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The Strain and the Clinical Outcome of *Clostridioides difficile* Infection: A Meta-analysis

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Background. The association between bacterial strains and clinical outcomes in *Clostridioides difficile* infection (CDI) has yielded conflicting results across studies. We conducted a systematic review and meta-analyses to assess the impact of these strains.

Methods. Five electronic databases were used to identify studies reporting CDI severity, complications, recurrence, or mortality according to strain type from inception to June 2022. Random effect meta-analyses were conducted to assess outcome proportions and risk ratios (RRs).

Results. A total of 93 studies were included: 44 reported recurrences, 50 reported severity or complications, and 55 reported deaths. Pooled proportions of complications were statistically comparable between NAP1/BI/R027 and R001, R078, and R106. Pooled attributable mortality was 4.8% with a gradation in patients infected with R014/20 (1.7%), R001 (3.8%), R078 (5.3%), and R027 (10.2%). Higher 30-day all-cause mortality was observed in patients infected with R001, R002, R027, and R106 (range, 20%–25%).

NAP1/BI/R027 was associated with several unfavorable outcomes: recurrence 30 days after the end of treatment (pooled RR, 1.98; 95% CI, 1.02–3.84); admission to intensive care, colectomy, or CDI-associated death (1.88; 1.09–3.25); and 30-day attributable mortality (1.96; 1.23–3.13). The association between harboring the binary toxin gene and 30-day all-cause mortality did not reach significance (RR, 1.6 [0.9–2.9]; 7 studies).

Conclusions. Numerous studies were excluded due to discrepancies in the definition of the outcomes and the lack of reporting of important covariates. NAP1/BI/R027, the most frequently reported and assessed strain, was associated with unfavorable outcomes. However, there were not sufficient data to reach significant conclusions on other strains.

Keywords. *Clostridioides difficile*; complications; mortality; recurrence; strain.

In the early 21st century, an outbreak of *Clostridioides difficile* infection (CDI) was first reported in Canada and the United States and thereafter in Europe [1]. In addition to the large number of cases, CDI has been associated with severe and recurrent symptoms and high mortality rates [2, 3]. One bacterial strain, NAP1/BI/027, has been frequently detected in complicated cases [4]. This strain has been shown to have high virulence, which is attributed to genetic mutations in the toxin-encoding loci and resistance to fluoroquinolones [1]. The CDI outbreak was successfully managed via sustained infection control and stewardship interventions, and therapeutic options are now available [5, 6]. Several *C difficile* virulence factors and mechanisms of action have been identified [7]. However, CDI remains the most frequent health care–associated infectious diarrhea, with an important clinical burden, including recurrence of the disease [8], longer hospital stay, and higher costs [9, 10].

Pulsed-field gel electrophoresis is the reference technique for CDI typing in North America, while ribotyping is the reference technique in Europe. More than 200 ribotypes have been identified to date, and some have been systematically associated with the presence of the binary toxin (CDT), such as R027, R078, and R023 [11]. More recent typing methods include multiple-locus variable number tandem repeat analysis, multilocus sequence typing, and amplified fragment-length polymorphism for the flagellin gene (*fli*C) and surface protein precursor (*slp*A), as well as whole genome sequencing; all of which are being used to provide a higher level of discrimination than traditional techniques [12, 13].

However, the association between strain type and unfavorable clinical outcomes has been inconsistent across studies. While some authors have asserted that clinical outcomes are related to particular strains [14–18], other large studies

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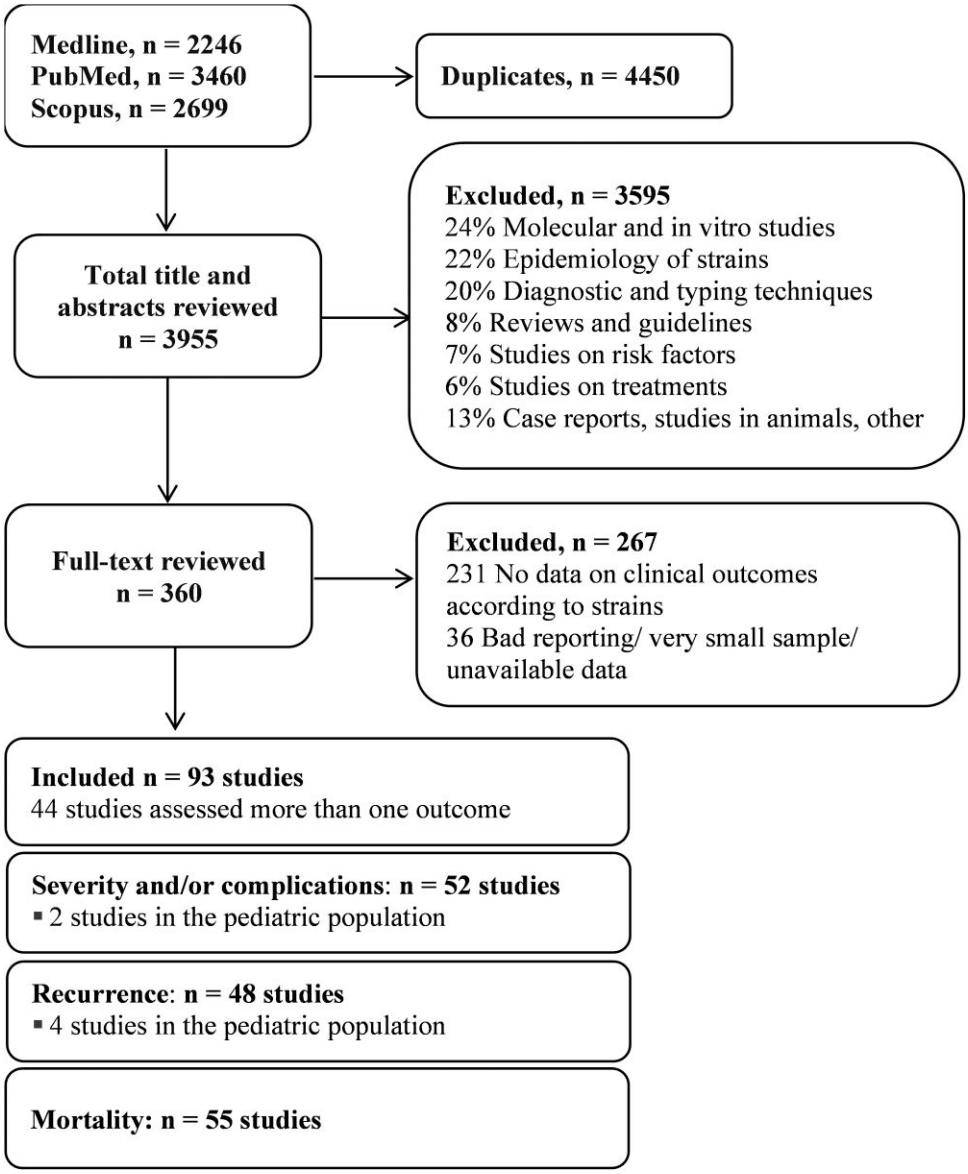

