Is *Clostridium difficile* infection influenced by antimicrobial use density in wards?

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This study was performed to elucidate the relationship between antimicrobial use density (AUD) and *Clostridium difficile* infection (CDI) manifesting as antimicrobial-associated diarrhea (AAD) in hospital wards during a 4-year period. Case definition of CDI was an adult exhibiting AAD with a daily stool frequency of three or more, arising at least 48 hours after ward admission, and fecal samples testing positive for toxin (A and/or B). Metronidazole or vancomycin was orally administered as treatment. AUDs were calculated for a total of 21 antimicrobials in a span of 48 months and nine wards. We included the average value of AUDs, representing two succeeding months of sample submission into the sample information. We also entered data on the 2-year division and intensified contact precaution for statistical analysis. Of a total of 463 cases, 95 (20.5%) were CDI-positive. Multivariate regression analysis showed odds ratios [OR] of 1.739 (95% confidence interval [CI] of 1.050 – 2.881, $P=0.032$) and 1.598 (95% CI of 1.006 – 2.539, $P=0.047$) for clindamycin and piperacillin, respectively in AUD. Thus increased ward AUDs of clindamycin and piperacillin may run the risk of CDI.

Treatment with a variety of drugs is known to cause *Clostridium difficile* infection (CDI) as antimicrobial-associated diarrhea (AAD)[1]. Specifically, fluoroquinolones, which are effective against anaerobes, have an association with CDI[2]. Restricted use of fluoroquinolones has also been associated with a reduction in CDI incidence[3].

Few studies have described the relationship between CDI and antimicrobial use density (AUD) representing antimicrobial pressure in hospital wards. Previous studies have investigated the influence of AUD on the incidence of extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae*[4], *Pseudomonas aeruginosa*[5], and methicillin-resistant *Staphylococcus aureus* (MRSA)[6]. We attempted to clarify the relationship between *C. difficile* and ward AUD.

Materials and Methods

Case definition

We defined CDI as AAD in an admitted adult patient with loose or watery fecal samples, in the absence of laxative uses, enteral feeding, or antineoplastic agents, who also met three additional prerequisites; (1) admission in the ward for at least 48 hours, (2) positive stool test for toxin A and/or B (TOX A/B QUIK CHEK, Nissui Pharmaceutical Co., Tokyo), and (3) loose or watery stool with a frequency of three or more per day.

Materials

From a total of nine wards in the hospital, attending physicians and infection control professionals submitted fecal samples from all inpatients presenting with AAD. All of the samples were tested for toxin whereas a portion of samples was cultured for *C. difficile*. We excluded samples from patients who had already tested positive within the past month and those that did not meet the case definition. Antimicrobials in patients positive for CDI were discontinued except for parenteral vancomycin to treat co-existent MRSA infection and agents for life-threatening co-infection. Oral administration of metronidazole or vancomycin was combined with probiotic agents, such as of lactobacilli for treatment.

Patients with CDI were isolated, and the room and equipment were disinfected with sodium hydrochlorite. The use of gloves was re-enforced during the care in the patients positive for CDI or MRSA. MRSA isolated before the onset of CDI was recorded into a database and noted as a preventing event by intensified contact precaution. Positive CDI cases were reported to hospital infection control professionals such as board-certified infectious disease physicians, bacteriologists, and nurses. They inspected compliance to the hospital guidelines by house staff.

Analysis

AUDs were calculated for a total of 21 agents, 48 months, and nine wards by the formula:

$$\text{AUD} = (\text{Total antimicrobial dose})/(\text{DDD} \times \text{Monthly inpatients}) \times 1000$$

DDD stands for defined daily dose by WHO (Geneva, Switzerland)[7]. We included the averaged AUDs for two months, which included the month of sample submission and the month preceding it. In a given antimicrobial AUD, the median value was used to assign a value of 0 or 1 for data higher or lower than the median, respectively.

