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Surveillance and Correlation of Antibiotic Prescription and Resistance of Gram-Negative Bacteria in Singaporean Hospitals▽†

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A surveillance study was performed in four Singapore public hospitals from 2006 to 2008 to determine the correlation between antibiotic prescription and Gram-negative bacterial antimicrobial resistance. Targeted organisms included ceftriaxone- and ciprofloxacin-resistant *Escherichia coli* and *Klebsiella pneumoniae*, as well as imipenem-resistant *Pseudomonas aeruginosa* and *Acinetobacter* spp. Antibiotic prescription data were collated in the WHO anatomical therapeutic chemical (ATC)/defined daily dose (DDD) format, while antibiotic resistance was expressed as incidence density adjusted for total inpatient-days every quarter. Individual trends were determined by linear regression, while possible associations between antibiotic prescription and resistance were evaluated via cross-correlation analysis. Results over 3 years indicated significantly rising incidence densities of ceftriaxone- and ciprofloxacin-resistant *E. coli* and imipenem-resistant *Acinetobacter* spp. (blood isolates only). Antimicrobial-resistant *Klebsiella pneumoniae* rates declined. The prescription rates of piperacillin-tazobactam, ertapenem, meropenem, ciprofloxacin, and levofloxacin increased significantly, while imipenem and moxifloxacin prescription decreased. Cross-correlation analysis demonstrated possible associations between prescription of fluoroquinolones and ciprofloxacin-resistant *E. coli* ($R^2 = 0.46$), fluoroquinolones and ceftriaxone-resistant *E. coli* ($R^2 = 0.47$), and carbapenems and imipenem-resistant *Acinetobacter* spp. ($R^2 = 0.48$), all at zero time lag. Changes in meropenem prescription were associated with a similar trend in imipenem-resistant *Acinetobacter* blood isolates after a 3-month time lag. No correlation was found between cephalosporin use and resistance. In conclusion, our data demonstrated correlation between prescription of and Gram-negative bacterial resistance to several, but not all, key antimicrobial agents in Singapore hospitals. In areas where Gram-negative bacterial resistance is endemic and prescription of broad-spectrum antimicrobial agents is high, factors other than antimicrobial usage may be equally important in maintaining high resistance rates.

Antimicrobial resistance is an escalating global public health threat (3, 19). Gram-negative bacterial resistance is of particular importance as there is a dearth of novel antibiotics directed against these organisms (1), which are increasing in prevalence worldwide (8, 12, 15). Logically, an association should exist between antibiotic consumption and Gram-negative bacterial resistance, and this was demonstrated in multiple studies in both the hospital and community settings (2, 7, 10, 13, 18). Causal relationships have been difficult to establish, however, and there are studies that have failed to show any interdependence (1, 6). Even in several supportive studies, the association between antimicrobial consumption and resistance was not universal among all the antibiotics and organisms tested (2, 10, 18). This has been attributed to various reasons, including failure to control for confounding factors such as infection control measures, study biases, and lack of uniformity in susceptibility testing methods and definitions of resistance (14).

A review of various individual hospital studies highlighted the general trend of progressively increasing prevalence of quinolone-, cephalosporin-, and carbapenem-resistant Gram-negative bacilli in Singapore hospitals over the past 3 decades (11). No attempt had previously been made to investigate the association between antimicrobial consumption and Gram-negative bacterial resistance locally. Since 2006, the Network for Antimicrobial Resistance Surveillance (Singapore) has conducted laboratory- and pharmacy-based surveillance of antibiotic resistance and prescription in local public hospitals. The aim of this study is to report surveillance results over the past 3 years and to investigate the relationship between Gram-negative bacterial antimicrobial resistance and broad-spectrum antibiotic prescription in local institutions. This is the first systematic evaluation of the problem of antimicrobial resistance and broad-spectrum antibiotic prescription in Singapore.

