MAJOR ARTICLE

Cumulative Antibiotic Exposures Over Time and the Risk of *Clostridium difficile* Infection

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Background. *Clostridium difficile* infection (CDI) is a major cause of hospital-acquired diarrhea and is most commonly associated with changes in normal intestinal flora caused by administration of antibiotics. Few studies have examined the risk of CDI associated with total dose, duration, or number of antibiotics while taking into account the complex changes in exposures over time.

Methods. A retrospective cohort study conducted from 1 January to 31 December 2005 among hospitalized patients 18 years or older receiving 2 or more days of antibiotics.

Results. The study identified 10,154 hospitalizations for 7,792 unique patients and 241 cases of CDI, defined as the detection of *C. difficile* toxin in a diarrheal stool sample within 60 days of discharge. We observed dose-dependent increases in the risk of CDI associated with increasing cumulative dose, number of antibiotics, and days of antibiotic exposure. Compared to patients who received only 1 antibiotic, the adjusted hazard ratios (HRs) for those who received 2, 3 or 4, or 5 or more antibiotics were 2.5 (95% confidence interval [CI] 1.6–4.0), 3.3 (CI 2.2–5.2), and 9.6 (CI 6.1–15.1), respectively. The receipt of fluoroquinolones was associated with an increased risk of CDI, while metronidazole was associated with reduced risk.

Conclusions. Cumulative antibiotic exposures appear to be associated with the risk of CDI. Antimicrobial stewardship programs that focus on the overall reduction of total dose as well as number and days of antibiotic exposure and the substitution of high-risk antibiotic classes for lower-risk alternatives may reduce the incidence of hospital-acquired CDI.

Clostridium difficile infection (CDI) is a major cause of hospital-acquired diarrhea, the incidence and severity of which has been increasing in recent years [1]. The changing epidemiology has partially been attributed to the emergence of a new bacterial strain, designated BI/NAP1/027, which produces elevated levels of toxins A and B and is highly resistant to fluoroquinolones [2–3]. Given the increased burden and severity of CDI, there is an increasing need to identify modifiable risk factors for nosocomial CDI.

Antibiotic exposure is the most common factor predisposing patients to CDI. Most classes of antibiotics have been associated with the risk of CDI [4], particularly cephalosporins [5], clindamycin [6], and more recently, fluoroquinolones [7]. However, there is considerable variability in the quality of the studies. The majority of previously published studies on this topic are limited by the use of case-control methodology and retrospective assessment of antibiotic exposures, improper control groups, or small sample sizes [5, 8]. Furthermore, antibiotic exposures for hospitalized patients may vary during the course of admission, and prior prospective studies investigating the relationship between antibiotics and CDI have not fully taken into account these complex changes in exposure [7, 9]. While some studies have examined risk associated with the duration of specific antibiotic treatment regimens, only

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1 has evaluated the effects of total duration for all courses on the risk of CDI [10]. Currently, there are no measures to assess cumulative dose of antibiotic on the individual patient level, most likely a result of the inability to simply sum doses across antibiotic regimens due to differences in potency. Consequently, data on the association between total dose of antibiotic and the risk of CDI are lacking. Preliminary evidence suggests that the risk of CDI increases with increasing number of antibiotics used, but this finding has not been confirmed [6, 11–13]. Exposure to antibiotics is the most readily modifiable risk factor for the development of CDI; however, the majority of previous studies have focused on class of antibiotic rather than considering more complex patterns of use. A recent editorial [14], addressing consequences of unrestricted antibiotic use with respect to healthcare-associated infections, emphasized the need for antimicrobial stewardship programs to preserve the effectiveness of currently available therapies. The development of such programs should be informed by research that delineates the risks associated with cumulative antibiotic exposures. Accordingly, we examined the relationship between changes in cumulative antibiotic exposures over time and the risk of CDI.