Antimicrobials of parenteral use for AUD calculation are as follow: ampicillin, sulbactam-ampicillin, cefazolin, ceftazidime, cefmetazole, sulbactam-cefoperazone, cefotaxime, cefotiam, cefozopran, cefpirome, ceftriaxone, clindamycin, flomoxef, fosfomycin, imipenem-cilastatin, meropenem, minocycline, panipenem-betamipron, piperacillin, tazobactam-piperacillin, and paren-

teral vancomycin. Quinolones were not included because of their limited intravenous use.

The Chest Medicine ward was selected as an index ward because of the high incidence of CDI shown in a pilot study. The prevalence rate of CDI in a given year and ward was defined as follows:

$$\text{CDI prevalence rate} = (\text{Positive cases})/(\text{Sum of inpatients}) \times 1000$$

We included background information after the second year of data collection to see factor in historical bias and intensified glove use due to pre-CDI isolation of MRSA. Data underwent logistic regression analysis where the outcome was determined as a positive CDI. Any variables significant in univariate regression analysis underwent multivariate regression analysis. Statistical significance was defined as $P < 0.05$. SPSS statistical software (IBM Inc., Armonk, NY) was used for the analysis.

In select samples, CCMA Medium EX (Nissui Pharmaceutical, Tokyo) was used as media for anaerobic culture. After 48 hours, colony was extracted and cultured using Rabbit ABCM Blood Agar Medium (Eiken Chemical, Tokyo). BBL CRYSTAL ANR (Japan Becton-Dickinson, Tokyo) was used to type *C. difficile.* The antibiotic susceptibility of *C. difficile* isolates was defined by the Clinical and Laboratory Standards Institute (Wayne, PA)[8].

Results

In a total of 463 cases, 95 (20.5%) were CDI-positive with a median duration of 27 days after admission. Among these, 15 cases (15.8%) were preceded by the isolation of MRSA with enhanced contact precaution.

The univariate analysis for background factors of CDI revealed significance in the AUDs of clindamycin and piperacillin alone (Table 1). Using these factors, multivariate analysis showed that both were significant, with the odds ratio for clindamycin and piperacillin at 1.739 ($P = 0.032$) and 1.598 ($P = 0.047$), respectively in AUD (Table 2).

Among the wards, chest medicine ward 6A showed the highest CDI prevalence rate of 0.301, with a median of four years (Fig. 1). In 2010, neurosurgery ward 4A documented the highest prevalence rate of 0.703. In both cases, patients with recurrent CDI across several months were included in the study and stayed in separate rooms, making an outbreak less likely.

From 2008 through 2011, median AUD levels of clindamycin peaked in 2010, whereas mean AUD levels of piperacillin tended to decrease (Fig. 2).

The values of AUDs for 21 agents showed increased median values for clindamycin in the neurosurgery ward and sulbactam-ampicillin in both the chest medicine ward and the intensive care unit (Fig. 3).

Out of a total of 29 specimens isolated with *C. difficile*, 16 (55.2%) tested positive for toxin

Table 1. Univariate logistic regression analysis on risk factors for *Clostridium difficile* infection during a 4-year period.

Factors	Odds ratio	95% confidence interval	*P*
AUD:			
Ampicillin	1.350	0.817 – 2.231	0.242
Cefazolin	1.121	0.713 – 1.762	0.622
Cefmetazole	1.149	0.747 – 1.768	0.527
Cefotaxime	0.700	0.352 – 1.393	0.310
Cefotiam	0.910	0.579 – 1.429	0.682
Cefozopran	1.054	0.671 – 1.655	0.820
Cefpirome	0.770	0.166 – 3.574	0.738
Ceftazidime	0.967	0.616 – 1.519	0.885
Ceftriaxone	1.318	0.837 – 2.074	0.233
Clindamycin	1.780	1.077 – 2.941	0.025*
Flomoxef	1.054	0.671 – 1.655	0.820
Fosfomycin	0.887	0.549 – 1.432	0.623
Imipenem-cilastatin	0.860	0.509 - 1.259	0.335
Meropenem	0.809	0.514 – 1.272	0.358
Minocycline	1.410	0.897 – 2.218	0.136
Panipenem-betamipron	0.952	0.591 – 1.534	0.841
Piperacillin	1.780	1.077 – 2.941	0.021*
Tazobactam-piperacillin	0.959	0.611 – 1.507	0.857
Sulbactam-ampicillin	0.873	0.555 – 1.373	0.557
Sulbactam-cefoperazone	0.969	0.617 – 1.521	0.890
Vancomycin	0.913	0.581 – 1.435	0.693

Note: AUD, antimicrobial use density of the month and ward being more than the median of all the values; *, significant at $P < 0.05$.

