



Risk Factors for Recurrence, Complications and Mortality in *Clostridium difficile* Infection: A Systematic Review

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Abstract

Background: *Clostridium difficile* infection (CDI) can lead to complications, recurrence, and death. Numerous studies have assessed risk factors for these unfavourable outcomes, but systematic reviews or meta-analyses published so far were limited in scope or in quality.

Methods: A systematic review was completed according to PRISMA guidelines. An electronic search in five databases was performed. Studies published until October 2013 were included if risk factors for at least one CDI outcome were assessed with multivariate analyses.

Results: 68 studies were included: 24 assessed risk factors for recurrence, 18 for complicated CDI, 8 for treatment failure, and 30 for mortality. Most studies accounted for mortality in the definition of complicated CDI. Important variables were inconsistently reported, such as previous episodes and use of antibiotics. Substantial heterogeneity and methodological limitations were noted, mainly in the sample size, the definition of the outcomes and periods of follow-up, precluding a meta-analysis. Older age, use of antibiotics after diagnosis, use of proton pump inhibitors, and strain type were the most frequent risk factors for recurrence. Older age, leucocytosis, renal failure and co-morbidities were frequent risk factors for complicated CDI. When considered alone, mortality was associated with age, co-morbidities, hypo-albuminemia, leucocytosis, acute renal failure, and infection with ribotype 027.

Conclusion: Laboratory parameters currently used in European and American guidelines to define patients at risk of a complicated CDI are adequate. Strategies for the management of CDI should be tailored according to the age of the patient, biological markers of severity, and underlying co-morbidities.

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Introduction

Highly associated with exposure to antibiotics, *Clostridium difficile* infection (CDI) causes 20 to 30% of antibiotic-associated diarrhoea and is the most common cause of nosocomial diarrhoea [1–4]. The risk of CDI increases up to 6-fold during antibiotic therapy and in the subsequent month [5,6]. In the early 2000s, a renewed interest in CDI followed the emergence of a hypervirulent strain (NAP1/BI/027) associated with frequent recurrences and higher severity [7,8]. Several novel treatments of CDI are being studied, some of which have been associated with a lower risk of recurrence [9–11].

Identifying clinical parameters or host-related factors associated with adverse outcomes would improve the management of CDI in the early stage of the disease. In a previous systematic review [12], we showed that several studies used empirically-defined risk factors for the derivation of clinical prediction rules for unfavourable outcomes of CDI, while others used univariate comparisons between CDI and non-CDI groups. Few clinical variables remained significant in multivariate analyses.

Risk factors for unfavourable outcomes of CDI have been studied before and after the emergence of NAP1/BI/027. To our knowledge, only one systematic review with a meta-analysis, published in 2008, has addressed risk factors for recurrence with a search limited to PubMed [13]. More recently, a systematic review of risk factors for mortality pooled results of univariate and multivariate analyses of hospital-based studies [14]. Two other reviews that ascertained CDI-related mortality were performed but specific risk factors were not reported [15,16]. Consequently, we performed a systematic review of all publications that identified risk factors for recurrence, treatment failure, complications and/or mortality in patients diagnosed with CDI.

Methods

Search strategy and selection criteria

A systematic review was performed according to PRISMA guidelines [17] (Checklist S1) using an electronic search of all studies published from January 1978 until October 2013. The search was limited to human studies and used the following online

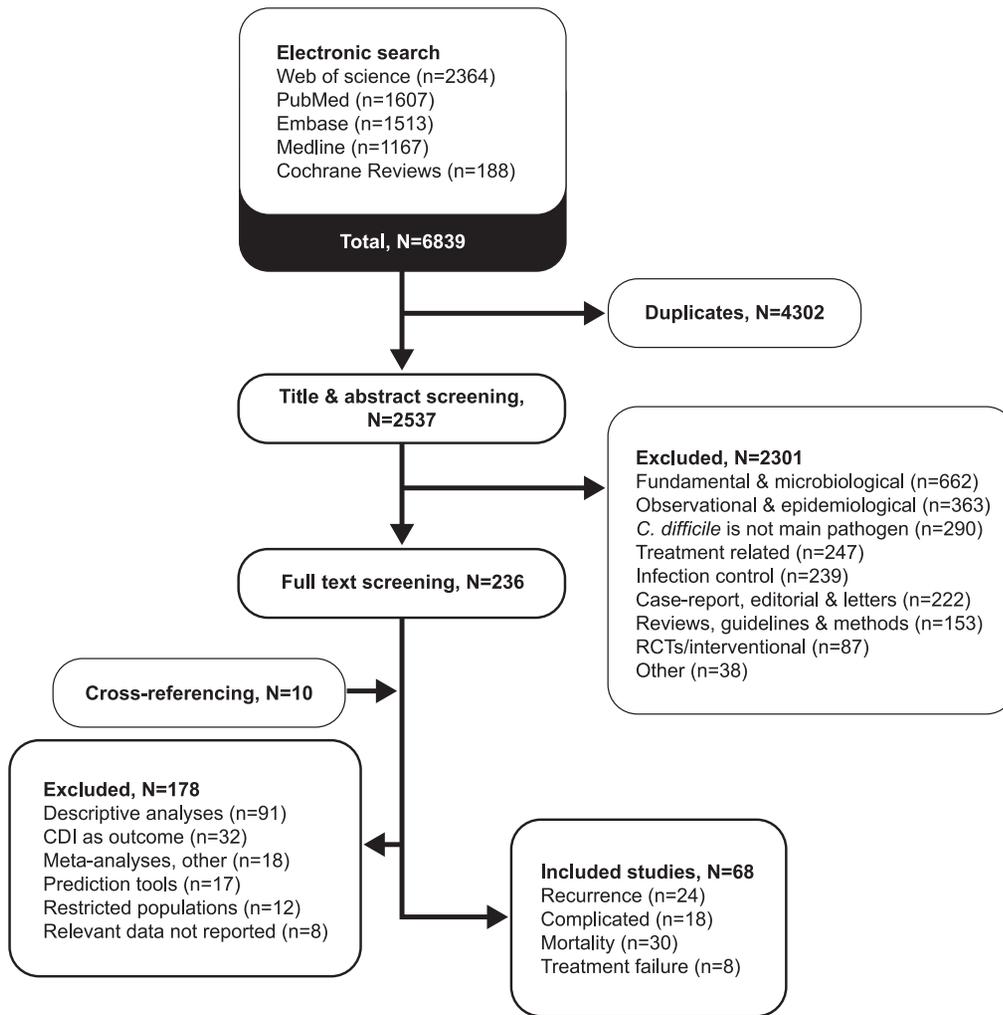


Figure 1. Flowchart of included and excluded publications.

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libraries and databases: MEDLINE, PubMed, Cochrane Library for evidence based-medicine, Embase and Web of Science (Text S1). The final electronic search was performed on 21 October 2013. Publications from all sources were merged into one file and duplicates were removed. A first screening of titles and abstracts followed by a full-text review were performed. In addition, the reference lists of identified studies were searched manually.

We included studies that: i) targeted *C. difficile* as the main pathogen; ii) measured at least one relevant outcome: severity, complications, mortality, treatment failure and/or recurrence; iii) identified risk factors for the main outcome(s) using risk assessment measures such as odds ratios (OR), relative risks or ratios (RR) and hazard ratios (HR). Any complication, fulminant colitis, ICU admission, shock, and/or death (when used as part of a composite outcome) were grouped under “complicated CDI”. We excluded all studies that used only univariate comparisons of groups, aimed to develop a risk stratification tool or a predictive model [12], and those conducted exclusively in children, in populations with selected pathologies or undergoing particular procedures (e.g. organ transplants, CT-scans, or endoscopies).

