiving increasing recognition. Disease severity ranges from asymptomatic infection to potentially fatal conditions including toxic megacolon, bowel perforation and sepsis.

In 2005, when performing a Europe-wide survey of 38 hospitals in 14 countries, the European Study Group of *C. difficile* found a novel ribotype (BI/NAP1/027) in Ireland, the Netherlands and Belgium[3]. Within 3 years this PCR ribotype had spread to at least 16 European countries[4] and was rapidly becoming one of the more prominent strains in North America[5].

Ribotype 027 was the causative agent of the largest *C. difficile* epidemic recorded to date, in which over 2000 fatalities occurred in Quebec, Canada during 2005. Early reports of this outbreak described

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higher-than-expected rates of morbidity and mortality associated with ribotype 027, giving rise to the term "hypervirulent" to distinguish this strain (and, subsequently, other strains such as ribotype 078) from "typical" endemic strains. Clear disambiguation between hypervirulent and typical strains is currently precluded by incomplete understanding of what causes some strains to generate outbreaks with substantial morbidity. We use ribotype 027 to demonstrate the invasion dynamics of hypervirulent strains because it was the causative agent of the largest recorded outbreak of *C. difficile* and because the considerable literature pertaining to this particular strain facilitates more accurate model parameterisation.

Over the past decade, research has been conducted to understand hypervirulence in *C. difficile* with no consensus reached on precise causative mechanisms. Here, three plausible explanations for the increased virulence associated with some newly emergent strains of the pathogen (including ribotype 027) are summarised.

Hypervirulent strains are more infectious than endemic strains. Pathogen transmission is via the fecal-oral route with new infections arising from the consumption of bacterial spores. *C. difficile* spores are highly desiccation resistant and can persist on hard surfaces for as long as 5 months[6,7]. *In vitro* studies conducted by Merrigan and colleagues[8] examined the accumulation of spores over the bacterial growth cycle and demonstrated that hypervirulent strains sporulated earlier and accumulated significantly more spores per total volume of culture than non-hypervirulent strains. This increased rate of sporulation may explain, at least in part, the observation of unusually high relapse rates associated with hypervirulent strains (in the order of 4-fold according to Marsh *et al.*[9]) because patients are more likely to contaminate their local environment and subsequently re-infect themselves. However, due to recent evidence to the contrary, the notion of enhanced sporulation in hypervirulent strains remains contentious[10].

Hypervirulent strains result in a higher rate of symptomatic disease. Following ingestion of the dormant bacterial stage, the *C. difficile* spore germinates on exposure to bile salts in combination with L-glycine[11,12]. Vegetative growth of the bacterium occurs during colonization of the host's gut. While colonization is a prerequisite of disease, most colonized individuals remain asymptomatic. Clinical manifestations of *C. difficile* disease are mediated through the production of toxins that are cytotoxic to epithelial cells of the large intestine, causing extensive colonic inflammation and epithelial tissue damage to the host[13]. Studies conducted by Pépin and colleagues[14] and Hubert *et al.*[15] both describe a doubling in the rate of complicated cases (severe disease) during the rise of ribotype 027 in Canada. Higher rates in symptomatic disease associated with hypervirulent strains have been postulated to result from increased toxin production[16,17] or possibly through heightened activity in variant forms of clostridial toxins[18]. It is important to note that there is also contention surrounding the notion of more disease (relative to asymptomatic carriage) and worse disease outcomes from hypervirulent infections[19,20].

Hypervirulent strains can outcompete endemic strains in the host's gut. Recently, Robinson and colleagues (2014) tested the hypothesis that vegetative cells of hypervirulent *C. difficile* strains could outcompete endemic strains for niche space. Four ribotype 027 clinical isolates and clinical isolates of four other strains (001, 002, 014 and 053) were pairwise tested in human fecal bioreactors and in a humanized microbiota mouse model. Ribotype 027 strains outcompeted endemic strains both *in vitro* and *in vivo* and the authors postulated that this competitive advantage is key to the overrepresentation of 027 in recent outbreaks[21].

To offer unique perspective to the critical epidemiological question of which mechanism underlies the rapid global spread (and for many regions, the subsequent clonal dominance) of ribotype 027, we analysed the simulated invasion of hypervirulent *C. difficile* following its introduction into a human community.

Methods

A Direct Gillespie algorithm was scripted in Matlab® software version 7.12 to simulate the epidemiological state transitions involved in *C. difficile* infection with endemic and hypervirulent strains. The simulated introduction of a hypervirulent strain into a community already harbouring endemic *C. difficile* used transmission parameters that were informed by the clinical literature (see Table 1). Following the numerical recipe outlined by Keeling and Rohani (2007), an exact stochastic analogue of the following set of ordinary differential equations was constructed:

$$\frac{dU}{dt} = \phi + (1-\varepsilon)\theta C + (1-\varepsilon_h)\theta C_h + (1-\sigma)\rho(1-\zeta)D + (1-\sigma)\rho(1-\zeta)D_h - \frac{\beta(C+D)U}{N} - \frac{\beta_h(C_h+D_h)U}{N} \quad (1)$$

Symbol	Definition	Value	Ref.
$\beta_h : \beta$	Transmission coefficient (hypervirulent:endemic strain) **MECHANISM 1**	1–1.5	Full range tested in simulations
$\varepsilon_h : \varepsilon$	Develop symptoms (proportion) (hypervirulent:endemic strain); **MECHANISM 2**	1–1.5	
α	Hypervirulent strain's ability to supplant colonized endemic; **MECHANISM 3**	0–1	
1-ε	Colonization self-clearance (proportion)	0.8	[40]
η	Develop into asymptomatic but Infectious state (day^{-1})	0.2	[41]
θ	Develop symptomatic CDI (day^{-1})	0.2	[40]
ζ	CDI self-resolve (proportion)	0.33	[42]
τ	CDI self-resolve rate (day^{-1})	0.5	[43]
ρ	CDI treatment (day^{-1})	0.1	[44]
σ	Treatment failure (proportion)	0.2	[45]
μ	Mortality rate (day^{-1})	0.0012	[40]

Table 1. Epidemiological model symbology and parameterisation.

$$\frac{dE}{dt} = \frac{\beta\,(C + D)U}{N} + \sigma\rho\,(1-\zeta)D - \eta E \tag{2}$$

$$\frac{dE_h}{dt} = \frac{\beta_h\,(C_h + D_h)U}{N} + \sigma\rho\,(1-\zeta)D_h - \eta E_h \tag{3}$$

$$\frac{dC}{dt} = \eta E + \tau\zeta D - \frac{\alpha\beta_h(C_h + D_h)C}{N} - \theta C \tag{4}$$

$$\frac{dC_h}{dt} = \frac{\alpha\beta_h\,(C_h + D_h)C}{N} + \eta E_h + \tau\zeta D_h - \theta C_h \tag{5}$$

$$\frac{dD}{dt} = \varepsilon\theta C - (\tau\zeta + \rho\,(1-\zeta) + \mu)D \tag{6}$$

$$\frac{dD_h}{dt} = \varepsilon_h\theta C_h - (\tau\zeta + \rho\,(1-\zeta) + \mu)\,D_h \tag{7}$$

The equations describe the rates of change between the different epidemiological categories as summarized in Fig. 1. These categories consist of people who are unexposed to *C. difficile* and who are susceptible to colonization (U); exposed to endemic strains (E) or to hypervirulent strains (E_h); colonized with endemic strains (C) or with hypervirulent strains (C_h); and suffering symptomatic disease from endemic strains (D) or hypervirulent strains (D_h). The rate at which individuals are infected is governed by the transmission coefficient (β). Once infected, individuals subsequently become colonized by the pathogen at rate 'η'. Most of these individuals will remain asymptomatic (determined by parameter ε) until the infection resolves and will re-enter the 'unexposed' category. The remaining individuals who go on to experience symptomatic CDI either self-resolve (re-entering the unexposed category), or revert to asymptomatically colonized or they die (according to rate μ). Births are set to perfectly balance deaths to maintain a stable human population, $\phi = \mu(D + D_h)$. The parameters governing the rates of change and the associated proportions are described in Table 1.

The key mechanisms by which hypervirulent strains differ from normal endemic strains are: 1) the rate of transmission is higher for hypervirulent strains ($\beta_h > \beta$); 2) the proportion that experience symptomatic disease is higher for hosts infected with hypervirulent strains ($\varepsilon_h > \varepsilon$); and 3) individuals that are already colonized with normal endemic strains can be colonized by hypervirulent strains ($\alpha > 0$). To ascertain the effects of these three alternative mechanisms, 1000 stochastic introductions of a hypervirulent strain into a community that already harboured normal endemic *C. difficile* at stable equilibrium was simulated. Several epidemiologically relevant metrics were evaluated: the proportion of introductions that elicited an epidemic; the speed at which the newly introduced strain equilibrated; and the

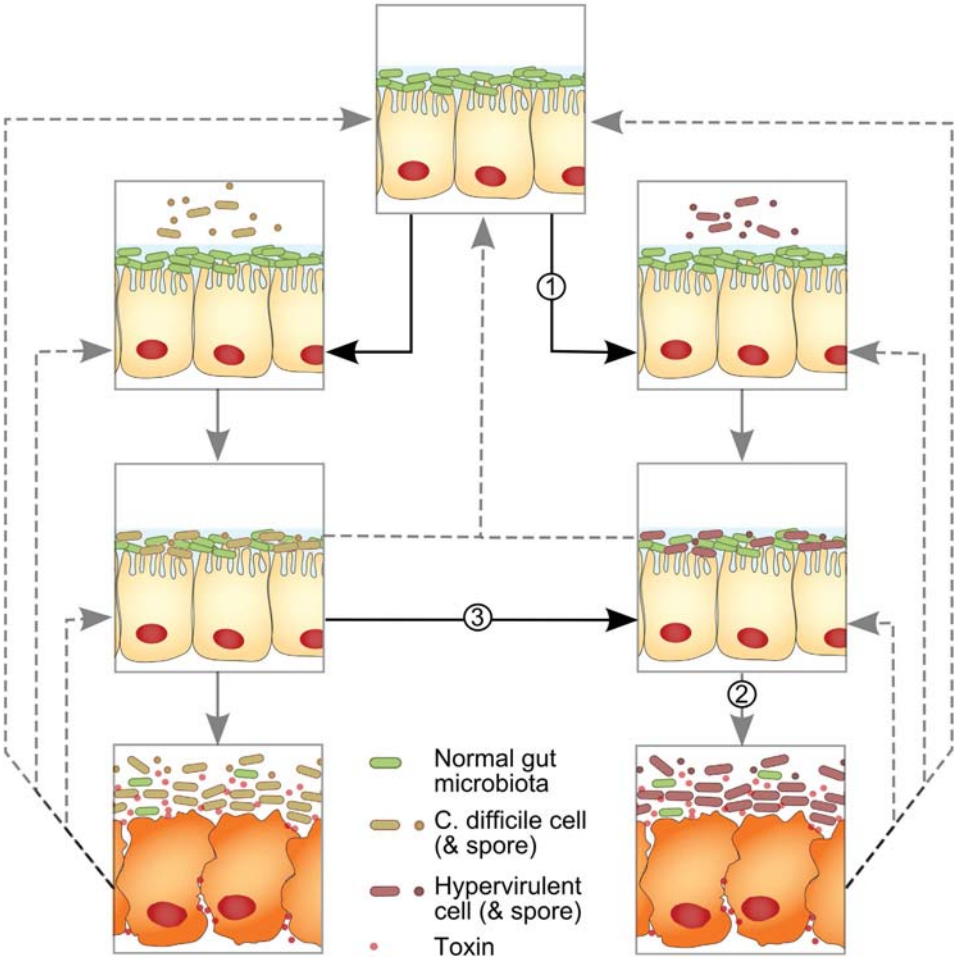

Figure 1. The *Clostridium difficile* epidemiological state transitions simulated by the stochastic model. The left column details colonisation with 'typical' endemic strains and the right column details colonisation with hypervirulent strains. From reviewing the literature, three possible areas were identified that differentiated transitions in hypervirulent strains from endemic strains: 1) Hypervirulent strains are more infectious (modelled by increasing the transmission coefficient); 2) Hypervirulent strains give rise to a higher symptomatic rate; and 3) Hypervirulent strains can outcompete endemic strains in the host's gut.

new equilibrium prevalence level following successful invasion, across a broad range of the parameters governing the different mechanisms of hypervirulence.

Results

Establishment (i.e. successful invasion) of hypervirulent strains was more likely for higher simulated levels of infectiousness (left axis, Fig. 2). For example, an invading strain that is 50% more infectious than endemic strains successfully established in 31.6% of simulations, compared with a strain that is only 20% more infectious which established in 13.8% of simulations. Similarly, hypervirulent strains that elicited a higher symptomatic rate in colonized individuals and that were capable of infecting individuals already colonized by normal endemic strains were more likely to establish. For example, 11.8% of invasions established for hypervirulent strains eliciting a 50% increase in the symptomatic rate relative to typical endemic strains whereas only 3.8% of invasions established with strains eliciting a 20% increase in the symptomatic rate relative to endemic strains. Seven percent of hypervirulent invasions became established when individuals who were colonized with endemic strains were equally susceptible to infection as uncolonized individuals, but only 2.4% of invasions established if endemic-colonized individuals were only one-fifth as susceptible to hypervirulent infection as uncolonized individuals.

Hypervirulence modelled through increased transmission potential, or through increased symptomatic infection rate, also had a positive relationship with the new equilibrium level established by the

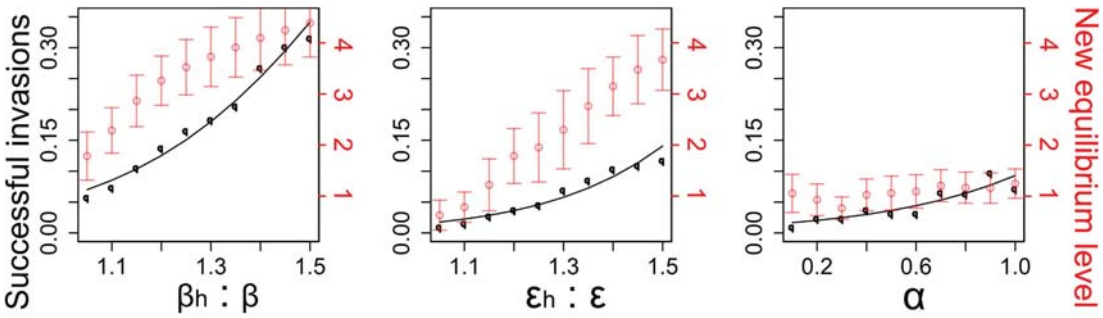

Figure 2. Proportion of hypervirulent *Clostridium difficile* introductions that successfully invade (along with fitted logistic regression curves; black left y-axis) and the new level to which they equilibrate (per 10,000 individuals, red right y-axis). Output from 1000 simulated introductions are displayed for each parameter level under all mechanistic scenarios. Left plot: hypervirulent strains are more infectious (modelled by increasing the transmission coefficient); Middle plot: hypervirulent strains give rise to a higher symptomatic rate; Right plot: hypervirulent strains can outcompete endemic strains in the host's gut.

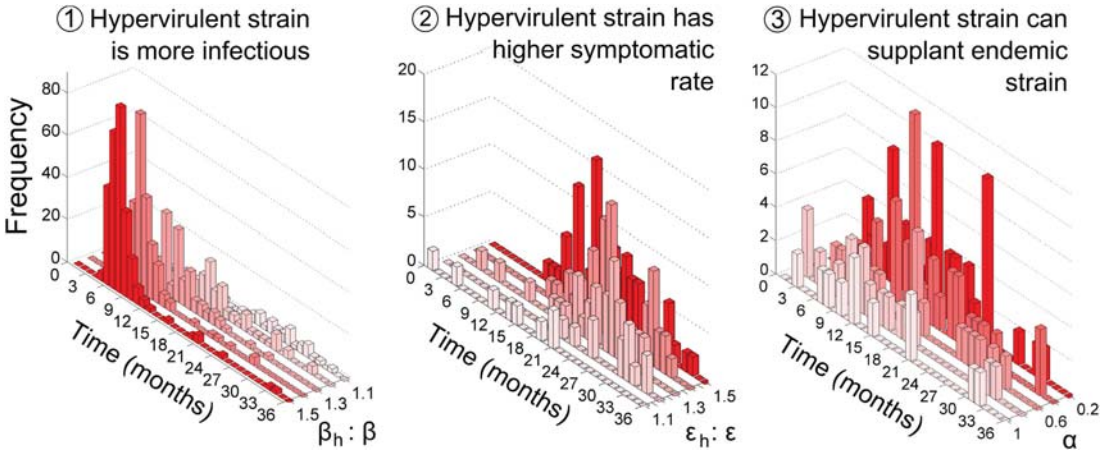

Figure 3. Frequency distribution of time (in months) taken for a newly introduced hypervirulent strain of *Clostridium difficile* to establish new equilibrium under the different mechanisms of hypervirulence. Output from 1000 simulated introductions are displayed for each parameter level under all mechanistic scenarios (note the reversed parameter axes for β and for α which was done to display results more clearly).

invading pathogen (right axis, Fig. 2). For example, an increased infectiousness associated with hypervirulence of 20% led to a new equilibrium prevalence of 3.2 symptomatic infections per 10,000 individuals (s.d. 0.5) compared to a 50% increased infectiousness which led to new equilibrium prevalence of 4.4 symptomatic infections per 10,000 individuals (s.d. 0.7). However, the third modelled mechanism of hypervirulence (an ability of hypervirulent strains to displace endemic strains within the host gut) showed no apparent relationship with the resultant new equilibrium prevalence.

The speed of establishment had a positive relationship with the level of hypervirulent strain infectiousness relative to endemic strains (Fig. 3); and a similar relationship was observed between establishment speed and hypervirulent strains eliciting higher symptomatic rates. Again, when hypervirulence was modelled by allowing an ability of hypervirulent strains to displace endemic strains within the host gut, the relationship between speed of establishment and displacement ability was obscured (Fig. 3).

Results demonstrate that regardless of the modelled mechanism of hypervirulence, the successful invasion of the introduced hypervirulent strain resulted in the exclusion of the extant endemic strain (Fig. 4).

Discussion

Through stochastic simulation, the invasion of a hypervirulent strain of *Clostridium difficile* into a human community already harbouring an endemic strain was explored. Several mathematical models of *C. difficile* transmission have been reported[22,23], most having been published in the last five years[24–29].

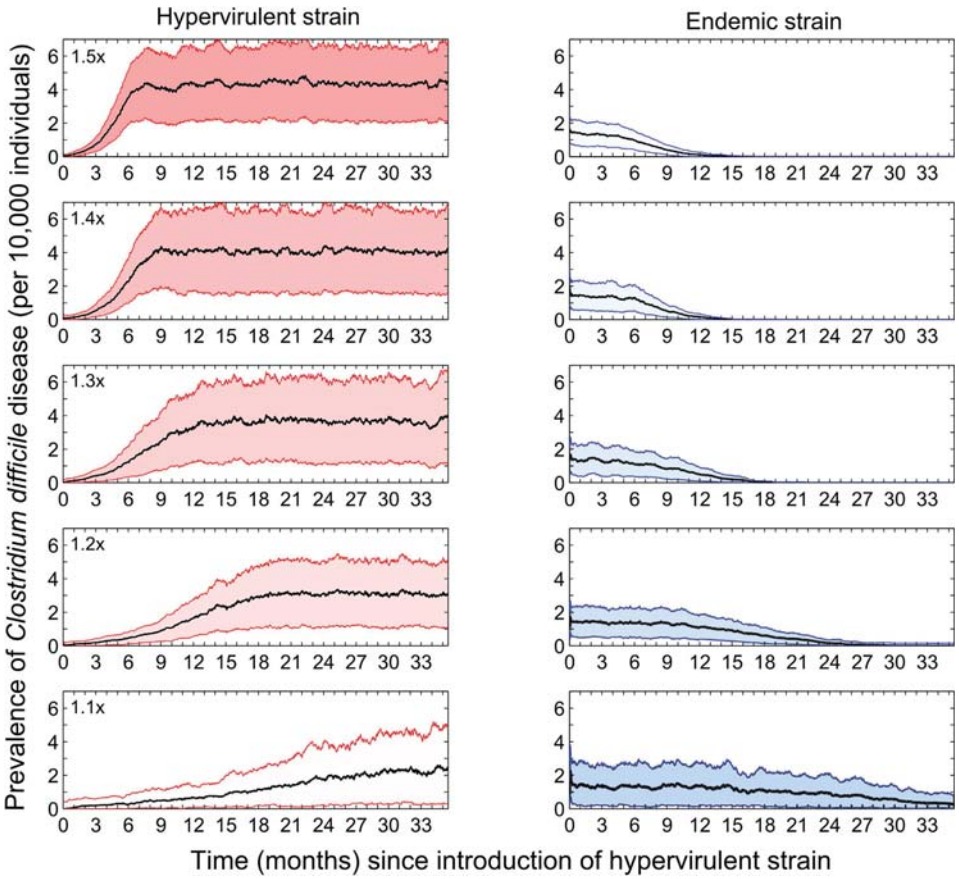

Figure 4. Successfully invading hypervirulent strains of *Clostridium difficile* (left column, red) competitively exclude pre-existing endemic strains (right column, blue). The fold increase in infectiousness relative to endemic strains is shown in the top left of the hypervirulent plots. A one-month moving average of the successful hypervirulent strain invasions from 1000 simulated introductions are displayed (with shaded 95% confidence intervals).

The rationale behind all these previous models was to strategize the control of infection in a hospital setting. However, *C. difficile* is increasingly recognised as a pathogen of the global community, rather than just the subset of the community housed within healthcare facilities. Additionally, recent studies have suggested that the community is a major source, if not the primary source, of infections experienced by the high-risk groups within healthcare settings[30,31]. To the best of our knowledge, the current study constitutes the first epidemiological model of *C. difficile* transmission within the wider community as well as the first comparative analysis of alternative mechanisms of hypervirulence.

Precise causes for the difference in virulence between hypervirulent stains and endemic strains remain unknown despite the fact that these newer 'atypical' strains now constitute the majority of infections in the community setting[32]. Consequently, the effects of three different mechanisms of heightened virulence were tested: increased infectiousness of the pathogen, an increased rate of symptomatic disease following colonization, and the ability of hypervirulent strains to displace endemic strains from a colonized gut. Intuitively, the parameters governing these different mechanisms all had positive relationships with the probability of an invading strain establishing in the community. However, comparing the influence of these parameters on the rate of invasion and the resultant equilibrium prevalence yielded strikingly different epidemiological patterns.

In line with classic epidemiological understanding[33], the rate at which an introduced pathogen spreads among a susceptible population is highly dependent on the transmission coefficient, which was modelled by increasing the infectiousness of a hypervirulent strain. Simulations showed that more infectious strains were more likely to establish, spread more rapidly, and equilibrated to a higher prevalence within the community. The likelihood of successful invasion and the new steady state prevalence were both less dramatically influenced by increasing the colonized proportion that went on to experience clinical disease. When individuals colonized with endemic strains were susceptible to colonization with

hypervirulent strains (the third modelled mechanism of hypervirulence) a much weaker relationship was found with likelihood of establishment, and no clear relationship was seen with the resulting equilibrium prevalence. This is because the spread of the newly introduced strain is essentially independent of the resident strain endemicity when a resident strain-colonized gut is colonized just as readily as an uncolonized gut. Consequences of this finding for the strategy to reduce hypervirulent spread through artificial infection with non-toxigenic strains require exploration.

Clinical reports during the past 15 years have described significantly increased rates of disease corresponding with a pronounced and rapid shift in *C. difficile* strain dominance. PCR-ribotyping of isolates from a Montreal area hospital demonstrated that NAP1/ribotype 027 was absent in 2000 and 2001 but represented more than 75% of all isolates corresponding with an outbreak in 2003–2004[34]. Increased disease prevalence has corresponded with the dominance of ribotype 027 in numerous countries across the world including in England where it peaked in 2007–2008[35], in Europe[36] and North America[37].

Tying this epidemiological picture in with the results of the current analysis, it appears that an ability of hypervirulent strains to displace endemic strains from the already-colonized host gut is the least likely mechanism facilitating dominance of ribotype 027. Despite testing a broad range of parameter values, from complete colonization resistance to susceptibility equivalent to an uncolonized individual, the newly introduced strain failed to reproduce the heightened prevalence level associated with emerging hypervirulent strains. This finding does not negate the possibility that hypervirulent strains are more competitive within-host than more typical strains; but it does suggests that this mechanism is not key to the successful invasion and clonal dominance of hypervirulent strains such as ribotype 027. Importantly, the current study demonstrated that direct competition between strains (inside the host's gut) is not a prerequisite for the sudden switching in prevailing strains; simulations of all alternative hypervirulence mechanisms clearly illustrated that previously dominant strains are not simply added to following new strain invasion, but are excluded through indirect (exploitative) competition.

Transmission dynamics of the remaining alternative hypervirulence mechanisms (increased infectiousness and increased symptomatic disease) are much more similar and, therefore, will be much more difficult to disentangle. It is likely that distinguishing between the remaining alternatives will not be possible from comparisons of simulation output with longitudinal, ribotyped infection data, and will necessitate a much clearer clinical picture of *C. difficile* infection. When these data become available in the future and/or there is increasing evidence derived through alternative means that favours a particular mechanism of hypervirulence, the current model formulation offers an important epidemiological tool for contributing towards infection control strategy. These developments will in turn allow for better refinement of the model to account for the interaction between host (as well as bacterial) factors involved in pathogenesis.

There are a number of limitations to the current study that warrant discussion. Despite burgeoning interest in this pathogen of global health significance, basic metrics of the infection process, such as latent periods, are scant in the literature[38]. Due to limited information on the life history of *C. difficile* infection, parameterisation of the current model has depended on numbers amassed from multiple studies across multiple epidemiological settings. This is a common issue with biologically realistic simulation modelling[39]. While a substantial effort was made in preferentially selecting recent studies that better reflected the pathogen's modern epidemiology (published within the past 5 years) as sources of parameter estimates, this was not always possible. Another important limitation is that it has been assumed that the alternative hypervirulence mechanisms operate in a mutually exclusive manner when, in reality, several mechanisms might interact synergistically. Perhaps the most important limitation is the absence of longitudinal ribotype data for a newly invading hypervirulent strain with which to fit our simulation model. The current study using the most up-to-date clinical and microbiological information demonstrates that a complete switch in the dominant ribotype can take place in as little as 6 months. This highlights not only the frequency at which ribotype data would require collection to capture invasion dynamics but also the necessity for an extremely rapid, active surveillance response following initial hypervirulent detection.

