ORIGINAL ARTICLE

Rates of susceptibility of carbapenems, ceftobiprole, and colistin against clinically important bacteria collected from intensive care units in 2007: Results from the Surveillance of Multicenter Antimicrobial Resistance in Taiwan (SMART)

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Introduction

The gradual increase in minimum inhibitory concentrations (MICs) of glycopeptides for strains of Staphylococcus aureus (i.e., glycopeptide creep) is a worrisome concern worldwide. Infections caused by S. aureus strains with vancomycin MIC levels ≥2 mg/L are almost always associated with a reduction in clinical efficacy of vancomycin as well as high morbidity and mortality rates. Multidrug-resistant (MDR) gram-negative bacteria (GNB) are also an important concern in intensive care units (ICUs) around the world. Because of the coexistence of methillin-resistant S. aureus (MRSA) and MDR-GNB in most hospitals and nursing homes, physicians often need to prescribe multiple antibiotics for the management of hospital-acquired and health care-associated infections.

Ceftobiprole, a novel cephalosporin that is effective against MRSA and many derepressed AmpC β-lactamase-producing enteric GNB species, but not against extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae spp. has been shown to be as effective as cefepime against GNB, including Pseudomonas aeruginosa isolates. In addition, ceftobiprole exerted less potential of selecting single-step P. aeruginosa mutants of AmpC hyperproducer than ceftazidime. Ceftobiprole has high binding affinity for penicillin-binding protein 2a (PBP2a) and PBP2x, which renders it highly active against penicillin- and cephalosporin-resistant Streptococcus pneumoniae (regardless of the mecA expression level, and daptomycin susceptibility), and vancomycin-nonsusceptible S. aureus isolates. In Taiwan, there is a lack of in vitro susceptibility data regarding ceftobiprole and colistin. The Surveillance of Multicenter Antimicrobial Resistance in Taiwan (SMART), launched in 2000, is designed to longitudinally monitor the in vitro susceptibility profiles of clinical pathogens to important and future promising antibiotic agents, particularly the pathogens isolated from ICUs over time throughout Taiwan. Because the data regarding susceptibility to colistin and ceftobiprole and serial profiles in carbapenem nonsusceptibilities for the important clinical isolates still lacked in our country, we conducted an in vitro survey to evaluate the distributions of MIC values of ceftobiprole and colistin against S. aureus, important nonfermentative GNB and Enterobacteriaceae isolates, and...
compare the trends in MICs of carbapenem agents against isolates of important ESBL-producing and non-ESBL-producing Enterobacteriaceae species using the MIC breakpoints recommended by the Clinical and Laboratory Standards Institute (CLSI) in 2014.11

Methods

Bacterial isolates

From July 1, 2007 through December 31, 2007, a total of 1088 consecutive, nonduplicate isolates of Enterobacteriaceae, along with 200 isolates of S. aureus, 100 isolates of Acinetobacter baumannii, and 403 isolates of P. aeruginosa (1 isolate per patient) were collected from patients in ICUs at 10 major teaching hospitals (5 in the northern part, 1 in the central part, and 4 in the southern part) throughout Taiwan. The Enterobacteriaceae isolates comprised Escherichia coli (n = 344), Klebsiella pneumoniae (n = 359), Enterobacter cloacae (n = 103), Citrobacter freundii (n = 36), Serratia marcescens (n = 102), Morganella morganii (n = 66), and Proteus mirabilis (n = 78), as reported in our previous study.12 Among the sources of clinical specimens in this study, nearly one-fourth (24.5%) of specimens were collected from sterile sites (16.6% from bloodstream and 7.9% from other sterile sites), whereas 43.1%, 9.4%, and 23% of all specimens were collected from the respiratory tract, wounds, and other nonsterile sites, respectively. In the current survey, the number of clinical isolates submitted by each participating hospital was nearly even. The MIC values of tested antibi-

Detection of ESBL production, determination of MICs, and nonsusceptibilities to carbapenem agents