Data extraction

Two reviewers (CAC and SS) extracted the following data into a standardized matrix: year of publication, location, year of diagnosis, type of tests for the laboratory diagnosis of CDI, definition and frequency of the outcome(s) of interest, study design, duration of follow-up, population and comparison groups, sample size, statistical analyses, number of variables and number of events per variable (EPV) in the final model, and main results in relation with the objectives of the review. Correspondences requesting clarifications were sent to authors in case of missing or incomplete data (n = 9).

Studies that assessed two or more outcomes were allocated to each category of outcomes. Results from included studies were plotted using GraphPad Prism 6.01 (GraphPad Software, San Diego, CA). Due to the small number of studies assessing common risk factors for defined outcomes, ORs, RRs and HRs with their confidence intervals (CI) are reported in the same forest plots. Some factors such as multi-organ failure or other severe medical status immediately preceding mortality were considered too closely related to death in the pathogenic pathway and were therefore not considered as risk factors in this review.

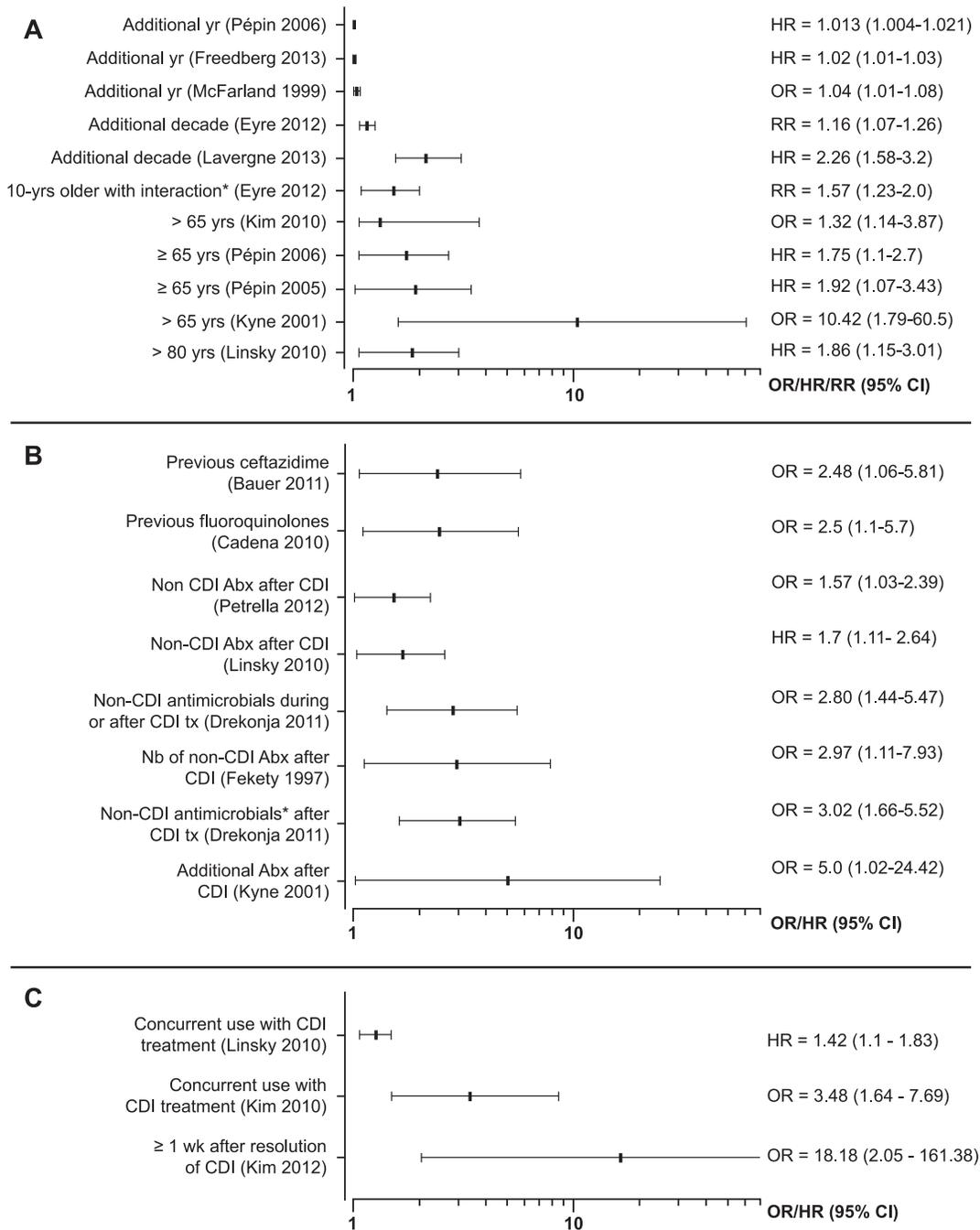


Figure 2. Forest plots of associations of age, antibiotic use and PPIs with recurrence of CDI. *Effect of age in deciles in interaction with previous dialysis/chemotherapy. *Non-CDI antimicrobial within 30-days of completing treatment for CDI. doi:10.1371/journal.pone.0098400.g002

Risk of bias assessment

A quality control process was performed on 10% of the first screening of abstracts (LV), as well as on included studies. Reviewers had a good agreement concerning eligible studies and final inclusion (87%). Disagreements were resolved by a third party (JP).

Two methods were used for the assessment of the individual and overall risk of bias across studies: i) the number of EPV (recurrence, treatment failure, complications, and/or death) in the final multivariate model of each study, assuming that at least

10 EPV are necessary [18,19]; ii) relevant clinical and epidemiological variables in relation with CDI in each study, and adjustment for these variables in multivariate analyses. The main variables were: confirmed diagnosis of CDI, age, gender, the site of acquisition of the infection (SI), co-morbidities, occurrence and number of previous episode(s) of CDI (PE), recent antibiotic therapy (AB), immunosuppression (IS), use of anti-ulcer medication (AU), recent surgery or procedure (RS), and blood tests (white cell count, haematocrit, serum lactate, serum albumin, serum creatinine, and C-reactive protein).

Table 1. Association between unfavourable outcomes and strain type in multivariate analyses.

