Antibiotics and hospital-acquired *Clostridium difficile*-associated diarrhoea: a systematic review

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A systematic review of studies that investigated the association of antibiotics with hospital-acquired *Clostridium difficile*-associated diarrhoea (CDAD) was undertaken to summarize the strength of the evidence for this relationship. The results from the studies identified were considered after critically reviewing the design and conduct of each study. Although the majority of studies found an association with various antibiotics, antibiotic classes or components of antibiotic administration, most were limited in their ability to establish a causal relationship by the use of incorrect control groups, the presence of bias, inadequate control of confounding and small sample sizes. The limitations identified in this review prevented the pooling of results in a meta-analysis. Two studies of reasonable quality suggested an association between clindamycin, cephalosporins, penicillins and CDAD. Well-designed studies grounded in epidemiological principles are needed to identify true risk factors for CDAD and to provide reliable estimates of the strength of association.

**Keywords:** *Clostridium difficile*, systematic review

**Introduction**

*Clostridium difficile*-associated diarrhoea (CDAD) is the most commonly diagnosed infectious hospital-acquired diarrhoea in developed countries.\(^1\) Infection with *C. difficile* may produce a spectrum of outcomes that range from asymptomatic colonization to acute diarrhoea and pseudomembranous colitis, which can result in colonic perforation and death if left untreated.\(^2\) Although *C. difficile* was identified as the causative agent of CDAD during the late 1970s,\(^3,4\) it has only been since the late 1980s that it has received greater attention due to an increased incidence worldwide and outbreaks of CDAD in hospitals.\(^5-9\) Antibiotics are believed to be the most important risk factor for CDAD by reducing ‘colonization resistance’ of the bowel, allowing subsequent colonization and infection with *C. difficile*.\(^10\)

Despite publication of numerous articles implicating almost all antibiotics with CDAD in hospitalized patients, it is still not clearly understood which antibiotics, or antibiotic classes, in particular are important and how these interact with other risk factors. Many narrative reviews of the subject have been published in recent years; however, only one systematic review that included a meta-analysis of the data has been published.\(^11\) It was noted in this review that most of the studies were small, indicating that the quality of CDAD epidemiological studies may be questionable. The pooling of data from observational studies of low internal validity can produce spurious results, particularly if bias and confounding are present.\(^12\) The aim of our study was to conduct a systematic review of epidemiological studies in order to determine the validity of reported associations of antibiotics with CDAD.

**Materials and methods**

We identified all published epidemiological studies that investigated the association between antibiotics and hospital-acquired CDAD. Studies were included in the review if they met the following criteria: measurement of antibiotic use in...
hospitalized patients as an exposure, an outcome of laboratory-confirmed symptomatic CDAD and the inclusion of a comparison group without CDAD. Case reports and descriptive studies were excluded. Studies that used other CDAD cases as a comparison group, for example mild versus severe cases or single episodes versus recurrent CDAD, were also excluded. Non-English articles were excluded; however, their titles and abstracts were reviewed to assess eligibility based on the remaining criteria.

Computed searches of MEDLINE (1966–2001) and EMBASE (1988–2001) were undertaken and the articles identified were downloaded to Endnote version 4.0. Search keywords used in the extraction were: Clostridium difficile; human; diarrhoea or colitis; antibiotic or antimicrobial; case control studies, cohort studies, prospective studies or retrospective studies. Literature cited in identified articles was examined for further studies. Assessment of the overall quality of the eligible studies was undertaken by critically reviewing each study in terms of study design, selection and information bias, confounding, precision and external validity.

Results

Forty-eight articles, out of a total of 673 identified, fulfilled our eligibility criteria: 23 case–control, 22 cross-sectional and three cohort studies, all published between 1978 and 2001. One cohort study was designed to measure the incidence of C. difficile colonization and infection in tube-fed and non-tube-fed patients, but undertook a multivariate analysis of risk factors of cases and non-cases.13 All eligible studies measured symptomatic CDAD as an outcome, and defined CDAD cases based on the results of microbiology tests of stool samples and the presence of diarrhoeal symptoms. Eighty-five articles were excluded from the review because they were published in a language other than English. Of these, only four were eligible for inclusion based on the remaining criteria.14–17

Study quality

The use of inappropriate control groups, the presence of bias, confounding, misclassification and lack of precision in the effect estimates were common problems identified in the study. Several studies used controls selected from populations that did not necessarily represent the source population from which the cases had come. Most commonly, patients that had been tested for C. difficile during their hospital admission and were negative were used.18–30 One study used C. difficile carriers31 and another used patients with other nosocomial infections.32 Table 1 summarizes the results from the remaining 33 studies judged to have used appropriate comparison groups.