METHODS

Study Design and Participants

A retrospective cohort study was conducted among patients hospitalized at Strong Memorial Hospital (SMH), a tertiary care academic hospital in Rochester, New York, during the period from 1 January to 31 December 2005. Patients were allowed to contribute more than 1 hospitalization to the study, and hospitalization was the unit of analysis. Patients were included if they were: (1) 18 years or older on admission, (2) admitted to an adult, nonpsychiatric inpatient unit, and (3) prescribed 2 or more consecutive days of antibiotics during their stay. Patients with a positive assay for *C. difficile* in the period from 60 days prior to admission through 2 days after admission and those who received oral vancomycin within 2 days of admission were excluded based on the possible presence of CDI at the start of follow-up. All data were obtained electronically from SMH administrative and clinical databases. Information was collected on patient demographics, admission and discharge dates, *International Classification of Diseases, Ninth Revision* (ICD-9) procedural and diagnostic codes, prior hospitalizations at SMH, all medications prescribed, and inpatient unit(s) visited for each hospital stay. Approval for this study was obtained from the Institutional Review Boards at SMH, Rochester General Hospital (RGH), and Unity Health System.

Outcome Assessment

Patients were followed for up to 60 days post discharge; therefore we obtained information on all positive *C. difficile* assays from SMH and 2 other laboratories that serve the Rochester region (RGH laboratory and ACM Clinical Laboratory) in order to maximize the sensitivity of case ascertainment. A case patient was defined as any patient meeting cohort eligibility criteria with a positive stool toxin enzyme immunoassay (EIA) for *C. difficile* (Premier Toxin A&B, Meridian Bioscience) at SMH during inpatient stay, or at any of the 3 laboratory systems within 60 days following discharge. The 3 laboratories routinely test only unformed stools, and we assumed that patients with positive EIA tests had symptomatic disease. The outcome of interest was the time to occurrence of CDI, and was defined as the time from hospital admission to the time of the first positive EIA for cases. Those who did not experience CDI by the end of follow-up were considered noncases.

Exposure Assessment

Information was available for the timing, dose, and route of administration for all antibiotic prescriptions written during hospitalization. For cases, metronidazole orders written within 2 days of a positive EIA and oral vancomycin orders written at any time were considered presumptive treatment for CDI and were excluded from analysis. Antibiotics were grouped into the following classes: aminoglycosides, first- and second-generation cephalosporins, third- and fourth-generation cephalosporins, clindamycin, macrolides, metronidazole, penicillins, β-lactamase inhibitor combinations (85% of which were piperacillin-tazobactam), fluoroquinolones, sulfas, vancomycin, and miscellaneous (including antituberculosis agents not otherwise classified, tetracyclines, and other uncommonly prescribed agents). Carbapenems were infrequently prescribed (<2% of all orders) and were grouped with the third- and fourth-generation cephalosporins. For each day of antibiotic exposure, the total dose for each individual antibiotic was calculated. Because 1 mg of antibiotic A is not necessarily the same as 1 mg of antibiotic B, daily doses were standardized according to the World Health Organization (WHO) Defined Daily Dose (DDD) system. DDDs are determined by the WHO according to the standard dose of a particular antibiotic per day for the main indication of that drug based on an assumed patient weight of 70 kg [15]. In our study, standardization was accomplished by dividing the calculated total daily dose by the DDD for each antibiotic. The number of DDDs received was then summed for all antibiotics prescribed on a given day to arrive at a total daily dose. Total dose of antibiotic was allowed to vary over the course of hospitalization such that on any given day of follow-up, the value for total dose reflects the sum of all DDDs prescribed for all antibiotics up to and including that day. Similar to total dose, both the number and days of antibiotic exposure were calculated daily. Total cumulative duration was measured in days of therapy (DOT), where multiple antibiotics given on the same day are counted as multiple antibiotic days [16]. The cumulative measures of antibiotic exposure

did not demonstrate a linear relationship with the log of the hazard and were therefore divided into quartiles.

Covariates

The following potential confounders were assessed: the presence of comorbidities based on the Chronic Disease Score–Infectious Diseases (CDS-ID), including peptic ulcer disease, diabetes, kidney disease, respiratory disease, transplant, and cancer [17]; recent surgery and gastrointestinal (GI) procedures using ICD-9 codes for the current and previous hospitalizations; HIV infection based on ICD-9 codes or the prescription of HIV-specific medications; dichotomous nonantibiotic medication exposures, including chemotherapy, immune suppressants, antacids, laxatives, and enemas; use of proton pump inhibitors (PPIs) and histamine-2 (H2) blockers as acid suppressive agents; history of CDI defined as any previous positive EIA that occurred more than 60 days prior to admission based on positive assay results reported from all 3 laboratories; and ''colonization risk'' (low, medium, and high risk) as a time-dependent measure of ''*Clostridium difficile*–Associated Disease (CDAD) pressure''[18] based on tertiles of the rate of CDI per 1000 patient-days for each of the 29 included inpatient units during the study, and accounting for patient transfer from 1 unit to another during hospitalization. Unit rankings were calculated using monthly and yearly rates; variation in monthly rates was observed by unit, but relative rankings of units were consistent when using either monthly or yearly rates. Only those exposures, including length of stay, occurring prior to the development of CDI were considered for case patients.