Study	Typing method	Strain type	Period of data collection	N of strains	% of strain (n)	OR/HR/RR (95% CI)
Recurrence						
Petrella 2012 [33]/ Louie 2013 [36]	REA	BI vs. non-BI	2007–2009	719	34 (247) ^a	1.57 (1.01–2.5)/1.6 (1.03–2.5)
Stewart 2013 [37]	PCR toxinotyping/ribotyping and tcdC genotyping	Tox A ⁺ B ⁺ CDT/Tox A ⁺ B ⁺ CDT + tcdC deletion	NR	69	61 (42) ^{**} / 56 (39) ^{**}	3.1 (2.97–3.3)/5.3 (3.5–6.1)
Marsh 2012 [38]	MLVA and tcdC genotyping	tcdC1 genotype (ribotype 027) vs. other	2001–2009	82	45 (37)	6.9 (1.7–28.2)
Eyre 2012 [25]	MLST	Ribotype 027 vs. clade 1 ^a	2006–2010	1076	28 (300)	1.2 (0.9–1.5)
Complicated CDI						
Bauer 2011 [46]	PCR toxinotyping and ribotyping		2008	389		
		Ribotype 018 vs. others			6 (23)	6.2 (1.28–29.8)
		Ribotype 056 vs. others			2 (6)	13.0 (1.1–148.3)
		Ribotype 015 vs. others			3 (13)	4.6 (0.98–21.2)
		Ribotype 027 vs. others			5 (19)	2.6 (0.6–10.2)
		Ribotype 014/020 vs. others			16 (61)	0.6 (0.2–2.2)
Sees 2012 [49]	PCR toxinotyping/ribotyping and tcdC genotyping	Tox A ⁺ B ⁺ CDT ⁺ vs. A ⁺ B ⁺ CDT ⁻	2006–2007	82	26 (21)	6.0 (1.5–23.8)
Walk 2012 [48]	PCR toxinotyping/ribotyping	Ribotype 027/078 vs. others	2010–2011	310	14 (43)	0.8 (0.07–10.0)
Rao 2013 [47]	PCR toxinotyping/ribotyping	Ribotype 027 vs. others	2010–2012	22	32 (7)	2.7 (0.3–25.3)
30-day mortality						
Inns 2013 [21]	PCR	Ribotype 027 vs. infrequent ^b	2009–2011	1426	10 (147)	1.3 (1.02–1.7)
Labbé 2008 [55]	PCR toxinotyping and ribotyping	Ribotype 027 vs. others	2000–01 & 2003–04 (outbreak)	230 175	61 (141) 29 (41)	2.1 (1.0–4.2)/7.5 (1.6–35.5)
Walker 2013 [23]	MLST, correlation with ribotypes	Ribotype 027 vs. clade 1 ^a	2006–2011	1893	20 (560)	3.4 (2.5–4.7)
Huttunen 2012 [56]	PCR	Ribotype 027 vs. others ^c	2008–2010	780	14 (111)	4.6 (1.4–15)
Goorhuis 2011 [57]	MLVA and STRD	Ribotype 027 vs. others ^d	2005–2007	168	27 (46)	10.5 (1.2–92)
Inns 2013 [21]	PCR	Ribotype 015 vs. infrequent ^b	2009–2011	1426	8 (111)	0.5 (0.3–0.8)
Goorhuis 2011 [57]	MLVA and STRD	Ribotype 017 vs. others ^d	2005–2007	168	34 (57)	8.9 (1.04–75.8)
Walker 2013 [23]	MLST, correlation with ribotypes	Ribotype 078 vs. clade 1 ^b	2006–2011	1893	2 (63)	5.4 (3.1–9.3)
Sees 2012 [49]	PCR toxinotyping/ribotyping and tcdC genotyping	Tox A ⁺ B ⁺ CDT ⁺ vs. A ⁺ B ⁺ CDT ⁻	2006–2007	82	26 (21)	1.0 (0.2–5.1)

REA = Restriction endonuclease analysis. PCR = Polymerase chain reaction. MLVA = Multiple-Locus Variable number tandem repeat. STRD = Summed Tandem-Repeat Difference. MLST = Multilocus Sequence Typing. NR = not reported;

^aOverall % of strain BI in the cohort, the % in the sub-population used for multivariate analyses was not reported. ^{**}% of binary toxin gene and tcdC mutation respectively, the % of combinations were not reported.

^cComparison of clade 2 with 99% PCR ribotype 027 vs. clade 1, and clade 5 with 100% PCR ribotype 078 vs. clade 1. ^b Compared to infrequent ribotypes in the study (other than R01, 02, 05, 015, 016, 023, 027, 064, 078 and 106). ^c Hypervirulent strain vs. non-hypervirulent, ribotype 027 was prevailing during the study period. ^d Other ribotype: non-027 and non-017.

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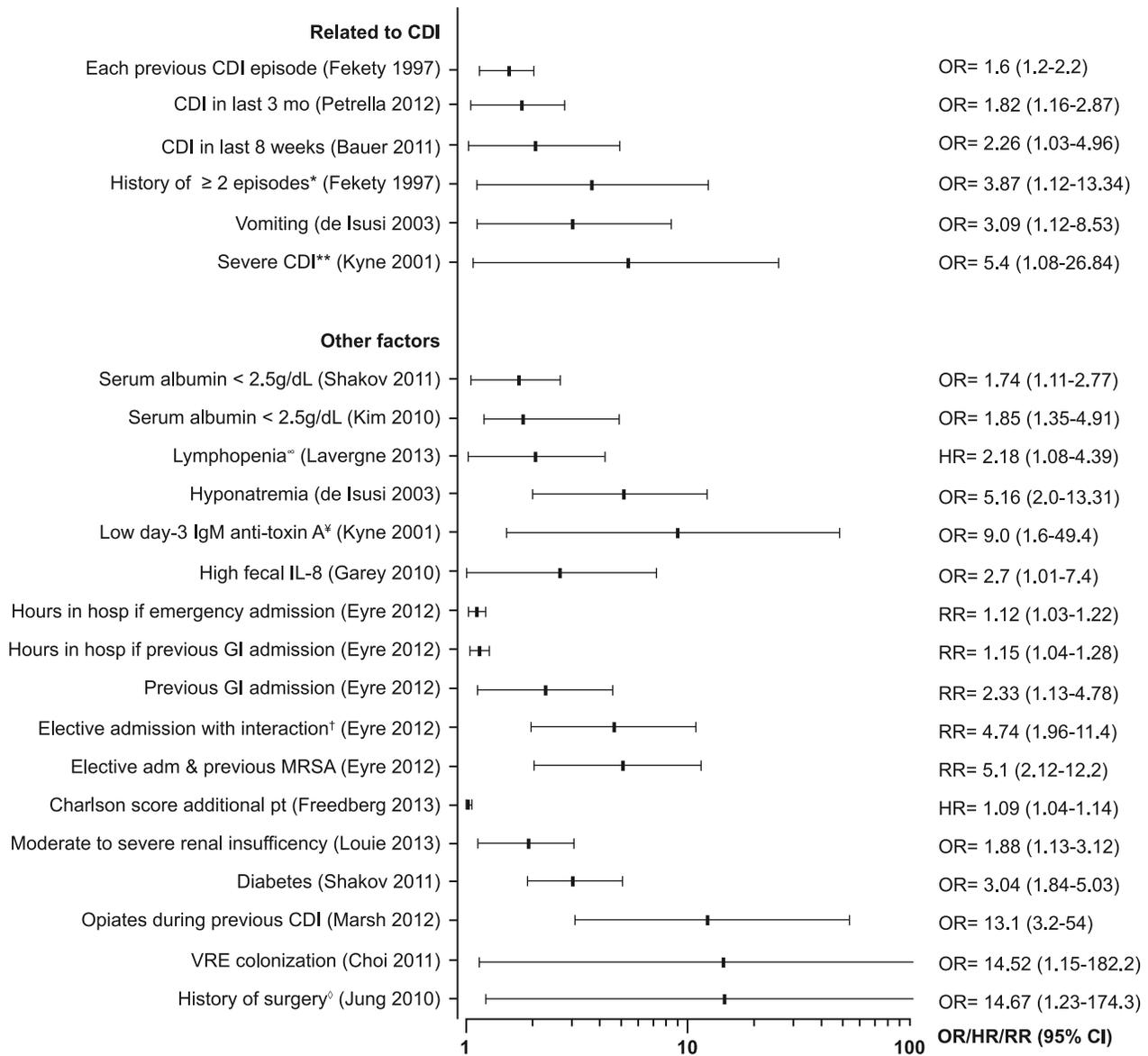