Diagnostic suspicion bias can lead to an overestimate of effect, and may have been present in studies that identified CDAD cases diagnosed through clinical management of patients, if the physician was aware of the relationship between antibiotics and CDAD. Of the 48 eligible studies, only 14 did not identify cases through the clinical management of patients.13,28,31–42 The common feature of these 14 studies was the prospective identification of cases using objective diagnostic criteria; however, response rates were not reported in several studies.13,28,34,39–42 Selection bias was also of concern in two studies that included antibiotic exposure in the case definition.26,43 Some studies were unclear in their method of control selection and therefore had potential for selection bias if the controls had not been selected randomly.19,43–49 In particular, Thibault et al.44 used a ‘convenience sample’ of cases, and Nelson et al.45 used the first 33 cases in an outbreak of 195 cases in their case–control study.

Studies commonly failed to report sources of exposure information, raising the possibility of observer bias.24,34–37,39,40,46,50–55 Of the remaining studies, all obtained exposure information from medical records. The routine recording of antibiotic and medication exposures, procedures and diagnoses in medical records allows accurate information to be obtained; however, variation in the quality of the medical record may result in missing data, as acknowledged by Katz et al.22 Non-differential misclassification of exposures could not be excluded from most studies due to the method of data collection used. For example, the use of standardized data collection instruments, awareness of the purpose of the study by the abstractor, the use of single or multiple abstractors and whether the abstractors were blind to disease status, was poorly reported.

Misclassification of disease could not be excluded in many studies owing to the sensitivity and specificity of laboratory tests used to diagnose C. difficile infection. The most common test used was the direct detection of cytotoxin from faecal samples, which may have resulted in the inclusion of false-negatives among the controls, particularly in studies that used C. difficile-negative patients from laboratory records for comparison.18–23,25–27,29,30 Other studies included patients that were identified through the culture of C. difficile from stool samples, enhancing sensitivity but potentially including false-positives owing to the low specificity of culture.33,36,39,41,44,46 Others only included as cases those that had both positive cultures and cytotoxin results.34,37,43,53,54 Only three studies used culture of a toxigenic isolate of C. difficile as part of their case definition.13,24,38 This definition is considered to be the most appropriate for epidemiological studies.55 Others used enzyme immunoassay (EIA) tests to detect either toxin A40,47,49,56–60 or toxins A and B28,31,50,61 from faecal samples. Several studies failed to use a definition of diarrhoeal symptoms, or ‘diarrhoea’ was stated with no clarification.19,20,24,25,32,37,45–47,54,57,58,61,62 Of particular concern