Figure 1. Flowchart of inclusion and exclusion.

have not shown any statistically significant associations [19–21].

To obtain a clear picture of the virulence of *C difficile* strains and their effects on clinical outcomes, we conducted a systematic review of the literature and meta-analyses.

METHODS

The PRISMA [22] and COSMOS-E [23] guidelines were followed. Electronic databases were searched from inception to 30 June 2022 without language restrictions: PubMed and Ovid MEDLINE, Cochrane Database of Systematic Reviews, Embase, and Web of Sciences. The following keywords with Booleans were used: ("*Clostridium difficile*" OR "*Clostridium difficile*–associated diarrhea" OR "*Clostridium difficile*–associated disease" OR "Clostridioides" OR "colitis" OR "pseudomembranous") AND ("strain" OR "type" OR "ribotype" OR "typing" OR "binary" OR "toxin").

Studies were included if they (1) focused on *C difficile* as the main pathogen, (2) reported the frequency of bacterial strains with at least 1 typing technique, and (3) measured at least 1 relevant clinical outcome: severity, complications, mortality, treatment failure, and/or recurrence of CDI. Data from the clinical trials were analyzed in subgroups when relevant.

Case reports, conference proceedings, surveillance data reports without clinical outcomes or diagnostic techniques, and studies involving <50 patients were excluded (Figure 1).

Data Collection

Two independent reviewers screened the titles and abstracts and assessed full-text eligibility using EndNote software. Data from the studies were extracted in double to a standardized matrix. For studies with missing data, authors were contacted via email (28 publications) and 16 replied.

Detailed descriptions of the studies are provided in Supplementary Tables 2, 3, and 4.

Age distribution, comorbidities indices, and prescribed treatments were extracted according to strains. For recurrent CDI (rCDI), the diagnostic criteria of the initial and recurrent episodes were documented and used as stratification covariates.

The clinical outcomes were extracted as reported and grouped as appropriate. We included 4 CDI complications (cCDIs)—pseudomembranous colitis, intestinal perforation, ileus, and toxic megacolon—all of which are severe events that lead to admission to the intensive care unit (ICU). For recurrence, the definitions were separated according to occurrence after the diagnosis of the initial CDI episode and after the end of treatment for the initial episode. Attributable mortality was considered if reported as such by the authors of the studies.

According to the discrimination levels of the typing techniques [24], R014 and R020, R053 and R163, and R078 and R126 were considered similar and so were grouped.

Meta-analyses

Raw data were extracted based on the assumption that each patient was infected by 1 strain. For each outcome and strain, we assessed the pooled proportion (number of patients with outcome/number of strains) with a 95% CI and crude risk ratio (RR). For the RR, each strain was compared with all other strains in the same study.

All analyses were conducted with the *meta* package in R software (R Core Team) and the functions *metaprop* for meta-analysis of single proportions and *metabin* for that of binary outcome data. The codes are shown in the Supplementary material.

Random effects and inverse variance weighting were considered for pooling the studies in all analyses. To take account of the distribution of proportions, Freeman-Tuckey double-arcsine, logit, or logarithmic transformations were used as appropriate, and the results are shown after back-transformation. An increment of 0.001 was used in case of 0 events. For estimation of pooled RRs, a mixed-effects logistic regression model with random study effects was used.

A DerSimonian and Laird, maximum likelihood, or Sidik-Jonkman estimator was used to estimate the between-study variance (τ). Heterogeneity within the studies was estimated with Cochran Q and I^2 statistics. The analyses were stratified according to available data.

RESULTS

Characteristics of Studies

A total of 93 studies were included in the review: 48 reported recurrence according to strain type, 52 reported CDI severity or complications, and 55 reported mortality (Figure 1). Four studies assessed recurrence in the pediatric population [25–28], and 2 assessed severity and complications [25, 27]. These studies did not assess the effects of common strains and were therefore not in the meta-analysis.

A summary of the study characteristics is presented in Table 1. The reported outcomes are summarized in Supplementary Table 1.

Data were collected during overlapping periods (Supplementary Figure 1). In most studies (54% reporting severity or complications, 44% recurrence, and 62% mortality), patients were of any age, including pediatrics and elderly, and no data were reported about patients' age in 8%, 7%, and 4% of studies, respectively. Ribotyping was the most frequently used technique, followed by CDT gene detection or deletion techniques to identify the strains, mainly with Cepheid Xpert *C difficile* Epi assay.