Figure 3. Other risk factors for recurrence of CDI. * History of recurrence vs. new CDI. ** Modified Horn's index (3 pts). [∞] Lymphopenia at completion of CDI treatment: Absolute cell count $<1.0 \times 10^9/L$. [‡] <2.22 ELISA units, adjusted on disease severity. [†] Elective admission vs. emergency if previous dialysis/chemotherapy (interaction). [◊] History of surgery within 1 month before CDI treatment. MRSA = previous methicillin-resistant *Staphylococcus aureus* (interaction). VRE = vancomycin-resistant enterococci.
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Results

The electronic search led to 6839 publications. After excluding duplicates, 2537 were reviewed by their title and abstract (Figure 1), among which 2301 were excluded at the first screening and 178 after full-text verification. We included in this review 68 studies that examined risk factors for one or more outcomes: 19 assessed risk factors for recurrence only, 11 for complicated CDI only (including or not mortality), two for treatment failure only, 23 for mortality alone (among them six in patients needed colectomy), and 13 for multiple outcomes (including six for treatment failure). The characteristics of included studies are shown in tables S1 to S4. The majority of included studies used retrospective cohorts (45; 66%), 15 used prospective cohorts (22%), four were retrospective case-control studies (6%), and four were clinical

trials (6%). Except for six studies using administrative databases [20–26], sample sizes were small with a median of 128 patients (range 13–2042). Most studies (14/18) on complicated CDI included death (mostly all-cause 30-day mortality) within a composite outcome. The method used for CDI toxin detection was reported in 94% (n = 64) of studies: Toxin A and B enzyme immunoassay (EIA) was used in 39% (n = 25), direct cytotoxin assay (CTA) in 19% (n = 12), toxin A EIA in 9% (n = 6), polymerase chain reaction (PCR) in 9% (n = 6), toxigenic culture in 5% (n = 3), unspecified toxin assay in 11% (n = 7), and combined approaches in 12% (n = 8).

Overall, the risk of experiencing at least one recurrence ranged between 12% and 64% (median 22%; n = 26 studies). Risk of a complicated CDI (including or not death) ranged between 7% and

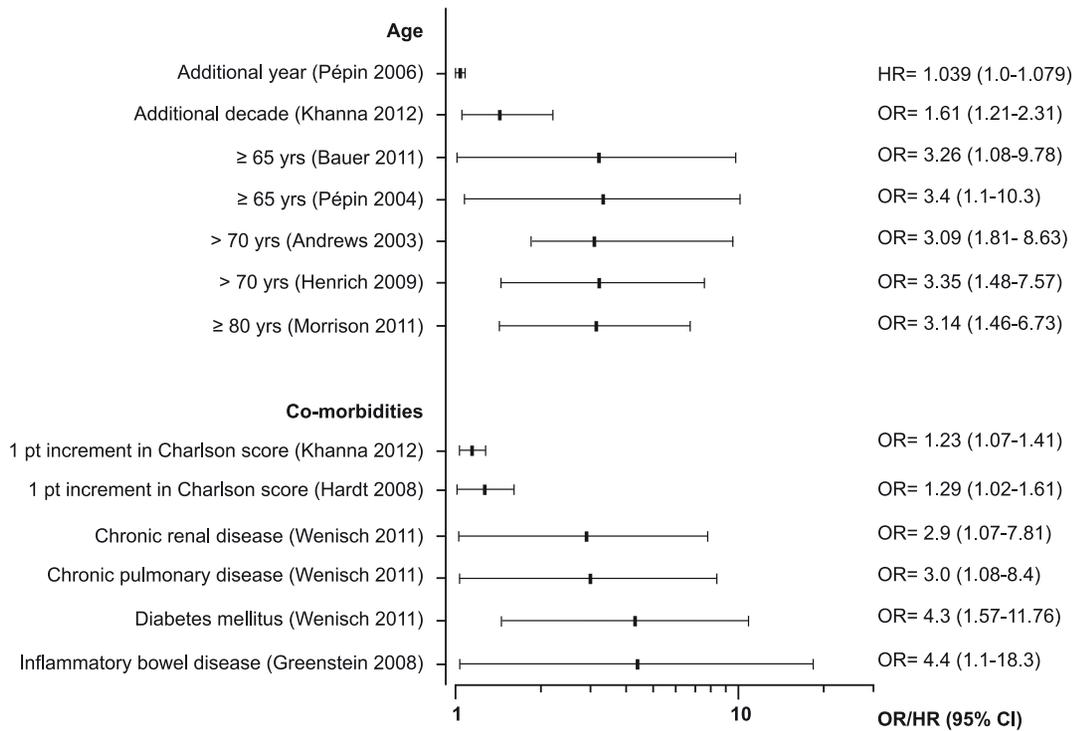


Figure 4. Forest plots of reported associations with complicated CDI: age and co-morbidities or health status.
doi:10.1371/journal.pone.0098400.g004

48% (median 18%; n = 15), and treatment failure between 5% and 50% (median 21%; n = 9). In studies on mortality alone, risk of 30-day mortality ranged between 8% and 53% (median 19%; n = 14).

Predictably, mortality was higher in selected patients who needed an emergency colectomy (median 38%, range = 31–46%; n = 6) or ICU admission (median 36%, range = 28%–53%; n = 4).

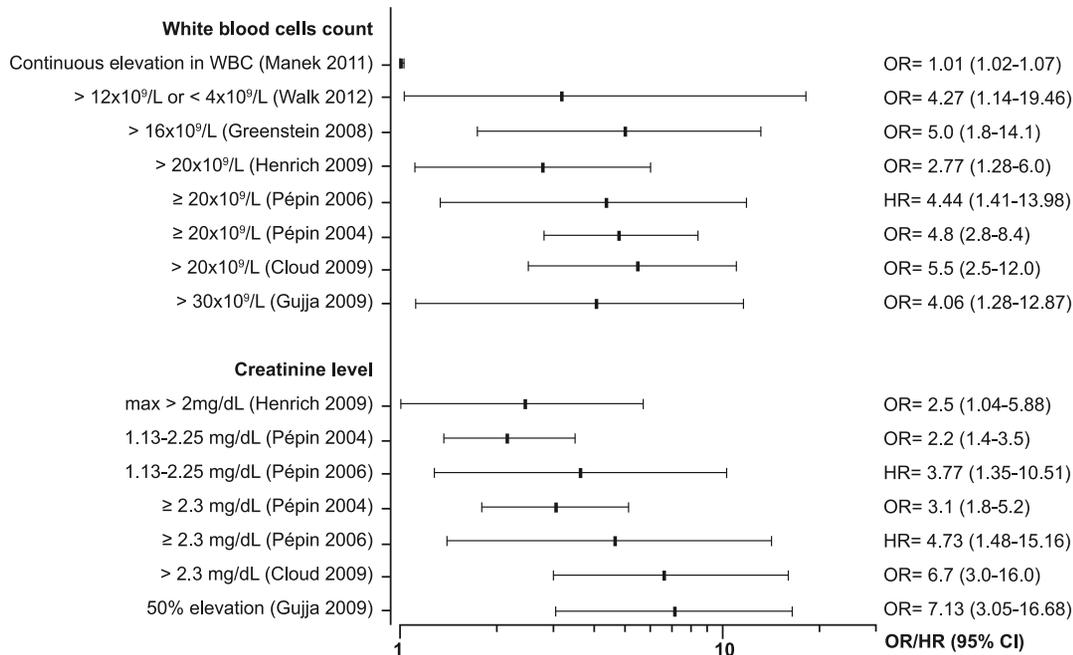


Figure 5. Forest plots of reported associations with complicated CDI: white blood cells count (WBC) and creatinine levels. WBC units were converted to the international system unit (10⁹/L). Creatinine levels were converted to the conventional unit using the formula: Creatinine [mg/dL] = creatinine/88.4 [μmol/L].
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Table 2. Infrequent risk factors for complicated CDI and 30-day mortality.