Over the past 15 years, morbidity and mortality resulting from *C. difficile* has steadily increased worldwide as a function of the emergence of hypervirulent strains (most notably, ribotype 027). There is contention surrounding all currently proposed mechanisms distinguishing hypervirulent strains from more typical (less virulent) predecessor strains; how this pathogen has become the leading cause of infectious nosocomial diarrhoea remains unknown. In addition to providing new evidence that clearly favours certain hypervirulence mechanisms over others, the current analysis constitutes the first epidemiological model to explore the dynamics of *C. difficile* outside of a healthcare setting by simulating pathogen spread within the wider human community – an aspect that is widely regarded to be critical to the pathogen's modern epidemiology. Methods described in this foundational study provide an important contribution to future outbreak analysis of this disease of increasing global relevance.

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Author Contributions

L.Y. conceived of the study, wrote and analysed the model. T.V.R., D.L.P. and A.C.A.C. consulted on model construction. L.Y., T.V.R., D.L.P., J.M., R.J.S.M., L.F.K. and A.C.A.C. contributed to the interpretation of model output, drafted the manuscript and approved the final manuscript.

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Appendix 1.2

WSES guidelines for management of *Clostridium difficile* infection in surgical patients

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REVIEW Open Access

WSES guidelines for management of *Clostridium difficile* infection in surgical patients

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Abstract

In the last two decades there have been dramatic changes in the epidemiology of *Clostridium difficile* infection (CDI), with increases in incidence and severity of disease in many countries worldwide. The incidence of CDI has also increased in surgical patients. Optimization of management of *C difficile*, has therefore become increasingly urgent. An international multidisciplinary panel of experts prepared evidenced-based World Society of Emergency Surgery (WSES) guidelines for management of CDI in surgical patients.

Executive summary

In the last two decades, the dramatic increase in incidence and severity of *Clostridium difficile* infection (CDI) in many countries worldwide [1], has made CDI a global public health challenge [2–5]. Recently two comprehensive sets of guidelines for management of CDI were published [6, 7] that do not address issues specifically with regard to surgeons. CDI in surgical patients is of particular interest. Surgery, especially gastrointestinal surgery, may predispose patients to the development of CDI. Surgery is also a treatment option in severe cases of CDI [8–11]. Optimization of the perioperative CDI patient management is therefore necessary for reduction in health care costs, as well as patient morbidity and mortality. To provide empirical guidelines for the surgeon called upon to assist in the care of the CDI patient, an international multidisciplinary panel of experts worldwide have prepared these evidenced-based guidelines for the management of *C. difficile* infection. In constituting the expert panel, the board of World Society of Emergency Surgery

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(WSES) involves many of the world's leading surgical experts in management of CDI. This expert panel includes professionals who treat CDI patients on a daily basis as well as those with research interests in the condition. These guidelines outline clinical recommendations based on the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) hierarchy criteria summarized in Table 1 [12, 13].

Recommendations

Diagnosis

1) Stool testing should only be performed on diarrhea stools from at-risk patients with clinically significant diarrhea (Recommendation 1 C).

2) For patients with ileus who may be unable to produce stool specimens, polymerase chain reaction testing of perirectal swabs may be an accurate and efficient method to detect toxigenic *C. difficile* in patients with symptoms of CDI (Recommendation 2B).

3) Nucleic acid amplification tests (NAAT) such as polymerase chain reaction (PCR) for *C. difficile* toxin genes appear to be sensitive and specific and may be used as a standard diagnostic test for CDI. NAAT as single-step algorithm can increase detection of asymptomatic colonization therefore it should only be performed in patients with clinical suspicion for CDI (Recommendation 1 B).

4) Glutamate dehydrogenase (GDH) screening tests for *C. difficile* are sensitive but do not differentiate between toxigenic and non-toxigenic strains. They may be used in association with toxin A and B EIA testing. Algorithms involving screening with an EIA for GDH followed by a toxin assay may be used (Recommendation 1 B).

5) Enzyme immunoassay (EIA) for toxin A/B is fast and inexpensive and has high specificity but it is not recommended alone due to its relatively low sensitivity. (Recommendation 1 B).

6) *Clostridium difficile* culture is relatively slow but sensitive. It is rarely performed today as a routine diagnostic test. *C. difficile* culture is recommended for subsequent epidemiological typing and characterization of strains (Recommendation 1 C).

7) Repeat testing within 7 days should not be performed on patients who previously tested negative unless the clinical picture has changed significantly (Recommendation 1 C).

Table 1 Grading of recommendations from Guyatt and colleagues [12, 13]

Grade of recommendation	Clarity of risk/benefit	Quality of supporting evidence	Implications
1A			
Strong recommendation, high-quality evidence	Benefits clearly outweigh risk and burdens, or vice versa	RCTs without important limitations or overwhelming evidence from observational studies	Strong recommendation, applies to most patients in most circumstances without reservation
1B			
Strong recommendation, moderate-quality evidence	Benefits clearly outweigh risk and burdens, or vice versa	RCTs with important limitations (inconsistent results, methodological flaws, indirect analyses or imprecise conclusions) or exceptionally strong evidence from observational studies	Strong recommendation, applies to most patients in most circumstances without reservation
1C			
Strong recommendation, low-quality or very low-quality evidence	Benefits clearly outweigh risk and burdens, or vice versa	Observational studies or case series	Strong recommendation but subject to change when higher quality evidence becomes available
2A			
Weak recommendation, high-quality evidence	Benefits closely balanced with risks and burden	RCTs without important limitations or overwhelming evidence from observational studies	Weak recommendation, best action may differ depending on the patient, treatment circumstances, or social values
2B			
Weak recommendation, moderate-quality evidence	Benefits closely balanced with risks and burden	RCTs with important limitations (inconsistent results, methodological flaws, indirect or imprecise) or exceptionally strong evidence from observational studies	Weak recommendation, best action may differ depending on the patient, treatment circumstances, or social values
2C			
Weak recommendation, Low-quality or very low-quality evidence	Uncertainty in the estimates of benefits, risks, and burden; benefits, risk, and burden may be closely balanced	Observational studies or case series	Very weak recommendation; alternative treatments may be equally reasonable and merit consideration

8) Immunocompromised patients (including patients in chemotherapy, chronic corticosteroid therapy or immunosuppressive agents, and post-transplant patients) should be always tested for CDI if they have a diarrheal illness (Recommendation 1 C).

9) CT imaging is suggested for suspected severe-complicated *C. difficile* colitis, however its sensitivity is not satisfactory for screening purposes (Recommendation 2 B).

10) Ultrasound may be useful in critically ill patients suspected to have pseudomembranous colitis who cannot be transported for CT scan (Recommendation 2 C).

11) Flexible sigmoidoscopy may be helpful for the diagnosis of *C. difficile* colitis (CDC) when there is a high level of clinical suspicion for *C. difficile* despite repeated negative laboratory assays (Recommendation 2 B).

Antimicrobial therapy

12) Unnecessary antimicrobial agent(s) and proton pump inhibitors should be discontinued if CDI is suspected (Recommendation 1 C).

13) Empirical therapy for CDI should be avoided unless there is a strong suspicion for CDI. If a patient has a strong suspicion for CDI, empirical therapy for CDI should be considered while awaiting test results (Recommendation 1 B).

14) Metronidazole is recommended for the treatment of mild-moderate disease (Recommendation 1 A).

15) Oral vancomycin is recommended for treatment of patients with severe disease, or for patients with mild-moderate disease who do not respond to metronidazole. (Recommendation 1 A).

16) In patients in whom oral antibiotics cannot reach the colon, vancomycin may be administered by enema and metronidazole can be given intravenously (Recommendation 1 B).

17) Fidaxomicin may be used to treat CDI, especially in the patients at higher risk for recurrence (e.g. elderly patients with severe underlying disease or those requiring receiving concomitant antibiotics) (Recommendation 1 A).

Surgical management

18) Patients with severe CDI who progress to systemic toxicity should undergo early surgical consultation and evaluated for potential surgical intervention (Recommendation 1 C).

19) Resection of the entire colon should be considered to treat patients with fulminant colitis (FC) (Recommendation 1 B).

20) Diverting loop ileostomy with colonic lavage may be a useful alternative to resection of entire colon (Recommendation 2 C).

21) Patients with FC should be treated with high dose oral or by enema vancomycin (500 mg, 6 hourly) in combination with intravenous metronidazole (500 mg, 8 hourly). (Recommendation 1 C).

Supportive care

22) Supportive measures, including intravenous fluid resuscitation and electrolyte replacement, should be provided to all patients with severe *C. difficile* infection (Recommendation 1 C).

23) Early detection of shock and aggressive management of underlying organ dysfunction are essential for optimum outcomes in patients with fulminant colitis (Recommendation 1 C).

Recurrent C. difficile infection (RCDI)

24) Agents that may be used to treat the first recurrence of CDI include metronidazole, for non-severe RCDI, and vancomycin for severe RCDI. (Recommendation 1 B).

25) Fidaxomicin may be used as an alternative agent (Recommendation 1 B).

26) In subsequent recurrence of CDI (2nd or later) oral vancomycin or fidaxomicin is recommended (Recommendation 1 B).

Probiotics

27) Probiotics may be considered as an adjunctive treatment to antibiotics for immunocompetent patients with RCDI (Recommendation 2 B).

Faecal microbiota transplantation

28) Intestinal or faecal microbiota transplantation (IMT or FMT) may be an effective option for the treatment of RCDI (Recommendation 1 B).

29) FMT may be effective in immunocompromised patients and patients who have had solid organ transplants (Recommendation 2 B).

Intravenous immunoglobulin (IVIG)

30) IVIG should only be used as adjunct therapy in patients with multiple recurrent or fulminant CDI until results from large, randomized controlled trials are available (Recurrence 2 C).

Monoclonal antibodies

31) Infusion with monoclonal antibodies may be of use to prevent recurrences of CDI, particularly in patients with CDI due to the 027 epidemic strain (Recommendation 2 C).

Enteral nutrition in CDI

32) Tube feeding patients should be clinically assessed due to their risk for developing CDI (Recommendation 2 C).

Anti-motility agents

33) The use of anti-peristaltic agents for the treatment of CDI should be discouraged. If anti-peristaltic, if used in isolation agents, are used to control persistent symptoms in patients with CDI they must always be accompanied by medical therapy (Recommendation 2 C).

Prevention

34) Proper antimicrobial stewardship in selecting an appropriate antibiotic and optimizing its dose and duration to cure an infection may prevent the emergence of *C. difficile* (Recommendation 1 B).

35) Patients with suspected or proven CDI should be placed in contact (enteric) precautions (Recommendation 1 B).

36) Hand hygiene with soap and water is a cornerstone of the prevention of *C. difficile*. Hand hygiene, contact precautions and good cleaning and disinfection of the environment and patient care equipment, should be used by all health-care workers contacting any patient with known or suspected CDI (Recommendation 1 B).

Introduction

C. difficile is an anaerobic, spore forming Gram-positive bacillus, which may form part of the normal intestinal microbiota in healthy newborns but which is rarely present in the gut of healthy adults [14–16]. The organism is spread via the oral-fecal route and in hospitalized patients may be acquired through the ingestion of spores or vegetative bacteria spread to patients by healthcare personnel's hands or from the environment [17, 18]. It is the most common cause of diarrhea in hospitalized patients.

Pathogenesis

Clostridium difficile spores survive the acidic environment of the stomach and germinate in the intestine [19]. They act as an environmental reservoir for *C. difficile* and can facilitate spread among patients, as well as contributing to the high recurrence rates observed in CDI. The primary toxins produced by this bacterium are toxins A and B [20]. Some strains of *C. difficile* also produce binary toxin. Toxins A and B act as glucosyltransferases, promoting the activation of Rho GTPases leading to disorganization of the cytoskeleton of the colonocyte, and eventual cell death [21]. Since CDI is a toxin mediated infection, non toxigenic *C. difficile* strains are non-pathogenic. Over the years the respective roles and importance toxins A and B has been debated. Toxin A was thought to be the major virulence factor for many years, [22–24]. It is now established that both toxins A and B are important for inducing colonocyte death and colitis. In addition to toxins A and B, some strains produce a third toxin known as binary toxin [25–29]. Binary toxin has an ADP-ribosyltransferase function, which also leads to actin depolymerization [30, 31]. It has been demonstrated in *C. difficile* strains associated with nosocomial outbreaks of CDI with increased clinical severity [32, 33].

Typing is useful to differentiate *C. difficile* strains and to obtain epidemiological information. Different typing methods for *C. difficile* are actually available: restriction endonuclease analysis (REA), pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), repetitive-element PCR typing, toxinotyping, multilocus variable-number tandem-repeat analysis (MLVA) and PCR- ribotyping [34].

C. difficile strains with increased virulence traits (hyper virulent), have been described in the last 10 years. In particular, PCR-ribotype 027, also known as North American pulsed-field gel electrophoresis type 1 (NAP1) or restriction endonuclease analysis group BI, has been associated with increased disease severity, recurrence and significant mortality [35].

Asymptomatic colonization may occur in 6 to 50 % of long-term care facility residents depending on whether CDI is endemic [36, 37]. In a 15-month prospective study of 4143 patients performed in six Canadian hospitals in Quebec and Ontario [38], 184 (4.4 %) had asymptomatic colonization at the time of unit admission, and 123 (3.0 %) had health care–associated *C. difficile* colonization.

Risk factors

Risk factors for CDI may be divided into three general categories: host factors (immune status, co-morbidities), exposure to CD spores (hospitalizations, community sources, long-term care facilities) and factors that disrupt normal colonic microbiome (antibiotics, other medications, surgery) [39].

Host factors

Risk factors identified to date include, age more than 65 years, comorbidity or underlying conditions, inflammatory bowel diseases, immunodeficiency (including human immunodeficiency virus infection, hematologic malignancies and chemotherapy), malnutrition, and low serum albumin level [3, 40]. Diabetes mellitus is increasingly recognized as a risk factor for hospital and community-acquired CDI [41]. More recently, gene polymorphisms (e.g. IL-8) may be associated with increased risk for CDI but further studies are needed [42].

The effect of prior appendectomy on the development of *C. difficile* colitis has been debated in literature [43].

A recent review by Seretis et al. [44] of five studies conducted retrospectively was published in 2014. Although the results were conflicting regarding the impact of prior appendectomy on the occurrence or relapse of CDI, it appeared that an *in situ* appendix did not impact on the development of CDI.

In the retrospective analysis by Clanton et al. [45] on 55 patients who underwent colectomy for CDI between 2001 and 2011, a prior appendectomy was noted in 24 of 55 specimens (44, 99 % CI: 0.280–0.606). This was compared to an observed lifetime rate of appendectomy of 17.6 %. The rate of appendectomy in the cohort of patients who later underwent colectomy for CDI was significantly higher than would be expected in the general population (44 % vs 18 %, P < 0.01).

In a second retrospective study [46], of 388 patients with an intact appendix, 20 (5.2 %) developed fulminant infection and required colectomy, whereas of the 119 patients with a previous appendectomy, 13 (10.9 %) required colectomy. An increased severity of disease, indicated by increased rate of colectomy, occurred for the group with a history of appendectomy ($P = 0.03$).

A sub-group analysis of a large population based study published in 2013 [47] showed that appendectomy was not associated with adverse outcomes in CDI. Patients with appendectomy before CDI had no differences in risk factors, treatment, or outcomes including treatment failure, development of severe or severe-complicated CDI, and recurrence rates as compared with patients without appendectomy.

Larger prospective studies are needed to assess the impact of prior appendectomy on development and severity of CDI.

Exposure to *Clostridium difficile* spores

Factors that increase risk of exposure to *C. difficile* spores, such as increased duration of hospital stay may increase the risk of CDI. A length of stay > 2 weeks has been shown to be a risk factor for CDI [48]. Hospitals with well implemented infection prevention and control measures may reduce the risk of patients of developing CDI [49].

Normal flora disruption

The indigenous gut microbiota is the complex community of microorganisms that populates the gastrointestinal tract. This micro-ecosystem plays a crucial role in protecting the intestines by providing resistance to colonization and infection by pathogenic organisms [50]. Gut microbiota also has immeasurable effects on homeostasis in the host [51]. Under normal conditions, the human gut microbiota may impede pathogen colonisation through general mechanisms such as direct inhibition through bacteriocins, nutrient depletion (consuming growth-limiting nutrients) or stimulation of host immune defences [38] though the exact mechanism by which the microbiota protects against CDI is unknown [52]. Disruption of the normal balance of colonic microbiota as a consequence of antibiotic use or other stressors, is, however, likely to be important [53].

Antibiotic exposure

It is presumabed that disruption of the normal gut flora provides a perfect setting for *C. difficile* to proliferate and produce toxin.

The risk of CDI is increased up to 6-fold during and in the subsequent month after antibiotic therapy [54]. Although nearly all antibiotics have been associated with CDI, clindamycin, third-generation cephalosporins, penicillins and fluoroquinolones have traditionally been considered to pose the greatest risk [55–61]. An association between CDI and antimicrobial treatment > 10 days has also been demonstrated [62, 63]. Antibiotics which have been less commonly associated with CDI include macrolides, sulfonamides and tetracyclines [64]. Even very limited exposure, such as single-dose surgical antibiotic prophylaxis may increase patients risk for both *C. difficile* colonization [65, 66] or infection.

Other medications

Exposure to gastric acid-suppressive medications, such as histamine-2 blockers and proton pump inhibitors (PPIs) may be a potential risk factor for development of CDI. Recent studies have suggested the association between use of stomach acid–suppressive medications, primarily PPIs, and CDI [67, 68]. In 2012 a systematic review of [69] 42 observational studies (30 case–control, 12 cohort) totalling 313,000 participants were evaluated for incident and recurrent CDI in PPI users. Despite the substantial statistical and clinical heterogeneity, the findings indicated a probable association between PPI use and incident and recurrent CDI. This risk was further increased by concomitant use of antibiotics and PPI. Other studies suggested that this association may be the result of confounding with the underlying severity of illness and duration of hospital stay [70]. Given that acid suppression drugs, especially PPIs, may be over- prescribed in surgical settings consideration should be given to stopping PPIs in patients at high risk of CDI.

Surgery

Recent reports have linked the development of CDI in surgical patients with widespread use of broad-spectrum antibiotics, increasing numbers of elderly and immunocompromised patients undergoing surgical interventions and the emergence of more virulent strains of *C. difficile* [8, 71, 72].

Abdelsattar et al. [11] prospectively identified patients with laboratory-confirmed postoperative CDI after different general, vascular, or gynaecological surgeries at 52 academic and community hospitals in the state of Michigan, USA between July 2012 and September 2013. The highest rates of CDI occurred after lower-extremity amputation (2.6 %), followed by bowel resection or repair (0.9 %) and gastric or esophageal operations (0.7 %). Gynaecological

and endocrine operations had the lowest rates (0.1 and 0 %, respectively). Using multivariable analyses, older age, chronic immunosuppression, hypoalbuminemia (≤3.5 g/dL) and preoperative sepsis were associated with CDI. Use of prophylactic antibiotics was not independently associated with CDI, neither was sex, body mass index (BMI), surgical priority, weight loss, or comorbid conditions.

Zerey et al. [8] performed a five-year retrospective analysis of the Agency for Healthcare Research and Quality's National Inpatient Sample Database representing a stratified 20 % sample of hospitals in the United States, from 1999 to 2003. Patients undergoing an emergency operation were at higher risk of CDI than those having operations performed electively. Colectomy, small-bowel resection, and gastric resection were associated with the highest risk of CDI. Patients undergoing cholecystectomy and appendectomy had the lowest risk.

In 2010, Rodriguez et al. [73] published a retrospective analysis of all general surgery inpatients admitted to a large tertiary referral general surgical unit in the United Kingdom, between March 2005 and May 2007. Multivariate analysis identified malignancy, gastrointestinal disease, anemia, respiratory disease, circulatory disease, diabetes mellitus, those undergoing gastrointestinal surgery and increasing age to be independently associated with *C. difficile*.

To assess risk factors associated with CDI on a surgical ward, in 2012 Kim et al. conducted a retrospective chart review of all patients admitted between January 2010 and July 2011 [74]. The rate of CDI occurrence was 0.4 % (19/4,720 patients). Multivariate analysis showed that colectomy and hospital stays longer than 10 days were the highest risk factors for CDI occurrence in the surgical ward.

Using the Japanese Diagnosis Procedure Combination inpatient database, Yasunaga et al. [75] analyzed factors affecting the occurrence of CDI and the outcomes of CDI following digestive tract surgery. Of 143,652 patients undergoing digestive tract surgery, CDI was identified in 409 (0.28 %) patients. High mortality, long hospital stay and high costs were associated with postsurgical CDI.

Colo-rectal surgery is known as risk factor for CDI in surgical patients [76, 77]. Recently Damle et al. [78] published a retrospective analysis of patients who developed CDI following colorectal resection. Utilizing the U.S. University Health System Consortium database the authors identified adult patients undergoing colorectal surgery between 2008 and 2012. A total of 84,648 patients met study inclusion criteria. CDI occurred in 1,266 (1.5 %) patients during the study period. The strongest predictors of CDI were emergency procedure, inflammatory bowel disease, and severity of illness score. CDI was associated with a higher rate of complications, intensive care unit (ICU) admission, longer preoperative inpatient stay, 30-day readmission rate, and death within 30 days compared to non-CDI patients.

In 2008 Lumpkins et al. [79] published a retrospective observational study about the incidence of CDI in the critically injured trauma population. Five hundred eighty-one consecutive critically injured trauma patients were followed prospectively for development of CDI, diagnosed by toxin assay. Among 581 patients 19 cases of CDI were diagnosed (3.3 %). Intensive care unit length of stay, ventilator days, and hospital length of stay were significantly higher in the CDI patients. The diagnosis was made at mean of 17 days after admission; however, in four patients (21 %), the infections were diagnosed within six days of admission. Fourteen patients (74 %) had received therapeutic antibiotics for confirmed or suspected infection prior to the appearance of colitis; four patients (21 %) received only intraoperative prophylaxis, and one patient had no antibiotic exposure.

Recently Egorova et al. [80] reviewed the trend, hospital variability in CDI rates, in vascular surgery in USA. The rates of CDI after major vascular procedures including aortic abdominal aneurysm (AAA) repair, carotid endarterectomy or stenting, lower extremity revascularization (LER), and LE amputation were identified using Nationwide Inpatient Sample database for 2000–2011. During the study period the rates of CDI after vascular procedures had increased by 74 % from 0.6 in 2000 to 1.05 % in 2011. In 2011, the highest rates were after ruptured AAA repair (3.3 %), followed by lower extremity amputations (2.3 %), and elective open AAA (1.3 %).

Iinflammatory bowel disease (IBD)

Patients with inflammatory bowel disease (IBD) may have increased risk of developing CDI, along with worse outcomes, higher rates of colectomy and higher rates of recurrence [81–84]. Patients with IBD also appear to have higher rates of asymptomatic carriage of *C. difficile* [85]. They receive various types of immunosuppressive drugs including steroids that has been found to increase the risk of CDI [86, 87].

The clinical presentation of an IBD exacerbation and CDI often is indistinguishable and requires a high index of suspicion for adequate treatment [6]. As the symptoms of CDI and an exacerbation of IBD (diarrhea, abdominal pain, fever and leukocytosis) overlap, the diagnosis of CDI may be delayed if it is not tested for [88]. In addition, in IBD patients with ileostomies, the development of acute enteritis as manifested by an increase in ileostomy output, nausea, fever and leukocytosis may also indicate CDI. The same is true for pouchitis, which presents as an increase in the number of stools per day [89]. In one study 10.7 % of patients with ileal pouch anal anastomosis, presenting with pouchitis, were found to have CDI [90].

In patients with IBD and severe colitis, empirical therapy directed against both CDI and treatment of an IBD flare should be started simultaneously while awaiting results of *C. difficile* testing [6].

Due to high rates of asymptomatic colonization of *C. difficile* in patients with IBD, only patients with increased diarrhea or new symptoms attributable to CDI should be tested for *C. difficile* toxin. Typical findings of CDI on colonoscopy are often absent in patients with IBD (0–13 % of cases) [91] which may be attributed to a weakened inflammatory response. There is no evidence from prospective studies to suggest that one antibiotic regimen is better than another for the treatment of CDI in IBD patients. Considering the worse outcomes seen in patients with IBD and CDI, some institutions use vancomycin as first line therapy. In a survey of North American gastroenterologists, there was no agreement on combination of antibiotics and immunomodulators in patients with an IBD flare and CDI [92]. The American College of Gastroenterology recommended with low quality supporting evidence, that ongoing immunosuppression can be maintained in patients with CDI but escalation of immunosuppression should be avoided.

Physicians should remain alert to the possibility of CDI in a patient with an IBD exacerbation to ensure rapid diagnosis and treatment. Early surgical consultation is also key for improving outcomes of patients with severe disease. Colectomy with preservation of the rectum may need to be considered for severely ill IBD patients with CDI.

Immunocompromise patients

It is well known that the rate of CDI in the post-transplant setting is higher [93]. It has also been reported that cancer patients have a higher risk compared with non-cancer patients [94] due to chemotherapy causing the immunosuppression. Recently two retrospective studies were published on CDI in cancer patients [95, 96].

In the first a total of 225 patients were included, and 39 of them (17.3 %) were diagnosed with CDI. Type of tumor significantly differed between CDI patients, thus relative risk in each type of cancer was calculated after adjusting for age, antibiotic exposure, corticosteroid, and proton-pump inhibitor use. Patients with gastrointestinal tumors were less prone to CDI. Conversely, breast cancer patients have a greater predisposition to CDI. Antibiotic treatment was found to be associated with an increasing risk for CDI in breast cancer patients [95].

In the second study of 277 cancer patients with diarrhea 41 (14.8 %) were *C. difficile* toxin-positive. Multivariate analysis showed that chemotherapy (OR, 8.308; 95 % CI, 1.997–34.572; $P = 0.004$) and a positive result of fecal occult blood test (OR, 8.475; 95 % CI, 1.463–49.109; $P = 0.017$) were independent risk factors for acquisition of CDI among cancer patients [96].

Patients with HIV/AIDS are at a high risk of being infected with *C. difficile* too. This relationship is stronger in those with low absolute CD4 T cell counts or who meet clinical criteria for an AIDS diagnosis [97].