Data Analysis

In order to account for the clustering of patients with multiple hospitalizations within the cohort, all hospitalization-specific bivariate and multivariable analyses were conducted using marginal Cox proportional hazards (PH) models with robust covariance estimation. Because models include exposures that change with time, hazard ratios (HRs) reflect comparisons between individuals with and without exposures at the time of each event. For time-varying exposures, reported frequencies reflect exposure at any time during hospitalization. Four individual multivariable models were constructed; 1 for each of the antibiotic exposure classifications (dose, DOT, number, and class; the multivariable model for class included all 12 antibiotic class variables). All multivariable models were stratified on the receipt of antacids, laxatives, or enemas due to nonproportionality of hazards over time. Because we did not have exposure information for the period following discharge, we performed a sensitivity analysis to determine whether the results were comparable between those who developed CDI during hospitalization and those who were diagnosed after discharge. Similar estimates and patterns of risk were observed for both groups (data not shown). Statistical analyses were performed in SAS V9.2 (SAS) with a 2-sided alpha of .05.

RESULTS

The number of hospitalizations that qualified for entry into the study was 10154, contributed by 7792 unique patients. Approximately 80% of patients contributed 1 hospitalization to the cohort, and 94% contributed less than 3 (maximum: 9). During the study period, there were 587,659 days of follow-up and 241 CDI events from 240 patients, resulting in an incidence rate of 4.34 per 10,000 patient-days (95% CI 3.6, 4.7). The median age of the 7792 patients was 59.3 (interquartile range [IQR]: 30.7); 81% were white, 14% were black, and 4% were other races. Approximately 51% were male. PPI or H2 blockers were prescribed to 69% of patients. Fifty-six (0.6%) patients had a history of CDI greater than 60 days prior to the current admission.

Case patients were significantly older than noncase patients (median age 68.4 years vs 59.1 years, respectively; $P < .0001$), but there was no difference in race or sex. Clinical characteristics associated with the development of CDI on bivariate analysis are shown in Table 1. Factors associated with an elevated risk of CDI include increasing CDS-ID, GI procedures, HIV infection, history of CDI, increasing length of stay, and greater colonization pressure. Medications associated with increased risk include PPI and H2 blockers, antacids, laxatives, enemas, immune suppressive agents, and chemotherapy.

A comparison of the antibiotic exposure profiles for case and noncase patients is presented in Table 2. Overall, patients were prescribed a median (IQR) of 2.0 (2.0) antibiotic orders for 7.0 (9.0) days during hospitalization. The most frequently prescribed classes of antibiotics were first- and second-generation cephalosporins (39.2%), fluoroquinolones (35.5%), β-lactamase inhibitor combinations (30.9%), and vancomycin (28.2%) (data not shown).

Overall, case patients received greater cumulative doses, numbers, and days of antibiotics relative to noncases. We observed dose-dependent increases in the risk of CDI with increasing cumulative dose, number, and days of antibiotic exposure. After taking into account the effects of age, CDS-ID, GI procedures, HIV, previous CDI, colonization risk, acid suppressive agents, immune suppressive agents, chemotherapy, length of stay, and receipt of antacids, laxatives, and enemas, patients who received 2 antibiotics were at a 2.5-fold increased risk of CDI when compared with those who received only 1 antibiotic (95% CI 1.6–4.0). Among patients that received 3 or 4 antibiotics or 5 or more antibiotics, the estimates increased to 3.3 (95% CI 2.2–5.2) and 9.6 (95% CI 6.1–15.1), respectively. Similar patterns were observed for cumulative dose and duration of antibiotic exposure. Cephalosporins, β-lactamase inhibitor combinations, fluoroquinolones, sulfas, and intravenous