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<table>
<thead>
<tr>
<th>Ref.</th>
<th>Study [first author (year), country, type]</th>
<th>Participants</th>
<th>Bias</th>
<th>Confounders considered (method)</th>
<th>Results</th>
<th>Comments</th>
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</thead>
<tbody>
<tr>
<td>65</td>
<td>Aziz (2001), UK, case-control</td>
<td>Inpatients of a general hospital (35 cases, 67 inpatient controls)</td>
<td>Diagnostic, exposure misclassification, disease misclassification</td>
<td>Age (matched), gender (multivariate)</td>
<td>Cephalosporins (OR 25.81, 95% CI 3.01–221.6) and other antimicrobials except penicillins (6.43, 1.01–41.11) significant in LR model adjusted for gender, presence of another case in same ward, exposure to antacids or immunosuppressants</td>
<td>No adjustment for severity of illness/co-morbidity (more LRTI and UTI in cases) or LOS (cases longer LOS)</td>
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<tr>
<td>48</td>
<td>Barbut (1997), France, case-control</td>
<td>HIV inpatients of a 30-bed infectious disease unit (34 cases, 66 diarrhoea-free/ C. difficile-negative with diarrhoea controls)</td>
<td>Diagnostic and other selection bias, exposure misclassification, disease misclassification</td>
<td>LOS (matched), severity/co-morbidity (HIV) (restricted)</td>
<td>Clindamycin (OR 5.0, 95% CI 1.3–18.3), penicillin (OR 4.6, 95% CI 1.1–18.8) use significant in multivariate model. No other antibiotics significant by univariate analysis</td>
<td>Used χ^2 rather than McNemar’s test for univariate analysis of matched data. Age not significant by univariate analysis</td>
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<td>57</td>
<td>Barthram (1995), UK, cross-sectional</td>
<td>Admission to a geriatric ward of a university hospital (31 cases, 201 diarrhoea-free plus C. difficile-negative controls)</td>
<td>Diagnostic, information, disease misclassification</td>
<td>Age (restricted, multivariate)</td>
<td>Cases were exposed to a higher average 'spectrum of activity' than non-cases, 7.74 species versus 6.29 species (P&lt;0.001, t-test). LR model: only spectrum of activity was significant</td>
<td>No LR model results presented</td>
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<td>51</td>
<td>Bilgrami (1999), USA, cross-sectional</td>
<td>200 consecutive BMT recipients (14 CDAD, 186 diarrhoea-free controls)</td>
<td>Diagnostic, information, disease misclassification</td>
<td>Severity/co-morbidity (BMT) (restricted)</td>
<td>Prophylactic ampicillin exposure significant, P = 0.24 (χ^2) (OR 2.21, 95% CI 0.64, 8.0) (calculated from article)</td>
<td>Patient groups found to be similar with respect to age and gender, but fewer cases with haematologic versus solid organ malignancies</td>
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<td>13</td>
<td>Bliss (1998), USA, prospective cohort</td>
<td>Consecutive adult inpatients at a Veterans Affairs medical centre (76 tube fed, 76 non-tube fed). Analysis of 8 CDAD and 144 diarrhoea-free patients</td>
<td>Exposure misclassification</td>
<td>Gender, age, severity of illness (matched for cohort analysis only), age (multivariate)</td>
<td>3GCs (OR 4.1, 95% CI 0.9–18.6), iv metronidazole (OR 0.45, 95%CI 0.08–2.5) non-significant, tube-feeding only significant factor (OR 9.0, 95% CI 1.02–79.8, P = 0.049) in LR model. Cephalosporin, aminoglycosides significant by univariate analysis only</td>
<td>Matching lost when analysed by disease status. No comparison of LOS or co-morbidities between cases and non-cases or inclusion in multivariate analysis.</td>
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<tr>
<td>64</td>
<td>Brown (1990), USA, case-control</td>
<td>Adult inpatients of a tertiary care hospital (37 cases, 37 diarrhoea-free controls)</td>
<td>Diagnostic, information, disease misclassification</td>
<td>Age, severity/co-morbidity (ICU) (multivariate)</td>
<td>&gt;10 antibiotic days (OR 16.1, 95% CI 2.2–117), &gt;65 years of age (OR 14.1, 95% CI 1.4–141), ICU stay (OR 39.2, 95% CI 2.2–713), GI procedure (OR 23.2, 95% CI 2.2–255) significant in LR model. Any antibiotic, clindamycin, 3GC, aminoglycosides, vancomycin significant by univariate analysis</td>
<td>Individual antibiotics not entered into logistic model</td>
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<tr>
<td>37</td>
<td>Cartmill (1992), UK, cross-sectional (outbreak)</td>
<td>18 geriatric inpatients (7 CDAD, 9 diarrhoea-free plus C. difficile negative)</td>
<td>Information, disease misclassification</td>
<td>Age (restricted)</td>
<td>7/7 cases compared with 4/9 controls received antibiotics, P = 0.003 (Fisher’s exact test), OR undefined</td>
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<td>Study [first author (year), country, type]</td>
<td>Participants</td>
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<td>52 Chakrabarti (2000), UK, cross-sectional</td>
<td>BMT recipients (10 CDAD, 65 C. difficile-negative controls with or without diarrhoea)</td>
<td>Diagnostic, information, disease misclassification</td>
<td>Severity/co-morbidity (BMT) (restricted)</td>
<td>GVHD grade 3–4 (OR 9.8, 95% CI 2.1–43) significant in LR model. Metronidazole not significant by univariate analysis (OR 0.73, 95% CI 0.14–3.44) (calculated from paper).</td>
<td>Age not significant in univariate analysis</td>
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<td>63 Chang (2000), USA, cross-sectional</td>
<td>Inpatients of a 305-bed urban hospital (68 cases, 2603 diarrhoea-free controls)</td>
<td>Diagnostic, exposure misclassification, disease misclassification</td>
<td>Age, LOS (multivariate)</td>
<td>Clindamycin (OR 4.22, 95% CI 2.11–8.45), no. antibiotics (OR 1.49, 95% 1.23–1.81), LOS (OR 1.03, 95% 1.02–1.04), age (OR 1.02, 95% 1.01–1.04) and physical proximity to patients with CDAD (OR 1.86, 95% 1.05–3.28) significant in LR model.</td>
<td>Co-morbidities not measured</td>
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<td>50 Ferroni (1997), France, case–control (outbreak)</td>
<td>Inpatients of a 50-bed paediatric orthopaedic unit (6 cases, 27 inpatient controls)</td>
<td>Diagnostic, information, disease misclassification</td>
<td>Age (restricted), severity/co-morbidity (orthopaedic) (restricted)</td>
<td>Treatment with lincomycin + aminoglycoside significantly associated with outbreak (P &lt; 0.05, Fisher’s exact test), along with bone and joint infection, and surgery, by univariate analysis only.</td>
<td>Confounders such as age, gender and LOS not significantly different</td>
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<td>34 Gerding (1986), USA, case–control</td>
<td>Inpatients of a 700-bed medical-surgical teaching hospital (109 cases, 108 diarrhoea-free controls)</td>
<td>Information, disease misclassification</td>
<td></td>
<td>Clindamycin (OR 3.05, 95% CI 1.56–6.0) (calculated from paper) only significantly associated antibiotic. Others significant differences included LOS, underlying infection, underlying cardiovascular illness.</td>