The increased risk may be partially attributed to frequent hospitalization, exposure to antibiotics and antibiotic prophylaxis for opportunistic infections, but HIV related alterations in fecal microbiota, gut mucosal integrity, and humoral and cell mediated immunity may be also likely to play a role [98].

Community-acquired *C. difficile* infection (CA-CDI)

Community-acquired CDI (CA-CDI) has been demonstrated in populations previously thought to be at low-risk, including younger patients not previously exposed to antibiotics [99]. Suggested risk factors include increasing outpatient antibiotic prescriptions, greater use of acid-suppression medications, an increase in the proportion of asymptomatic carriers in the community and novel risk factors like food and water contamination [100]. A sub-group analysis of a population-based epidemiological study of CDI in Olmsted County, Minnesota from 1991–2005 was published in 2012 [101]. Of 157 CA-CDI cases, the median age was 50 years and 75.3 % were female. Among CA-CDI cases, 40 % required hospitalization, 20 % had severe and 4.4 % had severe-complicated infection, 20 % had treatment failure and 28 % had recurrent CDI.

Recently a systematic review and meta-analysis investigated the association between commonly prescribed medications and comorbidities with CA-CDI [41]. Twelve publications ($n = 56{,}776$ patients) met inclusion criteria. Antimicrobial (odds ratio, 6.18; 95 % CI 3.80–10.04) and corticosteroid (1.81; 1.15–2.84) exposure were associated with increased risk of CA-CDI. Among the comorbidities, inflammatory bowel disease (odds ratio, 3.72; 95 % CI, 1.52–9.12), renal failure (2.64; 1.23–5.68), hematologic cancer (1.75; 1.02–5.68), and diabetes mellitus (1.15; 1.05–1.27) were associated with CA-CDI. By location, antimicrobial exposure was associated with a higher risk of CA-CDI in the United States, whereas proton-pump inhibitor exposure was associated with a higher risk in Europe. By life stages, the risk of CA-CDI associated with antimicrobial exposure greatly increased in adults older than 65 years.

Risk factors for recurrent CDI

In a meta-analysis by Garey et al. [102] found that continued use of non-*C. difficile* antibiotics after diagnosis of CDI (OR: 4.23; 95 % CI: 2.10–8.55; $P < 0.001$), concomitant receipt of antacid medications (OR: 2.15; 95 % CI: 1.13–4.08; $P = 0.019$), and older age (OR: 1.62; 95 % CI: 1.11–2.36; $P = 0.0012$) were significantly associated

with an increased risk of recurrent CDI. Other factors identified in individual studies include age, hospital exposure, comorbid conditions, severe underlying illness, poor quality of life scores, initial disease severity and previous recurrent CDI [103, 104].

A recent systematic review and meta-analysis [105] was published to evaluate current evidence on the risk factors for recurrent CDI. A total of 33 studies (n = 18,530) met the inclusion criteria. The most frequent independent risk factors associated with recurrent CDI were age ≥65 years (risk ratio [RR], 1.63; 95 % confidence interval [CI], 1.24–2.14; P = .0005), additional antibiotics during follow-up (RR, 1.76; 95 % CI, 1.52–2.05; P < .001), use of proton-pump inhibitors (PPIs) (RR, 1.58; 95 % CI, 1.13–2.21; P = .008), and renal insufficiency (RR, 1.59; 95 % CI, 1.14–2.23; P = .007). The risk was also greater in patients previously on fluoroquinolones (RR, 1.42; 95 % CI, 1.28–1.57; P < .001).

Clinical manifestations

The spectrum of symptomatic CDI ranges from mild diarrhea to severe disease or fulminant colitis and as many as 30 % of patients may develop recurrent CDI [106, 107].

Though diarrhea is the hallmark symptom of CDI it may not be present initially, possibly due to colonic dysmotility either from previous underlying conditions or possibly from the disease process itself [108].

This is especially important in surgical patients who may have a concomitant ileus. Therefore, in surgical patients it is important to have a high index of suspicion for the development of CDI.

Mild-moderate CDI

Diarrhea may be accompanied by mild abdominal pain and cramps and if prolonged may result in altered electrolyte balance and dehydration. When this occurs in patients with severe comorbidity, particularly after surgery, non-severe CDI may increase morbidity significantly [109].

Severe CDI

Severe CDI is associated with increased abdominal cramping and pain and constitutional features such as fever, leukocytosis, and hypoalbuminemia. The absence of diarrhoea in these patients may signal a progression to fulminant infection [110]. Though a wide variety of severity predictors for severe CDI has been described [111–115] international consensus for the definition of severe CDI is lacking [6, 7, 116].

One systematic review identifying risk factors for adverse outcomes of CDI was published by Abou Chakra et al. in 2012 [114]. Except for leukocytosis, albumin and age, there was much heterogeneity in the data and most studies were limited by small sample sizes.

To investigate the prognostic value of fever, leukocytosis, and renal failure, Bauer et al. [113] in 2012 analyzed the database of two randomized controlled trials, which contained information for 1105 patients with CDI. They found that both leucocytosis and renal failure were useful predictors of a complicated course of CDI. Miller et al. [115] in 2013 subsequently published an analysis of the same two clinical therapeutic trials to validate a categorization system to stratify CDI patients into severe or mild-moderate groups. A combination of five simple and commonly available clinical and laboratory variables (ATLAS) measured at the time of CDI diagnosis were able to accurately predict treatment response to CDI therapy. The ATLAS criteria included: age, treatment with systemic antibiotics, leucocyte count, albumin and serum creatinine [115].

Any of the following may be predictors of severe CDI:

- WBC >15 × 10^9/L
- Acutely rising serum creatinine
- Temperature >38.5 °C
- Albumin <2.5 mg/dL

The progression to fulminant *C. difficile* colitis is relatively infrequent [109] (1–3 % of all CDI) although mortality in this group of patients remains high due to the development of toxic megacolon with colonic perforation, peritonitis and septic shock and subsequent organ dysfunction. Systemic symptoms may result from toxin-induced inflammatory mediators released locally in the colon [117–119]. Studies have demonstrated a significant rise in the number of cases of fulminant colitis associated with multiple organ failure and increased mortality in recent years associated with the hypervirulent 027 strain of *C. difficile* [120, 121]. Early diagnosis and treatment is therefore important in reducing the mortality associated with fulminant colitis. Patients who present with organ failure including increased serum lactate or vasopressor requirements, should be assessed immediately with regard to early operative intervention [121].

Recurrent CDI (RCDI)

Recurrence of symptoms after initial therapy for *C. difficile*, develops in 10–30 % of cases, and this often presents a clinical challenge. Patients may have several episodes of recurrence that may occur over a period of years [122–127]. Recurrence and reinfection are therefore difficult to distinguish by symptoms alone, but may be distinguished if the strain of *C. difficile* is typed.

RCDI may be either a consequence of germinating resident spores remaining in the colon after antibiotic treatment has stopped, or re-infection from an environmental source.

Even though consensus regarding factors associated with CDI recurrence is not universal learning algorithms

have been developed to predict CDI recurrence with good sensitivity [128].

Ultimately distinction between recurrence and reinfection can only be achieved if the strain of *C. difficile* is 'typed' using molectular epidemiology [129].

Wider consequences of CDI

Patients who develop CDI have increased hospital length-of-stay, higher medical care costs, more hospital re-admissions, and higher mortality [130–132].

These consequences are also found for surgical patients with CDI.

In the Zerey et al. analysis [8] epidemiologic data suggested that the infection was most prevalent after emergency operations and among patients having intestinal tract resections. Infection with *C. difficile* was an independent predictor of increased length of stay, which increased by 16.0 days (95 % CI 15.6, 16.4 days; $p < 0.0001$) in the presence of infection. Total charges increased by $77,483 (95 % CI $75,174, $79,793; $p < 0.0001$), and there was a 3.4-fold increase in the mortality rate (95 % CI 3.02, 3.77; $p < 0.0001$) compared with patients who did not acquire *C. difficile*.

In the Abdelsattar et al. study [11] three procedure groups had higher odds of postoperative CDI: lower-extremity amputations (adjusted odds ratio [aOR], 3.5; $P = .03$), gastric or esophageal operations (aOR, 2.1; $P = .04$), and bowel resection or repair (aOR, 2; $P = .04$). Postoperative CDI was independently associated with increased length of stay (mean, 13.7 days vs 4.5 days), emergency department presentations (18.9 vs 9.1 %) and readmissions (38.9 vs 7.2 %, all $P < .001$).

Data from Nationwide Inpatient Sample database in patients who underwent vascular surgery [79], showed that in 2011 patients who had experienced CDI had median length of stay 15 days (IQR 9, 25 days) compared with 8.3 days for matched patients without CDI, in-hospital mortality 9.1 % (compared to 5.0 %), and $13,471 extra cost per hospitalization. The estimated cost associated with CDI in vascular surgery in the United States was about $98 million in 2011. Data from the National Inpatient Sample examined just patients with lumbar surgery and found CDI increased length of stay by 8 days, hospital costs by 2-fold and increased inpatient mortality by 36-fold [133].

Higher mortality was also observed for liver transplant recipients (from 2000 to 2010) at Detroit hospital [134].

The ACS-NSQIP database from 2005 to 2010 was used by Lee et al. to study emergently performed open colectomies for a primary diagnosis of *C difficile* colitis in US [135]. The overall mortality was 33 % (111/335). Age 80 years or older, preoperative dialysis dependence, chronic obstructive pulmonary disease), and wound class III were associated to patients mortality. Thrombocytopenia (platelet count $< 150 \times 10^3/mm^3$), coagulopathy (International Normalized Ratio > 2.0), and renal insufficiency (blood urea nitrogen > 40 mg/dL) were associated with a higher mortality as well.

Recently a study was performed to quantify additional hospital stay attributable to CDI in four European countries, by analyzing nationwide hospital-episode data [5]. Patients in England had the longest additional hospital stay attributable to CDI at 16.09 days, followed by Germany at 15.47 days, Spain at 13.56 days, and The Netherlands at 12.58 days, derived using regression analysis. Propensity score matching indicated a higher attributable length of stay of 32.42 days in England, 15.31 days in Spain, and 18.64 days in The Netherlands. Outputs from this study consistently demonstrate that in European countries, for patients whose hospitalization is complicated by CDI, the infection causes a statistically significant increase in hospital length of stay.

Recommendations for the management of CDI

Diagnosis

1) Stool testing should only be performed on diarrhea stools from at-risk patients with clinically significant diarrhea (Recommendation 1 C).

2) For patients with ileus who may be unable to produce stool specimens, polymerase chain reaction testing of perirectal swabs may be an accurate and efficient method to detect toxigenic *C. difficile* in patients with symptoms of CDI (Recommendation 2B).

Prompt and precise diagnosis is important for the effective management of CDI.

Early identification of CDI allows early treatment and can potentially improve outcomes. Rapid isolation of infected patients is important in controlling the transmission of *C. difficile* [136].

The diagnosis of CDI is based on the presence of a clinical picture compatible with CDI and microbiological evidence of free toxin and/or the demonstration of toxigenic *C. difficile* in a diarrhea stool sample [136]. Clinical features include: diarrhea (defined as by passage of 3 or more unformed stools in 24 h), abdominal pain and cramps, abdominal distension, ileus (signs of severely disturbed bowel function) and toxic megacolon.

Since *C. difficile* can colonize the intestinal tract of healthy individuals, diagnostic testing for CDI should be performed only on diarrhea stools from symptomatic patients. Testing of formed stool can result in false positive tests, which may result in unnecessary antibiotic therapy.

One limitation of the reliance on stool specimens are the patients with suspected severe CDI complicated by ileus as these patients may be unable to produce specimens for testing. For these patients testing of perirectal swabs may be an accurate and efficient method to detect

toxigenic *C. difficile*. In 2012 Kundrapu et al. [137] described the results of a prospective study of 139 patients being tested for *Clostridium difficile* infection by polymerase chain reaction. The sensitivity, specificity, positive predictive value, and negative predictive value of testing perirectal swabs were 95.7, 100, 100, and 99.1 %, respectively. The authors concluded that for selected patients, perirectal swabs provided an acceptable alternative to stool specimen analysis. Clinical context such as a history of recent antibiotic administration and/or residence in hospital are useful in selecting patients for testing. Other signs such as fever, abdominal pain, leukocytosis, in combination with other laboratory tests (e.g. creatinine and serum lactate) are useful for defining severity of infection.

3) Nucleic acid amplification tests (NAAT) such as polymerase chain reaction (PCR) for *C. difficile* toxin genes appear to be sensitive and specific and may be used as a standard diagnostic test for CDI. NAAT as single-step algorithm can increase detection of asymptomatic colonization therefore it should only be performed in patients with clinical suspicion for CDI (Recommendation 1 B).

4) Glutamate dehydrogenase (GDH) screening tests for *C. difficile* are sensitive but do not differentiate between toxigenic and non-toxigenic strains. They may be used in association with toxin A and B EIA testing. Algorithms involving screening with an EIA for GDH followed by a toxin assay may be used (Recommendation 1 B).

5) Enzyme immunoassay (EIA) for toxin A/B is fast and inexpensive and has high specificity but it is not recommended alone due to its relatively low sensitivity. (Recommendation 1 B).

6) *Clostridium difficile* culture is relatively slow but sensitive. It is rarely performed today as a routine diagnostic test. *C. difficile* culture is recommended for subsequent epidemiological typing and characterization of strains (Recommendation 1 C).

7) Repeat testing within 7 days should not be performed on patients who previously tested negative unless the clinical picture has changed significantly (Recommendation 1 C).

The best standard laboratory test for diagnosis of CDI has not been clearly established [138]. In the past, toxigenic culture (TC) was accepted by many microbiologists as the method of choice for diagnosis of CDI. The procedure includes stool culture for *C. difficile* on a selective differential medium (cycloserine, cefoxitin, fructose agar or CCFA) and an assay to test the colonies for the ability to produce toxins. Despite the fact that TC is considered a gold standard method, there are significant issues including slow turnaround time and it's inability to detect the presence of toxins in stool. This may also lead to false positive results given up to 7 % of asymptomatic hospitalized patients may be colonized with toxigenic *C. difficile* [139].

However, TC can still be used as a confirmatory test in symptomatic patients with toxin positive/GDH assay(s)-negative stool samples. *C. difficile* culture is also necessary for subsequent epidemiological typing and characterization of strains.

The EIA for toxin A/B has been adopted by most clinical laboratories because it is fast, convenient and inexpensive [140]. However, studies have shown that sensitivity can be low. Toxin A + B EIA tests have a described sensitivity of 32–98 % and a specificity of 84–100 % [141].

Glutamate dehydrogenase (GDH) is an enzyme produced by *C. difficile* in relatively large amounts compared with toxins A and B [142, 143]. A positive GDH assay only documents the presence of *C. difficile* but it does not discriminate between toxigenic and non-toxigenic strains (about 20 % of the *C. difficile* population). Therefore, a second test for toxin production is necessary for confirmation. GDH screening tests for *C. difficile* used in association to toxin A + B EIA testing gives an accurate test result quickly [140, 141] even if the sensitivity of such strategy is lower than nucleic acid amplification tests (NAATs).

NAATs such as PCR for CD toxin genes have a high sensitivity and specificity, but not all laboratories routinely perform this assay [143]. A current topic of debate is whether a stool sample that was positive by a molecular assay needs to be tested with a confirmatory toxin assay [144] given it can also identify toxigenic *C difficile* in asymptomatic patients. This underscores the importance of only testing patients with symptoms. There is no evidence suggesting that surgical patients should be diagnosed any differently than general medical patients.

8) Immunocompromised patients (including patients in chemotherapy, chronic corticosteroid therapy, or immunosuppressive agents, and post-transplant patients) should be always tested for CDI if they have a diarrheal illness (Recommendation 1 C).

It has already been highlighted that immunocompromised patients including those on glucocorticoids, or chemotherapy and post-transplant patients are at increased risk for CDI.

9) CT imaging is suggested for suspected severe-complicated *C. difficile* colitis, however its sensitivity is not satisfactory for screening purposes (Recommendation 2 B).

CT has been studied as an imaging modality for diagnosing *C. difficile* colitis [145–148]. Typical CT findings of CDC include colonic wall thickening, dilation, pericolonic stranding, "accordion sign" (high-attenuation oral contrast in the colonic lumen alternating with low-attenuation inflamed mucosa), "double-halo sign, target

sign" (intravenous contrast displaying varying degrees of attenuation caused by submucosal inflammation and hyperemia), and ascites [149]. However, the most common finding, colonic wall thickening is non-specific and can be found in other forms of colitis, although it may be more pronounced in that caused by *C. difficile*.

In the Kirkpatrick et al. study [150], CT diagnosis of CDC was made with a sensitivity of 52 %, a specificity of 93 %, and positive and negative predictive valued 88 %, and 67 % respectively. Sensitivity would have been increased to 70 % with no change in specificity if a colon wall thickness of greater than 4 mm had been used, in conjunction with the presence of colon wall nodularity, accordion sign, peri-colonic stranding, or otherwise unexplained ascites.

10) Ultrasound may be useful in critically ill patients suspected to have pseudomembranous colitis who cannot be transported for CT scan (Recommendation 2 C).

Point-of-care ultrasound may be useful in diagnosing and managing critically ill patients who cannot be moved to the radiology department [151].

Ultrasound findings of pseudomembranous colitis in severe cases include a thickened colonic wall with heterogeneous echogenity and narrowing of the colonic lumen [152]. Pseudomembranes can also be visualised as hyperechoic lines covering the mucosa [152–155].

In the early stages of pseudomembranous colitis, the texture of the colonic wall is preserved. The hypoechoic edematous mucosa and muscularis propria may be thickened with the echogenic submucosa sandwiched between them. The presence of submucosal gaps may indicate extension of tissue damage into deeper structures. Intraperitoneal free fluid is seen in more than 70 % of cases [153–155].

11) Flexible sigmoidoscopy may be helpful for the diagnosis of *C. difficile* colitis (CDC) when there is a high level of clinical suspicion for *C. difficile* despite repeated negative laboratory assays (Recommendation 2 B).

Endoscopy should be used sparingly to confirm the diagnosis of *C. difficile* colitis since the diagnosis can be usually made by laboratory tests, clinical findings and imaging. Moreover colonoscopy may be hazardous in the setting of fulminant colitis where there may be increased risk of perforation [156].

A study by Johal et al. [157] described the use of flexible sigmoidoscopy as a tool for the diagnosis of *C. difficile* colitis when stool assays were negative. Of 136 patients with *C. difficile* associated diarrhea (CDAD) 56 patients had pseudomembranous colitis at sigmoidoscopy. The stool *C. difficile* cytotoxin test was negative in 29 (52 %) but toxigenic *C. difficile* was isolated from all of nine stool samples cultured. Of patients with pseudomembranous colitis, 30.4 % relapsed over the subsequent 57.7 days. The authors concluded that sigmoidoscopy should be considered in all hospitalised patients with diarrhea in whom the stool tests for *C. difficile* cytotoxin and enteric pathogens are negative.

Emergency colonoscopy or sigmoidoscopy may also reveal pseudomembranous colitis in patients too ill to wait for laboratory results.

Antimicrobial therapy

12) Unnecessary antimicrobial agent(s) and proton pump inhibitors should be discontinued if CDI is suspected (Recommendation 1 C).

13) Empirical therapy for CDI should be avoided unless there is a strong suspicion for CDI. If a patient has a strong suspicion for CDI, empirical therapy for CDI should be considered while awaiting test results (Recommendation 1 B).

In cases of suspected severe CDI, antimicrobial agent(s) should be discontinued, if possible [158].

A meta-analysis addressing factors associated with prolonged symptoms and severe disease due to *Clostridium difficile* showed that continued use of antimicrobials for infections other than CDI is significantly associated with an increased risk of CDI recurrence [159].

When antimicrobial therapy is indicated for symptomatic cases with a positive *C. difficile* toxin result, options include metronidazole, oral or intraluminal vancomycin and fidaxomicin [160–166].

14) Metronidazole is recommended for the treatment of mild-moderate disease (Recommendation 1 A).

Given at a dose of 500 mg orally 3 times a day for 10 days, metronidazole has been shown to be an inexpensive and effective treatment of non-severe CDI [167]. Metronidazole can also be administrated intravenously with or without intraluminal vancomycin in patients unable to take oral medication e.g. those with post-surgical ileus.

A Cochrane analysis published in 2011 [167] reviewed 15 studies on the antibiotic treatment for CDI in adults. In three randomized controlled trials comparing symptomatic cure between metronidazole and vancomycin, no statistically significant difference was found [167]. Symptomatic cure was achieved in 79 % of patients who received vancomycin compared with 71 % of patients who received metronidazole (three studies; 335 patients; RR 0.91; 95 % CI 0.81–1.03, p 0.14).

15) Oral vancomycin is recommended for treatment of patients with severe disease, or for patients with mild-moderate disease who do not respond to metronidazole. (Recommendation 1 A).

Vancomycin orally 125 mg four times daily for 10 days is considered superior to metronidazole in severe *C. difficile* disease [168–170]. This may reflect the superior

pharmacokinetic properties of vancomycin which is concentrated in the gut lumen. Doses of up to 500 mg have been used in some patients with severe CDI [7] although there is little evidence for this in the literature.

16) In patients in whom oral antibiotics cannot reach the colon, vancomycin may be administered by enema and metronidazole can be given intravenously (Recommendation 1 B).

Intravenous vancomycin has no effect on CDI since the antibiotic is not excreted into the colon. Vancomycin enema may be an effective therapy for patients who cannot tolerate the oral preparation or patients with ileus who have delayed passage of oral antibiotics from the stomach to the colon. Trans-stoma vancomycin may also be effective in surgical patients with Hartmann resection, ileostomy, or colon diversion. A single-hospital, retrospective chart review on 47 consecutive patients with *C. difficile* colitis treated with intra-colonic vancomycin (ICV) was published by Kim PK et al. in 2013 [171]. Thirty-three of 47 patients (70 %) with severe *C. difficile* colitis responded to adjunct ICV with complete resolution without surgery. Multivariable analysis suggested that failures to intra-colonic vancomycin enemas occurred in patients who were older and frail with albumin < 2.5 g/dl and early surgery should be considered for those patients. Early surgery should also be offered to those patients who are failing maximal medical therapy that include ICV enemas.

17) Fidaxomicin may be used to treat CDI, especially in the patients at higher risk for recurrence (e.g. elderly patients with severe underlying disease or those requiring receiving concomitant antibiotics) (Recommendation 1 A).

Fidaxomicin orally 200 mg twice daily for 10 days may be an alternative to vancomycin in some patients with CDI [172, 173].

Fidaxomicin was non-inferior to vancomycin for initial cure of CDI in two prospective trials [164, 165]. In a first double-blind, randomized, non-inferiority trial [164] 629 adults with acute symptoms of *C. difficile* infection and a positive result on a stool toxin test were enrolled and randomly assigned to receive fidaxomicin (200 mg twice daily) or vancomycin (125 mg four times daily) orally for 10 days. The rates of clinical cure with fidaxomicin were non-inferior to those with vancomycin in both the modified intention-to-treat analysis (88.2 % with fidaxomicin and 85.8 % with vancomycin) and the per-protocol analysis (92.1 % and 89.8 %, respectively). Significantly fewer patients in the fidaxomicin group than in the vancomycin group had a recurrence of the infection, in both the modified intention-to-treat analysis and the per-protocol analysis. In a second multi-centre, double-blind, randomized, non-inferiority trial [165] 535 patients, 16 years or older with acute, toxin-positive C difficile infection were randomly allocated (1:1) to receive oral fidaxomicin (200 mg every 12 h) or oral vancomycin (125 mg every 6 h) for 10 days. Non-inferiority was shown for both the modified intention-to-treat analysis (15.4 % vs. 25.3 %, $P = 0.005$) and the per-protocol analysis (13.3 % vs. 24.0 %, $P = 0.004$). Patients receiving concomitant antibiotics for other infections had a higher cure rate with fidaxomicin (46 [90 · 2 %] of 51) than with vancomycin (33 [73 · 3 %] of 45; $p = 0 \cdot 031$). Fidaxomicin may be useful for treating patients who are considered at high risk for recurrence (elderly patients with multiple comorbidities who are receiving concomitant antibiotics). However, it is important to note that there are no data available on the efficacy of Fidaxomicin in severe life-threatening disease.

The use of other antibiotics such as tigecycline [174, 175] fusidic acid, teicoplanin, rifamixin [167] and nitazoxanide [176], has been described in the literature, but they are not currently recommended for general use.

Surgical management

Patients with fulminant colitis (FC) who progress to systemic toxicity require surgical intervention.

To determine clinical predictors for the development of fulminant colitis in patients with CDI a 10-year retrospective review of FC patients who underwent colectomy was performed and compared with randomly selected age- and sex-matched non-fulminant CDI patients at a single institution study by Girotra in 2012 [177]. Predictive clinical and laboratory features included: old age (>70 years), prior CDI, profound leukocytosis (>18,000/mm^3), hemodynamic instability, use of antiperistaltic medications, and a clinical triad of increasing abdominal pain, distention and diarrhea.

18) Patients with severe CDI who progress to systemic toxicity should undergo early surgical consultation and evaluated for potential surgical intervention (Recommendation 1 C).

Patients with severe CDI who progress to systemic toxicity are likely to have serious comorbidities. Delaying surgery in this group leads to increased likelihood of adverse outcomes [178], although some reports show that a short period of medical optimization can improve outcomes before colectomy [179].

There are no reliable clinical and/or laboratory findings that can predict those patients who will respond to medical therapy and those who will subsequently need surgery [180].

Data comparing mortality rates between surgical and medical treatment for fulminant *C. difficile* colitis were published in a recent systematic review by Stewart et al. [181]. Five hundred and ten patients with FC were identified in six studies. Emergency colectomy for patients

with FC provided a survival advantage compared with continuing antibiotics. When all six studies numbering 510 patients were analysed, the pooled adjusted odds ratio of mortality comparing surgery with medical therapy, and weighted by the contribution of each study, was 0.70 (0.49–0.99) leading the authors to conclude that emergency colectomy has a therapeutic role in treating complicated *C. difficile* colitis.

Patients presenting with organ failure (acute renal failure, mental status changes, or cardiopulmonary compromise) also need prompt intervention.

The timing of surgical intervention is the key for survival of patients with FC [182–185].