Factor	OR/HR/RR (95% CI)
Complicated CDI	
Hospital-acquired CDI [42]	4.6 (2.4–8.6)
Severe diarrhoea [85]	3.6 (1.2–11.1)
Small bowel obstruction or ileus [41]	3.1 (1.0–9.4)
Recurrent CDI [44,45]	2.7 (1.2–5.8), 4.1 (1.5–9.4)
Serum albumin <2.5g/dL [41]	3.4 (1.6–7.6)
Increase in C-reactive protein* [86]	1.15 (1.08–1.2)
Increase in procalcitonin level [47]	3.1 (1.5–6.3)
Abnormal abdominal CT-scan [41]	13.5 (5.7–32.1)
Confusion [44]	2.0 (1.05–3.8)
Abbreviated mental score <7 [87]	11.0 (2.3–58.8)
Endoscopy [87]	4.0 (1.2–14.9)
Tube feeding within prior 2 months [42]	2.4 (1.5–3.9)
Any operative therapy within prior 30 days [88]	3.5 (1.1–10.8)
Surgery in the previous two months [42]	0.6 (0.4–0.9)
Immunosuppression** [42]	2.3 (1.5–3.6)
Prior corticosteroid use [58]	2.1 (1.01–4.35)
Prior acid suppression use [58]	2.4 (1.2–4.8)
Prior intravenous immunoglobulin therapy [88]	8.9 (2.2–36.1)
Prior use of fluoroquinolones [43]	2.0 (0.98–4.1)
Use of exacerbating Abx after CDI [44]	3.0 (1.6–5.8)
30-day mortality	
Colectomy [73,89]	0.2 (0.1–0.7); 40 (2.8–576.4)
Prolonged hospitalization before CDI (> 15 days) [90]	0.13 (0.03–0.6)
Hospital-acquired CDI [21]	1.9 (1.5–2.6)
ICU care [91]	2.8 (1.5–5.4)
Response failure to treatment [91]	3.9 (1.4–10.7)
Occult blood in stool [92]	0.32 (0.11–0.9)
Positive stool occult blood test [90]	6.3 (1.13–35.3)
Peak lactate \geq 5 mmol/L [73]	12.4 (2.4–63.7)
Low peak day 12 anti-toxin A IgG [93]	0.97 (0.95–0.99)
Immunosuppression [89]	35.8 (2.8–464.5)
Immunosuppression *** for at least 1 month [73]	7.9 (2.3–27.2)
Any glucocorticoid use [94]	1.8 (1.62–1.98)

Abx = antibiotics. ICU = intensive care unit. IgG = immunoglobulin G.

*For each increment of 10 mg/mL.

**Chemotherapy, HIV infection, neutropenia, organ transplantation, or use of immunosuppressive drugs.

*** Systemic corticosteroids, leukaemia, lymphoma, organ transplant, or neutropenia.

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Analysis of risk factors

1. Risk factors for recurrence (24 studies). Recurrent CDI was assessed through pre-defined follow-up performed at 60 and 90 days after diagnosis in only four prospective cohorts and four clinical trials (Table S1). The interval between the recurrent and the first episodes varied between 2 and 180 days after completion of therapy. Frequent risk factors for recurrence are shown in Figure 2: age (9 studies) (Figure 2A), antibiotics during or after CDI diagnosis (7 studies) (Figure 2B), and use of PPIs (3 studies) (Figure 2C). The relative risk for recurrence ranged between 1.01 and 1.04 for each additional year of age [26–29], ENREF_27 between 1.3 and 10.4 with age >65 years [26,30–32], between 1.6 and 5.0 with use of antibiotics after CDI

[20,32–34], and between 1.4 and 18.2 with use of PPIs [20,30,35]. In four studies with different typing methods [33,36–38], the hypervirulent strain (NAP1/BI/027) was associated with recurrence (Table 1), but this association was not significant in a study using genome sequencing [25].

Risk of recurrence was inconsistently associated with the site of acquisition: community-acquisition of CDI was highly associated with recurrence in one study (OR = 11.2; $p = 0.02$) [39], while acquiring CDI in hospital and each additional day of hospitalization were risk factors in two others (HR = 1.5; 95% CI = 1.1–2.1 and HR = 1.01; 95% CI = 1.0–1.02, respectively) [26,31]. Many other risk factors were examined, and among them three were considered as related to recurrent CDI, but each in only one or

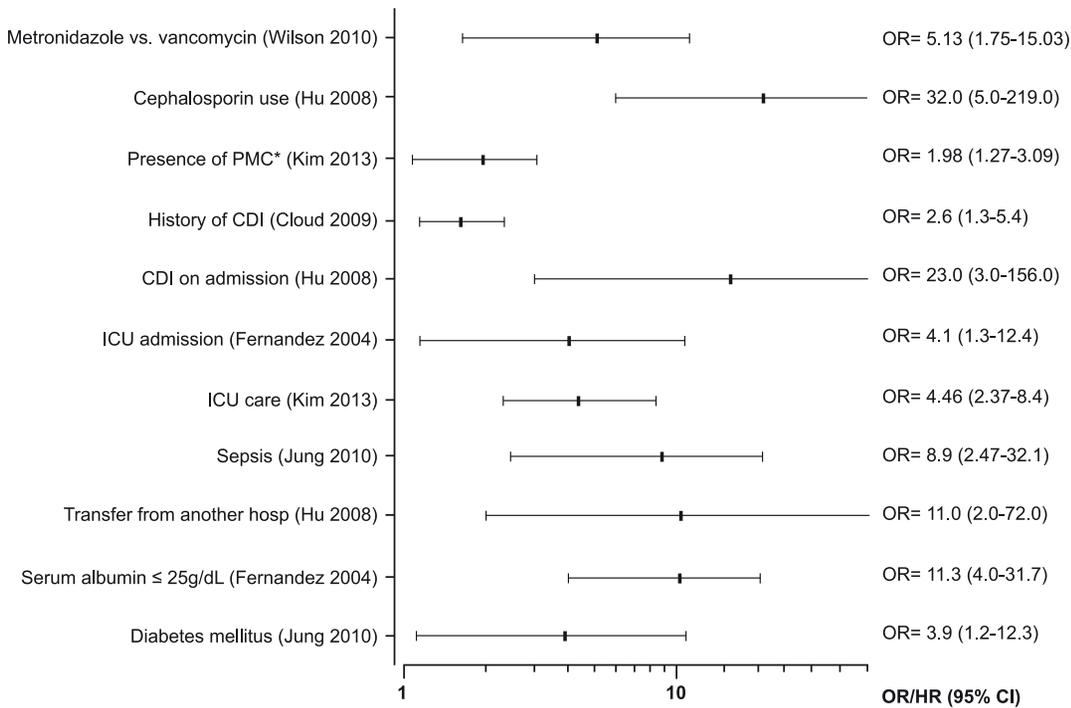


Figure 6. Forest plots of reported associations with treatment failure. *PMC = pseudomembranous colitis. doi:10.1371/journal.pone.0098400.g006