Table 1. Univariate logistic regression analysis on risk factors for *Clostridium difficile* infection during a 4-year period. (Continued)

Factors	Odds ratio	95% confidence interval	*P*
Ward:			
Chest medicine	1.008	0.579 – 1.753	0.978
Contact precaution:**			
Intensified vs. not	1.045	0.562 – 1.943	0.890
2-year division:			
Latter vs. Former	1.105	0.700 – 1.744	0.667

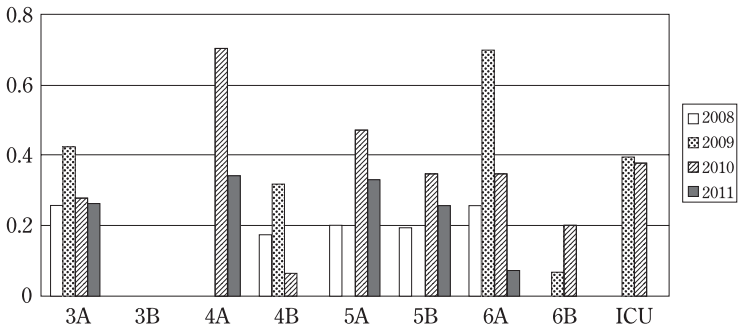
Note: **; intensified contact precaution as determined by preceding isolation of methicillin-resistant *Staphylococcus aureus* (MRSA).

Table 2. Multivariate logistic regression analysis on risk factors for *Clostridium difficile* infection.

Factors	Odds ratio	95% confidence interval	*P*
AUD:			
Clindamycin	1.739	1.050 - 2.881	0.032*
Piperacillin	1.598	1.006 – 2.539	0.047*

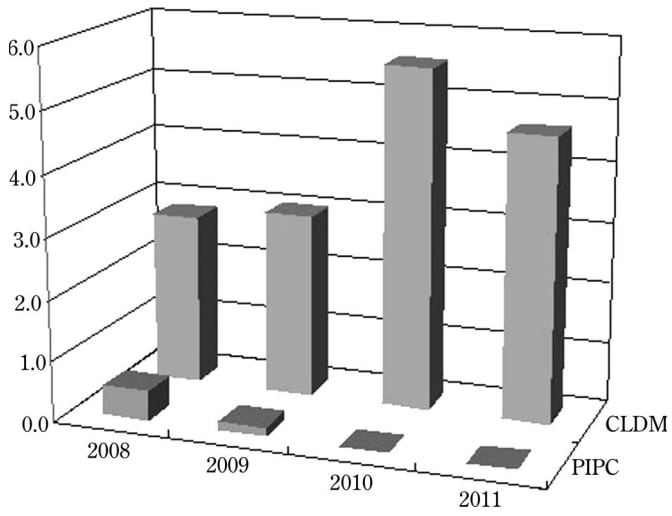
Note: AUD, antimicrobial use density of the month and ward being more than the median of all the values; *, significant at $P<0.05$.

Fig. 1. Annual prevalence rates of *Clostridium difficile* infection (CDI) in wards.



Note: ICU, intensive care unit; 6A, for the chest medicine; 4A, for the neurosurgery.

Fig. 2. Antimicrobial use density (AUD) of clindamycin (CLDM) and piperacillin (PIPC) by years.



Median values are shown in the vertical axis.
AUD of CLDM shows a peak in 2010 while AUD of PIPC is decreasing.