Table 1. Clinical Characteristics Associated With the Development of *Clostridium difficile* Infection

Characteristic	CDI positive *n* (%)	CDI negative *n* (%)	Crude hazard ratio[a,b] (95% CI)
No. of hospitalizations	241	9913	—
CDS-ID score, median (IQR)[c]	2.8 (1.6)	1.8 (3.2)	1.3 (1.2, 1.4)
Cancer	14 (6)	290 (3)	—
Diabetes	81 (34)	2149 (22)	—
Transplant	26 (11)	987 (10)	—
Kidney disease	28 (12)	503 (5)	—
Respiratory illness	63 (23)	2009 (20)	—
Peptic ulcer disease	182 (76)	5960 (60)	—
GI procedure	79 (33)	1360 (14)	2.6 (2.0, 3.5)
HIV	8 (3)	174 (2)	2.1 (1.1, 4.2)
Previous CDI	5 (2)	51 (1)	4.5 (1.8, 11.2)
Patient location (colonization risk)[d]	—	—	—
Low-risk unit	30 (12)	2753 (28)	Ref
Medium-risk unit	107 (44)	4253 (43)	2.3 (1.5, 3.4)
High-risk unit	104 (43)	2907 (29)	3.1 (2.1, 4.7)
Length of stay, median (IQR)[c]days	12 (17)	7 (8)	1.1[b](1.1, 1.1)
PPI/H2 blockers	208 (86)	6757 (68)	2.8 (1.9, 4.0)
Antacids, laxatives, and enemas	185 (77)	6000 (61)	2.0 (1.5, 2.7)
Immune suppressants	88 (37)	2810 (28)	1.4 (1.1, 1.8)
Chemotherapy	26 (11)	520 (5)	1.9 (1.3, 2.9)

NOTE. CDI indicates *Clostridium difficile* infection; CI, confidence interval; CDS-ID, Chronic Disease Score–Infectious Diseases; IQR, interquartile range; GI, gastrointestinal; HIV, human immunodeficiency virus; Ref, reference; PPI, proton pump inhibitor; H2, histamine-2.

[a] Hazard ratios and 95% CI from single covariate Cox proportional hazards models with robust covariance estimation.

[b] Hazard ratio for 7-day increments in Length of Stay.

[c] IQR is defined as the mathematical difference between the 75th percentile and the 25th percentile.

[d] Hazard ratio for time-dependent variables reflect comparisons between individuals with and without exposure at each event time. Frequencies reflect exposure at any time during hospitalization.

vancomycin were associated with an increased risk of CDI, while metronidazole was associated with a decreased risk of CDI.

DISCUSSION

Antibiotics are well established in the etiology of healthcare-associated CDI [4]. Much of the focus has been on particular classes or individual drugs, however, and little attention has been paid to the role of cumulative exposures across classes. Furthermore, antibiotic exposures for hospitalized patients change over time, and no previous studies have been undertaken to account for these complex changes in exposure.

Cumulative dose, number, and duration of antibiotics were independently associated with the development of CDI, with higher levels of exposure corresponding to greater risk. There is preliminary evidence to support an association between the number and days of antibiotics and the risk of CDI [6, 10, 12–13]. However, empiric biological data on the relationship between measures of dose, number, and days of antibiotics and the development of CDI are sparse. We surmise that larger doses and numbers and greater duration of antibiotics all result in a greater degree of normal flora depletion relative to shorter treatment courses comprising fewer antibiotics and smaller overall doses. The degree of depletion depends on the concentrations of drug achieved, duration of exposure, and susceptibility of the microorganisms in the intestine, and likely influences the ability of *C. difficile* to overgrow and cause disease [19–20]. Treatment with higher doses may result in higher drug concentrations, while longer durations of therapy allow for sustained periods of elevated concentrations and susceptibility to spores. Empiric use of antibiotics usually involves the use of more than 1 drug because the etiologic agent is unknown, potentially resulting in wider overall coverage of the possible pathogen as well as the enteric flora relative to monotherapy treatment. This may hold true regardless of whether antibiotics are given concurrently or sequentially, and may explain in part why larger numbers of antibiotics are associated with greater risk in our and previous studies.