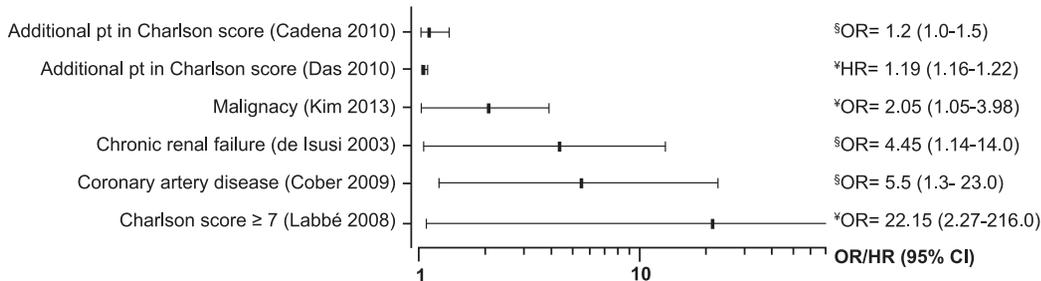
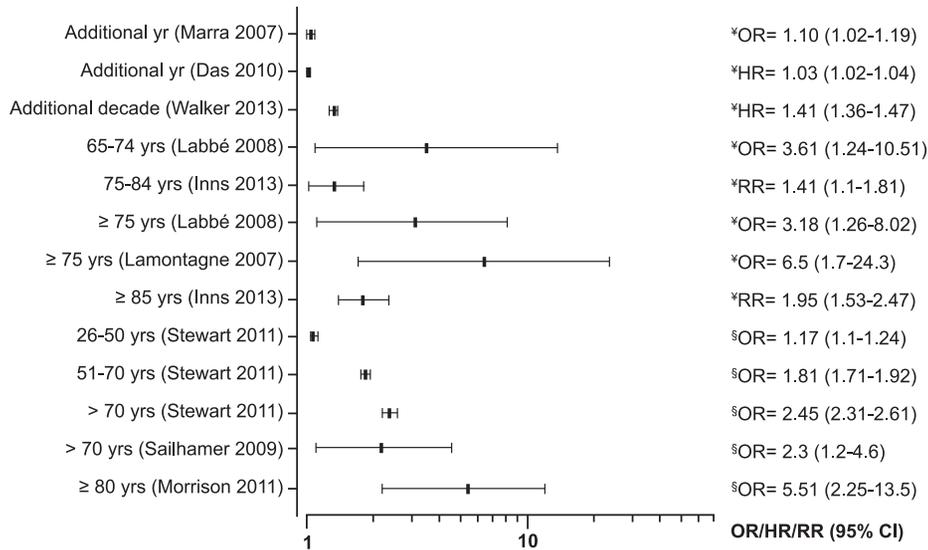


Figure 7. Forest plots of associations of age and co-morbidities with mortality. (¶ ≤30-day mortality; § >30-day). doi:10.1371/journal.pone.0098400.g007

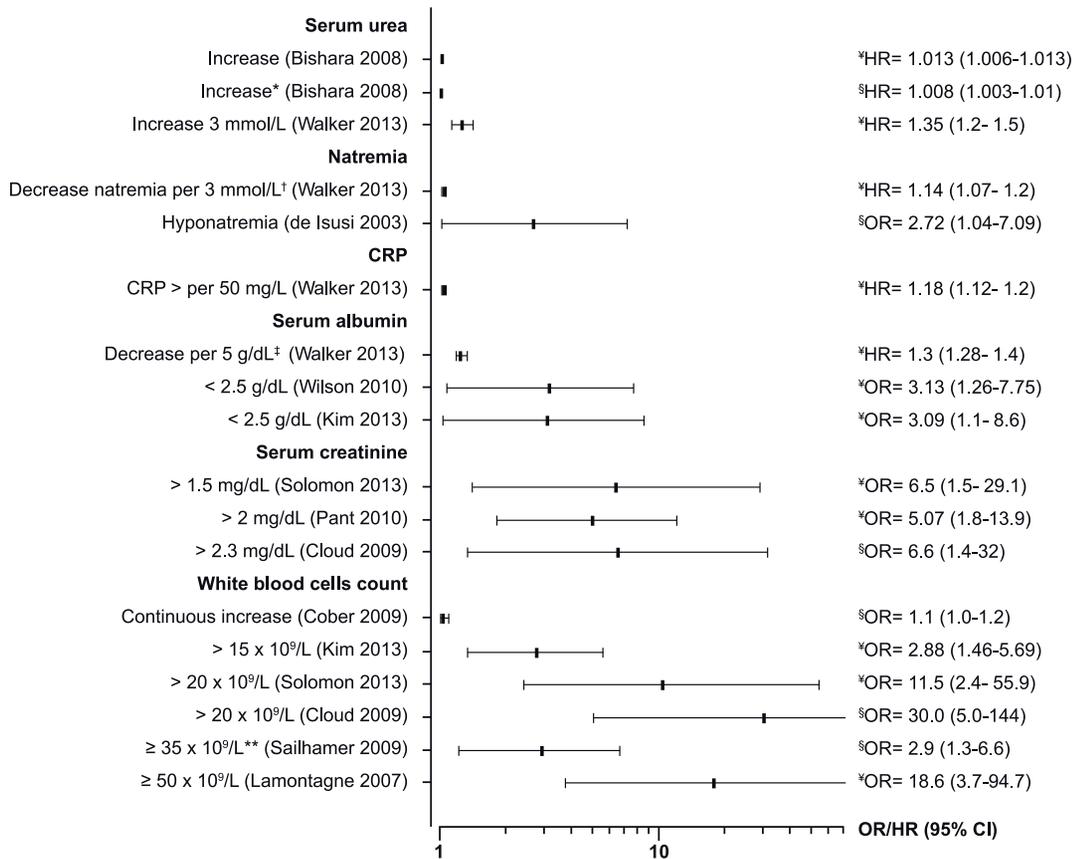


Figure 8. Forest plots of associations of blood tests with mortality. (¶≤30-day mortality; § >30-day). *Increase in serum urea associated with 28-days and long-term mortality. †Original value: Sodium per 3 mmol/L higher <136; HR=0.88 (0.83–0.93). **Leucocytosis: WBC≥35×10⁹/L or leucopenia: WBC<4×10⁹/L. ‡Original value: Albumin per 5 g/dL higher; HR=0.74 (0.71–0.78). doi:10.1371/journal.pone.0098400.g008

two studies (Figure 3). The role of the immune response was addressed in only three studies (Figure 3) [29,32,40], but all showed that recurrence was associated with low antibody titres (IgM and IgG anti-toxin A, and IL-8) [32,40], and a positive *C. difficile* antitoxin serology (HR = 0.17; 95% CI = 0.05–0.59) [29].

2. Risk factors for complicated CDI (18 studies). The definition of complicated CDI varied between studies, resulting in much heterogeneity (Table S2 and Table S3). Frequent risk factors identified in several studies were: older age and underlying comorbidities (7 and 4 studies respectively) (Figure 4), high leucocyte count (8 studies) and acute renal failure (5 studies) (Figure 5). The relative risk of complicated CDI ranged between 2.7 and 5.5 with leucocytes count >20×10⁹/L [41–43], and between 3.1 and 6.7 with creatinine >2.3 mg/dL [26,42,43].