<td>Included non-nosocomial cases; used $\chi^2$ instead of McNemar’s for univariate analysis of matched data</td>
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<td>62 Gorecki (1999), USA, cross-sectional</td>
<td>Surgical inpatients of a 560-bed university affiliated private hospital (156 inappropriate antibiotic prophylaxis, 55 appropriate)</td>
<td>Diagnostic, exposure misclassification, disease misclassification</td>
<td>Severity/co-morbidity (surgical) (restricted)</td>
<td>Overall antibiotic use significantly associated with CDAD (OR 2.86, 95% CI 1.38–5.99, calculated). Cefotaxime was only individual antibiotic reported to be significantly associated (P = 0.04, $\chi^2$), but not when checked (OR 5.0, 95% CI 0.78–33.5). No other antibiotics significant. Other significant factors included severity of illness, antineoplastic agents, assisted feeding.</td>
<td>Eight patients with diarrhoea in inappropriate group, but only two positive for C. difficile.</td>
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<td>35 Grube (1987), USA, cross-sectional</td>
<td>Burn centre ICU admissions (9 CDAD, 11 symptomatic C. difficile-negative patients, 92 diarrhoea-free controls)</td>
<td>Information, disease misclassification</td>
<td>Severity/co-morbidity (burn ICU) (restriction)</td>
<td>CDAD patients greater number of antibiotic days (mean 54.1) than cytotoxin-negative diarrhoea patients (40.7) and diarrhoea-free patients (6.6) but hypothesis test not performed.</td>
<td>Burn size greatest in CDAD group. Only total LOS measured, not up to diarrhoea onset. No significant difference in age of patients</td>
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<td>59 Halim (1997), Australia, case–control</td>
<td>Inpatients of an acute care 500-bed teaching hospital (64 cases, 120 diarrhoea-free controls)</td>
<td>Diagnostic, exposure misclassification, disease misclassification</td>
<td></td>
<td>Overall antibiotic use significantly associated with CDAD (OR 2.86, 95% CI 1.38–5.99, calculated). Cefotaxime was only individual antibiotic reported to be significantly associated (P = 0.04, $\chi^2$), but not when checked (OR 5.0, 95% CI 0.78–33.5). No other antibiotics significant. Other significant factors included severity of illness, antineoplastic agents, assisted feeding.</td>
<td>No difference in age or gender between groups, but cases had a higher severity of disease index (Horn’s index), and no information was available on LOS.</td>
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<td>Study</td>
<td>Design</td>
<td>Setting</td>
<td>Study Population</td>
<td>Outcome</td>
<td>Analysis</td>
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<td>Harbath (2001), USA</td>
<td>Cross-sectional</td>
<td>Inpatients of a 320-bed teaching hospital who underwent cardiovascular surgery (31 cases, 260 non-cases)</td>
<td>Diagnostic, exposure misclassification, disease misclassification</td>
<td>Severity/co-morbidity (cardiovascular surgery) (restricted, multivariate), age, LOS (&gt;8 days) (multivariate)</td>
<td>3GC (OR 5.9, 95% CI 12.2–16), LOS (OR 1.03, 95% 1.01–1.05), β-lactamase-resistant β-lactams (OR 4.6, 95% 1.7–12.3) independent predictors in LR model. Age, renal or GI disease, anaemia, no co-morbidities, ICU stay, emergency operation, hospital transfer, antibiotic prophylaxis &gt;8 days not significant in LR model.</td>
<td>Kreisel (1995), USA, 8/9 CDAD patients vs 119/222</td>
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<td>Impallomeni (1995), MacGowan (1997), Kent (1998), Keighley (1978), UK</td>
<td>Prospective cohort</td>
<td>Patients undergoing GI surgery at an urban general hospital (119 exposed to antibiotics, 122 not exposed)</td>
<td>Exposure misclassification, disease misclassification</td>
<td>Severity/co-morbidity (GI surgery) (restricted)</td>
<td>Cefotaxime (RR 7.2, 95% CI 3.9–13.2), cefuroxime (OR 5.2, 95% CI 2.9–9.45), erythromycin (OR 2.8, 95% CI 1.5–5.2) all related to CDAD. CDAD cases had longer LOS (62 days vs 21 days) compared with the whole group.</td>
<td>Hutin (1993), France, No information on other</td>
</tr>
<tr>
<td>Johnson (1990), USA</td>
<td>Cross-sectional</td>
<td>282 long-stay inpatients on three wards: surgical, medical and orthopaedic (9 CDAD, 222 diarrhoea-free controls)</td>
<td>Information, disease misclassification</td>
<td>LOS (&gt;7 days) (restricted)</td>
<td>Cefotaxime (OR 2.48, 95% CI 0.67–9.2) found to be significantly different (P &lt; 0.01, Mann–Whitney U-test).</td>
<td>61 patients admitted to surgical services over a 5 month period (21 CDAD, 354 C. difficile negative with or without diarrhoea)</td>
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<tr>
<td>Keighley (1978), UK</td>
<td>Prospective cohort</td>
<td>Patients undergoing GI surgery at an urban general hospital (119 exposed to antibiotics, 122 not exposed)</td>
<td>Exposure misclassification, disease misclassification</td>
<td>Severity/co-morbidity (GI surgery) (restricted)</td>
<td>In LR model, cefoxitin along with admission from nursing home or rehabilitation centre, operation for bowel obstruction were significant, but results not presented. Inappropriate antibiotic use (&gt;24 h, &gt;48 h for cardiac, orthopaedic, post-operative) (OR 5.1, 95% CI 1.1–23.64), duration of prophylaxis longer for cases (3.1 vs 1.7 days, P &lt; 0.05, t-test) iv administered cephalosporins (1.5 vs 1.0 g/day), all cephalosporins (1.8 vs 1.1), β-lactams (1.8 vs 1.2) and all antimicrobials (2.4 vs 1.7) found to be significantly different (P &lt; 0.01, Mann–Whitney U-test)</td>
<td>1643 patients admitted to surgical services over a 5 month period (21 CDAD, 354 C. difficile negative with or without diarrhoea)</td>
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<td>Kreisel (1995), USA</td>
<td>Case-control</td>
<td>Surgical inpatients of a tertiary care medical centre receiving antibiotic prophylaxis (23 CDAD, 39 non-exposed controls)</td>
<td>Diagnostic and other selection bias, exposure misclassification, disease misclassification</td>
<td>Age, gender (matched), severity/co-morbidity (surgery) (restricted)</td>
<td>OR not provided but able to be calculated. No analysis of difference in risk for surgical vs non-surgical patients. No confounder control except LOS.</td>
<td>Surgical inpatients of a tertiary care medical centre receiving antibiotic prophylaxis (23 CDAD, 39 non-exposed controls)</td>
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<td>MacGowan (1997), UK</td>
<td>Case-control</td>
<td>Inpatients of an urban hospital with community-acquired pneumonia (28 CDAD, 56 non-exposed controls)</td>
<td>Diagnostic, exposure misclassification, disease misclassification</td>
<td>Age (matched), severity/co-morbidity (pneumonia) (restricted)</td>
<td>Antibiotics and hospital-acquired C. difficile-associated diarrhoea</td>
<td>Inpatients of an urban hospital with community-acquired pneumonia (28 CDAD, 56 non-exposed controls)</td>
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<td>Ref.</td>
<td>Study [first author (year), country, type]</td>
<td>Participants</td>