In our study, patients who received cephalosporins, β-lactamase inhibitor combinations, fluoroquinolones, sulfa drugs, and intravenous vancomycin were significantly more likely to develop CDI relative to patients who did not receive these antibiotics, independent of all other antibiotics received. Cephalosporins are well known to predispose patients to CDI [5, 7–8, 12, 21]. Recently, fluoroquinolones have also been recognized as an important risk factor [7, 9, 21], likely as a result of the frequency of use [22], broad spectrum of activity, and the increasing proportion of CDI caused by the highly fluoroquinolone-resistant North American pulsed-field gel electrophoresis type 1 (NAP1) strain [2]. In our study, fluoroquinolones demonstrated the strongest association with CDI of any class investigated.

Sulfa drugs and β-lactamase inhibitor combinations have not been commonly associated with CDI [7, 21], possibly reflecting differences in prescribing patterns. In a study by Dubberke and colleagues [9], intravenous vancomycin courses 7 days or longer were independently associated with CDI. Intravenous vancomycin has rarely been associated with CDI in previous studies [23], and our results support this association. It has been suggested that vancomycin may be excreted into the stool in levels sufficient to cause disruption of normal flora but insufficient to prevent *C. difficile* overgrowth [9]. In both the present study and that of Dubberke and colleagues [9], intravenous vancomycin was associated with the risk of CDI independent of other classes of antibiotic received and other underlying conditions,

Table 2. Comparison of Cumulative Antibiotic Exposures for Case and Noncase Hospitalizations

Characteristic	CDI positive *n* (%)	CDI negative *n* (%)	Crude hazard ratio[a,b] (95% CI)	Adjusted hazard ratio[a,c,d] (95% CI)
Defined daily doses[e], median (IQR)	14.8 (21.2)	7.2 (12.3)	—	—
<3.0	18 (7)	1502 (15)	Ref	Ref
3.0 to 7.79	49 (20)	3702 (37)	1.1 (.7, 2.1)	1.2 (.7, 2.1)
7.80 to 21.0	89 (37)	2952 (30)	2.9 (1.8, 4.8)	2.8 (1.7, 4.6)
>21.0	85 (35)	1757 (18)	5.3 (3.2, 8.8)	5.3 (3.1, 9.0)
Antibiotic days, median (IQR)[f]	14.0 (23.0)	7.0 (9.0)	—	—
<4	22 (9)	2208 (22)	Ref	Ref
4 to 7	41 (17)	3071 (31)	1.5 (.9, 2.4)	1.4 (.8, 2.4)
8 to 18	87 (36)	3097 (31)	3.4 (2.1, 5.4)	3.0 (1.9, 5.0)
>18	91 (38)	1537 (16)	9.8 (6.0, 16.0)	7.8 (4.6, 13.4)
Number of antibiotics, median (IQR)[f]	3.0 (4.0)	2.0 (2.0)	—	—
1	31 (13)	3744 (38)	Ref	Ref
2	54 (22)	2507 (25)	2.7 (1.8, 4.3)	2.5 (1.6, 4.0)
3 or 4	70 (29)	2505 (25)	3.7 (2.4, 5.7)	3.3 (2.2, 5.2)
5 or more	86 (36)	1157 (12)	11.6 (7.7, 17.4)	9.6 (6.1, 15.1)
Class—any during hospitalization[g]				
Aminoglycosides	22 (9)	837 (8)	0.8 (.2, 2.6)	0.9 (.3, 3.0)
Cephalosporins				
First- and second-generation	94 (39)	3883 (39)	2.4 (1.4, 4.0)	2.4 (1.4, 4.1)
Third- and fourth-generation	74 (31)	1527 (15)	3.1 (1.9, 5.2)	3.1 (1.9, 5.2)
Clindamycin	34 (14)	876 (9)	1.7 (.8, 3.9)	1.9 (.8, 4.4)
Macrolides	48 (20)	1266 (13)	1.7 (.8, 3.4)	1.5 (.7, 3.1)
Metronidazole	37 (15)	981 (10)	0.3 (.1, .9)	0.3 (.1, 0.9)
Penicillins	30 (12)	993 (10)	1.7 (.8, 3.6)	1.9 (.9, 4.0)
β-Lactamase inhibitor combinations	120 (50)	3013 (30)	2.4 (1.6, 3.7)	2.3 (1.5, 3.5)
Quinolones	132 (55)	3471 (35)	4.5 (3.1, 6.5)	4.0 (2.7, 5.9)
Sulfas	33 (14)	1158 (12)	1.8 (1.0, 3.0)	1.9 (1.1, 3.4)
Vancomycin	120 (50)	2741 (28)	2.6 (1.7, 3.9)	2.6 (1.7, 4.0)
Miscellaneous[h]	31 (13)	772 (8)	1.4 (.7, 2.7)	1.3 (.7, 2.6)

NOTE. CDI indicates *Clostridium difficile* infection; CI, confidence interval; IQR, interquartile range; Ref, reference.