Recurrent CDI was strongly associated with an increased likelihood of complicated CDI (OR = 2.7; 95% CI = 1.2–5.8 and OR = 4.1; 95% CI = 1.5–9.4, respectively) [44,45], as well as exposure to particular treatments (Table 2). Ribotypes 018 and 056 were risk factors for complicated CDI (OR = 6.2; 95% CI = 1.3–23.8 and OR = 13.0; 95% CI = 1.1–148.3, respectively), in a pan-European study (Table 1) [46]. Ribotype 027 was not significantly associated with complicated CDI in multivariate analysis nor with indices of severity in other studies [47,48], while *_ENREF_48* strains harbouring binary toxin gene were associated with complicated CDI in one study (OR = 5.9; 95% CI = 1.5–23.8) [49]. Other factors were associated with complicated CDI in one or two studies each (Table 2).

3. Risk factors for treatment failure (8 studies). The definition of this outcome was heterogeneous, corresponding to a lack of improvement of symptoms after 5 to 10 days of the initial treatment (Table S2 and Table S4). Only need of intensive care was associated with treatment failure (mainly during metronidazole treatment) in more than one study (Figure 6). Increasing age (in decades OR = 1.14; 95% CI = 1.01–1.29) and increasing WBC in elderly patients (OR = 1.1; 95% CI = 1.0–1.2) were significant factors in one study each [50–52].

4. Risk factors for mortality (30 studies). Most included studies (73%) measured mortality within the 30-day interval after diagnosis, as per the current recommendations for CDI surveillance [1]. In the other studies, follow-up ranged between 14 [23,53], 60 [43], and 90 days [51,54], while nine studies did not specify any duration (Table S3 and Table S4).

Mortality, overall or due to CDI, was mainly associated with age (9 studies), underlying co-morbidities (6 studies) (Figure 7), and laboratory parameters (overall 11 studies): leucocytosis, increased serum urea, increased serum creatinine, elevated C-reactive protein, hypo-natremia and serum albumin (Figure 8). Ribotype 027 was associated with 30-day mortality in 5 studies with a relative risk ranging between 1.3 and 10.4 (Table 1) [21,23,55–57].

A severe CDI defined by two or more of age >60, leucocytosis, albumin <2.5 mg/dL or ICU admission almost doubled the risk of 90-day overall mortality after adjustment for co-morbidities (OR = 1.8; 95% CI = 1.2–2.6) [54]. Laboratory parameters were

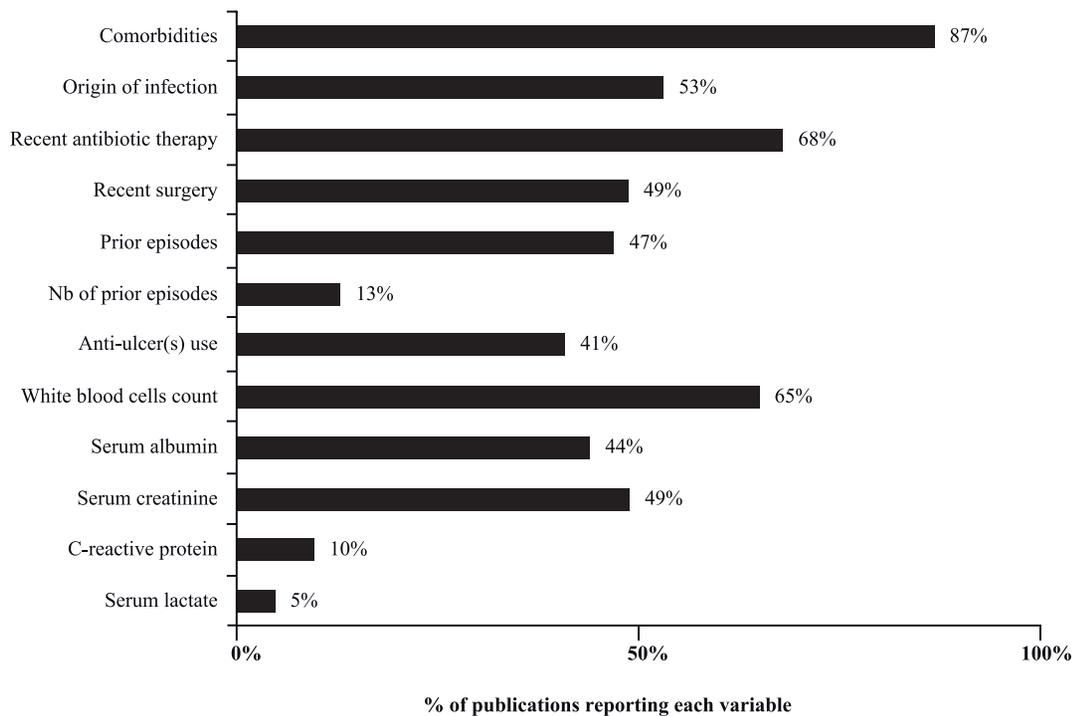


Figure 9. Quality evaluation of included studies (n = 68) according to reported clinical data.
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associated with all-cause 30-day mortality in one study each (Figure 8). High levels of WBC ($>20 \times 10^9/L$ and $\geq 50 \times 10^9/L$) were more strongly associated with death than with complicated CDI (Figure 5) [43,54]. Other factors associated with 30-day mortality reported in one study or two studies are shown in Table 2. Continuous increase in WBC was associated with 90-day mortality in one study [51], and prior exposure to acid suppression therapy was associated with mortality in one study where the delay was not reported [58]. In one study [43], death with CDI as contributor was associated with WBC $>20 \times 10^9$ cells/L, serum creatinine >2.3 mg/dL and exposure to fluoroquinolones within 60 days.

Six other studies were conducted on patients requiring surgical treatment for CDI (colectomy or hemicolectomy) [24,59–64]. Risk factors associated with mortality were older age [62,63], high leucocytosis [61], preoperative hypo-albuminaemia [61,62], preoperative increase in serum lactate [62], and duration of treatment [59].

Risk of bias assessment

Almost all studies reported age and gender (96%) of their study populations, and the majority reported confirmed cases of CDI and co-morbidities (90% and 87% respectively). Only half (53%) of studies reported the site of acquisition (nosocomial versus community-acquired), recent surgical or other procedures (49%) and previous episodes of CDI (47%) (Figure 9). One third of included studies (n = 23) provided strain typing of *C. difficile*, but only 14 included the strain type in multivariate analyses. The association with outcomes and the period of data collection are presented in Table 1. Recent antibiotic (46; 68%) and immunosuppressive therapies (38; 56%) were frequently reported. Very few studies reported measures of serum lactate, C-reactive protein, and procalcitonin.

Among studies on mortality alone, only half of them reported the site of acquisition of CDI, as did around half of studies on recurrence and 64% on complicated CDI. Having experienced any previous episode of CDI was reported in only 53% of studies on recurrence and mortality (56%), and in only one third of studies on complicated CDI and multiple outcomes.

As for statistical analyses, the median number of variables in the final model (including statistically significant variables and all adjustments) was 7 (range 2–18). The median number of EPV was only 6.6 (range 0.6–430) (Table S1–S4). Only one-third (23; 34%) of studies had 10 EPV or more.