Many discrepancies were noted in the definitions of the outcomes (Supplementary Table 1): cCDI, including ICU admission, surgery, or all-cause 30-day death, was the most frequently retrieved outcome (13 studies), followed by severe CDI criteria per the Infectious Diseases Society of America (11 studies) and cCDI including CDI-associated death (8 studies; Supplementary Table 2). The definition of rCDI was not consistent (Supplementary Table 3), with the most frequent one being recurrence 60 days after diagnosis and 30 days after the end of treatment for the previous episode. Multiple recurrences with different delays were assessed in 4 studies [19, 29–31] and could not be included in the meta-analysis. All-cause 30-day mortality was assessed in 41 studies (Supplementary Table 4), while attributable 30-day mortality was assessed in only 14. The time of occurrence of mortality was not reported in 6 studies, and they had to be precluded.

Meta-analysis

Severity and Complications

Five strains (R001, R002, R014/020, NAP1/R027, R078/126) were frequently reported across studies assessing severity [32], CDI-associated ICU admission, and cCDI, including colectomy or associated/all-cause death (Table 2). The pooled proportions of severity in patients infected with R001, R014, and NAP1/R027 overlapped (Supplementary Figure 2). However, only NAP1/R027 was associated with a higher risk of severity (RR, 1.6; 95% CI, 1.2–2.1): 9% of patients infected with this strain required ICU admission vs 4% for other strains (Supplementary Figure 3), and the risk was 2-fold higher (2.0; 0.99–4.1).

cCDI including associated 30-day death was more frequent in patients infected with NAP1/027: 12% vs 3% and 5% in patients infected with R014 and R078, respectively (Supplementary Figure 4). The overall observed risk was higher

Table 1. Characteristics of Studies According to Clinical Outcomes, Excluding Studies in Pediatric Patients Only

Characteristic	Studies, No. (%)		
	Severity/cCDI (n = 50)	Recurrence (n = 44)	Mortality (n = 55)
Region of studies			
Europe	23 (46.0)	21 (47.73)	32 (58.18)
UK	4 (8.0)	4 (9.09)	9 (16.36)
America	20 (40.0)	14 (31.82)	18 (32.73)
Canada	6 (12.0)	2 (4.55)	5 (9.09)
US	11 (22.0)	7 (15.91)	10 (18.18)
Latin America	3 (6.0)	5 (11.36)	3 (5.45)
Asia/Australia/New Zealand	6 (12.0)	5 (11.36)	4 (7.27)
Multiple	1 (2.0)	4 (9.09)	1 (1.82)
Study design			
Prospective cohort	25 (50.0)	16 (36.36)	24 (43.64)
Surveillance data	13 (26.0)	5 (11.36)	14 (25.45)
Retrospective cohort	20 (40.0)	23 (52.27)	26 (47.27)
Case-control	3 (6.0)	2 (4.55)	4 (7.27)
Cross-sectional	1 (2.0)	…	…
Randomized controlled trial	1 (2.0)	3 (6.82)	1 (1.82)
No. of settings/centers			
1	24 (48.0)	28 (63.64)	27 (49.09)
2 or 3	2 (4.0)	3 (6.82)	4 (7.27)
≥4 (up to 322)	23 (46.0)	13 (29.55)	23 (41.82)
Not reported	1 (2.0)	…	1 (1.82)
Study population			
All CDI cases	27 (54.0)	35 (79.55)	27 (49.10)
Hospitalized CDI	23 (46.0)	9 (23.45)	17 (30.91)
HCFA	4 (8.0)	8 (18.18)	8 (14.55)
Inpatients only	12 (24.0)	…	9 (16.36)
CDI episodes			
Primary	4 (8.0)	5 (11.36)	6 (10.91)
Recurrent	…	3 (6.82)	…
Study population's age group			
All ages	27 (54.0)	18 (40.91)	34 (61.82)
Adults only	17 (34.0)	20 (45.45)	18 (32.73)
Elderly	2 (4.0)	3 (6.82)	1 (1.82)
Not reported	4 (8.0)	3 (6.82)	2 (3.64)
Typing technique			
Ribotyping	33 (66.0)	34 (77.27)	34 (61.82)
Gene detection or deletion	9 (18.0)	3 (6.82)	13 (23.64)
PFGE	5 (10.0)	1 (2.27)	3 (5.45)
EIA/REA	2 (4.0)	5 (11.36)	4 (7.27)
MLST	1 (2.0)	1 (2.27)	1 (1.82)

Abbreviations: cCDI, *C difficile* infection complication; CDI, *Clostridioides difficile* infection; EIA, enzyme immunoassay; HCFA, health care facility acquired; MLST, multilocus sequencing typing; PFGE, pulsed-field gel electrophoresis; REA, restriction endonuclease analysis.

in large studies (RR, 1.9; 95% CI, 1.1–3.2) but not according to design or country (Table 2). For cCDI including all-cause 30-day death, NAP1/R027 was less frequently found among typed strains (6%) than other strains: R014 (18%), R001 (15%), and R078 (11%). The pooled proportions of outcomes were comparable across all strains with overlapping 95% CIs (9%–19%; Supplementary Figure 5). However, only NAP1/R027 was significantly associated with a higher risk, mainly in large and recent studies (RR, 2.0 [95% CI, 1.3–3.1] and 1.8 [1.4–2.4], respectively).

Eight studies assessed the effect of *tcd*C/Δ117 gene deletion or harboring the CDT gene but without a common outcome for the meta-analysis [15, 33–39].