Seder et al. [186] described 6,841 patients with CDI and showed a decreased mortality associated with surgery performed before the need for vasopressor requirement, especially in the patients <65 years old. Hall et al. [184] reviewed 3,237 consecutive cases of CDI and showed an increased mortality rate when surgical exploration was performed after intubation or the development of respiratory failure and the use of vasopressors.

Recently a risk scoring system (RSS) for daily clinical practice was designed by van der Wilden et al. [187]. Age greater than 70 years was assigned 2 points, white blood cell count equal to or greater than 20,000 x 10^9/L or equal to or less than 2,000 x 10^9/L was assigned 1 point, cardiorespiratory failure was assigned 7 points, and diffuse abdominal tenderness on physical examination was assigned 6 points. A value of 6 points was determined to be the threshold for reliably dividing low-risk (<6) from high-risk (≥6) patients. Only patients with cardiorespiratory failure or diffuse abdominal tenderness were high risk.

Ferrada et al. [188] reviewed the existing literature on the treatment of CDI and published practice management guidelines (PMG) for the Eastern Association for the Surgery of Trauma (EAST). The authors strongly recommended, that adult patients with CDI undergo early surgery before developing shock and the need for vasopressors. Although timing remains controversial Ferrada et al. found that it was between 3 days and 5 days after diagnosis in patients who are worsening or not clinically improving [188].

Many factors have been described as predictors of mortality in patients who undergo emergency intervention.

Sailhamer et al. [189] reviewed the records of 4796 inpatients diagnosed with *C difficile* colitis. In 199 patients (4.1 %) with fulminant *C difficile* colitis the in-hospital mortality rate was 34.7 %. Independent predictors of mortality included age 70 years or older, severe leukocytosis or leukopenia (white blood cell count, >or = 35 000 × 10^9/L or <4000 × 10^9/L) or bandemia (neutrophil bands, >or = 10 %), and cardiorespiratory failure (intubation or vasopressors). Survival rates were higher in patients who were cared for by surgical vs nonsurgical departments.

The ACS-NSQIP database from 2005 to 2010 was used by Lee et al. to study emergency open colectomies performed for *C difficile* colitis in the USA [190]. The overall mortality was 33 % (111/335). Age 80 years or older, preoperative dialysis dependence, chronic obstructive pulmonary disease, and wound class III were associated high patient mortality. Thrombocytopenia (platelet count < 150 × 10^3/mm^3), coagulopathy (International Normalized Ratio > 2.0), and renal insufficiency (blood urea nitrogen > 40 mg/dL) were also associated with a higher mortality.

A systematic review and meta-analysis of outcomes following emergency surgery for *C. difficile* colitis was published by Banghu et al. [191]. Thirty-one studies were included, which presented data for 1433 patients undergoing emergency surgery for *C. difficile* colitis. It concluded that the strongest predictors for postoperative death were those relating to preoperative physiological status: preoperative intubation, acute renal failure, multiple organ failure and shock requiring vasopressors.

19) Resection of the entire colon should be considered to treat patients with fulminant colitis (FC) (Recommendation 1 B).

20) Diverting loop ileostomy with colonic lavage may be a useful alternative to resection of entire colon (Recommendation 2 C).

21) Patients with FC should be treated with high dose oral or by enema vancomycin (500 mg, 6 hourly) in combination with intravenous metronidazole (500 mg, 8 hourly). (Recommendation 1 C).

In the Bhangu et al. meta-analysis [191] the most commonly performed operation for treatment of FC was total colectomy with end ileostomy (89 %, 1247/1401). When total colectomy with end ileostomy was not performed, reoperation to resect further bowel was needed in 15.9 % (20/ 126). In the recent meta-analysis by Ferrada et al. [188], 17 studies comparing colectomy versus other procedures or no surgery as treatment for CDI were analyzed. The authors recommended that total colectomy (vs. partial colectomy or other surgery) is the procedure of choice for patients with *C. difficile* colitis.

To evaluate the role of emergency colectomy in patients with FC, and to identify subgroups of patients that may benefit Lemontagne et al. [192] published a retrospective observational cohort study of 165 cases of FC that required ICU admission or prolongation of ICU stay in 2 tertiary care hospitals of Quebec, Canada. Eighty-seven (53 %) cases died within 30 days of ICU admission, of which almost half (38 of 87, 44 %) died within 48 h of ICU admission. The independent predictors of 30-day mortality were leukocytosis > or = 50 × 10(9)/L, lactate > or = 5 mmol/L, age > or = 75 years, immunosuppression and

shock requiring vasopressors. Patients who underwent an emergency colectomy were less likely to die than those treated medically. Colectomy was more beneficial in patients aged 65 years or more, in immunocompetent patients and in patients with a leukocytosis > or = 20 × 10^9/L or lactate between 2.2 and 4.9 mmol/L.

Diverting loop ileostomy with antegrade colonic lavage may be a colon preserving alternative to total colectomy [193, 194]. To evaluate whether a minimally invasive, colon-preserving approach may be an alternative to subtotal colectomy in the treatment of FC, a historical control group study was performed at the University of Pittsburgh Medical Center or and the Veterans' Administration Healthcare System, Pittsburgh between June 2009 and January 2011 [193]. All patients with FC were managed by a loop ileostomy, intraoperative colonic lavage with warmed polyethylene glycol 3350/electrolyte solution via the ileostomy and postoperative antegrade instillation of vancomycin flushes via the ileostomy. Forty-two patients were treated during this time period. There was no significant difference in age, sex, pharmacologic immunosuppression, and Acute Physiology and Chronic Health Evaluation-II scores between the studied cohort and historical controls. The operation was accomplished laparoscopically in 35 patients (83 %). This treatment strategy resulted in reduced mortality compared to their historical population. Preservation of the colon was achieved in 39 of 42 patients (93 %). Of note, in this study vancomycin antegrade enemas were continued via the ileostomy every 6 h for 10 days after ileostomy formation and this likely augmented the effect of the defuctioning surgery.

Supportive care

22) Supportive measures, including intravenous fluid resuscitation and electrolyte replacement, should be provided to all patients with severe *C. difficile* infection (Recommendation 1 C).

Diarrhea results in significant volume depletion and electrolyte abnormalities, and fluid and electrolyte imbalance should be promptly corrected [119, 120].

23) Early detection of shock and aggressive management of underlying organ dysfunction are essential for optimum outcomes in patients with fulminant colitis (Recommendation 1 C).

Early detection and prompt aggressive treatment of the underlying organ dysfunction is an essential component of improving outcome of critical ill patients [120].

Severe CDI may present with a fulminant course and may be associated with great morbidity and high mortality. Physiologic support including close invasive monitoring in an intensive care unit setting and aggressive resuscitation are often necessary in fulminant colitis.

Recurrent *C. difficile* infection (RCDI)

Recurrence is diagnosed when CDI recurs <8 weeks after the onset of a previous episode, provided the symptoms from the previous episode resolved after completion of initial treatment and other causes have been excluded. Symptomatic recurrent *C difficile* infection (RCDI) occurs in approximately 20 % of patients and is challenging to treat [195]. Patients with recurrence of CDI should therefore be treated by clinicians who have experience in treating the infection.

24) Agents that may be used to treat the first recurrence of CDI include metronidazole, for non-severe RCDI, and vancomycin for severe RCDI. (Recommendation 1 B).

25) Fidaxomicin may be used as an alternative agent (Recommendation 1 B).

A systematic review on the treatment of RCDI was recently published [196]. Metronidazole and vancomycin have a good evidence base for use in RCDI but heterogeneity in treatment duration and treatment doses between the studies precluded robust conclusions. Fidaxomicin may also have a role in the treatment of first recurrence. Fidaxomicine was superior to vancomycin in terms of recurrences, with significantly less recurrence at 28 days. This was confirmed in some subgroup analysis [197].

26) In subsequent recurrence of CDI (2nd or later) oral vancomycin or fidaxomicin is recommended (Recommendation 1 B).

Vancomycin and fidaxomicin are equally effective in resolving CDI symptoms but fidaxomicin has been shown to be associated with a lower likelihood of CDI recurrence after a first recurrence [164, 165, 197]. However, there are no prospective randomized controlled trials investigating the efficacy of fidaxomicin in patients with multiple recurrences of CDI. Vancomycin is often administered using a prolonged tapered and/or pulsed regimen which may be more effective than a standard 10 to 14 day course although no RCTs have been reported [198].

Probiotics

27) Probiotics may be considered as an adjunctive treatment to antibiotics for immunocompetent patients with RCDI (Recommendation 2 B).

Little evidence exists to support the use of probiotics in the first episode of CDI [116]. Two randomized controlled trials showed some effectiveness for *Saccharomyces boulardii* CNCM I-745 in recurrent CDI. The first demonstrated a lower relapse rate compared with a placebo control group (35 vs 65 % in the placebo group) [199] and the second found that the combination of *S. boulardii* (1 g/d) with high dose vancomycin (2 g/d) was more effective than high dose vancomycin and placebo (17 vs 50 % recurrence rate) [200]. Other studies with

Lactobacillus strains (*L. rhamnosus* GG or *L. plantarum* 299v) were stopped prematurely due to enrollment problems [201]. Probiotics should not be administered to patients at risk of bacteraemia or fungaemia [116].

There is limited evidence to support the use of probiotics for the primary prevention of CDI from developing. A meta-analysis of 11 studies was in published 2012 [202]. Two studies showed significantly lower rates of CDI among the probiotic recipients. A meta-analysis of three studies that used the probiotic combination *Lactobacillus acidophilus CL1285* and *Lactobacillus casei LBC80R* and a combined analysis of those studies with four studies that used *Saccharomyces boulardii*, showed lower CDI rates in recipients of probiotics compared with recipients of placebo (risk ratio = 0.39; 95 % confidence interval 0.19–0.79. However, given the potential risk of bloodstream infection with these organisms further studies are warrented before their use can be recommended routinely.

Faecal microbiota transplantation

28) Intestinal or faecal microbiota transplantation (IMT or FMT) may be an effective option for the treatment of RCDI (Recommendation 1 B).

Intestinal or faecal microbiota transplantation (IMT or FMT) has been considered as an alternative therapy to treat RCDI [203–208]. It involves infusing intestinal microorganisms (in a suspension of healthy donor stool) into the intestine of patients to restore the intestinal microbiota.

The rationale of FMT is that disruption of the normal balance of colonic flora allows *C. difficile* strains to grow and produce CDI. By reintroducing normal flora via donor faeces, the imbalance may be corrected, and normal bowel function re-established [203].

FMT has not been widely adopted as a therapeutic tool probably due to concerns regarding safety and acceptability [204].

A systematic literature review of IMT treatment for RCDI and pseudomembranous colitis was published in 2011 by Gough et al. [205]. In 317 patients treated across 27 case series and reports, IMT was highly effective, showing disease resolution in 92 % of cases. In those studies, 35 % of patients received IMT via enema, with a response rate of 95; 23 % patients received IMT via naso-jejunal tube by gastroscope, with a response rate of 76; and 19 % via colonoscopy, with a response rate of 89 %. Effectiveness varied by route of instillation, relationship to stool donor, volume of IMT given, and treatment before infusion.

Recently a systematic review was published by Cammarota et al. [206]. Twenty full-text case series, 15 case reports, and 1 randomized controlled study were included for the final analysis. Almost all patients treated with donors' fecal infusion experienced recurrent episodes of CD-associated diarrhea despite standard antibiotic treatment. Of a total of 536 patients treated, 467 (87 %) experienced resolution of diarrhea. Diarrhea resolution rates varied according to the site of infusion: 81 % in the stomach; 86 % in the duodenum/jejunum; 93 % in the cecum/ascending colon; and 84 % in the distal colon. No severe adverse events were reported with the procedure.

In a recently published randomized clinical trial by van Nood et al. [208] patients with RCDI were randomised to three groups; 1) vancomycin regime only; 2) vancomycin with duodenal infused FMT and 3) vancomycin and bowel lavage. In the FMT treated group an 81% reduction in diarrhoea was observed. The FMT group were observed to have normalization of their intestinal bacterial composition which was similar to that of the donor. Although, this trial has shown exciting results, these need to be interpreted with caution as the trial included only small number of patients, was not blinded, and was aborted early due to profound differences in the groups. It has also been criticised for potentially having several potential biases.

FMT may be administered via enemas or as a slurry given via a nasogastric tube. In the fall of 2014, Youngster et al. [209] reported their experience on utilizing frozen FMT capsules in 20 patients who had RCDI. Fourteen patients (70 %) had resolution of diarrhea after the first treatment, and an additional 4 patients responded after a second treatment, for a clinical resolution rate of 90 %.

29) FMT may be effective in immunocompromised patients and patients who have had solid organ transplants (Recommendation 2 B).

Patients who are immunocompromised are at increased risk of CDI. During the last two years the first data on FMT in immunocompromised patients began to appear in the medical literature [210].

A multicenter retrospective series on the use of FMT in immunocompromised (IC) patients with CDI that was recurrent, refractory, or severe was published in 2014 [211]. Reasons for IC included: HIV/AIDS (3), solid organ transplant (19), oncologic condition (7), immunosuppressive therapy for inflammatory bowel disease (IBD; 36), and other medical conditions/medications (15).

This series demonstrated the effective use of FMT for CDI in IC patients with few serious adverse events or related adverse events.

Intravenous immunoglobulin (IVIG)

30) IVIG should only be used as adjunct therapy in patients with multiple recurrent or fulminant CDI until results from large, randomized controlled trials are available (Recommendation 2 C).

IVIG treatment has been proposed based on the evidence that the level of immune response to *C. difficile* colonization is a major determinant of magnitude and duration of clinical manifestations. Passive immunization with IVIG has been reported to be successful in several small series. A review by Abourgergi [212] of fifteen small, mostly retrospective and non-randomized studies documented success with IVIG in the treatment of protracted, recurrent, or severe CDI. The authors concluded IVIG should only be used as adjunct therapy until results from large, randomized controlled trials are available.

Monoclonal antibodies

31) Infusion with monoclonal antibodies may be of use to prevent recurrences of CDI, particularly in patients with CDI due to the 027 epidemic strain (Recommendation 2 C).

In a phase II clinical trial [213], the use of monoclonal antibodies to toxins A and B as an adjunct to antibiotics was shown to decrease recurrence rates in patients with CDI compared with placebo (7 vs. 25 % respectively; 95 % confidence interval, 7 to 29; $P < 0.001$). The recurrence rates among patients with the epidemic BI/NAP1/027 strain were 8 % for the antibody group compared with 32 % for placebo ($P = 0.06$); among patients with more than one previous episode of CDI, recurrence rates were 7 and 38 %, respectively ($P = 0.006$). The authors concluded that the addition of monoclonal antibodies against *C. difficile* toxins to antibiotic agents significantly reduced the recurrence of *C. difficile* infection. The findings of this study require confirmation before firm recommendations can be made.

Enteral nutrition in CDI

32) Tube feeding patients should be clinically assessed due to their risk for developing CDI (Recommendation 2 C).

It is widely accepted that enteral nutrition (EN) maintains gut mucosal integrity which leads to decreased intestinal permeability, decreased infections, and an improved immunological status. EN during episodes of diarrhea may be well tolerated and may improve enterocyte healing and maintenance of enzyme activity [214, 215]. EN, however, has also been associated with increased risk of CDI [216]. Bliss, et al. evaluated 76 tube-fed and non tube-fed hospital patients for the development of CDI [217]. Patients were controlled for age, severity of illness and duration of hospitalization. Patients who were tube-fed were statistically more likely to develop *C difficile* associated diarrhea (20 versus 8 % $p = 0.03$). One of the reasons may be prolonged use of elemental diets. It is known that critically ill patients tolerate feeding well if the feed is given in elemental form and delivered beyond the stomach into the jejunum because it is totally absorbed within the upper small intestine [218]. Elemental diets are completely absorbed within the small intestine and therefore deprive the colonic microbiota of their source of nutrition, such as dietary fiber, fructose oligosaccharides, and resistant starch [219]. The resultant suppression of colonic fermentation may therefore lead to the disruption of the normal gut flora and the creation of a "permissive" environment for *C. difficile* colonization and subsequent infection. In feeding tube patients the conversion of elemental diet feeding to a diet containing adequate indigestible carbohydrate after the first week of critical illness may, in theory, be useful.

Recently, Puri et al. [220] reported that daily concomitant treatment with 4 g cholestyramine in patients receiving long-term intravenous ceftriaxone (2 to 4 g ceftriaxone daily, for an average of >10 weeks) was associated with CDI in only three out of 46 patients (6.5 %) compared with 23.1 % of those receiving ceftriaxone alone [221]. Cholestyramine (or colestyramine) is a hydrophilic, water insoluble, non-digestible basic anion-exchange resin which can bind luminal TcdA and TcdB.

Studies have also investigated the possible value of exogenous Phosphatidylcholene (PC) administration for reinforcement of the mucus layer [222, 223]. Mucus or "exogenous" mucus in the form of PC may have a synergistic role with secretory IgA as a barrier against *C. difficile* toxin A though additional studies are needed to demonstrate its clinical benefit before recommendations can be made [222, 223].

Anti-motility agents

33) The use of anti-peristaltic agents for the treatment of CDI should be discouraged. If anti-peristaltic, if used in isolation agents, are used to control persistent symptoms in patients with CDI they must always be accompanied by medical therapy (Recommendation 2 C).

A review of the literature regarding anti-motility treatment of CDI found 55 patients with CDI who were exposed to anti-motility agents [224].

Nine patients (16 %) died, and 27 patients (49 %) had unknown outcomes. Seventeen patients (31 %) with CDI developed colonic dilation; 5 of these patients with severe CDI died. However, all patients who experienced complications or died were given anti-motility agents alone initially, without an appropriate antibiotic and 23 patients who received metronidazole or vancomycin co-administered with the anti-motility agent experienced no complications. Further study of the role of anti-motility agents in providing symptomatic relief and reducing environmental contamination with infectious stool may be warranted though, until there is clear evidence of benefit, their use in patients with CDI should be avoided [116].

Prevention

34) Proper antimicrobial stewardship in selecting an appropriate antibiotic and optimizing its dose and duration to cure an infection may prevent the emergence of *C. difficile* (Recommendation 1 B).

Despite vigorous infection control measures until recently, CDI was causing an increasing problem in healthcare facilities worldwide. As CDI is thought to follow disruption to the normal bacterial flora of the colon occurring as a consequence of antibiotic use [225], it is logical that antibiotic stewardship programs may be useful in preventing CDI [226]. Good antimicrobial stewardship involves ensuring appropriate antibiotic choice and optimizing antibiotic dose and duration to cure an infection while minimizing toxicity and conditions conducive to CDI. Recently, a systematic review [227] of interventions to improve antibiotic prescribing practices for hospital inpatients suggested that reducing excessive antibiotic prescribing can prevent hospital-acquired infections and that interventions to increase effective prescribing improve clinical outcome. It would appear that cephalosporin and quinolone antibiotics may be particularly high risk, in this context [116, 228].

35) Patients with suspected or proven CDI should be placed in contact (enteric) precautions (Recommendation 1 B).

Prompt identification of patients with symptomatic CDI is essential so that appropriate isolation precautions can be put into effect.

This is particularly important in reducing environmental contamination as spores can survive for months in the environment [229], despite regular use of environmental cleaning agents.

Contact (enteric) precautions patients with CDI should be maintained until the resolution of diarrhea, which is demonstrated by passage of formed stool for at least 48 h. Patients with known or suspected CDI should ideally be placed in a private room [116, 230] with ensuite hand washing and toilet facilities. If a private room is not available known CDI patients may be cohort nursed in the same area [231] though the theoretical risk of transfection with different strains exists.

This is supported by a retrospective cohort of 2859 patients by Chang et al. [232]. Patients who were roommates or neighbors of a patient with CDI were at risk of nosocomial acquisition of CDI (RR, 3.94; 95 % CI, 1.27–12.24).

36) Hand hygiene with soap and water is a cornerstone of the prevention of *C. difficile*. Hand hygiene, contact precautions and good cleaning and disinfection of the environment and patient care equipment, should be used by all health-care workers contacting any patient with known or suspected CDI (Recommendation 1 B).

Hand hygiene with soap and water and the use of contact precautions along with good cleaning and disinfection of the environment and patient equipment, should be used by all health-care workers contacting any patient with known or suspected CDI. Hand hygiene is a cornerstone of prevention of nosocomial infections, including *C. difficile.* Alcohol-based hand sanitizers are highly effective against non–spore-forming organisms, but they may not kill *C. difficile* spores or remove *C. difficile* from the hands [233, 234].

The most effective way to remove them from hands is through hand washing with soap and water.

For environmental cleaning, hypochlorite disinfection such as sodium hypochlorite solutions are suggested for regular use in patient areas where *C. difficile* transmission is ongoing [231].

Though disposable glove use during care of a patient with CDI may be effective in preventing the transmission of *C. difficile* [230], these must be removed at the point of use and hands thoroughly decontaminated afterwards through soap and water hand washing.

Abbreviations

CDI: *C. difficile* infection; RCDI: Recurrent *C. difficile* infection; FC: Fulminant colitis.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

MS wrote the manuscript. All Authors reviewed the manuscript and approved the final manuscript.

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Appendix 2.1

Supplement information for Section 2.2 – Asymptomatic *C. difficile* colonisation

Search strategy and selection criteria

References for this review were identified through searches of PubMed for articles published from January 1980 to February 2015 using search terms '*Clostridium difficile*' and 'colonization' or 'colonisation' or 'carriage'.

Search details: (((((("clostridium difficile"[MeSH Terms] AND "asymptomatic diseases"[MeSH Terms]) AND colonization[All Fields]) OR colonisation[All Fields]) OR carriage[All Fields]) AND "loattrfull text"[sb] AND English[lang])

The inclusion of studies was restricted to full-text articles written in English. Conference presentations and abstracts were excluded. Articles resulting from the search and relevant references cited in those articles were reviewed by LFK and JM. All the citation were initially screened by title and abstract, the full text version of the articles that met the inclusion criteria were then reviewed.

The search identified 11 489 publications. After screening the publications by title and abstract, 11 263 were excluded. Subsequently, full-text review of 226 publications was conducted, 125 met the eligibility criteria and were included in the review (Figure S1).

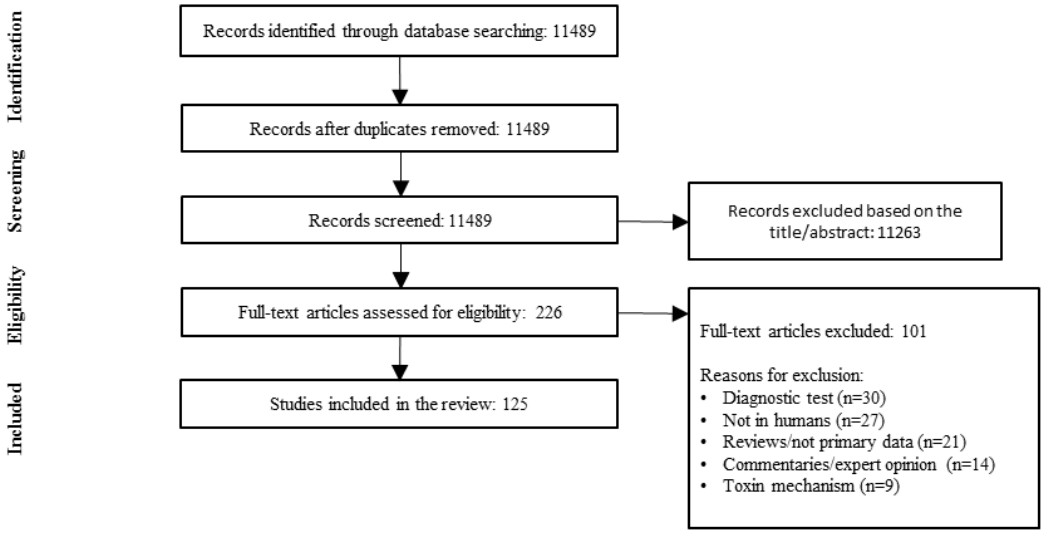


Figure S1.- PRISMA flow diagram.