MATERIALS AND METHODS

Study sites and period. Four of six Singapore public hospitals participated in the study. Hospital 1 is a 1,500-bed tertiary hospital; hospitals 2 through 4 are secondary general hospitals with 1,200, 900, and 400 beds, respectively. Data from January 2006 to December 2008 were analyzed on a quarterly basis for the

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purposes of the study. Denominator data in the form of hospital inpatient-days, i.e., the sum of each daily inpatient census every quarter, were obtained from the hospitals' administrative records.

Antibiotic prescription. Antibiotic prescription data were extracted from the electronic pharmacy records from each hospital. These figures comprise actual prescription data rather than purchase data. Antimicrobial agents tracked include carbapenems (imipenem, meropenem, and ertapenem), cephalosporins (ceftriaxone, ceftazidime, and cefepime only), fluoroquinolones (ciprofloxacin, levofloxacin, and moxifloxacin), and piperacillin-tazobactam. Defined daily dose (DDD) per 1,000 inpatient-days for each drug or drug category prescribed every quarter was calculated following the World Health Organization (WHO) anatomical therapeutic chemical (ATC) classification system of 2009 (20).

Antimicrobial resistance. Microbiologic data were extracted from the laboratory information system of each participating hospital and converted centrally into a standard format using WHONET 5.4 (WHO, Geneva, Switzerland), with duplicates eliminated according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) (4). The organisms tracked include ceftriaxone- and/or ciprofloxacin-resistant *Escherichia coli* and *Klebsiella pneumoniae* and imipenem-resistant *Pseudomonas aeruginosa* and *Acinetobacter* spp. Data were expressed as incidence density per 1,000 inpatient-days for every quarter. All hospital laboratories performed antimicrobial susceptibility testing predominantly through disk susceptibility testing, supplemented by the Vitek 2 system (bioMérieux, Marcy l'Etoile, France), following CLSI guidelines (5).

Statistical analysis. Each antibiotic prescription and resistance series was first explored independently for trend over time by linear regression. If a statistically significant ($P \leq 0.05$; $R^2 > 0.3$) trend was found, the presence or absence of associations between selected clinically meaningful antibiotic resistance and prescription series was further explored in pairs using cross-correlation analysis, where quarterly time lags of up to 1 year in both directions (i.e., time lag of -4 to 4) were applied to the antimicrobial resistance series during the analysis. An association between antibiotic prescription and resistance was deemed to be present and significant if the coefficient of determination (R^2) was >0.3 at any one time lag, and the highest correlation coefficient for each pair determined the most likely time lag where antibiotic prescription affected resistance or *vice versa* for that particular pair. A negative time lag for any result meant that antimicrobial resistance had preceded antibiotic prescription and *vice versa* for a positive time lag. All statistical analyses were performed using Systat version 12.0 (Systat Software, Inc., Point Richmond, CA).

RESULTS

Antibiotic prescription. Overall prescription of the different antimicrobial agents tracked is charted in Fig. 1, while the statistical trend for each antibiotic over 3 years is shown in Table 1. A significant increase in prescription was seen for the fluoroquinolones and carbapenems. However, the coefficient of determination for the carbapenems was poor ($R^2 < 0.5$). Cephalosporin prescription did not significantly increase over 3 years.

In terms of individual antimicrobial agents, there was increased prescription of ceftriaxone, ciprofloxacin, levofloxacin, meropenem, ertapenem, and piperacillin-tazobactam over this period, whereas cefepime, moxifloxacin, and imipenem prescription decreased significantly.

Antimicrobial resistance. The total number of organisms tested along with the percentage and incidence density of antimicrobial-resistant organisms over the 3-year period is shown in Table 2. The statistical trend for the incidence density of each antimicrobial-resistant organism is shown in Table 3. Over the study period, ciprofloxacin-resistant *E. coli* was the most prevalent antimicrobial-resistant organism. Virtually half of all *Acinetobacter* spp. were resistant to imipenem, compared to a far lower percentage of *P. aeruginosa.* However, the incidence densities of imipenem-resistant *Acinetobacter* spp. and *P. aeruginosa* were similar in view of the more frequent isolation of the latter. Ceftriaxone resistance of both *Enterobacteriaceae* tested was also common.