Recurrence

Regardless of the study design, period of data collection, region, types of patients, and sample size, rCDI occurred 30 days after the end of treatment in 1 out of every 4 cases of NAP1/R027 infection (overall, 24.3%; 95% CI, 16.5%–32.9%; Table 3, Supplementary Figure 6). This strain represented 45% of total

Table 2. Meta-analysis of Proportion and Risk Ratio of Severity and Complications by Strain Types and Subgroups

Outcome: Strain and Subgroup	No. of Studies	Sample Size, Range	Total Typed Isolates	Strain, %	Events/ Strain, No.	Outcome, % (95% CI)	Risk Ratio (95% CI)	Heterogeneity I^2, % (95% CI)
Severity[a]								
R001	3	254–1357	1626	7.81	45/127	36.05 (28.05–44.90)	1.00 (.79–1.28)	96.95 (93.83–98.49)
R014/020	4	150–1357	1936	8.68	62/168	37.43 (30.36–45.07)	0.84 (.20–3.56)	97.47 (95.62–98.54)
R027 or NAP1	6	57–1357	2367	36.97	405/875	50.68 (36.04–65.20)	1.55 (1.17–2.06)	92.62 (86.68–95.92)
CDI-associated ICU admission[b]								
R027 or NAP1	4	150–17202	3998	39.87	73/1594	9.26 (3.27–23.53)	2.00 (.99–4.06)	74.13 (27.73–90.74)
ICU admission, colectomy, or associated death[b]								
R014	3	133–1357	2097	12.30	14/258	3.13 (.87–6.27)	0.62 (.06–6.43)	93.37 (84.00–97.25)
R027 or NAP1	6	133–3084	5516	21.16	143/1167	12.41 (8.94–16.30)	1.50 (.80–2.81)	82.06 (58.63–92.22)
R027	5	133–3084	5280	20.98	129/1108	10.57 (8.10–13.28)	1.72 (.97–3.04)	70.11 (14.19–89.59)
N ≥ 1000 patients	4	1144–3084	5204	21.19	128/1103	11.86 (9.49–14.45)	1.88 (1.09–3.25)	72.93 (8.97–91.95)
Prospective design	4	133–1357	2333	30.56	85/713	12.45 (7.32–18.49)	1.43 (.65–3.18)	83.05 (56.74–93.36)
Canada and USA	4	272–3084	4763	21.79	120/1038	12.13 (9.22–15.37)	1.62 (.70–3.70)	86.71 (61.87–95.37)
R078/126	3	1144–1687	2698	4.11	7/111	4.69 (.40–11.96)	0.59 (.06–5.49)	85.54 (57.66–95.06)
ICU admission, colectomy, or 30-d all-cause death[b]								
R001	3	112–4387	5887	14.76	140/869	18.86 (11.70–28.97)	1.36 (.44–4.22)	95.18 (89.23–97.85)
R002	3	112–3333	2719	7.54	18/205	8.91 (5.50–14.13)	0.84 (.08–8.59)	87.45 (64.53–95.56)
R014/020	5	171–4387	8840	18.30	140/1618	10.71 (7.40–15.26)	0.95 (.27–3.31)	97.37 (95.75–98.38)
Europe	4	171–4387	8530	18.36	133/1566	10.49 (6.71–16.05)	0.88 (.20–3.81)	97.98 (96.64–98.79)
R027 or NAP1	5	171–4387	9358	6.57	86/615	14.65 (10.55–19.99)	1.73 (1.05–2.87)	75.28 (39.19–89.95)
R027 or data ≥2010	4	171–4387	8350	3.64	47/304	15.58 (10.22–23.04)	1.85 (1.40–2.45)	60.86 (0–86.90)
N ≥ 1000 patients	3	1150–4387	8877	6.05	73/537	13.90 (10.39–18.34)	1.99 (1.30–3.08)	63.71 (0–89.62)
Europe	3	171–4387	8040	3.23	37/260	13.94 (9.04–20.91)	1.73 (1.27–2.36)	69.97 (0–91.22)
R078/126	5	112–4387	8388	11.08	127/930	13.19 (9.53–17.98)	1.24 (.48–3.16)	87.88 (74.21–94.30)
Prospective design	4	112–4387	8078	11.34	126/916	13.79 [11.71–16.19)	1.37 (.49–3.76)	90.26 (78.04–95.68)
Europe	3	171–4387	8040	11.34	125/912	13.73 (11.65–16.13)	1.35 (.43–4.22)	93.41 (84.11–97.26)
Data ≥2010	4	171–4387	8350	11.09	126/926	13.47 (11.27–15.67)	1.20 (.42–3.39)	90.77 (79.44–95.86)

Abbreviations: CDI, *Clostridioides difficile* infection; EIA, enzyme immunoassay; ICU, intensive care unit; RR, risk ratio.

[a]White blood cell count ≥ 15×10^9 C/L or creatinine ≥1.5× baseline, as defined by the guidelines of the Infectious Diseases Society of America [32].

[b]Including pseudomembranous colitis, intestinal perforation, ileus, and toxic megacolon in some studies, which are considered rare events that lead to ICU admission.

typed strains, and R027 represented 50% in 5 studies. Although the proportion of rCDI seemed lower after 2010 (17% vs 30%), the studies had smaller sample sizes (135–288 patients) and 364 total typed isolates. R027 was significantly associated with a 2-fold higher risk of rCDI (RR, 1.98) in only 4 studies conducted in adult patients [19, 40–42].

When defined within 60 days of the index episode, the proportion of rCDI was assessed in patients infected with NAP1/R027, R014, R078, and R053 strains. The studies had larger sample sizes and more typed strains than those assessing recurrence within 60 days after the end of treatment (total typed isolates: 60 days after index episode, n = 5673 for R027; 60 days after end of treatment, n = 583 for R027/NAP1). The pooled proportion of rCDI was higher in patients infected with R027/NAP1 (21.6%) than in those infected with R014 and R078 (9.3% and 9.7%, respectively; Supplementary Figure 7). Only NAP1/R027 was significantly associated with a higher risk (RR, 1.82; 95% CI, 1.12–2.96), although this strain represented 10% of typed strains and R014 represented 16%.