Appendix 2.2

Supplement information for Section 2.3 – Risk factors for community-associated *C. difficile* infection

APPENDICES

Appendix 1.- Search strategies

PubMed

((((("Community-Acquired Infections"[MeSH Terms]) OR (Community OR Communities OR Residential OR Neighborhood OR Neighborhoods OR Neighbourhood OR Neighbourhoods)))

AND

("Clostridium"[Mesh] OR Clostridium))

AND

Difficile

Embase

('communicable disease'/exp OR community OR communities OR residential OR neighborhood OR neighborhoods OR neighbourhood OR neighbourhoods)

AND

'clostridium'/exp OR clostridium

AND

Difficile

CINAHL

(MH "Community-Acquired Infections+") OR Community OR Communities OR Residential OR Neighborhood OR Neighborhoods OR Neighbourhood OR Neighbourhoods

AND

(MH "Clostridium+") OR Clostridium

AND

Difficile

Cochrane CENTRAL

((((("Community-Acquired Infections"[MeSH Terms]) OR (Community OR Communities OR Residential OR Neighborhood OR Neighborhoods OR Neighbourhood OR Neighbourhoods)))

AND

("Clostridium"[Mesh] OR Clostridium))

AND

Difficile

Scopus

(TITLE-ABS-KEY(community OR communities OR residential OR neighborhood OR neighborhoods OR neighbourhood OR neighbourhoods)

AND

TITLE-ABS-KEY(clostridium)

AND

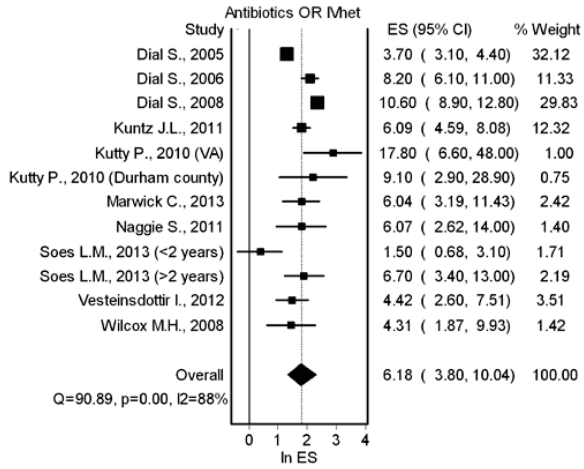
TITLE-ABS-KEY(difficile))

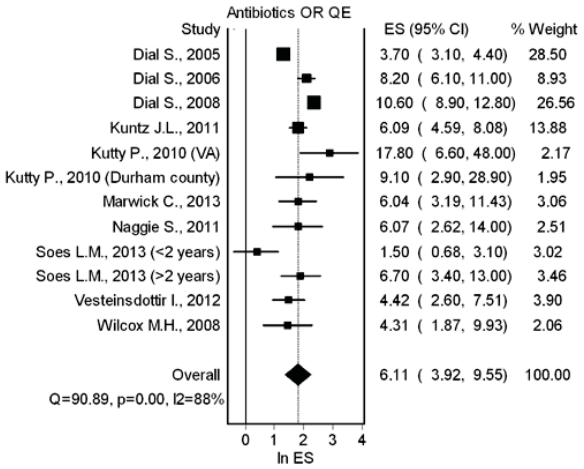
Appendix 2.- Data extraction tool

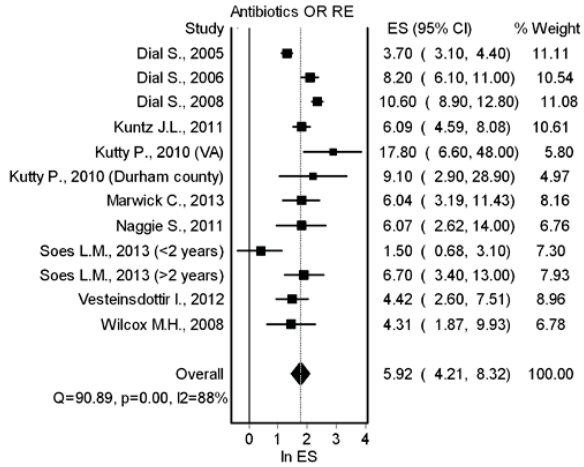
Select one medication exposure / comorbidity	Overall antimicrobials		Macrolides		Overall gastric supres.		NSAIDs		DM		Leukemia/lymphoma	
	Cephalosporins		Penicillins		H2RA		Corticosteroids		Diverticular disease		Peptic ulcer	
	Clindamycin		Tetracyclines		PPI		CHD		GERD		Renal failure	
	Fluoroquinolones		TMP-SMX		Aspiring		COPD		IBD		Solid cancer	

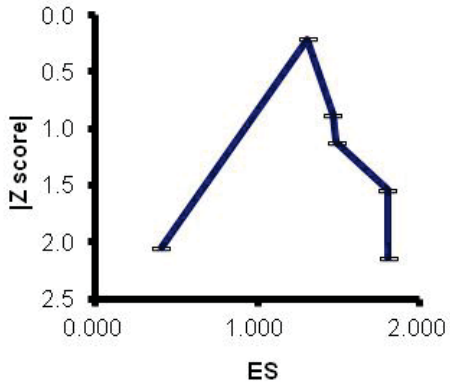
Study ID	Study characteristics								Demographic data					Exposed group characteristics		Control group characteristics	
Authors, year	Location / country	Sampling time frame	Follow-up (days)	Data source	Study design	Selection of cases / Inclusion criteria	Selection of controls	Definition of community acquired	Exposure (days)	Sample size	CDI Cases	Non CDI cases	Age (years), mean (SD)	Male, n(%)	Cases	Non-cases	Cases	Non-cases

Appendix 3.- Forest, Funnel and Doi plots

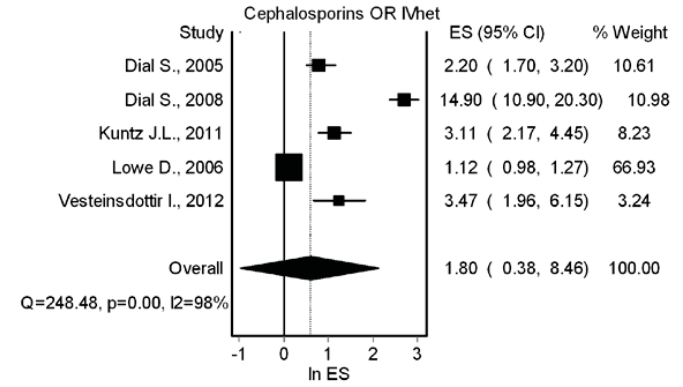


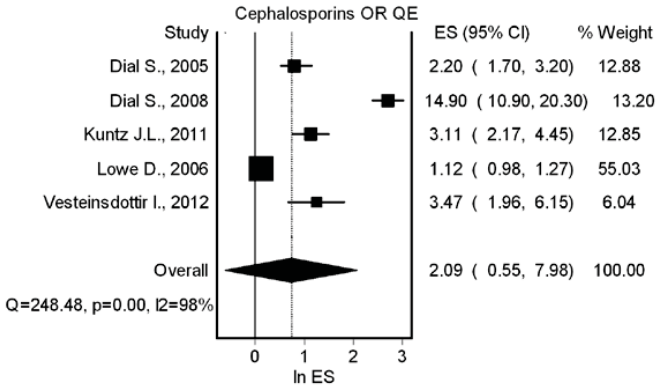


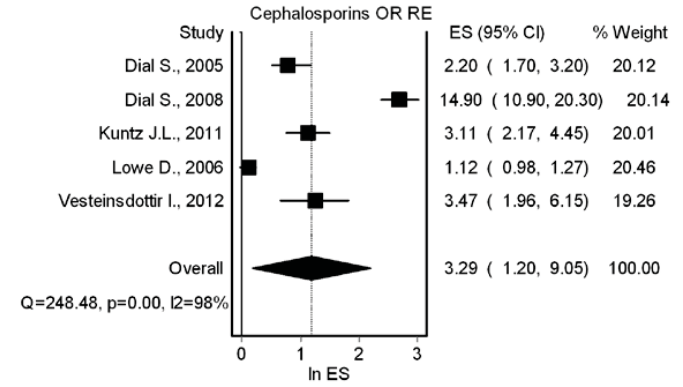


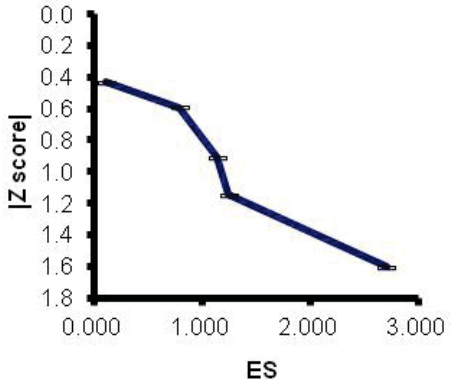


3.1.- Antimicrobials

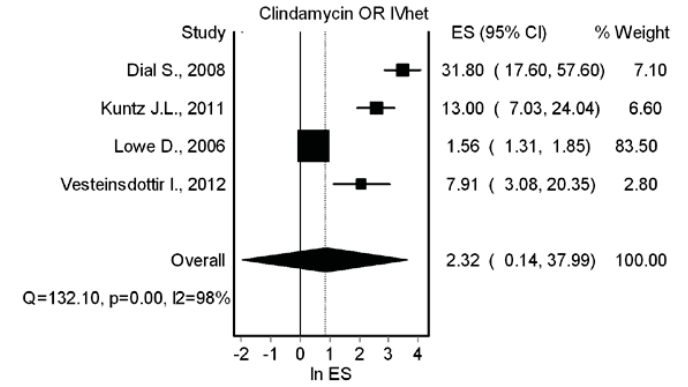


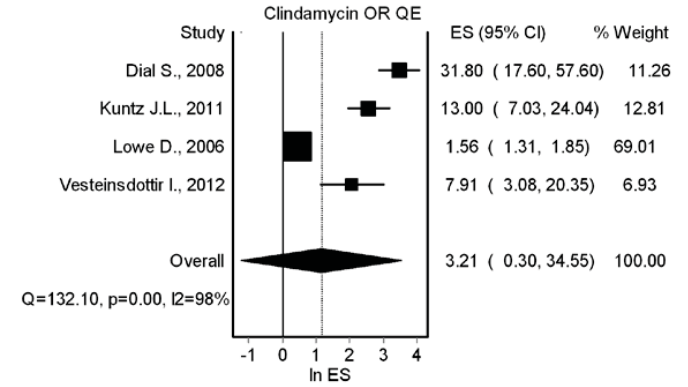


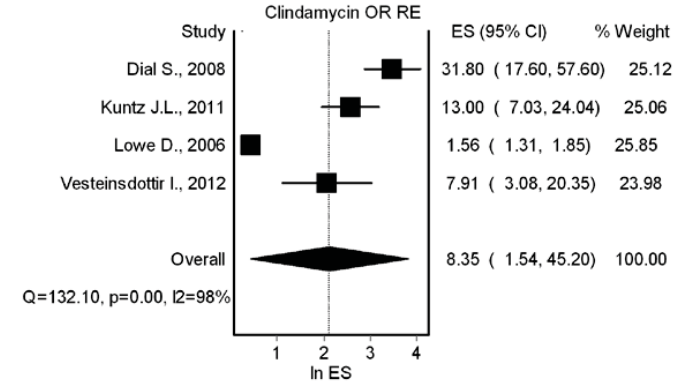




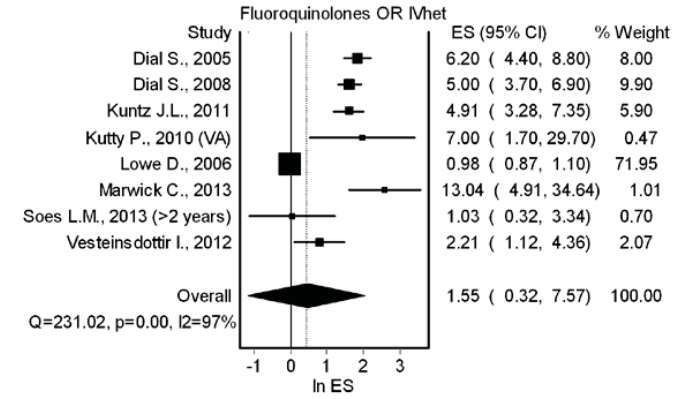
3.2.- Cephalosporins

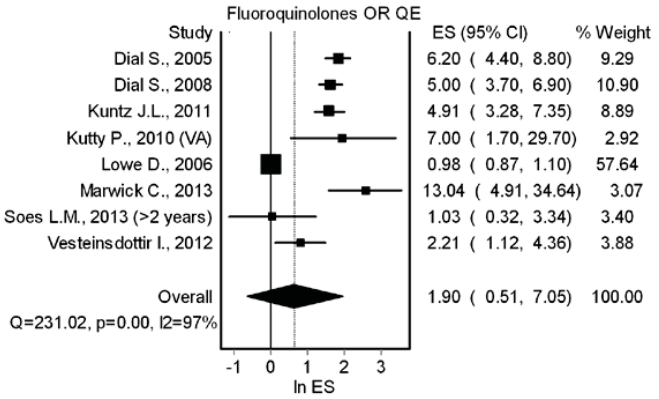


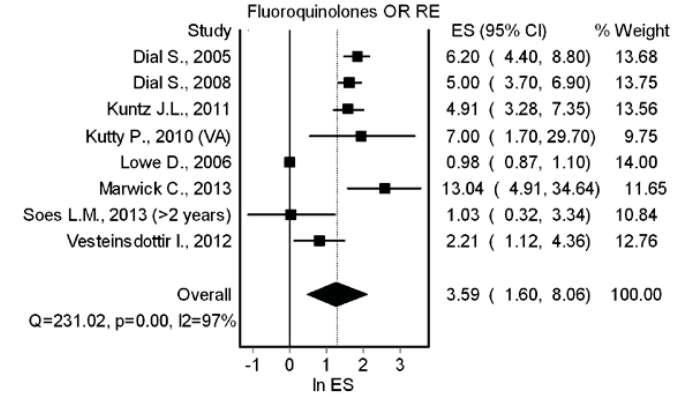


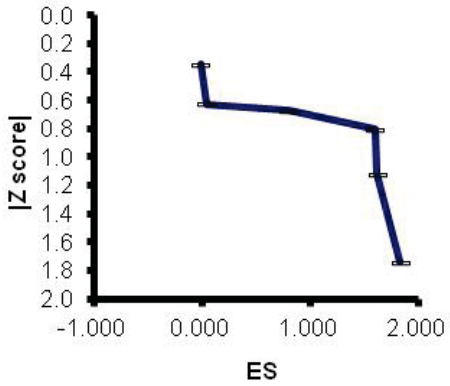


3.3.- Clindamycin









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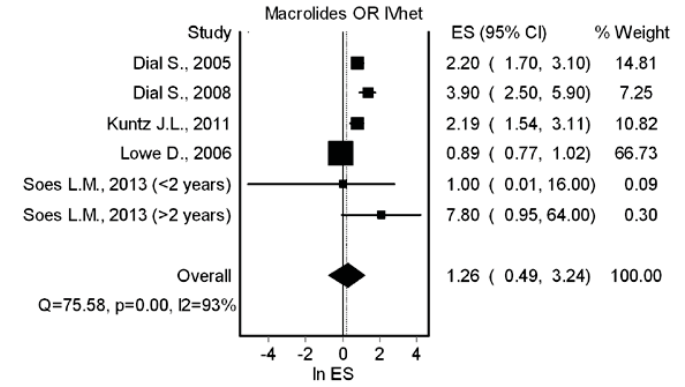
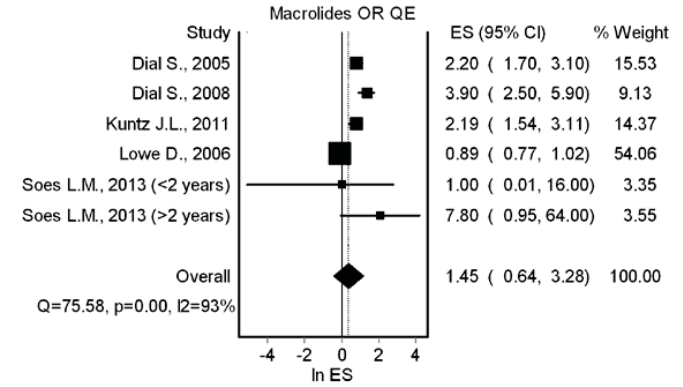
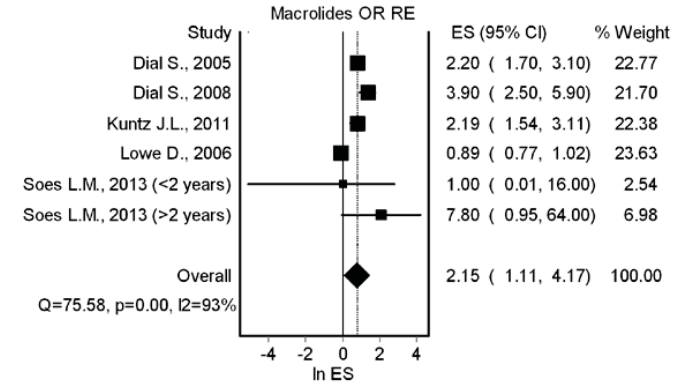
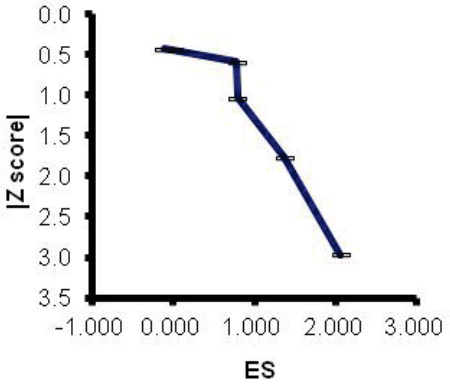
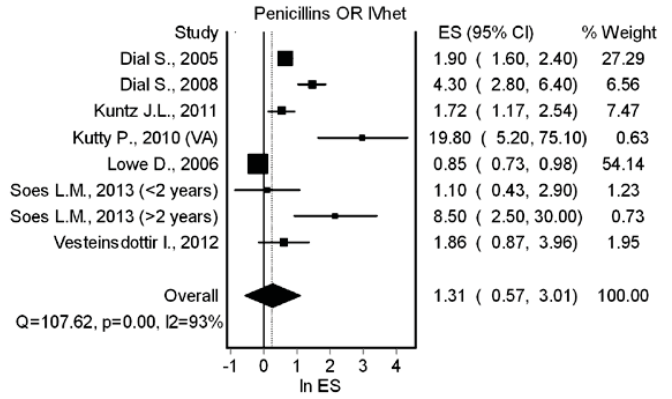