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<td>53</td>
<td>McFarland (1990), USA, cross-sectional</td>
<td>399 adults in general medicine ward (31 CDAD, 316 C. difficile-negative patients with or without diarrhoea)</td>
<td>Diagnostic, disease misclassification</td>
<td>Age, severity/co-morbidity (Horn's index) (multivariate)</td>
<td>Cephalosporins for 1–7 days (RR 2.07, 95% CI 1.06–6.62), penicillins for 8–14 days (RR 3.62, 95% CI 1.28–8.42) along with GI stimulants (RR 3.61, 95% CI 1.08–12.06) and enemas (RR 2.96, 95% CI 1.36–10.2) significant using Poisson regression including age and severity of disease</td>
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<td>56</td>
<td>Nath (1994), Canada, case-control (outbreak)</td>
<td>Medical and oncology inpatients of a 465-bed tertiary care teaching hospital (80 cases, 80 diarrhoea-free controls)</td>
<td>Diagnostic, exposure misclassification, disease misclassification</td>
<td>Age (matched)</td>
<td>Ceftazidime (OR 26, 95% CI 5.67–119.2), clindamycin (OR 15.16, 95% CI 2.93–78.44), cefuroxime (OR 5.17, 95% CI 1.86–14.36), ciprofloxacin (OR 3.81, 95% CI 1.05–13.79) as well as GI drugs (antacids) (OR 15.16, 95% CI 2.93–78.44) significant in LR model. Aminoglycosides, anti-staphylococcal penicillins significant by univariate analysis only. Model 2: 1 antimicrobial (OR 15, 95% CI 4.1–85.8) significant. Other factors not significant</td>
<td>Age, LOS not modelled. Cases had significantly greater LOS than non-diarrhoeal controls, no data reported on age. Congestive heart failure, only co-morbidity modelled, not significant in model 2</td>
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<td>45</td>
<td>Nelson (1994), USA, case-control (outbreak)</td>
<td>Inpatients of a 460-bed Veterans Affairs medical centre (33 cases, 32 other inpatient controls, 34 C. difficile-negative controls)</td>
<td>Diagnostic and other selection bias, exposure misclassification, disease misclassification</td>
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<td>46</td>
<td>Pear (1994), USA, case-control (outbreak)</td>
<td>Inpatients in a university affiliated Veterans Affairs medical centre receiving clindamycin (27 cases, 108 other inpatient controls)</td>
<td>Diagnostic and other selection bias, exposure misclassification, disease misclassification</td>
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<td>43</td>
<td>Pierce (1982), USA, case-control (outbreak)</td>
<td>Inpatients of a 500-bed acute care referral hospital (10 cases, 20 other inpatient controls)</td>
<td>Diagnostic plus other selection bias, exposure misclassification, disease misclassification</td>
<td>Age, gender, LOS (matched)</td>
<td>Aminoglycosides (P = 0.0003), cephalosporins (P = 0.018), clindamycin (P = 0.0022) significantly associated with CDAD</td>
<td>No effect estimates or 95% CIs provided, and could not be calculated. Use of Z instead of McNemar’s for matched data</td>
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<td>42</td>
<td>Schwaber (2000), Israel, cross-sectional</td>
<td>Adult inpatients of a tertiary care teaching hospital (13 CDAD, 38 diarrhoea-free patients, 85 symptomatic C. difficile-negative patients)</td>
<td>Exposure misclassification, disease misclassification</td>
<td></td>
<td>3G only significant antibiotic found (OR 3.84, 95% CI 10.98–16.2, calculated for CDAD versus cytotoxin negative. Analysis of CDAD vs diarrhoea-free patients found ampicillin--amoxicillin (OR 4.9, 95% CI 11.06–25.4), cephalexin (OR 6.8, 95% CI 1.2–68.8) to be significantly associated with CDAD</td>
<td>Comparison with diarrhoea-free patients not presented in paper, but able to be extracted. No confounder control, univariate analysis only</td>
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<td></td>
<td>Study (Year), Country, Design</td>
<td>Setting</td>
<td>Exposure Misclassification</td>
<td>Gender (multivariate)</td>
<td>RR/LR Model</td>
<td>Age, LOS, Severity of Illness</td>
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<td>38</td>
<td>Talon (1995), France, case-control (outbreak)</td>
<td>Inpatients of medical and geriatric wards of a 120-bed middle-term care facility (21 cases, 63 diarrhoea-free controls)</td>
<td>Exposure misclassification</td>
<td>Gender (multivariate)</td>
<td>( \beta )-Lactams (OR 4.92, 95% CI 1.58–16.19) and pristinamycin (OR 7.95, 95% CI 1.71–45.1) significant in LR model as well as 'presence of a feeding tube' (OR 19.7, 95% CI 11.88–118.15)</td>
<td>Age, LOS, severity of illness not considered as confounders</td>
</tr>
<tr>
<td>40</td>
<td>Thamlikitkul (1996), Thailand, prospective cohort</td>
<td>Inpatients of a tertiary care hospital (140 clindamycin, 140 ( \beta )-lactams, 140 no antibiotics)</td>
<td>Information, disease misclassification</td>
<td>Age, gender (matched)</td>
<td>For those receiving antibiotics (clindamycin or ( \beta )-lactams) reported RR 10 (95% CI 1.3–77)</td>
<td>RR re-calculated as 1.88, 95% CI 1.5–2.35</td>
</tr>
<tr>
<td>44</td>
<td>Thibault (1991), Canada, case-control</td>
<td>Inpatients of a university affiliated urban general hospital (26 cases, 26 diarrhoea-free controls)</td>
<td>Diagnostic plus other selection bias, exposure misclassification, disease misclassification</td>
<td>Age, gender (matched)</td>
<td>Only average number of antibiotics given (OR 1.6, 95% CI 1.1, 2.4) and GI surgery (OR 4.7, 95% CI 1.0–21.0) significant in LR model. Neomycin, clindamycin, metronidazole significant by univariate analysis but not in LR</td>
<td>Age and LOS not significant by univariate analysis. Only variables ( P &lt; 0.05 ) entered into LR. Did not use conditional LR</td>
</tr>
<tr>
<td>39</td>
<td>Tumbarello (1995), Italy, case-control</td>
<td>Inpatients of an infectious disease department of a university hospital (31 CDAD cases with HIV, 62 diarrhoea-free controls with HIV)</td>
<td>Information, disease misclassification</td>
<td>Age, gender (matched)</td>
<td>Antibiotic therapy only significant risk factor by univariate and Poisson analysis (OR 11.35, 95% CI 1.26–102). Trimethoprim–sulfamethoxazole, 3GCs and clindamycin significant by univariate analysis; multivariate data not provided</td>
<td>Age not significant by univariate analysis. LOS significant by univariate but not in multivariate model. Lacked full presentation of multivariate analysis</td>
</tr>
<tr>
<td>49</td>
<td>Yip (2001), Canada, case-control</td>
<td>Hospitalized patients in a 300-bed tertiary care hospital (27 cases, two groups of 27 inpatient controls)</td>
<td>Diagnostic and other selection bias, exposure misclassification, disease misclassification</td>
<td>Age, gender (control group 1), LOS (control group 2) (matched)</td>
<td>Control group 1: ciprofloxacin significant in LR model, data not supplied. Control group 2: cephalosporins (OR 6.7, 95% CI 1.3–33.7), ciprofloxacin (OR 9.5, 95% CI 1.01–88.4) significant in LR model (age not entered)</td>
<td>Conditional logistic regression not used. Use of two control groups not necessary because multivariate analysis was used</td>
</tr>
</tbody>
</table>