Within 60 days after the end of treatment, the proportion of rCDI was significantly higher in patients infected with NAP1/R027 than in those infected with R014 (32% vs 12%; Supplementary Figure 8). NAP1/R027 presented 42% of typed strains. However, the risk of recurrence was not associated with any of the tested strains.

Four studies assessed the effect of *tcd*C/Δ117 gene deletion or harboring the CDT gene, but without a common outcome for the meta-analysis [33, 34, 46, 47].

Mortality

Short-term Mortality. The pooled proportion of short-term mortality was similar (11%) in patients infected by NAP1/R027 and R078 strains (Table 4, Supplementary Figure 9; 5 studies) despite the 10-fold lower frequency of R078 vs R027 (34% vs 3.2%). Furthermore, only the NAP1/R027 strain showed a significant association with mortality (RR, 1.6; 95% CI, 1.0–2.5) in 3 large studies including 4821 typed isolates.

Attributable Mortality. Associations with 30-day attributable mortality could be assessed for R001, R014, NAP1/R027, and

Table 3. Meta-analysis of Proportion and Risk Ratio of CDI Recurrence by Strain Types and Subgroups

Outcome: Strain and Subgroup	No. of Studies	Sample Size, Range	Total Typed Isolates	Strain, %	Events/ Strain, No.	Outcome, % (95% CI)	Risk Ratio (95% CI)	Heterogeneity I^2, % (95% CI)
30 d after end of treatment								
R027 or NAP1	7	128–1380	2170	45.02	278/977	24.26 (16.55–32.91)	1.65 (.98–2.77)	82.36 (65.85–91.15)
R027	5	133–1380	1372	50.94	204/699	25.10 (9.33–40.87)	1.78 (.92 3.47)	80.55 (54.40–91.70)
Adult patients only[a]	4	133–1380	1757	46.61	244/819	29.57 (26.46–32.69)	1.98 (1.02–3.84)	86.59 (67.53–94.46)
All patients with CDI[b]	6	128–1380	2091	45.24	272/946	25.14 (16.42–33.87)	1.52 (.88–2.62)	85.09 (69.42–92.74)
Diarrhea for diagnosis of recurrence[c]	4	128–1380	1752	42.35	214/742	28.47 (24.98–31.96)	1.86 (.96–3.62)	87.76 (70.96–94.84)
Prospective design[d]	4	128–1380	1752	42.35	214/742	28.47 (24.98–31.96)	1.86 (.96–3.62)	87.76 (70.96–94.84)
Excluding clinical trial	3	128–1380	1033	47.92	146/495	27.26 (20.40–34.11)	2.06 (.68–6.29)	84.95 (55.48–94.90)
Retrospective design	3	133–150	418	56.22	64/235	23.26 (1.22–45.31)	1.32 (.40–4.37)	78.97 (32.75–93.42)
Data <2010	4	128–1380	1806	45.51	246/822	30.12 (27.13–33.43)	1.68 (.90–3.15)	87.83 (71.16–94.86)
Data ≥2010	3	135–288	364	42.58	32/155	16.88 (5.10–33.66)	1.66 (.36–7.57)	78.65 (31.52–93.34)
Isolates typed ≥50% of sample	6	128–1380	2091	45.24	272/946	25.23 (14.66–35.80)	1.52 (.88–2.62)	85.09 (69.42–92.74)
60 d after index episode								
R014/R020 (all data ≥2010)	3	600–3333	4544	15.93	68/724	9.37 (7.35–11.59)	0.92 (.72–1.18)	97.29 (94.66–98.63)
R027	5	111–3333 [e]	5673	10.06	124/571	21.62 (18.35–25.09)	1.82 (1.12–2.96)	84.06 (64.14–92.92)
R078	3	899–3333	4699	5.19	22/244	9.68 (5.37–15.08)	0.82 (.50–1.36)	94.63 (87.66–97.66)
R053/163[f]	3	50–899	1261	5.23	5/66	…	…	…
60 d after end of treatment								
R014/R020	4	60–490	744	9.41	9/70	12.16 (5.61–20.78)	1.06 (.30–3.70)	63.36 (0–87.64)
R027 or NAP1	4	60–324	583	42.19	73/246	31.51 (14.21–51.99)	2.04 (.79–5.22)	79.80 (46.42–92.38)

Abbreviations: CDI, *Clostridioides difficile* infection; RR, risk ratio.

[a]Overall analyses included 1 study in an elderly population [40].

[b]Excluding 1 study in primary CDI cases only [42].

[c]Defined as ≥3 loose stools/d for >24 hours as the main diagnostic criterion for the initial CDI episode. In most studies, these criteria defined recurrent episodes.

[d]A randomized clinical trial was considered a prospective design [41]. As the patients were enrolled in the same clinical trial, the study by Louie et al [41], with the largest sample size, was considered in the analyses and not the study by Petrella et al [21].

[e]For Neely et al [43], follow-up data were reported for 2698 of the 3333 patients included and typed. In this study, data on R014 and R020 were considered together for the assessment of the RR.

[f]No events were reported in 2 studies in 2 and 3 cases [44, 45]. Meta-analysis was not considered relevant.

R078. These strains were reported at similar frequencies (13%–19%), but the proportion of the outcome was much lower in patients infected with R014 (1.7%, n = 18 events) and R001 (3.8%, 34 events), whereas attributable mortality rates were higher in patients infected with NAP1/R027 (10.2%; 95% CI, 6.5–15.5) and R078 (5.3%; 4.1–6.7; Supplementary Figure 10). However, the risk of attributable mortality was 2-fold higher in patients infected with NAP1/R027 and 2-fold lower in patients infected with R014 (Table 4), with the proportion of deaths decreasing from 12% to 8% after 2008.