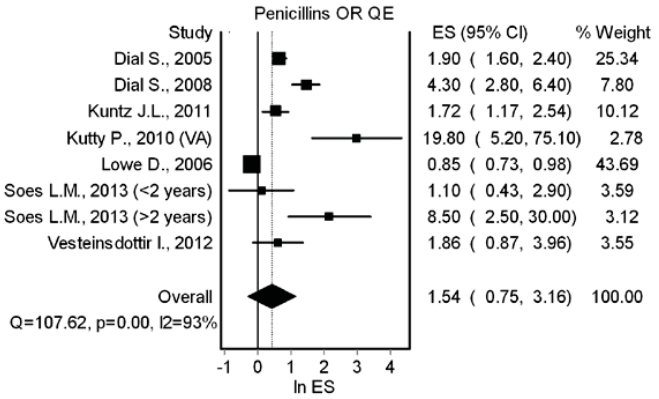


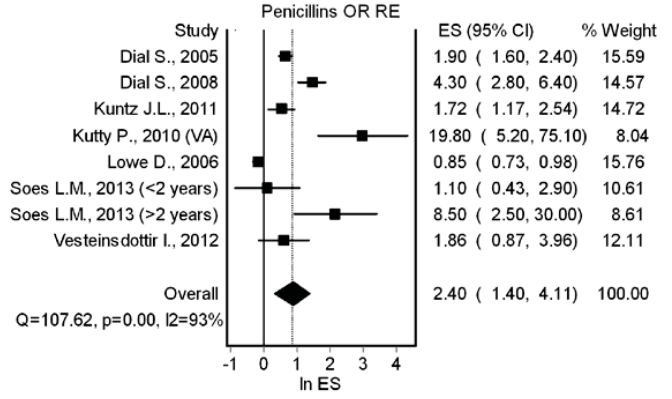


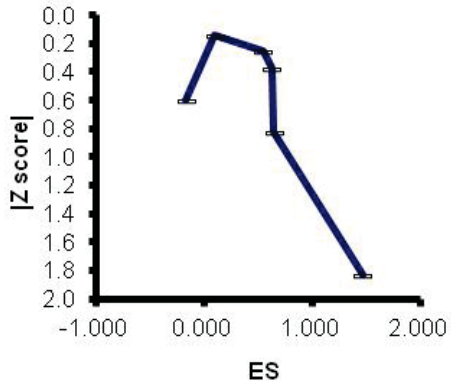


3.5.- Macrolides

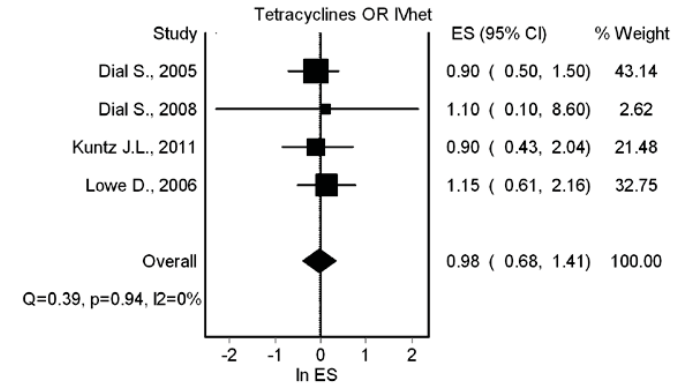


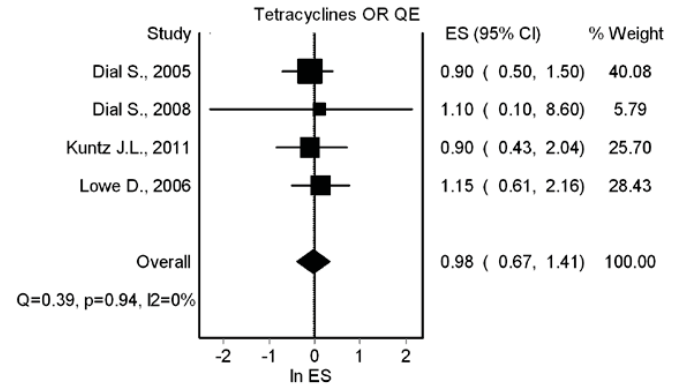


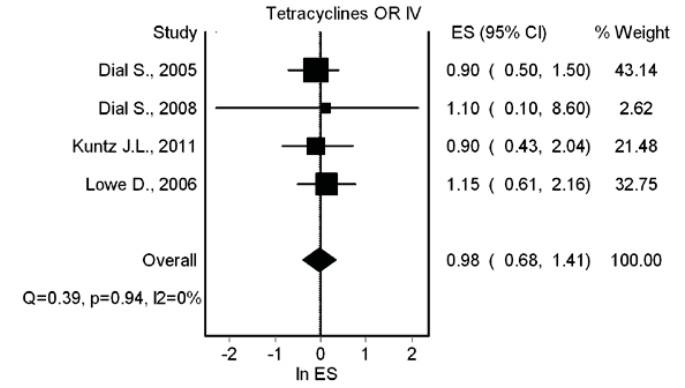




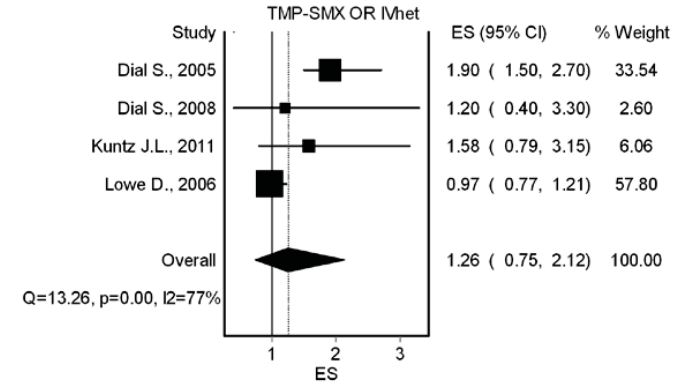
3.6.- Penicillins

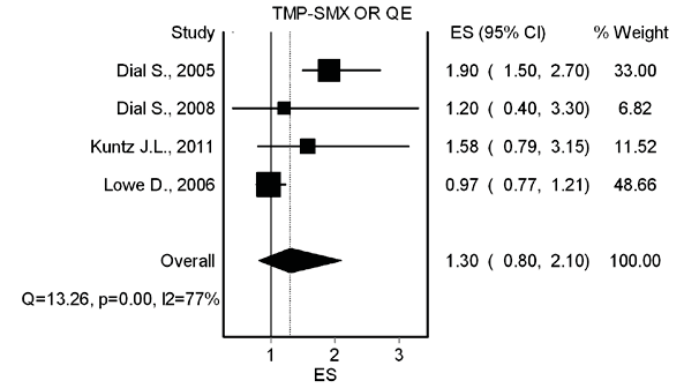


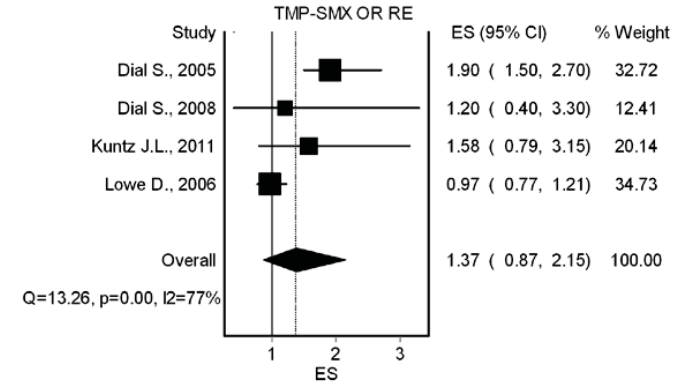




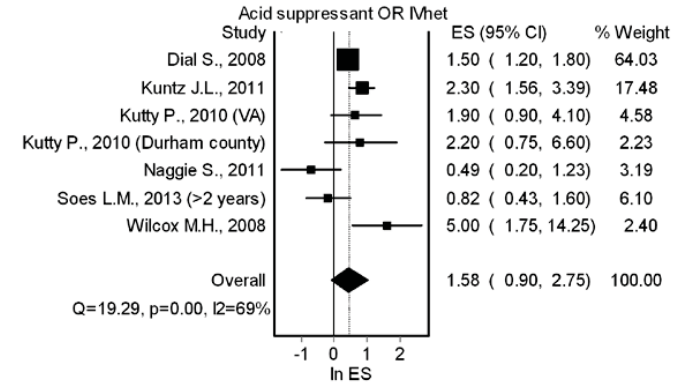
3.7.- Tetracyclines

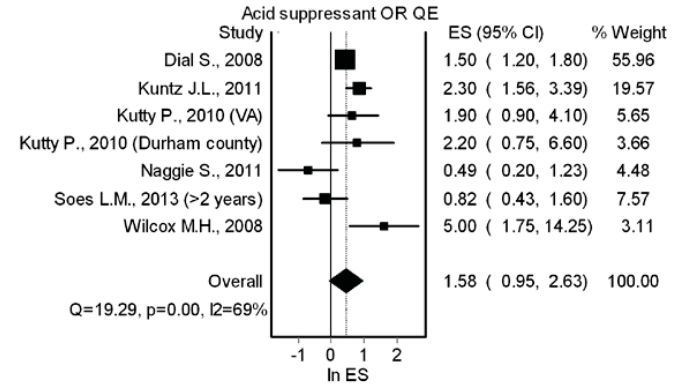


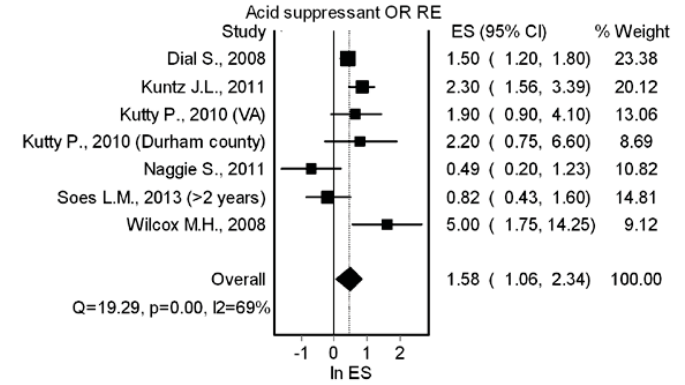


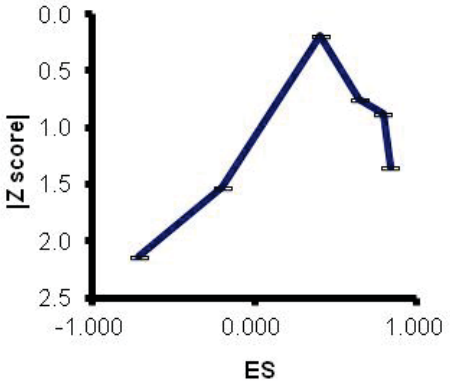


3.8.- Trimethoprim/sulfamethoxazole

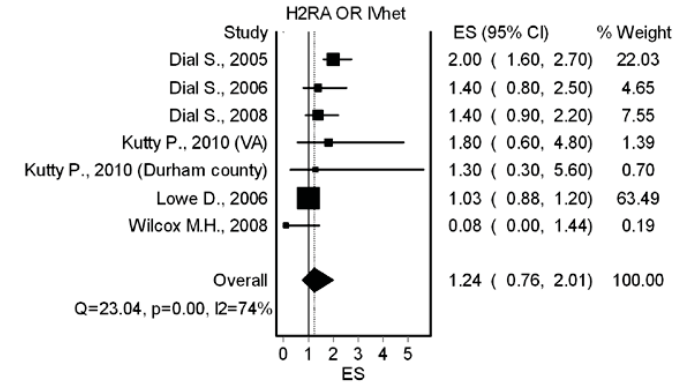


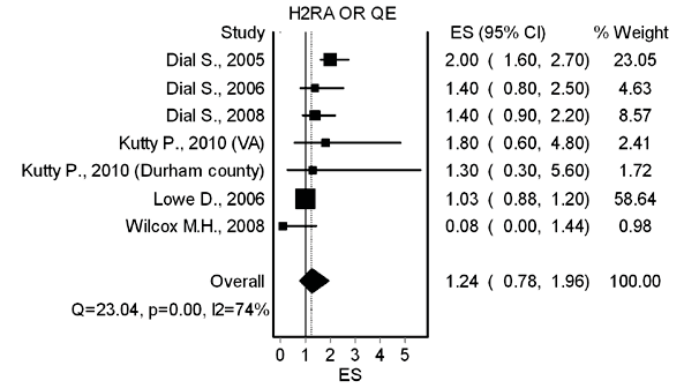


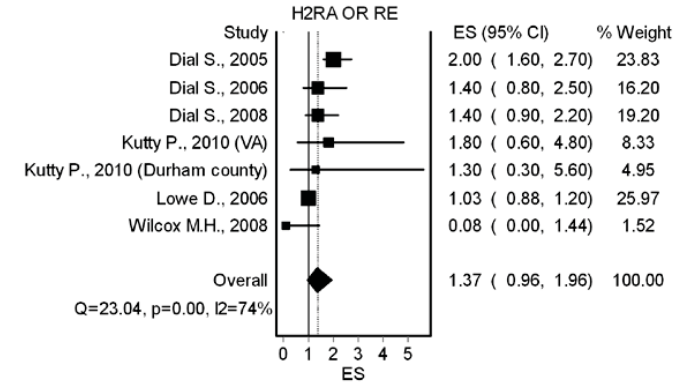


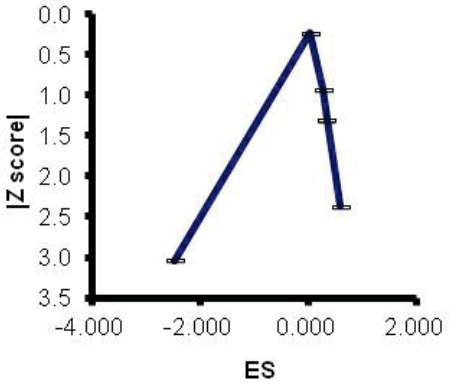


3.9.- Gastric acid suppressant

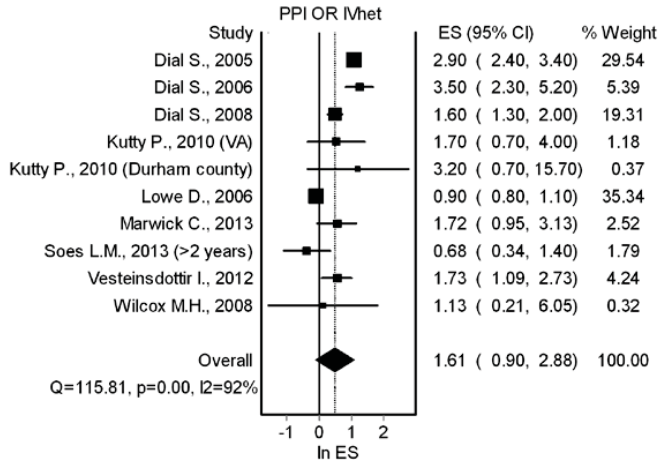


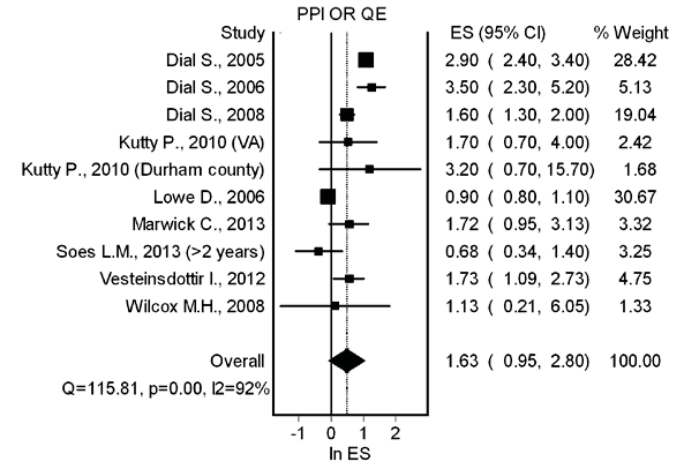


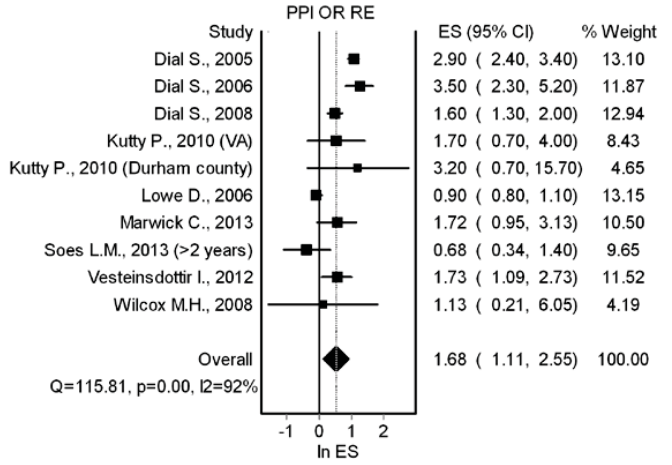


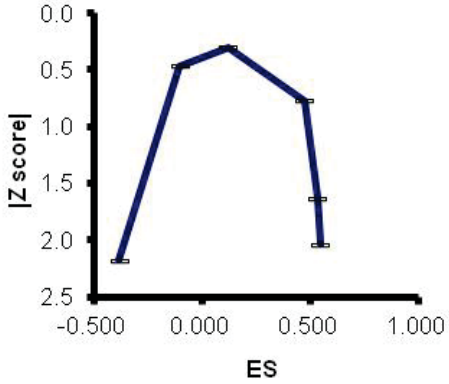


3.10.- Histamine-2 receptor antagonists

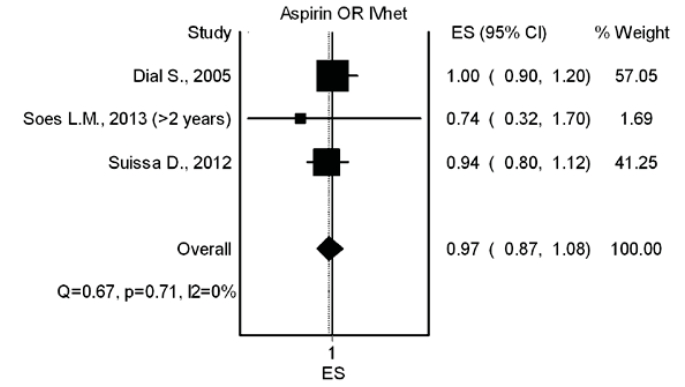


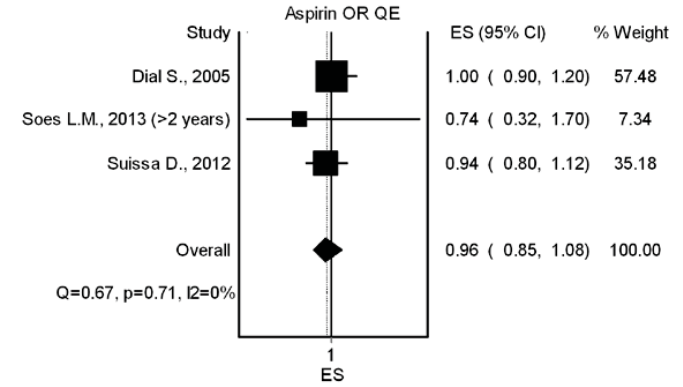


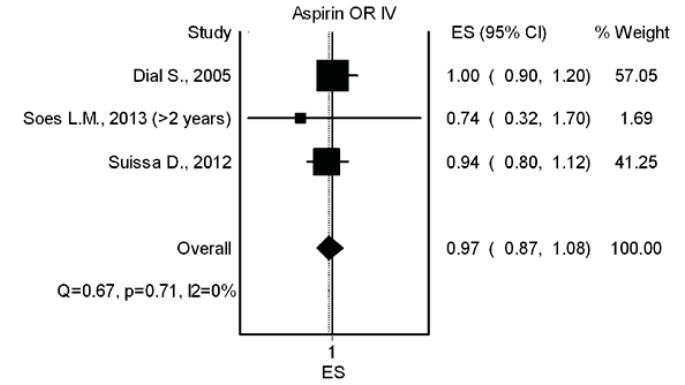




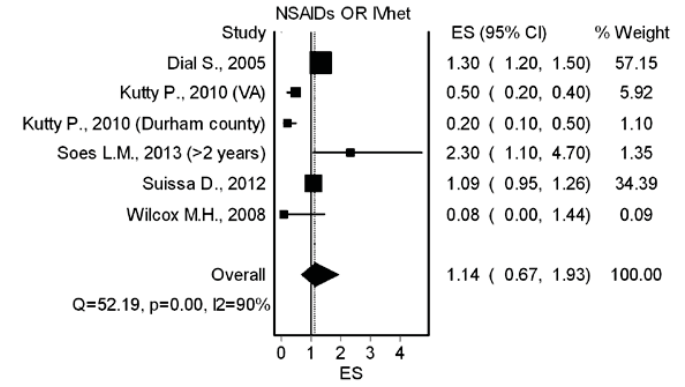
3.11.- Proton pump inhibitor

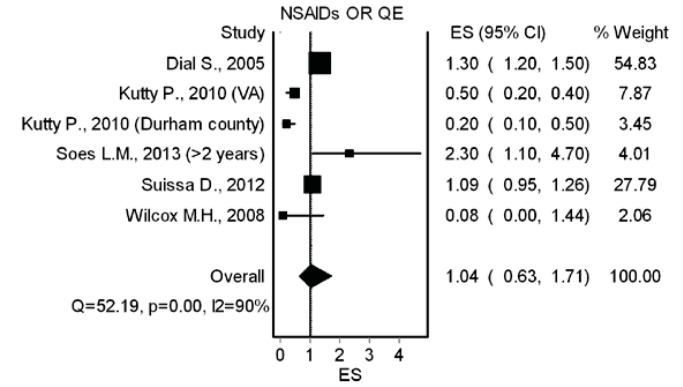

Aspirin OR Mhet
Study
ES (95% CI)
% Weight
Dial S., 2005
1.00 (0.90, 1.20) 57.05
Soes L.M., 2013 (>2 years)
0.74 (0.32, 1.70) 1.69
Suissa D., 2012
0.94 (0.80, 1.12) 41.25
Overall
0.97 (0.87, 1.08) 100.00
Q=0.67, p=0.71, I2=0%
1
ES

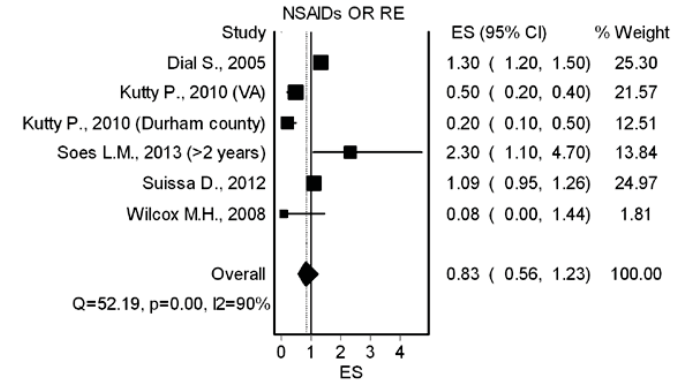

Aspirin OR QE
Study
ES (95% CI)
% Weight
Dial S., 2005
1.00 (0.90, 1.20) 57.48
Soes L.M., 2013 (>2 years)
0.74 (0.32, 1.70) 7.34
Suissa D., 2012
0.94 (0.80, 1.12) 35.18
Overall
0.96 (0.85, 1.08) 100.00
Q=0.67, p=0.71, I2=0%
1
ES

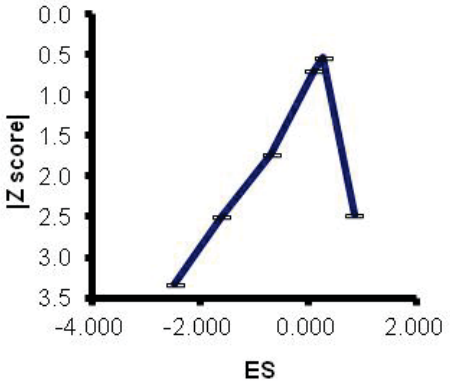

Aspirin OR IV
Study
ES (95% CI)
% Weight
Dial S., 2005
1.00 (0.90, 1.20) 57.05
Soes L.M., 2013 (>2 years)
0.74 (0.32, 1.70) 1.69
Suissa D., 2012
0.94 (0.80, 1.12) 41.25
Overall
0.97 (0.87, 1.08) 100.00
Q=0.67, p=0.71, I2=0%
1
ES