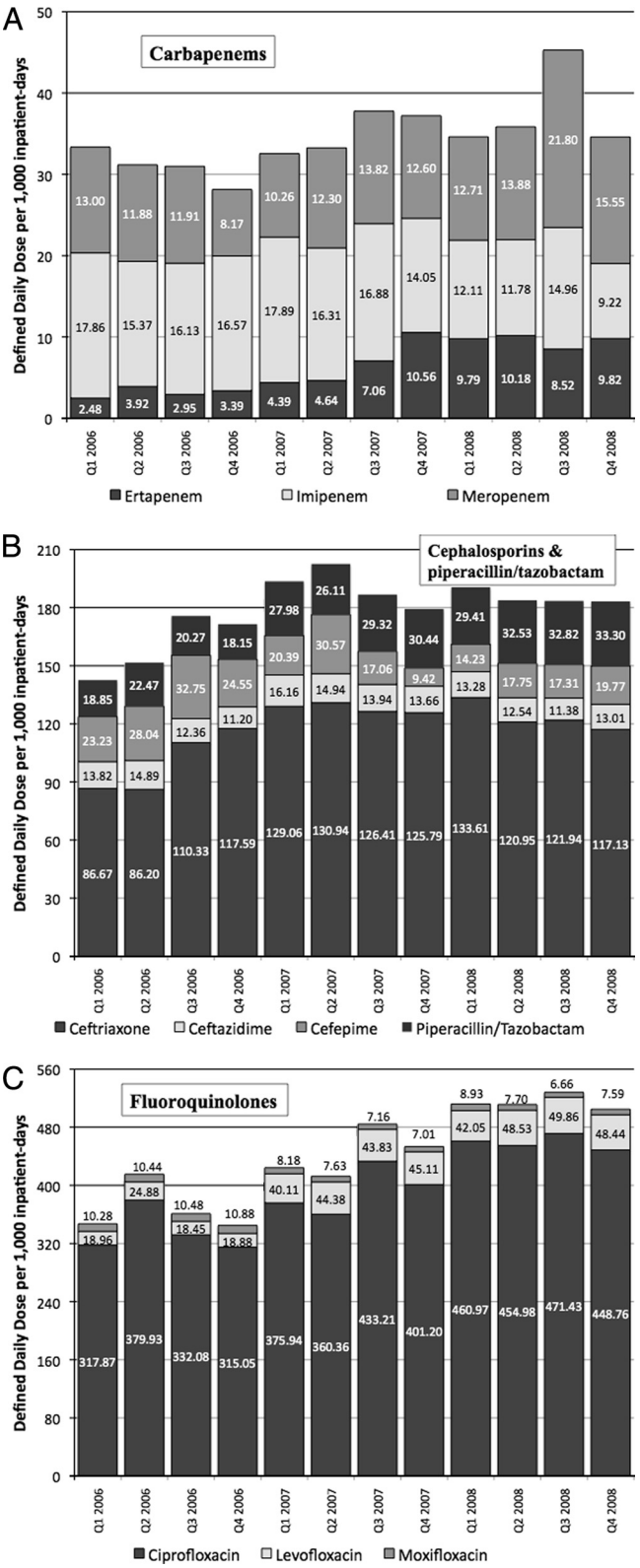

FIG. 1. Antibiotic prescription volumes in DDD/1,000 inpatient-days from four public Singapore hospitals over 3 years. (A) Carbapenems. (B) Cephalosporins and piperacillin-tazobactam. (C) Fluoroquinolones.