All-cause Mortality. The pooled proportion of 30-day all-cause mortality ranged between 20% and 25% in patients infected with R001, R002, R027, and R106 (Supplementary Figure 11) and between 10% and 16% in those infected with R014, R023, R053, and R078. With overlaps in 95% CIs, the proportion of mortality was lower in patients infected with R014, R023, R053, and R078 and higher and similar in patients infected with R001, R002, R027, and R106. NAP1/R027 was the most frequently reported strain (21 studies), with a median frequency of 37% in small studies and only 8% in large studies (n ≥ 500 patients, 9 studies). R078 was less frequently retrieved (median, 10% of typed strains) and mainly in large studies (n ≥ 1000 patients, 8 studies), with a maximum of 13%.

Only NAP1/R027 showed a higher risk of 30-day all-cause mortality (RR, 1.6; Table 4). However, when stratified by typing technique, NAP1 strains were not associated with mortality risk (RR, 1.3; 95% CI, 0.3–5.2). The risk remained higher (1.6; 1.2–1.96) in large studies and those that had typed more than half of patients, with lower heterogeneity across studies (63%). The risk of mortality increased in studies conducted on inpatients and health care facility–acquired CDI (RR, 2.3), European studies, and more recently collected data (≥2008).

The effects of harboring the CDT gene were reported in 9 studies [33, 35, 37, 38, 46, 47, 49–51]. Among the 1965 typed isolates, 29% harbored the CDT gene (Table 4). Although pooled 30-day all-cause mortality was higher in patients infected by these strains as compared with those who were not (18% vs 11%; Supplementary Figure 12), the pooled RR was not statistically significant (1.6; 95% CI, 0.9–2.9) overall or across possible stratifications.

Table 4. Meta-analysis of Proportion and Risk Ratio of Mortality Alone by Strain Types and Subgroups