OR, odds ratio; CI, confidence interval; RR, relative risk; 2GC, second-generation cephalosporin; 3GC, third-generation cephalosporin; LOS, length of stay; GVHD, graft-versus-host disease; LR, logistic regression; BMT, bone marrow transplant; LRTI, lower respiratory tract infection; UTI, urinary tract infection; ICU, intensive care unit; GI, gastrointestinal.
in some retrospective studies was the use of symptom definitions that required detail regarding the number of diarrhoeal episodes per day, which would be difficult to accurately obtain from notes in the medical record. Nonetheless, Katz et al.\textsuperscript{22} reported a 94\% success rate. Because such information is not consistently recorded in medical records, some retrospective studies used the stool description, e.g. uniform, to enhance case ascertainment.\textsuperscript{30,64}

The potential confounding effects of age, length of stay and severity of underlying illness, which may be associated with exposure to antibiotics and development of CDAD, were not adequately assessed in most studies. Age was most commonly considered either through restriction of the study population,\textsuperscript{18,24,28,29,37,58} matching\textsuperscript{27,40,43,44,47,49,56,60,65} or multivariate analysis.\textsuperscript{13,22,53,54,61,63,64} Some studies restricted the study population to those with a common underlying condition\textsuperscript{24,27,35,39,48,50,54,60} or an intensive care stay,\textsuperscript{20,31,64} or those who underwent particular procedures.\textsuperscript{32,33,41,47,51,52,61,62}