TABLE 1. Trends in antibiotic prescription in Singapore hospitals, 2006 to 2008

Antibiotic(s)	Gradient (DDD/1,000 inpatient-days per quarter)	R^2	*P* value	95% CI[a]	Trend
Cephalosporins	0.147	0.138	0.23	−0.111–0.407	Stable
Ceftriaxone[b]	0.279	0.406	0.03	0.041–0.516	Increasing
Ceftazidime	−0.013	0.104	0.31	−0.040–0.141	Stable
Cefepime[b]	−0.118	0.385	0.03	−0.223–−0.013	Decreasing
Piperacillin-tazobactam[b]	0.142	0.845	<0.01	0.099–0.184	Increasing
Fluoroquinolones[b]	1.677	0.807	<0.01	1.098–2.255	Increasing
Ciprofloxacin[b]	1.399	0.772	<0.01	0.864–1.935	Increasing
Levofloxacin[b]	0.311	0.780	<0.01	0.195–0.428	Increasing
Moxifloxacin[b]	−0.034	0.621	<0.01	−0.053–−0.015	Decreasing
Carbapenems[b]	0.079	0.431	0.02	0.015–0.143	Increasing
Imipenem[b]	−0.057	0.587	<0.01	−0.090–−0.023	Decreasing
Meropenem[b]	0.057	0.387	0.03	0.006–0.107	Increasing
Ertapenem[b]	0.079	0.815	<0.01	0.052–0.105	Increasing

[a] 95% CI, 95% confidence interval.
[b] Results where R^2 was >0.3 and *P* was <0.05.

A significant rising trend was found with regard to the incidence densities of ciprofloxacin- and ceftriaxone-resistant *E. coli* (overall and blood isolates) as well as imipenem-resistant *Acinetobacter* blood isolates, although the increment was gradual over 3 years. Surprisingly, the incidence density of overall ciprofloxacin-resistant *K. pneumoniae* fell, with a corresponding trend seen in ceftriaxone-resistant *K. pneumoniae* (overall and blood isolates). Figure 2 highlights the incidence density of antimicrobial-resistant *E. coli* and *Acinetobacter* spp. in conjunction with the prescription of respective classes of antibiotics.

TABLE 2. Incidence density and percentage of antimicrobial-resistant Gram-negative bacteria in Singapore hospitals, 2006 to 2008

Organism(s), drug susceptibility, and isolate type	No. of resistant isolates	% Resistance	Median incidence density of resistant isolates/1,000 inpatient-days (range)
Escherichia coli			
Ceftriaxone			
All isolates	6,629	20.0	1.87 (1.61–2.17)
Blood isolates	854	21.7	0.24 (0.18–0.30)
Ciprofloxacin			
All isolates	12,081	38.7	3.37 (3.18–3.74)
Blood isolates	1,285	31.0	0.36 (0.31–0.40)
Klebsiella pneumoniae			
Ceftriaxone			
All isolates	6,321	32.3	1.76 (1.42–2.27)
Blood isolates	685	27.4	0.19 (0.15–0.24)
Ciprofloxacin			
All isolates	6,285	30.1	1.72 (1.32–2.39)
Blood isolates	610	24.0	0.16 (0.13–0.25)
Acinetobacter spp., imipenem			
All isolates	2,000	46.2	0.56 (0.43–0.72)
Blood isolates	184	50.0	0.05 (0.03–0.08)
Pseudomonas aeruginosa, imipenem			
All isolates	1,139	7.5	0.32 (0.24–0.41)
Blood isolates	119	12.8	0.03 (0.02–0.07)

Correlation of antimicrobial consumption with resistance. In general, the rising incidence density of ciprofloxacin-resistant *E. coli* correlated at zero time lag with rising prescription trends of all quinolones ($R^2 = 0.46$ and 0.42 for all isolates and blood isolates, respectively) as well as ciprofloxacin and levofloxacin individually, piperacillin-tazobactam ($R^2 = 0.41$ and 0.51 for all isolates and blood isolates, respectively), and all carbapenems ($R^2 = 0.34$ and 0.35 for all isolates and blood isolates, respectively) as well as meropenem individually.

The rising ceftriaxone-resistant *E. coli* trend correlated at zero time lag with prescription trends of quinolones ($R^2 = 0.47$ and 0.52 for all isolates and blood isolates, respectively) including ciprofloxacin and levofloxacin individually, ceftriaxone ($R^2 = 0.31$ for all isolates only), piperacillin-tazobactam ($R^2 = 0.55$ and 0.63 for all isolates and blood isolates, respectively), and the carbapenems ($R^2 = 0.41$ and 0.51, respectively) including meropenem individually.