Outcome: Strain and Subgroup	No. of Studies	Sample Size, Range	Total Typed Isolates	Strain, %	Events/ Strain, No.	Outcome, % (95% CI)	Risk Ratio (95% CI)	Heterogeneity I^2, % (95% CI)
14-d all-cause mortality								
R027 or NAP1	5	319–2222	5405	34.43	206/1861	10.14 (5.94–15.30)	2.01 (.84–4.83)	93.06 (86.75–96.36)
Isolates typed ≥50% and N ≥ 1000 patients	3	1380–2222	4821	33.02	183/1592	10.78 (4.87–18.64)	1.58 (1.00–2.50)	72.02 (5.40–91.73)
R078	3	1380–2222	4821	3.22	20/155	11.99 (.18–23.81)	1.19 (.30–4.63)	79.99 (36.75–93.67)
30-d attributable mortality								
R001	4	230–4387	6814	13.15	34/896	3.81 (2.73–5.28)	0.80 (.13–4.91)	91.67 (81.85–96.18)
R014/020	3	335–4387	6584	16.16	18/1064	1.71 (1.08–2.71)	0.46 (.29–.75)	0 (0–89.60)
R027 or NAP1	8	57–17 202	11 997	17.87	165/2144	10.17 (6.51–15.55)	1.96 (1.23–3.13)[a]	57.45 (0–82.81)
R027	5	137–4387	9143	11.95	90/1093	9.18 (5.43–15.09)	1.89 (.86–4.14)	70.83 (16.63–89.79)
N ≥ 1000 patients	4	1380–17 202	11 477	16.81	124/1929	6.48 (5.46–7.68)	2.05 (1.24–3.39)	57.51 (0–87.89)
Data ≥2008	4	57–17 202	10 719	13.96	102/1497	8.21 (4.18–15.50)	1.67 (.77–3.63)	62.90 (0–89.40)
R078	4	1037–4691	11 073	13.65	77/1512	5.26 (4.08–6.75)	1.31 (.53–3.25)	90.65 (79.13–95.82)
Europe and data ≥2008	3	1037–4691	10 151	14.65	76/1487	5.27 (3.71–7.09)	1.36 (.51–3.66)	93.57 (84.58–97.31)
30-d all-cause mortality								
R001	7	114–11 571	11 421	11.50	228/1313	20.15 (14.61–27.11)	1.27 (.57–2.82)	97.02 ([95.1–98.03)
N ≥ 1000 patients	5	1350–11 571	11 004	11.28	208/1241	18.43 (12.75–25.88)	1.23 (.46–3.26)	97.93 (96.77–98.68)
Europe	6	114–11 571	10 499	11.72	214/1231	20.70 (14.37–28.88)	1.24 (.50–3.03)	97.45 (96.08–98.34)
UK and Scotland	4	114–11 571	4454	10.66	119/475	25.01 (21.22–29.0)	1.31 (.44–3.87)	96.70 (93.98–98.17)
Data ≥2008	5	114–11 571	9813	11.96	191/1174	21.42 (15.36–28.18)	1.22 (.44–3.33)	97.91 (96.72–98.66)
R002 (data ≥2009)	5	139–11 571	4750	9.77	82/464	22.66 (13.42–35.63)	1.25 (.79–1.98)	71.60 (28.25–88.76)
Isolates typed ≥50% of patients	4	139–1426	2139	8.79	48/188	26.26 (16.31–37.46)	1.49 (.88–2.52)	66.44 (1.76–88.53)
Europe	4	171–11 571	4658	9.51	72/443	17.62 (10.16–26.40)	1.05 (.27–4.08)	96.58 (93.76–98.12)
R014/020	8	142–11 571	11 140	15.94	165/1776	9.83 (7.02–13.61)	0.78 (.59–1.03)	66.28 (28.56–84.08)
Isolates typed ≥50% of patients	7	142–4387	8529	16.72	110/1426	8.18 (6.53–10.25)	0.67 (.51–.87)	37.62 (0–73.73)
Strains ≥500 and N ≥ 1000 patients	5	1350–11 571	10 677	15.69	154/1675	9.62 (6.95–13.32)	0.76 (.56–1.03)	66.28 (28.56–84.08)
Canada and US	3	150–1380	2171	13.91	25/302	8.47 (5.83–12.32)	0.83 (.15–4.57)	93.68 (84.93–97.35)
Europe	5	142–11 571	8969	16.43	140/1474	10.47 (7.31–14.99)	0.76 (.52–1.10)	79.67 (51.92–91.40)
Data ≥2008	5	150–11 571	9390	16.69	145/1567	10.01 (7.03–14.25)	0.80 (.56 1.15)	79.92 (52.63–91.49)
R023	3	1426–11 571	9396	3.07	31/289	11.71 (6.75–19.54)	0.72 (.52–1.01)	96.77 (93.40–98.42)
R027 or NAP1	21	57–17 202	17 187	18.04	604/3101	22.34 (18.24–27.06)	1.57 (1.15–2.16)	88.23 (83.40–91.66)
R027	17	86–11 571	14 186	21.88	421/1924	23.09 (18.43–28.11	1.63 (1.15–2.31)	87.52 (81.56–91.55)
NAP1 or BI	4	57–17 202	3087	16.15	194/1201	15.49 (13.49–17.62)	1.32 (.33–5.23)	92.81 (84.82–96.60)
Isolates typed ≥50% and N ≥ 100 patients	16	111–4387	11 381	20.0	345/1746	20.78 (16.56–25.35)	1.65 (1.14–2.39)	85.67 (78.21–90.57)
R027 only	14	111–4387	11 321	20.16	325/1606	21.71 (17.0426.78)	1.62 (1.31–2.01)	57.15 (22.27–76.37)
N ≥ 1000 patients/500 strains	5	1114–4387	9492	10.22	177/971	20.24 (12.36–29.49)	1.55 (1.22–1.96)	63.28 (3.03–86.09)
All patients with CDI	10	111–4387	8579	12.36	208/1060	20.95 (15.07–27.51)	1.34 (.77–2.31)	90.58 (84.83–94.16)
HCFA CDI/inpatients	6	111–1350	2802	25.05	147/702	20.75 (15.68–26.33)	2.35 (1.65–3.34)	45.08 (0–78.27)
Canada and US	6	111–1380	2655	37.06	156/984	16.71 (12.34–21.61)	1.55 (.78–3.09)	88.21 (76.85–93.99)
Europe	10	111–4387	8726	8.73	216/762	23.89 (17.99–30.33)	1.72 (1.06–2.79)	85.55 (75.23–91.57)
Data<2008	7	97–1380	2306	37.20	160/858	21.29 (15.11–28.21)	1.59 (.81–3.12)	88.15 (77.99–93.62)
Data ≥2008	9	111–4387	9172	10.16	208/1932	20.33 (14.74–26.57	1.71 (1.05–2.78)	85.19 (73.72–91.66)
Prospective design	9	124–4387	8821	12.20	184/1076	19.25 (14.28–24.76)	1.53 (.89–2.60)	88.20 (79.78–93.11)
Retrospective design	7	111–1426	2560	26.80	171/686	22.51 (15.95–29.83)	1.85 (.99–3.44)	83.73 (68.04–91.72)
R053/163	3	142–1114	1412	8.07	12/114	11.66 (5.26–23.89)	1.39 (.39–4.97)	72.83 (8.57–91.92)
R078/126	11	114–11 571	16 711	10.84	280/1812	16.20 (14.31–18.29)	0.94 (.47–1.85)	96.00 (94.33–97.18)
Isolates typed ≥50% of patients	9	114–4691	13 835	10.85	229/1501	10.35 (7.03–13.67)	0.91 (.41–2.02)	98.13 (95.69–99.19)
N ≥ 1000 patients	8	1114–11 571	16 123	10.84	270/1747	15.17 (7.04–23.30)	1.01 (.46–2.19)	96.99 (95.59–97.95)
R106	4	97–11 571	4260	9.08	86/387	24.56 (16.66–34.63)	1.02 (.64–1.62)	76.41 (35.34–91.39)
Binary toxin gene								
Yes	7	66–2299	1965	28.70	128/564	17.63 (11.41–24.76)	1.64 (.92–2.92)[b]	71.45 (33.74–87.69)

Table 4. Continued

Outcome: Strain and Subgroup	No. of Studies	Sample Size, Range	Total Typed Isolates	Strain, %	Events/ Strain, No.	Outcome, % (95% CI)	Risk Ratio (95% CI)	Heterogeneity I^2, % (95% CI)
No	6		1879	71.05	163/1335	10.72 (7.61–14.27)		
N ≥ 100 patients	5	107–2299	1813	27.74	122/503	12.25 (9.32–15.48)	1.75 (.91–3.36)	80.82 (49.69–92.68)
Isolates typed ≥50% patients	5	66–880	1488	20.09	54/299	10.41 (7.87–13.22)	1.63 (.80–3.35)	59.23 (0–84.78)
Data ≥2008	5	66–2299	1883	28.84	125/543	11.27 (7.82–15.18)	1.70 (.91–3.18)	75.24 (39.07–89.93)

Abbreviations: CDI, *Clostridioides difficile* infection; HCFA, health care facility acquired; ICU, intensive care unit.

[a]Risk ratio was assessed in 7 studies, as data on other strains were scarcely reported [48].

[b]Risk ratio was assessed in 6 studies, as data on the absence of the binary toxin gene were not reported [49].