[a] All hazard ratios for time-dependent variables reflect comparisons between individuals with and without exposure at each event time.

[b] Hazard ratios and 95% CI from single covariate Cox proportional hazards models with robust covariance estimation and time-dependent antibiotic exposures.

[c] Adjusted for antacids, laxatives, and enemas, age, CDS-ID, GI procedures, history of CDI, HIV, colonization risk, acid suppressive agents, immune suppressants, chemotherapy, and length of hospitalization.

[d] CDS-ID, colonization risk, age, and GI procedures were independent predictors of CDI in all models.

[e] World Health Organization Defined Daily Dose (DDD) system.

[f] IQR is defined as the mathematical difference between the 75th percentile and the 25th percentile.

[g] Hazard ratios account for all other classes of antibiotics received during hospitalization.

[h] Includes tetracyclines, nitrofurantoin, linezolid, daptomycin, antituberculosis agents such as isoniazid and rifampin, and other antibiotics not otherwise classified.

suggesting that there may be a true relationship. Interestingly, we did not find an increased risk of CDI associated with clindamycin, as has been frequently reported [5–7]. The lack of an association could be explained by the fact that 90% of clindamycin orders in our cohort were for a period of less than 7 days, or because of potentially insufficient statistical power.

In order to maximize the sensitivity of case ascertainment, we followed patients for outcome up to 60 days post discharge. This is consistent with the methods of a previous study [7] and with the observation that approximately 90% of cases of healthcare-associated CDI that occur after discharge are diagnosed within 60 days [24]. We were unable to obtain information on antibiotic exposures during this period, which may have resulted in some misclassification of exposures. However, there is unlikely to be a difference in the change in intensity of antibiotic exposures after discharge for patients with and without CDI, and sensitivity analyses demonstrated similar estimates and patterns of association for patients who developed CDI during hospitalization compared with those who were diagnosed after discharge. We limited our analysis to patients who received 2 or more days of antibiotics, despite evidence that prophylactic and short courses of antibiotics may be associated with increased risk

of CDI [25]. This is due to the difficulty in following these patients, many of whom are either not admitted to the hospital or are discharged soon after surgery.

This study was limited by the use of administrative hospital databases, which are used for the facilitation of patient care and are not necessarily intended for research use. In addition, EIA has demonstrated suboptimal sensitivity compared with the gold-standard cytotoxicity assay, which may have resulted in missing a substantial number of cases [26]. Incorrect classification of CDI cases as noncases would result in underestimation of our hazard ratios, because the misclassification is unlikely to be differential by antibiotic exposures. Finally, the use of DDD is potentially limited by differences in prescribing practices in the United States from those reflected in the DDD. While dosage adjustments related to renal failure and obesity are not reflected in the standards, we were able to account for this by adjusting each individual patient's dose rather than estimating antimicrobial use at the population level.

The current study also has several strengths, which represent improvements on methods employed in previous studies. The prospective study design allowed us to capture changes in antibiotic exposures over time. Our relatively large sample size enabled us to adjust for important covariates. We also employed a novel application of the DDD methodology, resulting in a measure of cumulative dose across antibiotics that predicts CDI.

The findings from this study support the overall principals of antimicrobial stewardship, which specify that in order to reduce undesirable outcomes associated with the inappropriate and excessive use of antibiotics (including but not limited to CDI), antimicrobial selection, dosing, and duration should all be taken into consideration and optimized [27]. Increasingly, Infectious Diseases Society of America (IDSA) clinical practice guidelines for common infections include recommendations to select shorter courses of antibiotics [28–30]. Our results suggest that reductions in duration of antibiotic exposure, in conjunction with formulary restriction [31–32] and evaluation of polypharmacy [33], could result in a reduction of nosocomial CDI.

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