3.12.- Aspirin

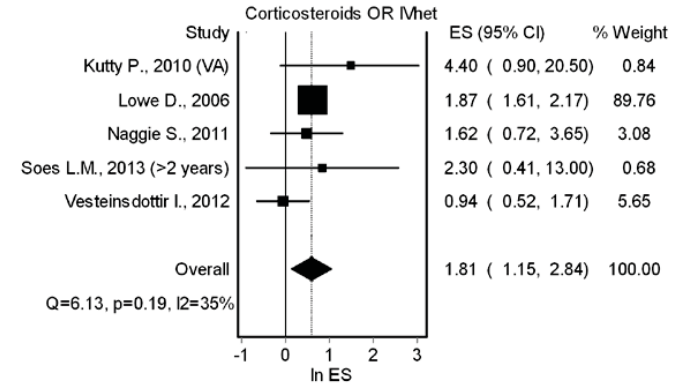


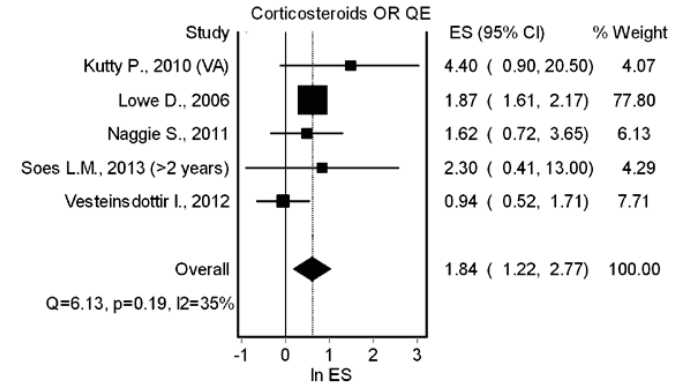


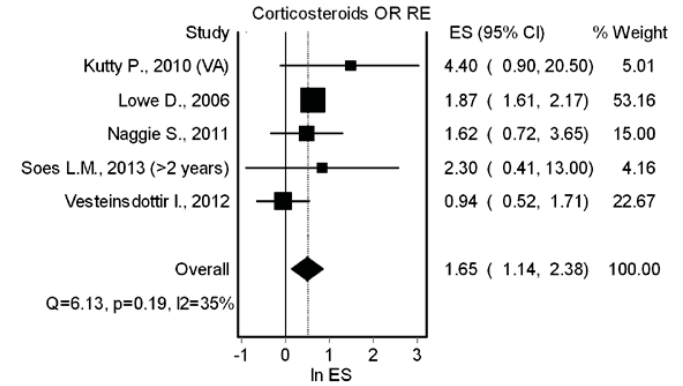


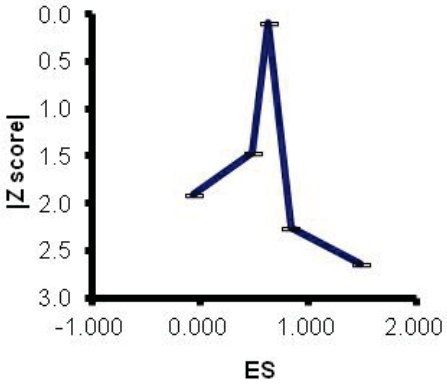


3.13.- Non-steroidal anti-inflammatory drugs

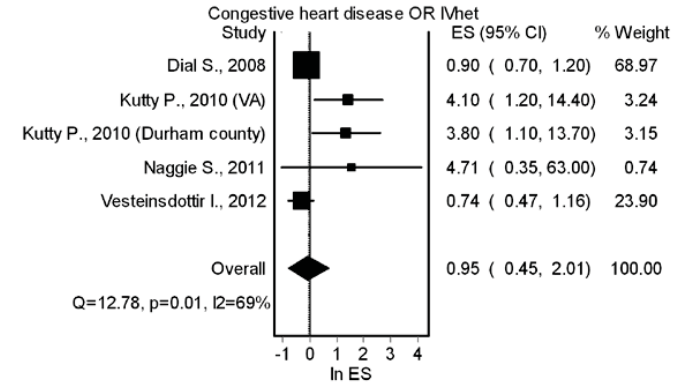


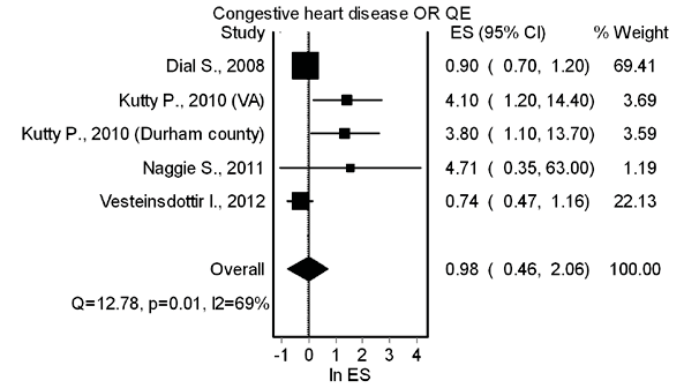


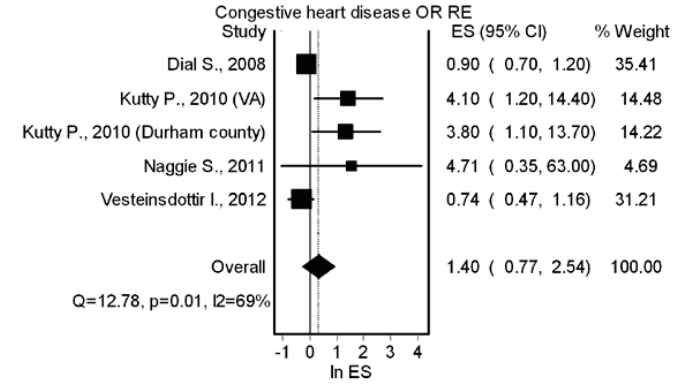


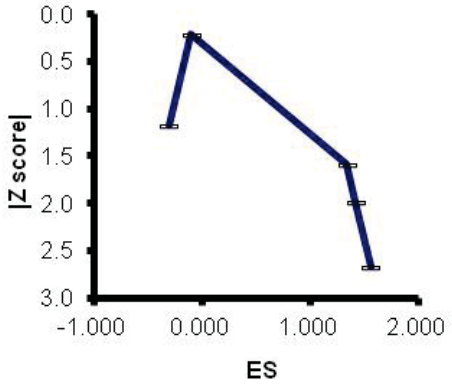


3.14.- Corticosteroids

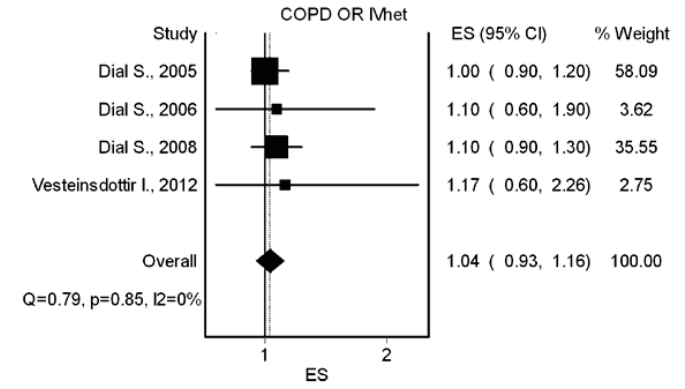


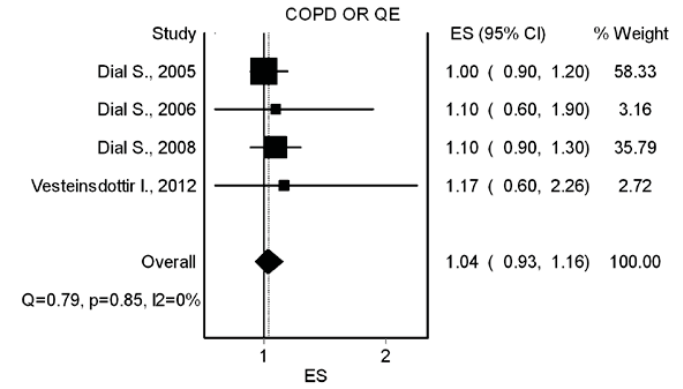


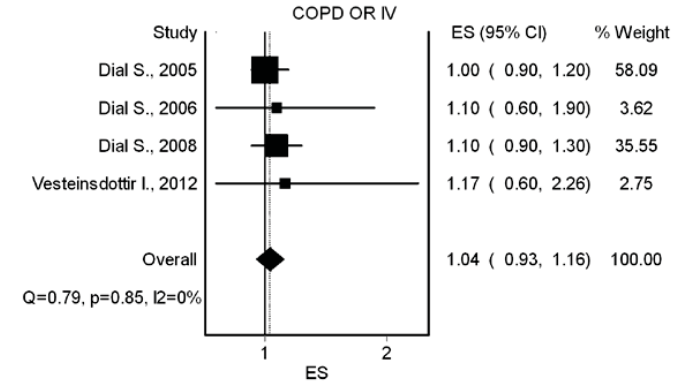




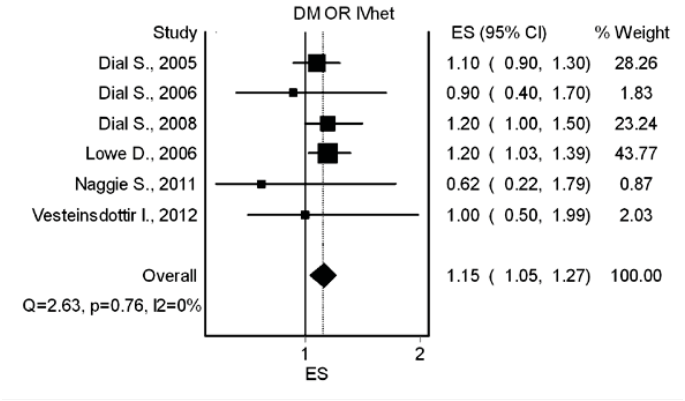
3.15.- Congestive heart disease

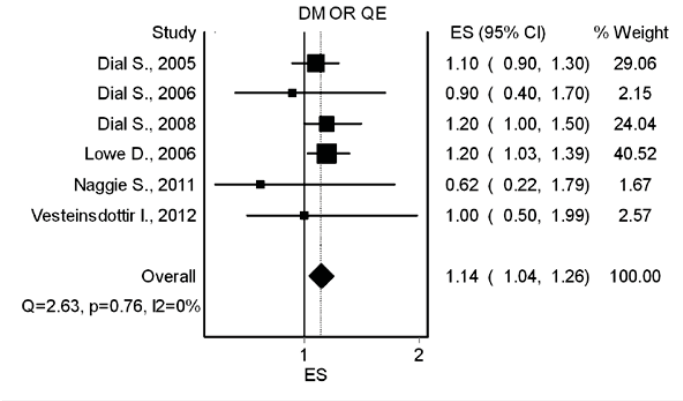


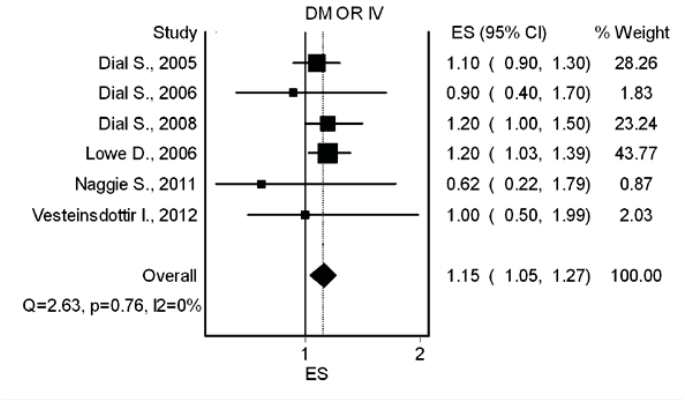


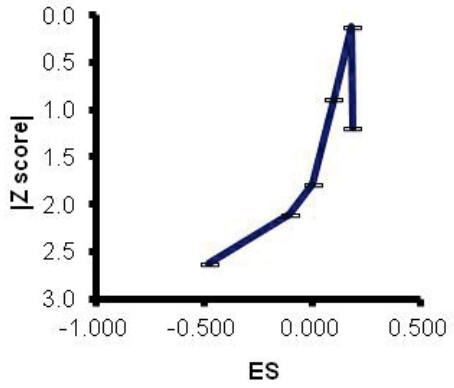


3.16.- Chronic obstructive pulmonary disease

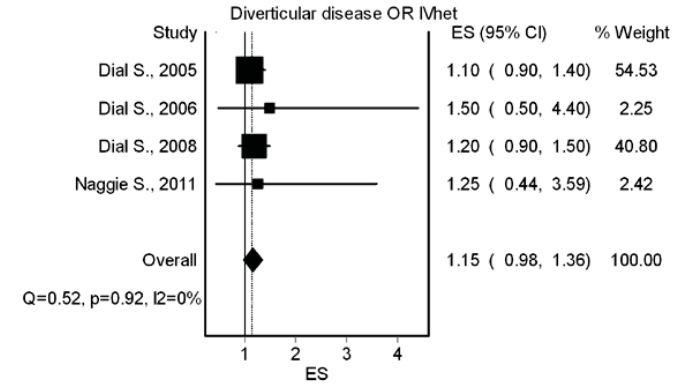


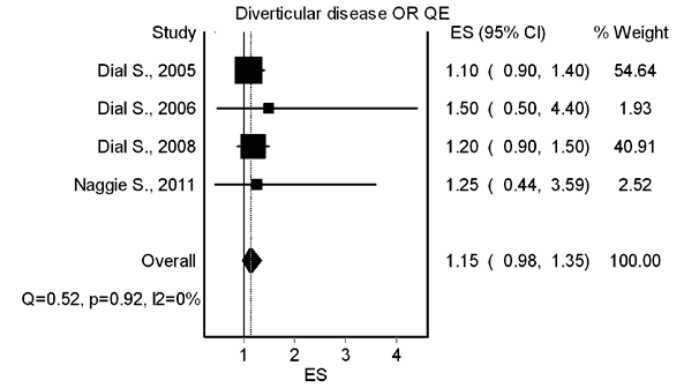


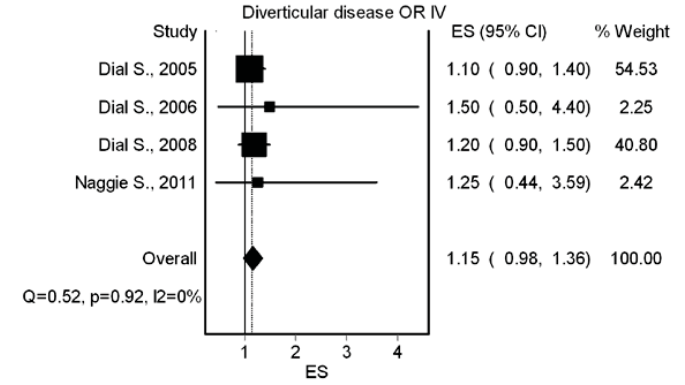




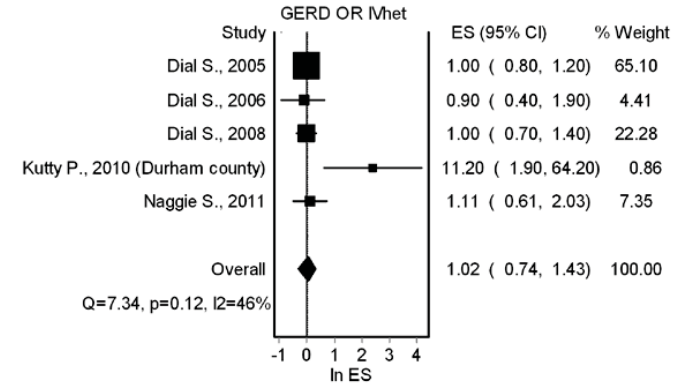
3.17.- Diabetes mellitus

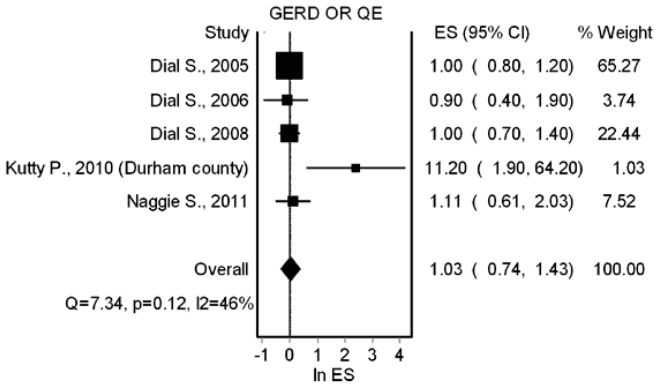


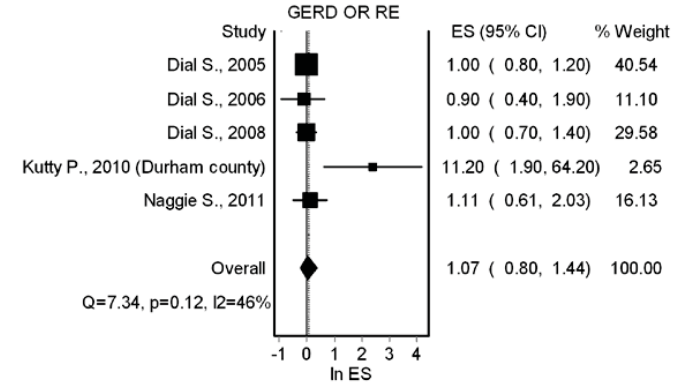


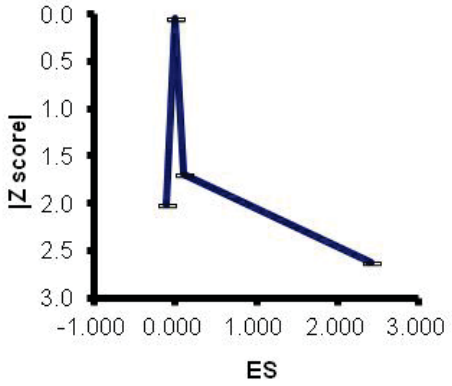


3.18.- Diverticular disease

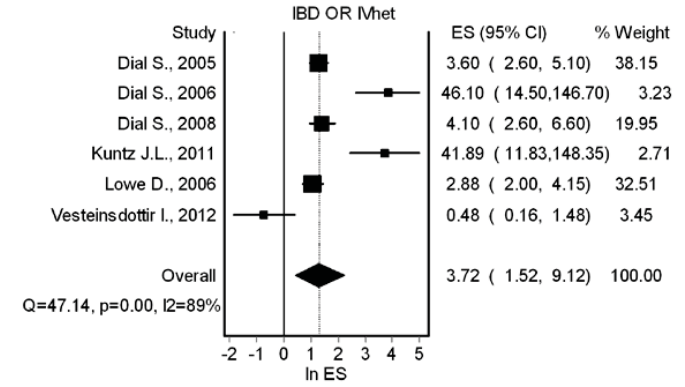


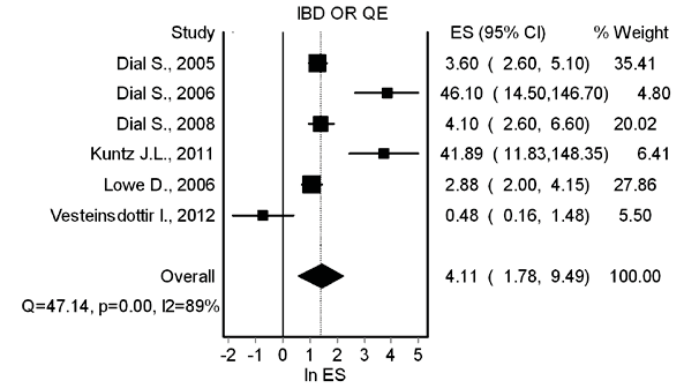


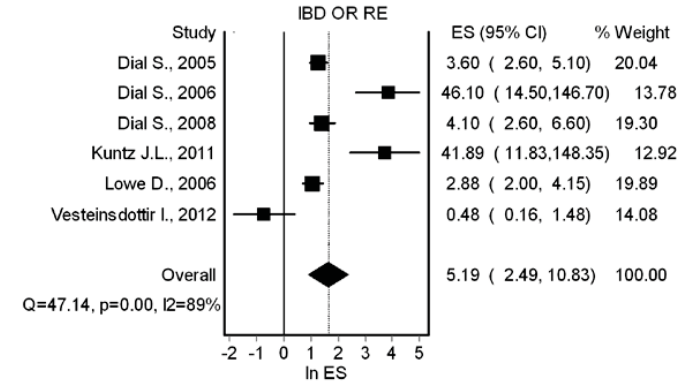


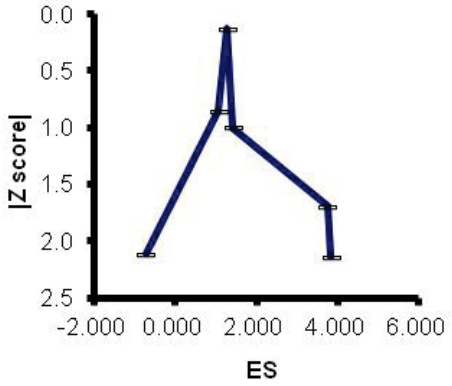


3.19.- Gastroesophageal reflux disease

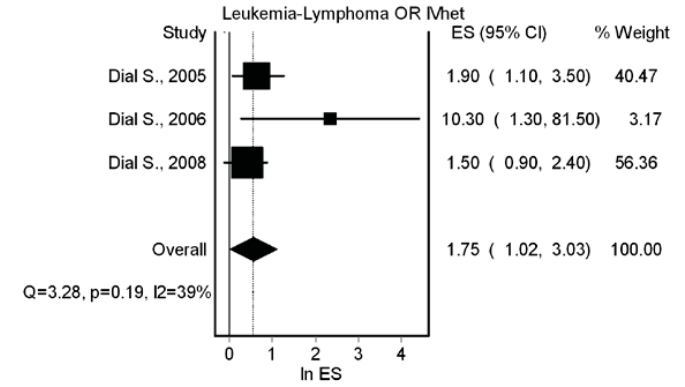


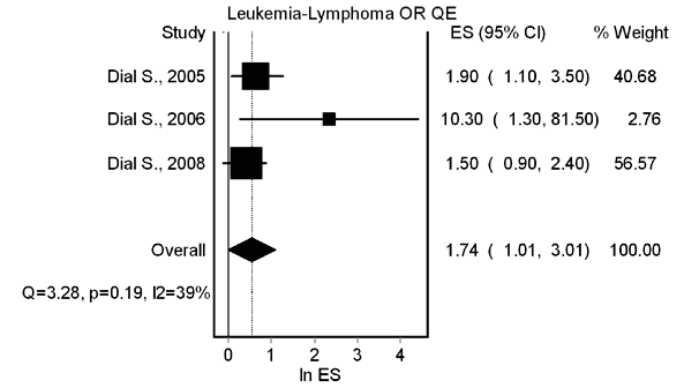


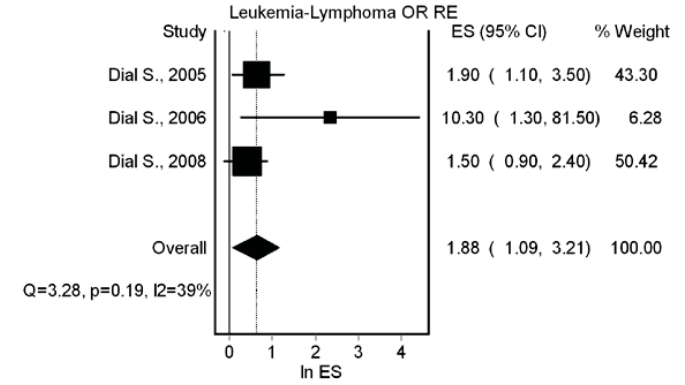




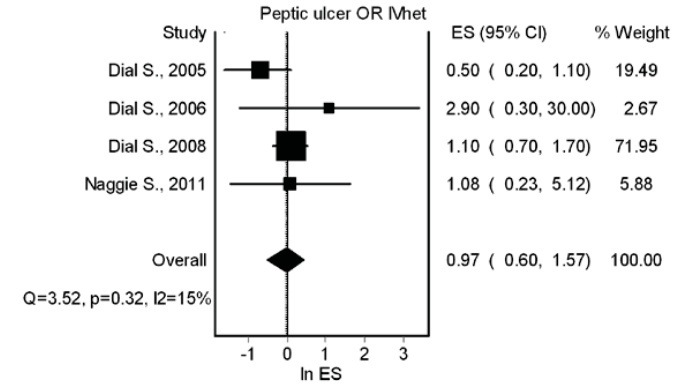
3.20.- Inflammatory bowel disease

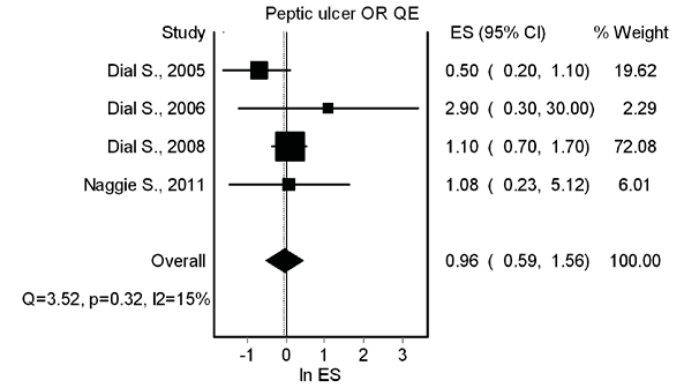


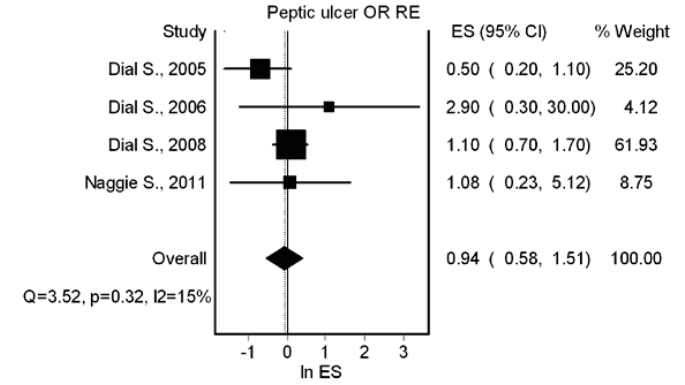




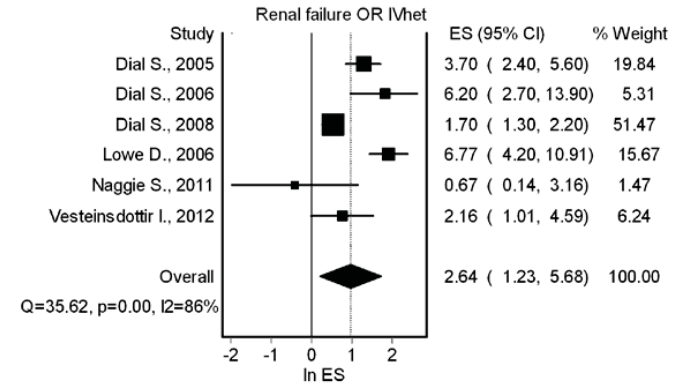
3.21.- Leukemia or Lymphoma

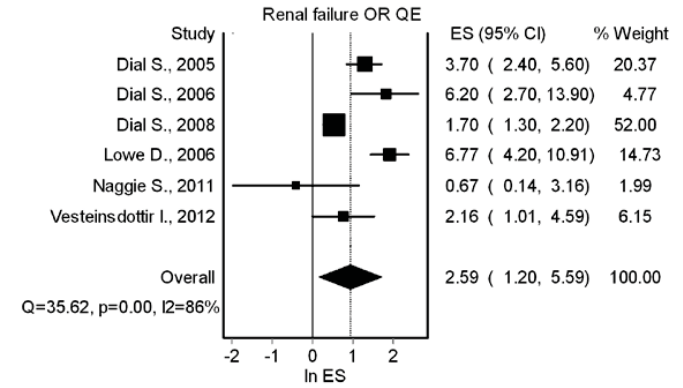


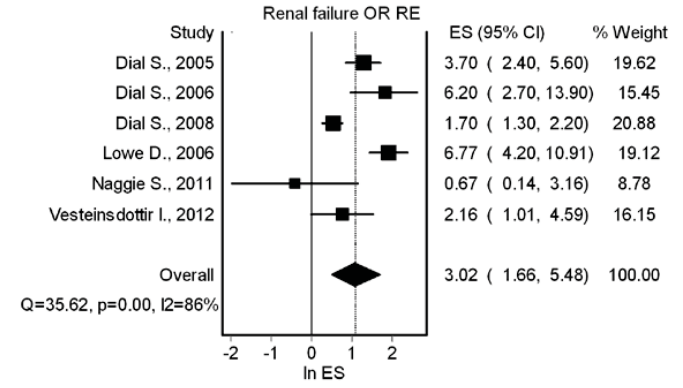


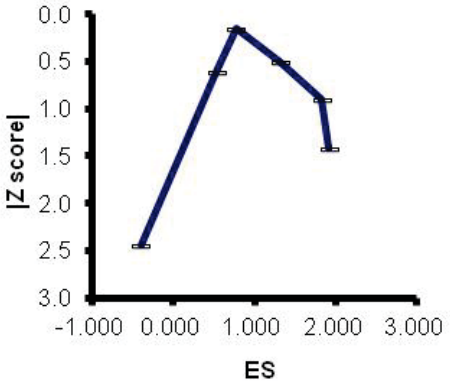


3.22.- Peptic ulcer

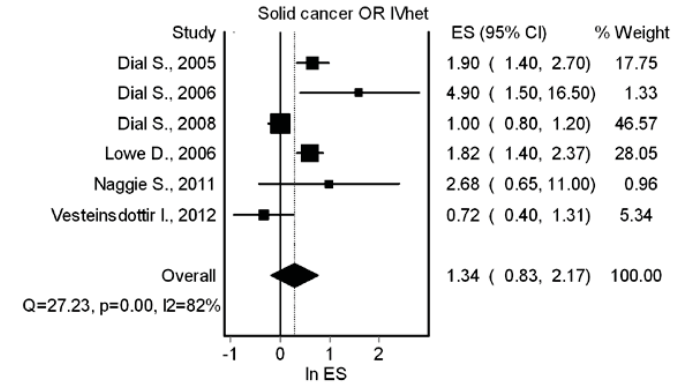


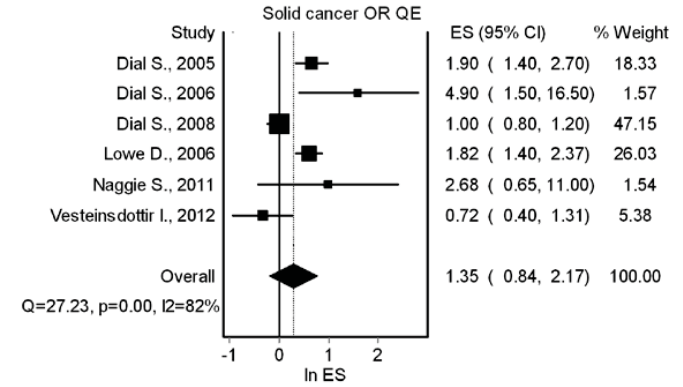


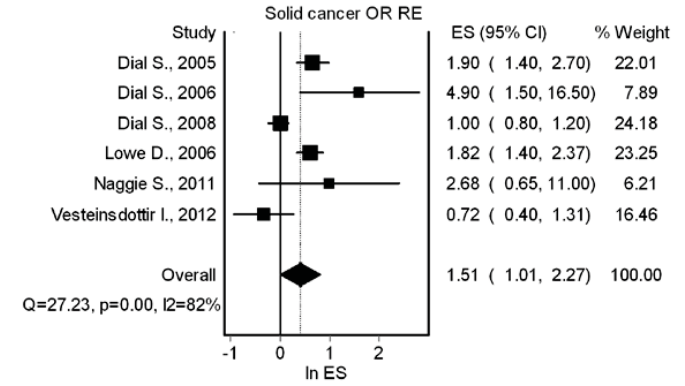


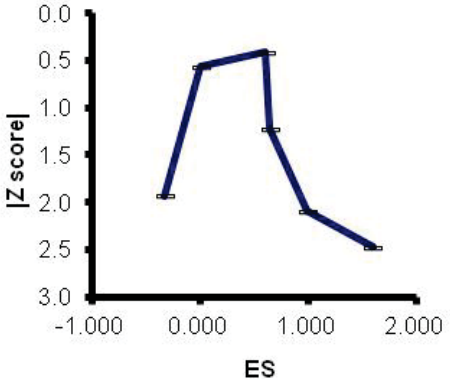


3.23.- Renal failure



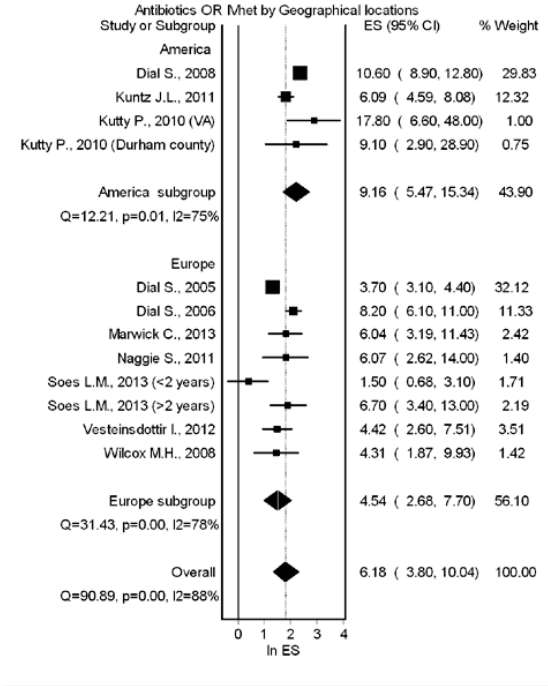


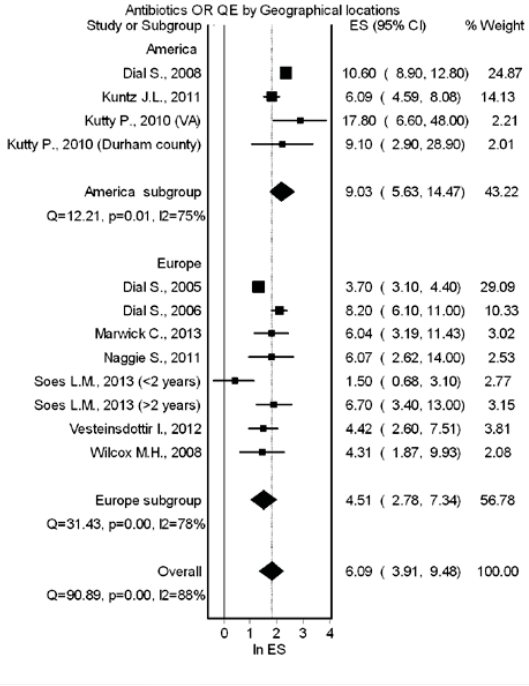


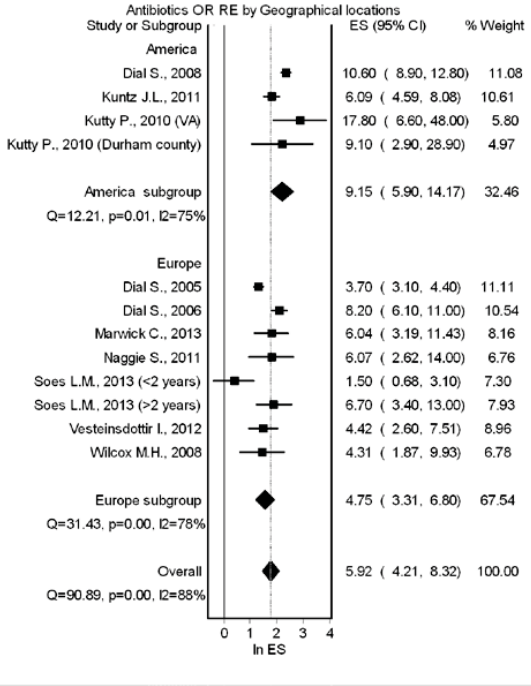


3.24.- Solid cancer

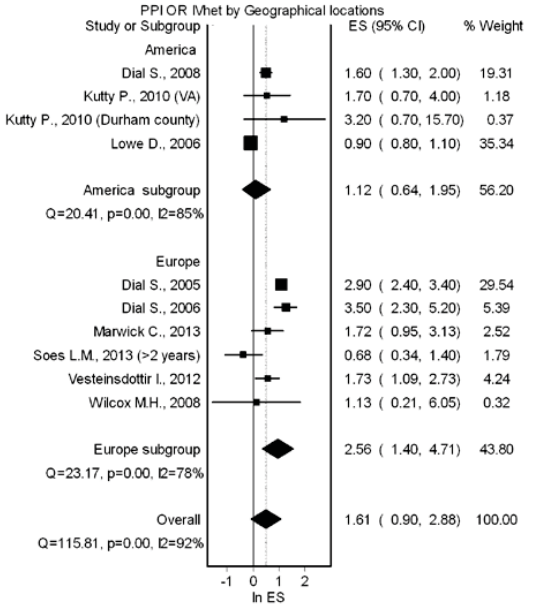
Appendix 4.- Sensitivity analysis

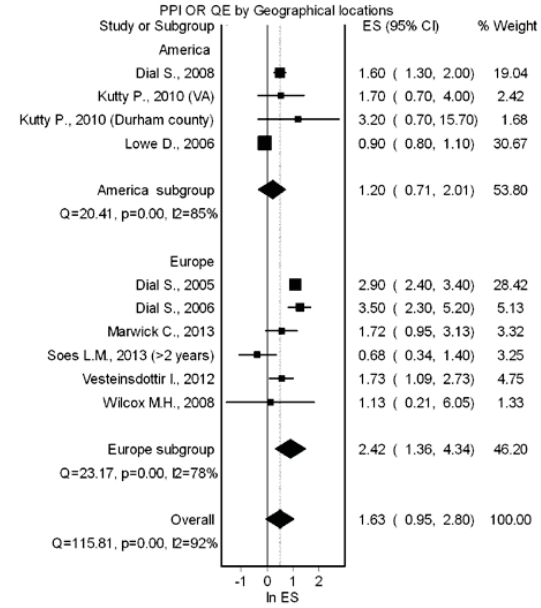


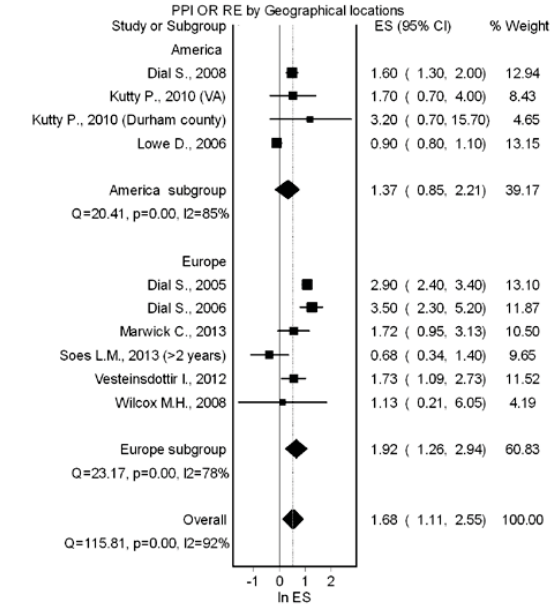




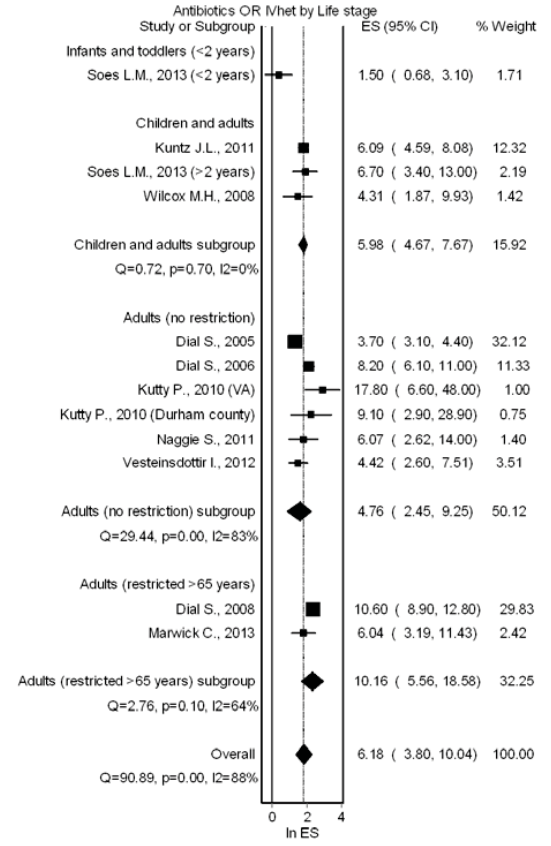
4.1.- Antimicrobials by location

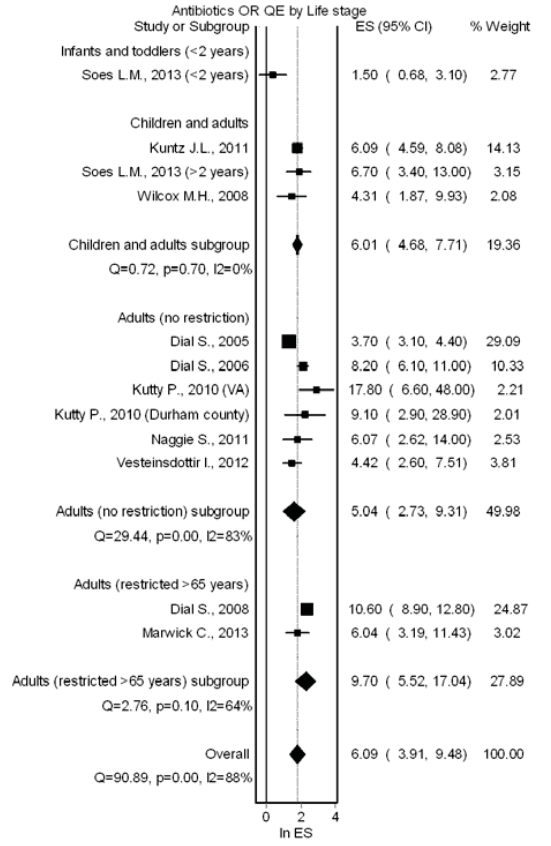


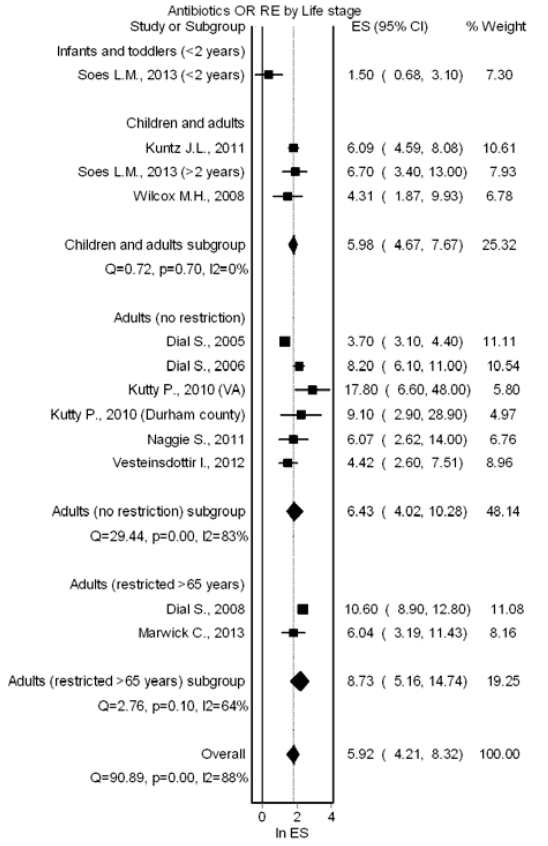




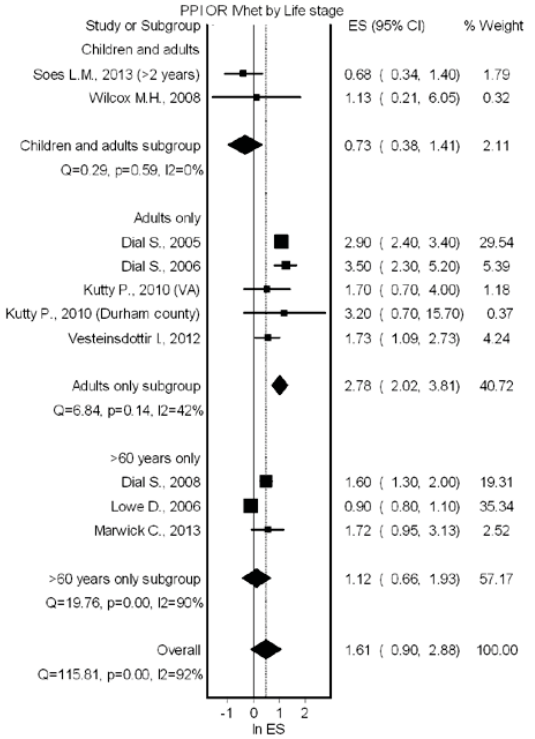
4.2.- Proton pump inhibitors by location

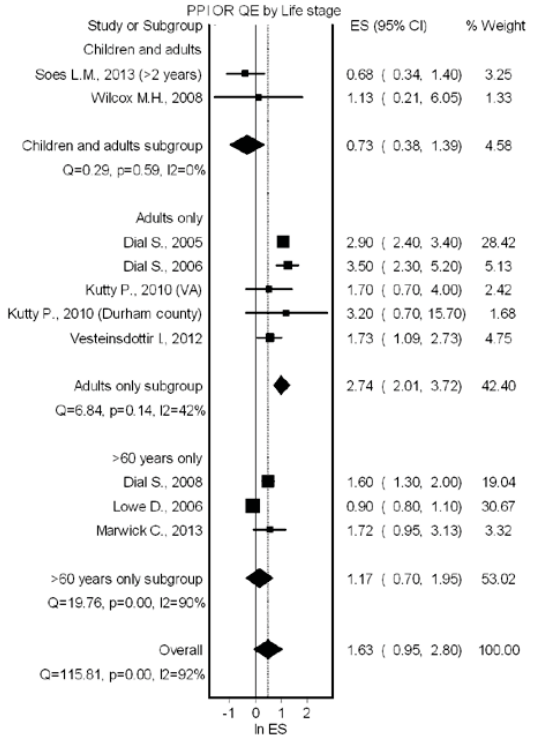


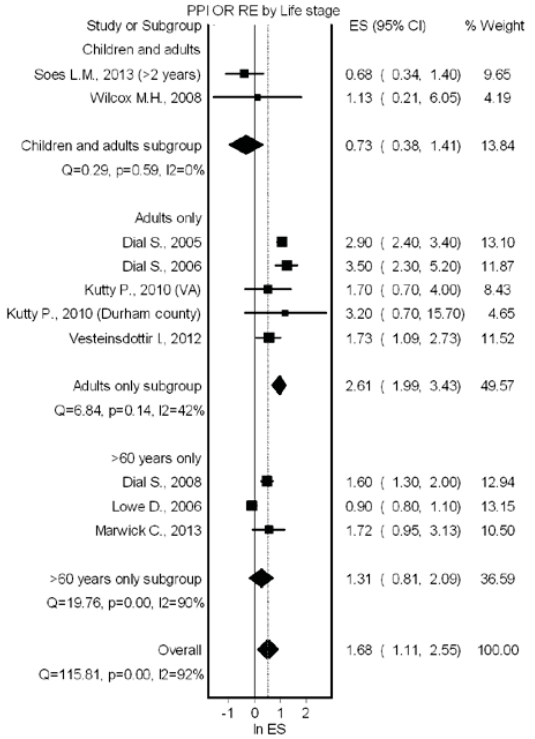




4.3.- Antimicrobials by life stage







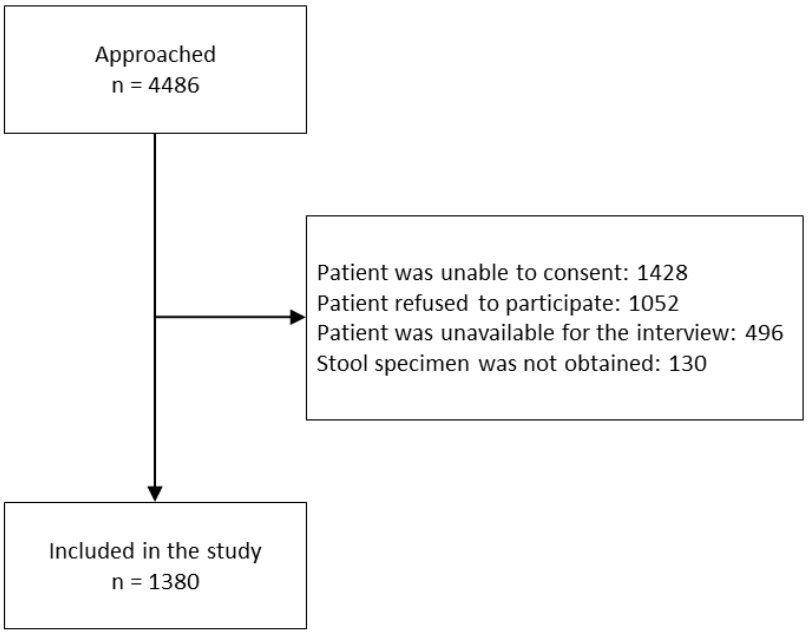
4.4.- Proton pump inhibitors by life stage

Appendix 3.1

Supplement information for Section 3.2 – Risk factors for asymptomatic *C. difficile* colonisation

Supplementary materials

S1. Patient enrolment flowchart.



S2. Prevalence of asymptomatic *C. difficile* colonisation and median current length of stay by hospital and by survey.

Survey	RBWH		SCGH	
	Prevalence of asymptomatic *C. difficile* colonisation (%)	Median current length of stay (IQR)	Prevalence of asymptomatic *C. difficile* colonisation (%)	Median current length of stay (IQR)
1 – Feb/Mar 12	8.1	5 (2-17)	14.4	5 (2-9)
2 – Aug/Sep 12	3.6	6 (3-21)	8.2	5 (2-11)
3 – Feb/Mar 13	3.6	4 (1-13)	11.4	4 (2-9)
4 – Aug/Sep 13	1.1	7 (3-14)	8.2	5 (2-9)
5 – Feb/Mar 14	4.8	6 (2-11)	8.2	4 (1-9)
6 – Aug/Sep 15	3.9	5 (2-8)	6.8	4 (1-8)

S3. List of all ribotypes isolated and their frequency.

Ribotype	Frequency
A+B+CDT-	
001	1
002	2
003	1
014/020	23
015	1
018	10
023	1
046	1
049	1
056	6
103	5
110	1
220	1
QX 001	2
QX 026	1
QX 051	1
QX 069	2
QX 087	1
QX 102	1
QX 150	1
QX 158	2
QX 161	1
QX 223	1
QX 412	3
QX 417	1
A+B+CDT+	
127	1
251	1
QX 220	1
A-B+CDT-	
QX 134	1
A-B-CDT+	
063	1

Ribotype	Frequency
A-B-CDT-	
009	3
010	5
026	1
039	2
051	2
286	1
QX 012	1
QX 077	1
QX 078	1
QX 083	1
QX 108	1
QX 141	3
QX 190	1
QX 210	1
QX 212	1
QX 213	1
QX 222	1
QX 298	1

Appendix 4.1

Supplement information for Section 4.2 – *C. difficile* ribotypes circulating in Australian hospitals and communities

Supplementary materials

S1. List of all ribotypes isolated and their frequency by year and source.

2012

Ribotype	Frequency
HA-CDI	
002	1
010	1
014/020	6
018	1
056	4
137	1
QX 121	1
QX 158	1
QX 170	1
CA-CDI	
002	3
014/020	6
015	1
018	2
053	1
054	1
056	2
070	1
137	2
247	1
297	1
QX new	1
QX 104	1
QX 158	2
QX 161	1
QX398	1

Ribotype	Frequency
TCDc	
001	1
002	2
003	1
014/020	10
015	1
018	9
056	1
103	1
QX 001	2
QX 087	1
QX 150	1
QX 158	2
QX 161	1
QX 220	1
QX 223	1
QX 412	1

2013

Ribotype	Frequency
HA-CDI	
001 and 137	1
002	1
003	1
005	1
009	1
010	1
014/020	19
015	2
017	1
056	7
064	1
070	2
081	1
220	1
251	1
QX new	1
QX 028	1
QX 076	1
QX 141	1
QX 158	1
QX 199	2
QX 214	1
QX 221	1
QX 366	2
QX 447	1
CA-CDI	
001	1
002	8
005	3
010	1
014/020	26
015	1
018	2
027	1
046	1
054	2
056	8
070	6
078	2
244	2
QX 001	2
QX 013	1
QX 020	1
QX 032	1
QX 047	1
QX 049	1
QX 058	1
QX 068	2
QX 104	1
QX 158	1
QX 176	1
QX 199	1
QX 365	1
QX 366	1
QX 446	1
QX 478	1

Ribotype	Frequency
TCDc	
014/020	9
023	1
046	1
056	3
103	2
110	1
220	1
251	1
QX 051	1
QX 102	1

2014

Ribotype	Frequency
HA-CDI	
002	2
005	2
014/020	5
018	2
046	1
054	1
056	1
106	1
131	1
244	1
247	1
297	1
QX 001	1
QX 005	1
QX 013	1
QX 026	1
QX 158	1
QX 193	1
QX 412	1
CA-CDI	
002	4
014/020	12
018	1
056	3
070	2
080	1
103	1
106	2
137	2
244	1
QX 001	1
QX 026	1
QX 070	1
QX 076	1
QX 086	2
QX 113	1
QX 148	1
QX 158	4
QX 197	1
QX 417	1

Ribotype	Frequency
TCDc	
014/020	4
018	1
049	1
056	2
063	1
103	2
127	1
QX 026	1
QX 069	2
QX 134	1
QX 412	2
QX 417	1

Appendix 5.1

Supplement information for Section 5.4 – Seasonality of *C. difficile* infection

S1.1.- Search strategies

PubMed