This approach may have controlled for severity of underlying illness to some extent.

Many of the studies had small sample sizes that may have limited the precision of their estimation of effect (see Table 1). This applied in particular to cross-sectional studies and cohort studies, which commonly had small numbers of CDAD outcomes.

### Evidence for the association of antibiotics with CDAD

Overall, 41 out of 48 studies found an increased risk of CDAD in hospitals associated with antibiotic exposure, but all had major weaknesses. For total antibiotics, exposure odds ratios (ORs) ranged from 2.86\textsuperscript{59} to 6.92\textsuperscript{76} in studies that used appropriate controls. A relative risk (RR) of 2.48 was recorded in one cohort study.\textsuperscript{33} For specific antibiotics, or antibiotic classes, ORs ranged from 2.12\textsuperscript{13} to 42.54\textsuperscript{24} for clindamycin, and from 3.84\textsuperscript{42} to 26.56\textsuperscript{43} for third-generation cephalosporins.

Only two studies were identified that had less serious threats to validity, and from which the reported results were considered the most reliable. McFarland et al.\textsuperscript{53} found an increased risk for CDAD after cephalosporin exposure for up to 1 week (RR 2.07, 95\% CI 1.06–6.62) and penicillin exposure for between 1 and 2 weeks (RR 3.62, 95\% CI 1.28–8.42) adjusted for age and severity of disease using Horn’s index. A moderately large cross-sectional study by Chang & Nelson\textsuperscript{63} provided precise estimates of effect for clindamycin (OR 4.22, 95\% CI 2.11–8.45) and increased numbers of antibiotics (OR 1.49, 95\% CI 1.23–1.81) adjusted for age, length of stay and proximity to patients with CDAD, but did not adjust for co-morbidities.

The ability to generalize study results to other populations was limited because of serious threats to internal validity in most of the studies. The association between CDAD and clindamycin, cephalosporins, penicillins and the number of antibiotics a patient received could be interpreted as representative of a more general association in other hospitalized patients.

### Discussion

Despite a large number of published studies supporting the association of various antibiotics with hospital-acquired CDAD, well-designed epidemiological studies are lacking. A few studies identified in this review were not designed to test causal hypotheses. For example, the studies by Hornbuckle et al.,\textsuperscript{27} Cooper et al.\textsuperscript{21} and Katz et al.\textsuperscript{22} were intended for use as clinical tools to predict the probability of a positive C. difficile test result in patients with hospital-acquired diarrhoea, and the study by Cheng et al.\textsuperscript{31} aimed to analyse differences in characteristics between patients with symptomatic CDAD and C. difficile carriers. However, the main objective of the majority of the studies was to estimate the risk of CDAD in hospitalized patients if exposed to various risk factors, notably antibiotics. The most serious threats to validity identified in this review were the use of incorrect control groups, lack of precision due to inadequate sample sizes and inadequate control of confounders. Diagnostic bias was a potential threat to many studies that identified cases through the clinical management of patients, but this is virtually unavoidable when laboratory databases are used for retrospective case identification. Information bias could not be excluded from many studies, mainly due to the provision of insufficient detail in the articles.