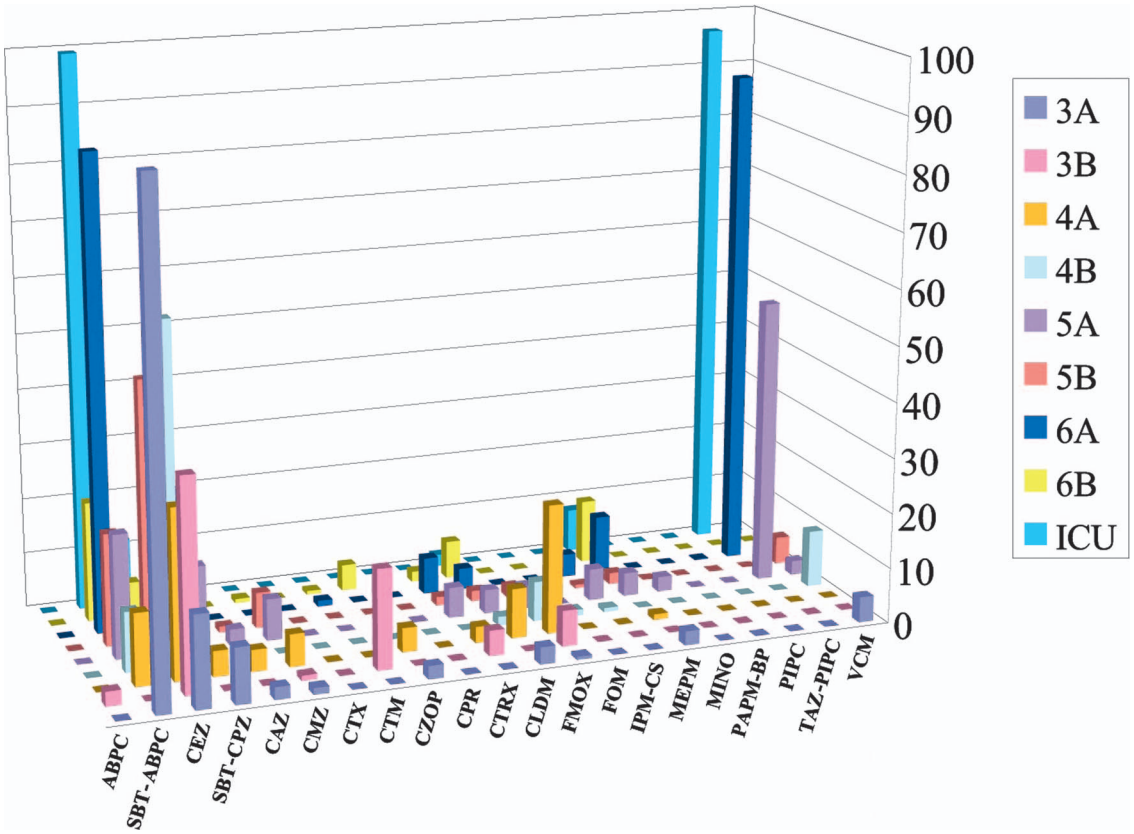
A and/or B. *C. difficile* strains were susceptible to clindamycin in nine of 16 (56.3%) samples, piperacillin in 16 of 16 (100.0%) samples (Table 3). MIC_{50} and MIC_{90} values were not suggestive of multi-drug resistance. No further examinations were performed including those for binary toxin.

Discussion

Antimicrobials associated with *C. difficile* diarrhea include clindamycin and cephalosporins, especially second- and third-generation agents such as ampicillin[1]. Similarly, the current study revealed that ward AUDs of piperacillin and clindamycin are risk factors for CDI. However, ureidopenicillins such as piperacillin alone or in combination with tazobactam have been described as less likely to cause CDI than ampicillin[9,10]. In fact, a shortage of tazobactam-piperacillin has been associated with an increase in CDI[11]. However, one study found hospital consumption of tazobactam-piperacillin to be associated with the occurrence of CDI[12].