The increasing imipenem-resistant blood *Acinetobacter* spp. trend correlated at zero time lag with prescription trends of levofloxacin ($R^2 = 0.42$), piperacillin-tazobactam ($R^2 = 0.46$), and the carbapenems ($R^2 = 0.48$) including ertapenem individually. The prescription trend of meropenem was associated after a lag of 3 months with the trend of imipenem-resistant *Acinetobacter* spp. ($R^2 = 0.58$).

No other significant positive correlations between antibiotic resistance and prescription up to a positive time lag of 1 year were present. The rising trend of ceftriaxone-resistant *E. coli* was associated with the rising trend in ertapenem prescription ($R^2 = 0.47$) after a time lag of 3 months (negative lag of one quarter), but there were no other clinically meaningful associations found for the negative time lag cross-correlation analyses.

DISCUSSION

Our study highlights the extensive prescription of broad-spectrum antimicrobial agents in Singapore hospitals, coupled with high resistance rates among the Gram-negative bacilli surveyed. Carbapenem prescription has increased mostly as a consequence of increasing ertapenem prescription, whereas

TABLE 3. Trends in antimicrobial resistance in Singapore hospitals, 2006 to 2008

Organism(s), drug resistance, and isolate type	Gradient (incidence density/1,000 inpatient-days per quarter)	R^2	*P* value	95% CI[a]	Trend
Escherichia coli					
Ceftriaxone					
All isolates[b]	0.032	0.609	<0.01	0.014–0.050	Increasing
Blood isolates[b]	0.007	0.572	<0.01	0.003–0.011	Increasing
Ciprofloxacin					
All isolates[b]	0.031	0.424	0.02	0.005–0.056	Increasing
Blood isolates[b]	0.007	0.518	<0.01	0.002–0.011	Increasing
Klebsiella pneumoniae					
Ceftriaxone					
All isolates[b]	−0.074	0.838	<0.01	−0.096–−0.051	Decreasing
Blood isolates[b]	−0.005	0.412	0.02	−0.009–−0.007	Decreasing
Ciprofloxacin					
All isolates[b]	−0.091	0.902	<0.01	−0.112–−0.070	Decreasing
Blood isolates	−0.004	0.264	0.08	−0.009–0.001	Stable
Acinetobacter spp., imipenem					
All isolates	−0.009	0.135	0.24	−0.263–0.007	Stable
Blood isolates[b]	0.003	0.394	0.03	0.003–0.005	Increasing
Pseudomonas aeruginosa, imipenem					
All isolates	0.0004	0.081	0.37	−0.006–0.014	Stable
Blood isolates	0.002	0.257	0.09	−0.004–0.005	Stable

[a] 95% CI, 95% confidence interval.
[b] Results where R^2 was >0.3 and *P* was <0.05.

imipenem prescription may be declining due to increased prescription of both ertapenem and meropenem. It is interesting that meropenem is considerably more expensive than imipenem in Singapore; thus, different marketing practices and changing physician perceptions may have accounted for declining imipenem usage, but this is beyond the scope of the present study.

Compared with previous studies, the prevalence of Gram-negative bacterial antimicrobial resistance in Singapore hospitals has remained relatively stable over the past 2 years (9, 11). Ciprofloxacin and ceftriaxone resistance rates in *E. coli* continued to increase, although antibiotic-resistant *K. pneumoniae* incidence densities surprisingly decreased over the study period. The reasons for this divergent trend between the *Enterobacteriaceae* could not be ascertained in a surveillance study, but it is plausible that an increase in incidence density of community extended-spectrum beta-lactamase (ESBL)-producing and quinolone-resistant *E. coli* isolates may have contributed to this phenomenon. Pada and coworkers had found that up to 12% of more than 1,000 emergency department attendees without previous health care association at hospital 2 in 2007 were colonized with ESBL-positive *Enterobacteriaceae*—the vast majority of which were *E. coli* (16). Unfortunately, we could not reliably distinguish between community- and hospital-associated infections in our study.