Almost half of the studies were described by their authors as ‘cohort’ or ‘prospective’ studies, although they were regarded in this review as cross-sectional studies because they did not meet the epidemiological classification of a cohort study.\textsuperscript{56} Cross-sectional studies are generally poor for testing causal hypotheses, owing to the potential for length-biased sampling (a person with a longer duration of illness will have a greater chance of being selected) and the use of measurements of current rather than past exposures, which may prevent the separation of cause and effect. Several cross-sectional studies identified in this review identified cases prospectively (incident cases), thus minimizing length-biased sampling, and collected risk factor information from the medical record rather than relying on self-reporting. However, the main limitation found with many of these studies was the low number of CDAD outcome events, which may have resulted in an imprecise estimate of effect.

The choice of controls in case–control studies of CDAD has been a contentious issue recently, with arguments for and against the use of symptomatic C. difficile-negative patients as controls.\textsuperscript{57,68} In order to accurately measure the risk of acquiring a disease if exposed to a particular factor, cases should be compared with controls selected from the source population, i.e. subjects who would have been included in the case group if they had developed the disease. Therefore,
Antibiotics and hospital-acquired C. difficile-associated diarrhoea

restricting the selection of controls to patients investigated for C. difficile is not representative of the source population from which the cases arose. This is likely to produce a result biased to the null either because exposures are likely to be similar for CDAD or other forms of nosocomial diarrhoea, or because of the classification of false-negatives as controls, depending on the sensitivity of the laboratory test used.

The different methods for laboratory diagnosis of CDAD used throughout the studies reviewed have implications for the misclassification of disease that can, as previously noted, produce a biased effect estimate. Direct detection of C. difficile cytotoxin (toxin B) from faecal specimens using mammalian tissue culture lines is considered the standard diagnostic test, and was the one used most commonly in the studies identified. However, this method lacks sensitivity due to inactivation of the cytotoxin in 20% of samples during storage and transport. Recent evidence indicates that C. difficile and its spores are affected relatively little by storage conditions compared with cytotoxin. In addition, ~60% of cytotoxin-negative faecal samples contain a cytotoxin-producing strain of C. difficile, suggesting a false-negative laboratory result. These methods are also time consuming, and EIAs that provide rapid results have been developed. Commercial EIAs for toxin A were used by several studies; however, this approach also requires culturing of C. difficile for adequate toxin detection. Furthermore, toxin A B+ variant C. difficile strains, found to account for 3% of isolates sent to a reference laboratory in the UK, would be missed using this method. Although toxin A B+ strains occur at a low prevalence, there is concern that these variant strains will spread through hospitals undetected where toxin A testing is used alone. To overcome this, EIAs that detect both toxins have been developed. These perform well against faecal cytotoxin tissue culture, but are still less sensitive than culture of toxigenic C. difficile. Culture of C. difficile followed by detection of cytotoxin, either by tissue culture or immunoassay, has recently been recommended for confirmation of diagnosis in patients with cytotoxin-negative faecal samples. Therefore, in epidemiological studies of CDAD, culture of C. difficile followed by toxin demonstration is important in order to accurately identify cases.

In this study, we systematically reviewed the quality of published studies that investigated antibiotics as risk factors for hospital-acquired CDAD in order to decide whether a meta-analysis could be reliably conducted. A previous systematic review concluded that ‘the meta-analysis approach enabled the ranking of antibiotics in relation to the risk of C. difficile infection’11. In our study, a critical review of studies that analysed antibiotics as risk factors for CDAD was undertaken in terms of study design, selection and information biases, control of confounding, precision of the effect estimate and external validity. Although our review was limited by including only published studies and excluding non-English articles, only two studies were identified that were considered to provide valid evidence for the role of antibiotics in hospital-acquired CDAD. Therefore, based on our findings we do not agree with Bignardi11 that a meta-analysis can be reliably conducted in order to assess the relationship between antibiotics and C. difficile.

Future studies designed to investigate the aetiology of hospital-acquired CDAD need to be aware of epidemiological principles. Investigators need to define a clear hypothesis when designing such studies so that appropriate controls are selected, an adequate sample size is used, bias minimized and confounding controlled. Adherence to these principles will ensure that risk factors are accurately identified and their association with CDAD validly and precisely estimated.

References


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C. Thomas et al.