DISCUSSION

This is the first review to assess the association between *C difficile* strains, disease severity, and unfavorable clinical outcomes via a meta-analysis. In contrast, previous reviews employed narrative approaches [52–54]. Our review included a large number of studies (n = 93) overall and for each outcome. The studies were published between 2004 and 2022 and encompass data spanning 1999 to 2019.

However, we faced substantial discrepancies that reduced the number of studies in the meta-analyses. Major limitations include the lack of a standard definition for the severity of CDI disease, the associated events that were considered complications, and the delay of occurrence of mortality. Studies that assessed all-cause mortality with a delay >30 days were also excluded due to the reduced possibility of attributing mortality to CDI. Studies on recurrence also had discrepancies in the index date, as well as in the criteria to consider a separate recurrent episode vs persistence of previous symptoms. The data were collected during various and overlapping periods, making it challenging to establish clear cutoffs for the eventual evolution of the strains. We could not conduct meta-regressions because several important factors were scarcely reported, such as follow-up duration for prospective studies, delay in the occurrence of outcomes, patient age, underlying diseases, and treatments. Stratifying the analyses by typing techniques is challenging. Most techniques were grouped under polymerase chain reaction, and only a few studies used advanced techniques. The definition of patients with CDI and the distinction between health care facility–acquired CDI and other conditions were not clearly stated across studies (eg, inpatients vs inpatients and outpatients admitted upon diagnosis).

NAP1/BI/R027 was the most frequently reported strain and is associated with almost all unfavorable outcomes. We showed that the proportions of outcomes were statistically comparable between this strain and other strains, such as R001, R078, and R106. However, few studies were included in the meta-analysis of other strains, and many studies grouped ribotypes without providing further details. The focus on a specific strain may have led to the oversight of other strains and contributed to the nonsignificant findings in RR. In this context, drawing definitive conclusions regarding the absence of association between other strains and the risk of unfavorable outcomes becomes challenging. NAP1/BI/R027 was associated with an 88% increased risk of cCDI, including the need for ICU admission, colectomy, and CDI-associated death in studies of ≥1000 patients. It was also associated with less specific outcomes, such as severity according to Infectious Diseases Society of America criteria [32] and cCDI including all-cause death. Only 3 studies [39, 55, 56] assessed the severity criteria of the European Society of Clinical Microbiology and Infectious Diseases [57] without common strains for meta-analysis. As highlighted by another review [58, 59], many other definitions used for severity are heterogenous and infrequent and include patient age (Supplementary Table 1).

Except for R002, the same patterns of results were observed for cCDI and across studies assessing 30-day all-cause death alone. All-cause death probably increased the frequency of the outcomes and led to misclassification bias. This is supported by lower frequencies retrieved in studies reporting early death, within 10 to 14 days after diagnosis.

Thirty days after the end of treatment, rCDI was estimated in 24% of patients infected with the NAP1/BI/R027 strain, which was significantly associated with a 2-fold higher risk (RR, 1.98) in adult patients. The proportion of patients with rCDI decreased by 40% after 2010 (from 30% to 16.8%). Across the studies [19, 40–42, 60–62], the NAP1/R027 strain represented 45% of all strains, and only 10% of the studies assessed recurrence within 60 days after the index CDI episode [29, 31, 34, 36, 43–45, 55, 63–65]. Nonetheless, this was associated with an increased risk of occurrence (RR, 1.8). Within 60 days of the index episode, rCDI occurred in 9% of patients initially infected with R014 (3 studies) and 10% of patients infected with R078. None of the strains were significantly associated with this outcome.

CDI-attributable mortality was less frequently reported, showing a lower overall frequency of 5% with an increase from 2% in patients infected with R014/20 to 4% in those infected with R001, 5% in R078, and 10% in NAP1/R027. The risk of mortality was 2-fold higher in patients infected with R027/NAP1 vs other strains overall and in large studies. A high and similar

frequency (23%) of 30-day all-cause mortality was observed in patients infected with R001, R002, R027, or R106. Lower frequencies were observed in R014, R053, and R078.

We were able to quantify only the association between strains harboring the CDT gene and 30-day all-cause mortality (RR, 1.6; 95% CI, 0.9–2.9) in 7 studies. A recent review highlighted the emerging epidemiology of CDT-producing strains [66]. Our findings showed that this gene is present in 35% of strains. While CDT is thought to be an additional virulence factor [11], it is not possible to link the higher risk of mortality to the actual production of the binary toxin.

Available data did not allow us to demonstrate whether more or less virulent strains have been circulating in recent years. A study analyzing a sample of 939 isolates in the United States between 2011 and 2016 showed a decline in R027 (35% to 13%), with R106 becoming the most common strain in 2016 [67]. The European Healthcare-Associated Infections Surveillance Network showed that, in 2016, R027 remained the most frequent strain (23%) in 20 countries [68]. R001 and R014 accounted for 7% each. Similar to R027, R002 strains showed higher in vitro sporulation rates and levels of produced toxins [69]. Although mostly induced by the action of toxins, other virulence factors have been discovered and associated with rCDI [7, 70]. The virulence of strains, mostly measured in vitro, could not be considered independently from host factors and risk factors for acquisition. The recent guidelines suggested fidaxomicin or vancomycin as first-line treatment regardless of CDI severity [6, 32], and there are no specific treatment recommendations based on *C difficile* strains. This is partly explained by limited real-time typing capacities in clinical settings. Overall, this review demonstrates the need for close surveillance of other emergent ribotypes.

CONCLUSION

The definitions of CDI clinical outcomes were heterogeneous, and important factors were scarcely reported, leading to the exclusion of many studies from meta-analysis. Thus, conducting meta-regressions was not possible. NAP1/BI/R027 was the most frequently reported and assessed strain, and it was associated with a higher proportion of unfavorable clinical outcomes. Data on other strains were lacking, precluding a comprehensive assessment of other strains.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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