As infection control measures in the study hospitals had not changed significantly over the study period, the drop in *K. pneumoniae* rates against the stable or rising trends of other antibiotic-resistant Gram-negative bacilli is less easy to explain, beyond postulating about the possible decline of a predominant nosocomial *K. pneumoniae* clone. No molecular typing had been performed on *K. pneumoniae* over this study period; hence, we are unable to verify or disprove this postulation at this time.

Unlike the majority of published studies, there were few significant correlations between antimicrobial prescription and subsequent resistance or *vice versa* (2, 7, 10, 13, 18). Overall carbapenem prescription was correlated with significant but slight changes in imipenem resistance in *Acinetobacter* blood isolates within the same time period. Perhaps the major finding was that up to 58% of the changes in imipenem resistance in *Acinetobacter* blood isolates might be explained by meropenem prescription following a 3-month interval between prescription and resistance development.

Carbapenem prescription showed significant correlation with antibiotic-resistant *E. coli* incidence densities at no time lag. This is intuitive, as most nosocomial *E. coli* isolates are resistant to both classes of antibiotics, and infections are most commonly treated with carbapenems in Singapore. However, quinolone and piperacillin-tazobactam prescription also correlated with both ciprofloxacin- and ceftriaxone-resistant *E. coli* isolates at no time lag, and analyzing the data at shorter monthly time intervals or the individual hospital level (data not shown) failed to demonstrate any significant time lag as well. Given that antibiotic-resistant *K. pneumoniae* rates failed to correlate positively with antibiotic prescription and coupling this with the potential influx of community quinolone-resistant and/or ESBL-producing *E. coli* isolates, it is difficult to state that either ciprofloxacin- or ceftriaxone-resistant *E. coli* rates are significantly associated with antibiotic prescription despite the significant coefficients of determination.

There are several possible explanations for the lack of significant correlation between antibiotic prescription and resistance in our study. As had previously been pointed out, resis-