("Clostridium"[Mesh] OR Clostridium))

AND

((Difficile)

AND

("Seasons"[Mesh] OR Season OR Seasons OR Seasonal)

Embase

'season'/exp OR season OR seasons OR seasonal

AND

'clostridium'/exp OR clostridium

AND

difficile

LILACS Virtual Health Library

Clostridium [Words]

AND

Difficile [Words]

S1.2.- Targeted search strategy for Southern hemisphere studies

PubMed

((((("Clostridium"[Mesh] OR Clostridium))

AND

Difficile

AND

((("Africa"[Mesh]) OR "Australia"[Mesh]) OR "South America"[Mesh] OR Africa OR

Australia OR “South America” OR “Southern Hemisphere”)))

S2.- Characteristics of excluded studies

Author, year	Reason for exclusion
Bignardi and Askew, 2000 [49] ‡	Reported the number of cases per month without the number of stool samples/patients tested
Cooper *et al.*, 2011 [50]	Hydrogen peroxide vapour intervention was implemented to reduce the incidence of *C. difficile*
Denno *et al.*, 2005 [51] §	Reported the number of cases per month without the number of stool samples/patients tested
Elumogo *et al.*, 2009 [52] ‡	Reported the number of cases per month without the number of stool samples/patients tested
Fekety *et al.*, 1997 [53] §	Reported the number of cases per season without the number of stool samples/patients tested
Fellmeth *et al.*, 2010 [54] ‡	Reported the number of cases per season without the number of stool samples/patients tested
Feuerstadt *et al.*, 2013 [55] ‡	Reported the number of cases per season without the number of stool samples/patients tested
Garcia *et al.*, 2007 [56]	Only 9 months follow-up
Gardilcic *et al.*, 2000 [57]	Only 4 months follow-up
Gulacsi *et al.*, 2013 [58]	In Hungarian
Hall *et al.*, 2012 [59]	Measured mortality rates of *C. difficile*
Kim *et al.*, 1989 [60] ¶	Reported the number of cases per month without the number of stool samples/patients tested
Kyne *et al.*, 1998 [61]	Only 7 months follow-up
Larang *et al.*, 2011 [62] ¶	Unable to extract data. Reported "A bimodal seasonal distribution of positive tests was noted with peaks in March and November".
Marco-Martinez *et al.*, 2014 [63] §	Reported the number of cases per season without the number of stool samples/patients tested
Pearson *et al.*, 2009 [64] §	Reported the number of cases per season without the number of stool samples/patients tested
Polgreen *et al.*, 2010 [17] ‡	Reported the number of cases per month without the number of stool samples/patients tested
Polgreen *et al.*, 2011 [65] ‡	Reported the number of cases per month without the number of stool samples/patients tested
Riley *et al.*, 1994 [66] §	Unable to extract data. Reported "A statistically significant seasonal variation in the isolation rate for *C. difficile* could not be demonstrated".
Souza Dias *et al.*, 2010 [67] §	Reported the number of cases per month without the number of stool samples/patients tested
van Kleef *et al.*, 2014 [68] §	Reported the number of cases per month without the number of stool samples/patients tested

‡ Corresponding author was contacted via email for further information regarding the number positive stool specimens for *C. difficile* and/or the total number of stool specimens/patients tested per months/seasons, no response was received.

§ Corresponding author replied, but was unable to provide the information before the manuscript was submitted for review.

¶ Unable to contact the corresponding author.

Appendix 6.1

Supplement information for Section 6.2 – Faecal microbiota transplantation for *C. difficile* infection

Supplementary material

S1.- Search strategy

PubMed

((((("Clostridium"[Mesh] OR "Clostridium Infections"[Mesh] OR Clostridium)) AND Difficile) AND ("Feces"[Mesh] OR "Intestines"[Mesh] OR Feces OR Faeces OR Fecal OR Faecal OR Stool OR Intestinal OR Intestine OR Intestines OR Microbiota)) AND ("Transplants"[Mesh] OR"Tissue Donors"[Mesh] OR Transplants OR Transplantation OR Transplant OR Bacteriotherapy OR Donor OR Donors)

Embase

#5. 'clostridium'/exp OR 'clostridium infection'/exp OR clostridium AND difficile AND ('feces'/exp OR 'intestine'/exp OR feces OR faeces OR fecal OR faecal OR intestinal OR intestine OR intestines OR microbiota) AND ('transplantation'/exp OR transplants OR transplantation OR transplant OR bacteriotherapy)

#4. 'transplantation'/exp OR 'donor'/exp OR transplants OR transplantation OR transplant OR bacteriotherapy OR donor OR donors

#3. 'feces'/exp OR 'intestine'/exp OR feces OR faeces OR fecal OR faecal OR stool OR intestinal OR intestine OR intestines OR microbiota

#2. difficile

#1. 'clostridium'/exp OR 'clostridium infection'/exp OR clostridium

Cochrane CENTRAL

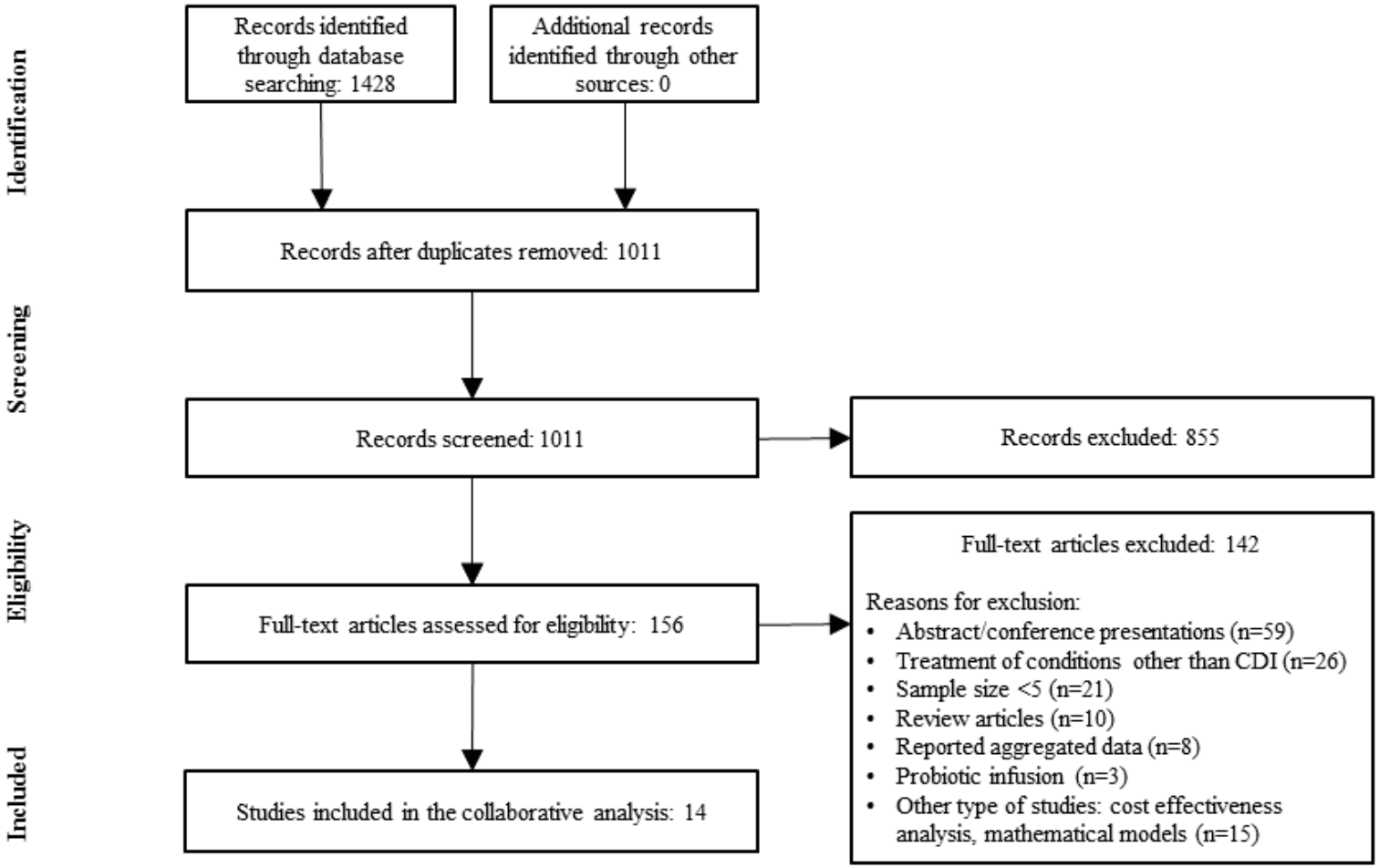
#1 [mh Clostridium] OR [mh "Clostridium Infections"] OR Clostridium

#2 Difficile

#3 [mh Feces] OR [mh Intestines] OR Feces OR Faeces OR Fecal OR Faecal OR Stool OR Intestinal OR Intestine OR Intestines OR Microbiota

#4 [mh Transplants] OR [mh "Tissue Donors"] OR Transplants or Transplantation or Transplant or Bacteriotherapy OR Donor OR Donors

#5 #1 AND #2 AND #3 AND #4



S2.- Preferred reporting items for systematic reviews and meta-analyses flow diagram

Appendix 6.2

Supplement information for Section 6.3 – Low concentration of vitamin D and the risk of *C. difficile* infection

Supplementary material - Search strategies.

PubMed

"Clostridium"[Mesh] OR "Clostridium Infections"[Mesh] OR Clostridium OR “C. difficile” OR CDI OR “C. difficile” OR CDI AND "Vitamin D"[Mesh] OR "vitamin d"[All Fields] OR "ergocalciferols"[MeSH Terms] OR "ergocalciferols"[All Fields]

Embase

'Clostridium'/exp OR 'Clostridium infection'/exp OR Clostridium OR “C. difficile” OR CDI OR “C. difficile” OR CDI

AND

Difficile

AND

'vitamin D'/exp OR vitamin d OR 'ergocalciferol'/exp OR "ergocalciferol" OR "ergocalciferols"

Web of Science

Clostridium OR “C. difficile” OR CDI OR “C. difficile” OR CDI

AND

Difficile

AND

vitamin d OR "ergocalciferol" OR "ergocalciferols"