Discussion

This review is the largest on unfavourable outcomes of CDI (68 studies), based on publications from 1978 until 2013, and the first to gather risk factors for CDI-related complications. It also represents an important update about risk factors for recurrence. Publications were subjected to two stages of screening before final inclusion and a quality control process was performed during all steps of the review. Studies with univariate comparisons were left out, as it would be irrelevant to consider clinical parameters, comorbidities and medications as independent factors when confounding and interaction were not addressed through multivariate analyses. A previous review of studies with a sample size ≥ 100 patients [14], used mortality as a keyword rather than an outcome for the search as suggested by Population Intervention Comparison Outcome (PICO) frameworks [17], which could have restricted the number of retrieved studies. Moreover, only inclusion criteria and study characteristics were used as markers of acceptable quality [14]. However, according to PRISMA guidelines [17], we qualitatively assessed the risk of bias across studies, but did not assess it individually. Recent reviews showed a lack in relevant tools such as scales, checklists, or quality criteria

for observational studies [65–67]. Available tools involve a subjective assessment of risk of bias, leading to inconsistent validity and reliability [65], and are more appropriate for interventional trials. Consequently, we assessed the quality of studies according to standard methodologies, and used an objective statement of bias through the measurement and reporting of relevant data.

As in a previous review, continued use of antibiotics, concomitant anti-ulcer medication and older age were risk factors for recurrence [13]. Concomitant use of antibiotics and PPIs have an additive effect on increasing susceptibility to CDI [68,69], which could explain the higher risk of recurrence. However, multivariate adjustment on use of antibiotics or PPIs was performed in only four [20,32,34,35], of the nine studies where those variables were associated with recurrence.

Several other limitations were observed across included studies. Small sample sizes (median 128) led to wide confidence intervals in estimations of relative risks. Adjustment for confounders was not always clear in included publications even if this represents an important factor for the validity of results [70]. Only 14 studies included the strain type as an independent variable in multivariate analysis. We could not use the year 2002 as a cut-off date for the introduction of NAP1/BI/027 strain [8,42], because the timing of its introduction varied considerably between countries and regions, and several studies collected data over long periods overlapping this date (Table 1).

Why we could not perform a meta-analysis

The quality of a meta-analysis depends heavily on the individual quality of pooled data. Hence, multiple methodological gaps and substantial heterogeneity across included studies would have led to an inappropriate meta-analysis. Most studies were conducted retrospectively with data gathered from medical charts and/or electronic databases. Although minimizing recall biases, this methodology is often hampered by missing data. Missing data in the original publications, mainly observational studies, was an important limitation for the estimation of the effect of risk factors. For instance, while previous episodes are likely to be a risk factor for recurrence [46,71], only half of included studies reported any previous episode of CDI and 13% reported the number of previous episodes. Except for mortality, the definition of the outcome, particularly complications, and the duration of follow-up differed between studies. Most studies accounted for all-cause mortality within their definition of complicated CDI. Risk of 30-day mortality ranged between 8 and 31% in studies having death as the main outcome where all CDI cases were considered, while four studies conducted on patients enrolled in ICU [53,72–74] reported risk of mortality ranging from 25 to 53%. In studies of patients who underwent a colectomy, where rates of mortality were particularly high, data were collected over 7 to 13 years, and except for one study [24], sample sizes were very small ($n = 13–130$). All of those studies recommended early surgery to prevent organ failure and to decrease mortality. Thus variations in overall mortality reflected either the selection of the sickest patients, causes of death unrelated to CDI, or perhaps differences in the pathogenicity of local *C. difficile* strains. Treatment failure was considered separately from complications in 8 studies, but without any common risk factors.

Poor reporting and considerable heterogeneity was noted in the diagnostic tests which defined cases of CDI, these tests differing in sensitivity and specificity [75,76]. Diagnosis was mostly confirmed with EIAs (toxin A alone, or A+B) despite their low sensitivity [77]. Only 33% of the studies used diagnostic tests of higher sensitivity and specificity: CTA in 19% ($n = 12$), PCR in 9% ($n = 6$), and toxigenic culture in 5% ($n = 3$). As a consequence, studies using

EIAs might have included sicker patients, while those based on PCR might have included patients merely colonized with *C. difficile* presenting an episode of diarrhea unrelated to this pathogen, and patients at an early stage of the disease [78,79]. In addition, the methods used for strain typing and the definition of some variables such as the scores for co-morbidities and severity of CDI were highly heterogeneous. The cut-off points considered for leucocytosis varied between 12 and $>50 \times 10^9/L$. A similar wide range was observed in the creatinine level. Evaluation of laboratory parameters as predictors was limited to frequently ordered tests: less than 10% of studies reported levels of serum lactate or C-reactive protein or procalcitonin.

Current guidelines for case-management

Currently, two American guidelines define patients with severe CDI (for whom the initial treatment should be vancomycin, a drug thought to lower the risk of complications) as those with a leucocytosis ($WBC >15 \times 10^9/L$) and/or a creatinine >1.5 times the baseline [80], and with $WBC >15 \times 10^9/L$ plus a serum albumin <3 g/dl or abdominal tenderness [75]. European guidelines use the same cut-offs of leucocytosis and creatinine, but include many other clinical, radiologic or laboratory criteria in their definition of severe CDI for whom vancomycin is recommended [81]. Whether age over 65 years or co-morbidities should by themselves be a criterion for severity is left to the discretion of the attending physician [81]. A recent meta-analysis on the treatment of recurrent CDI provided moderate evidence on the efficacy of available treatments [82]. Despite low to moderate evidence, vancomycin combined with metronidazole was recommended for severe and complicated cases [75]. Thus, while the three laboratory parameters (leucocytes, serum creatinine and albumin) identified by our systematic review are incorporated within current guidelines, older age remains to be properly addressed.

Conclusions

Currently available studies about risk factors and clinical parameters allowing the prediction of unfavourable outcomes in CDI are heterogeneous. Older age, antibiotics after the diagnosis of CDI, use of PPIs, and strain type are the most frequent risk factors for recurrence. Older age, leucocytosis, renal failure and underlying co-morbidities are frequent risk factors for complicated CDI, including mortality in many cases. As for mortality alone, in addition to age, it seems to be associated with co-morbidities, decreased serum albumin, leucocytosis, increased serum creatinine and/or urea and ribotype 027 (30-day mortality). Laboratory parameters used in American and European guidelines (high leucocytosis, acute renal failure) are adequate to define patients at risk of complications. The patient's age should be a key factor in the management of CDI. It would seem advisable for future iterations of these guidelines to incorporate age within their decisional algorithms, so as to offer to the elderly potentially more effective drugs such as vancomycin or fidaxomicin.

Addendum

While this manuscript was being evaluated, a study documented an association between low levels of vitamin D and increasing severity of CDI (defined as an abnormal CT scan and fulminant colitis) [83], and another one reported an association between low vitamin D levels and a composite outcome of all-cause 30-day mortality and/or recurrence [84]. Both were small studies and

further work is necessary to define whether or not vitamin D deficiency is genuinely associated with adverse outcomes of CDI.

Supporting Information

Table S1 Characteristics of included studies addressing risk factors for recurrence [20,25,27–40,71,95,96].

(PDF)

Table S2 Characteristics of included studies addressing risk factors for complicated CDI and treatment failure [41,42,44,45,47,48,50,85–88,97,98].

(PDF)

Table S3 Characteristics of included studies addressing risk factors for mortality considered alone [21–24,53,55–57,59–63,72–74,89,90,92,94,99–101].

(PDF)

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Table S4 Characteristics of included studies addressing risk factors for multiple outcomes [26,43,46,49,51,52,54,58,91,93,102–104].

(PDF)

Checklist S1 PRISMA checklist.

(DOC)

Text S1 Electronic search: databases and keywords.

(PDF)

Author Contributions

Conceived and designed the experiments: CNAC JP LV. Performed the experiments: CNAC SS LV. Analyzed the data: CNAC JP SS LV. Contributed reagents/materials/analysis tools: CNAC SS. Wrote the paper: CNAC JP SS LV.

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