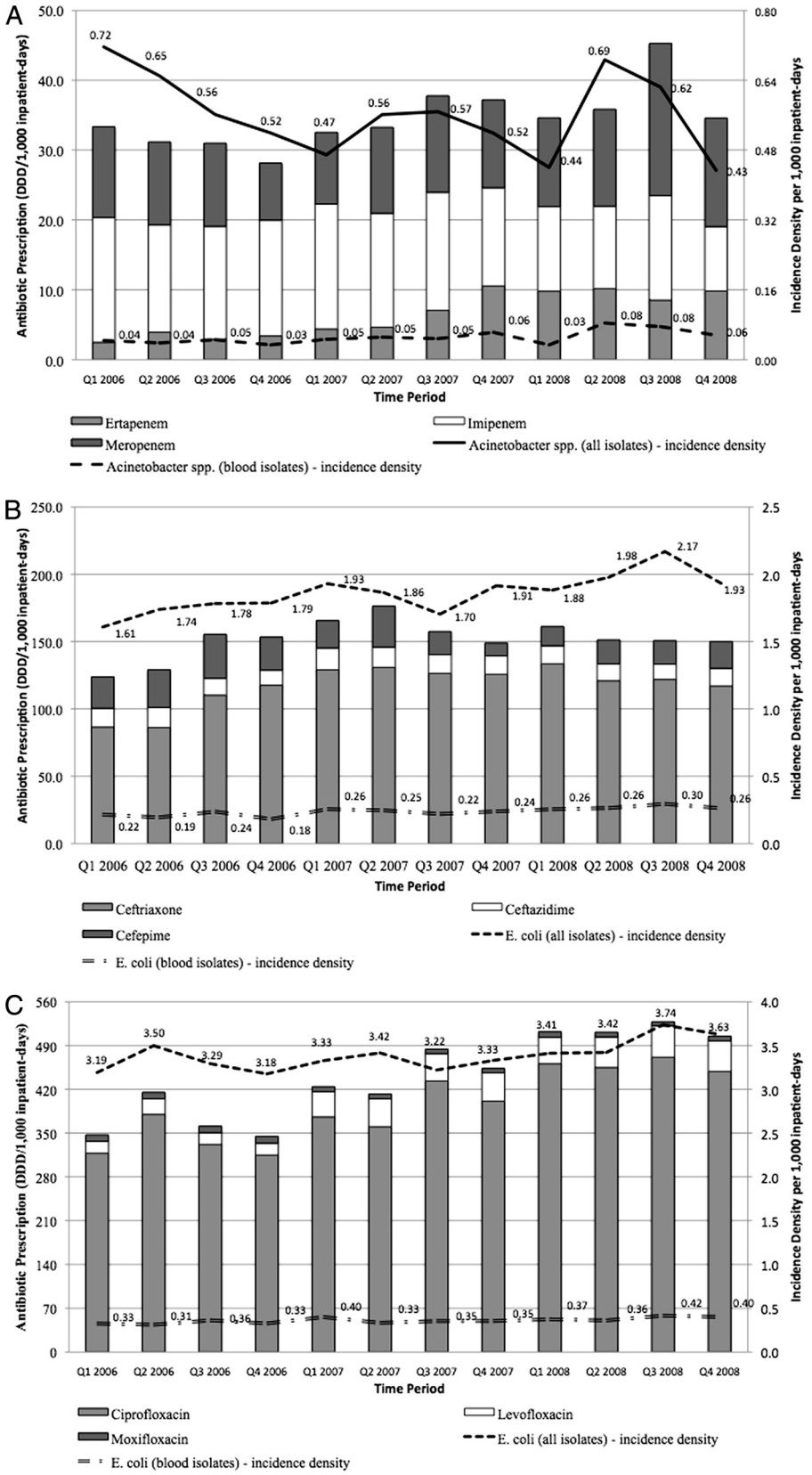

FIG. 2. Incidence density of antimicrobial-resistant Gram-negative bacteria and respective antibiotic prescription volumes over 3 years. (A) Carbapenem prescription and imipenem-resistant *Acinetobacter* spp. (B) Cephalosporins and ceftriaxone-resistant *E. coli*. (C) Fluoroquinolone prescription and ciprofloxacin-resistant *E. coli*.

tance selection pressure occurs at the individual level and calculating antibiotic prescription using DDD measurements does not measure individual exposure to antibiotics (17). A minority of patients are exposed to the majority of broad-spectrum antibiotic prescriptions in the hospital setting, and these are mainly the patients who are susceptible to infections by antibiotic-resistant pathogens. Hence, although DDD measurements are useful for comparison and benchmarking, they may not correlate well with subsequent antibiotic resistance development due to the inherent biases. In our study, although antibiotic prescriptions had fluctuated, the prescription volumes had generally remained high. It is possible that beyond a certain critical threshold of antibiotic use, antibiotic resistance becomes decoupled from prescription. However, such a threshold—if it exists—has not been defined. Finally, other factors besides antibiotic prescription impact on resistance in the hospital setting, including infection control and the presence of predominant bacterial clones. The interplay of these factors with antibiotic prescription has not been worked out.

There are several limitations of this work. First, the study period of 3 years is relatively short, although further data collection is ongoing. Second, differences in infection control measures in the hospitals over this period were not assessed, although anecdotally there had been no significant intensification of infection control efforts with regard to antimicrobial-resistant Gram-negative bacteria during this time. Third, because of the nature of the surveillance, we could not determine individual level or duration of exposure to antibiotics to further correlate prescription with antibiotic resistance.

In conclusion, the incidence of Gram-negative bacterial antibiotic resistance and broad-spectrum antibiotic prescription is high in Singapore hospitals. This first major attempt at correlating resistance with prescription in Singapore yielded several significant correlations over the 3-year study period.

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