Our study used AUD to reflect antimicrobial pressure and showed that piperacillin, but not ampicillin or tazobactam-piperacillin, was associated with CDI. Our series of *C. difficile* isolates, however, showed only a 50% susceptibility rate with ampicillin in contrast to 100% susceptibility rate with piperacillin, supporting existing literature on ampicillin-associated CDI (Table 3). However, AUDs in our study are presumed to reflect the ward-level effect of antimicrobials, which may explain the discrepancy with the literature on the susceptibility of *C. difficile* strains. The

Fig. 3. Antimicrobial use density (AUD) of 21 agents, *i.e.*, ampicillin (ABPC), sulbactam-ampicillin (SBT-ABPC), cefazolin (CEZ), sulbactam-cefoperazone (SBT-CPZ), ceftazidime (CAZ), cefmetazole (CMZ), cefotaxime (CTX), cefotiam (CTM), cefozopran (CZOP), cefpirome (CPR), ceftriaxone (CTRX), clindamycin (CLDM), flomoxef (FMOX), fosfomycin (FOM), imipenem-cilastatin (IPM-CS), meropenem (MEPM), minocycline (MINO), panipenem-betamipron (PAPM-BP), piperacillin (PIPC), tazobactam-piperacillin (TAZ-PIPC), and parenteral vancomycin (VCM) by wards.



Median values are shown in the vertical axis. Note: ICU, intensive care unit; 6A, for the chest medicine; 4A, for the neurosurgery.

AUD of parenteral vancomycin was not significant, probably as a result of its minimal effect on *C. difficile* in the gut.

Mean values of the two-month AUD were employed, because specimens submitted at the beginning of a month would be more influenced by the AUD of the previous month than of the current month. Therefore, the averaged AUDs represented antibiotic pressure with respect to ward volume. Despite our effort to prevent the propagation of *C. difficile*, the effect of mass clindamycin use may have influenced CDI in the wards as it does in an individual patient.

The association between AUD and MRSA incidence was previously determined as being in-

Table 3. Susceptibility of *Clostridium difficile* and minimum inhibitory concentration (MIC) against *C. difficile* strains (n=16) isolated in *C. difficile* infection.

Agents	Susceptibilities	MIC_{50} (μg/ml)	MIC_{90} (μg /ml)
Ampicillin	8 (50.0%)	1	2
Cefmetazole	14 (87.5%)	8	32
Clindamycin	9 (56.3%)	2	16
Flomoxef	N/A	2	8
Imipenem*	9 (56.3%)	2	32
Minocycline	N/A	<0.125	<0.125
Penicillin G	11 (61.5%)	0.5	2
Piperacillin	16 (100.0%)	2	8
Sulbactam-cefoperazone	8 (50.0%)	16	64
Vancomycin	N/A	0.5	1

Note: MIC_{50}, 50 percentile MIC; MIC_{90}, 90 percentile MIC; *, Without cilastatin as defined by Clinical and Laboratory Standards Institute (CLSI); N/A, not available by CLSI.

fluenced by the use of gloves[6]. Throughout the 10 years of our study, glove uses had been consistent once CDI was detected. However, the possibility of inconsistent glove use confounding the analysis remains. In the absence of multi-drug resistant Gram-negative rods in our hospital, pre-CDI detection of MRSA represented intensified use of gloves. However, logistic regression analysis showed no influence of this factor on CDI in our study.

In a previous study on extended-spectrum beta-lactamase-producing *K. pneumoniae*, an AUD for third-generation cephalosporin was employed as the index of control[4]. In two other studies investigating the susceptibility of *P. aeruginosa*, AUDs for meropenem and doripenem were used as indicators of antibiotic pressure[13]. In agreement with these studies[14], our work demonstrated that increased clindamycin AUD runs the risk of CDI in wards. The strains of *C. difficile* had low susceptibility to clindamycin, which may partially explain the CDI induction by microbial substitution. Our study supported a previous proposal to restrict hospital-wide use of clindamycin to regain *C. difficile* susceptibility to this antimicrobial[15].

The limitation of the current study is its retrospective design. To validate our hypothesis that ward AUD would influence CDI, a randomized control study should be performed.

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