For four species of Enterobacteriaceae (E. coli, K. pneumoniae, E. cloacae, and P. mirabilis) collected from ICU settings in 2007, the modified double-disc synergy test, which involves a disc containing ceftazidime 30 μg, with or without clavulanic acid 10 μg (at a center-to-center distance of 30 mm), instead of a disc containing 4 μg clavulanic acid, was applied to detect ESBL production if isolates (E. coli, K. pneumoniae, E. cloacae, and P. mirabilis) had cefotaxime, ceftriaxime, or cepfelipem MIC values ≥2 μg/mL. The production of ESBL was considered positive if the diameter of the cefepime disc increased by ≥5 mm, or the zone diameter expanded by ≥50% of the original size, as suggested by M’Zali et al.14 In addition, we determined the MIC values of carbapenem agents (imipenem and meropenem, as well as ertapenem specifically for P. mirabilis isolates, for the reasons described in the Discussion section) against these four species of Enterobacteriaceae isolates obtained as part of the SMART study in 2007 as well as their susceptibility profiles according to MIC breakpoints recommended by the CLSI in 2014.11

Investigations of MICs of colistin and ceftobiprole against important ICU bacteria

We investigated the rates of susceptibility of Enterobacteriaceae members, P. aeruginosa, and A. baumannii to colistin and surveyed the MIC values of ceftobiprole against ICU isolates of S. aureus and P. aeruginosa. Oxacillin MIC values were tested among all S. aureus isolates collected in 2007. All of the antibiotic agents investigated in this study were provided by their respective manufacturers. Ceftobiprole standard powder was provided by Johnson and Johnson Pharmaceutical Research and Development (Raritan, NJ, USA).

Statistical analyses

Categorical variables are presented as percentages and were compared using the Chi-square test. Continuous variables were compared using the Student t test or Mann–Whitney U test, depending on the validity of normality assumption. The coefficient of correlation between the MIC levels of oxacillin and ceftobiprole among the S. aureus isolates (overall vs. MRSA subgroup) was estimated by appropriate correlation methods. All statistical calculations were two-tailed and p < 0.05 was considered to represent statistical significance. All statistical analyses were performed using the statistical package SPSS for Windows (version 17.0; SPSS Inc., Chicago, IL, USA).

Results

Susceptibility of Enterobacteriaceae species, P. aeruginosa, and A. baumannii isolates to colistin

The in vitro data regarding susceptibilities to colistin among isolates of the Enterobacteriaceae members, P. aeruginosa, and A. baumannii obtained from patients hospitalized in ICUs in 2007 are listed in Table 1. Some of the species Enterobacteriaceae family evaluated in this ICU survey exhibited relatively high rates of nonsusceptibility (>25%) to this agent, with the exception of E. coli, K. pneumoniae, and C. freundii. By contrast, the rates of susceptibility to colistin were 76.7% for P. aeruginosa and 96% for A. baumannii isolates collected in ICU settings in 2007.

Comparison of MIC levels of ceftobiprole against isolates of P. aeruginosa and S. aureus

The MIC range, MIC50, and MIC90 values of ceftobiprole against P. aeruginosa isolates obtained from patients in ICUs in 2007 were 0.5–128, 2, and 16 μg/mL, respectively. Ceftobiprole inhibited 68% of P. aeruginosa isolates at a
concentration of 4 µg/mL, 81.1% of isolates at a concentration of 8 µg/mL, and 91.6% of *P. aeruginosa* isolates at a concentration of 16 µg/mL (Table 2). We also found that the MIC$_{50}$ and MIC$_{90}$ levels for methicillin-susceptible *S. aureus* isolates (n = 59) were 0.5 µg/mL and 0.5 µg/mL, respectively, and that the MIC$_{50}$ and MIC$_{90}$ levels for MRSA isolates (n = 141) were 2 µg/mL and 4 µg/mL, respectively. About 88.7% (125/141) of the MRSA isolates had ceftobiprole MICs $<2$ µg/mL, and only one MRSA isolate had a ceftobiprole MIC $>4$ µg/mL (64 µg/mL; Table 2). The overall MICs of oxacillin against *S. aureus* isolates correlated well with the overall MICs of ceftobiprole (Spearman correlation coefficient, $r = 0.876$, $p < 0.001$); however, there was a poor correlation between the MICs of those two agents against MRSA isolates ($r = 0.263$, $p = 0.245$). In addition, there was a significantly higher proportion of the MRSA isolates with oxacillin MICs $>128$ µg/mL among MRSA isolates with ceftobiprole MICs ranging from 2 µg/mL to 4 µg/mL than among isolates with ceftobiprole MICs $<2$ µg/mL ($p < 0.001$).

**Carbapenem susceptibility among four species of Enterobacteriaceae**

Comparisons of susceptibilities of non-ESBL and ESBL-producing isolates of four species of *Enterobacteriaceae* to carbapenem agents (imipenem, meropenem) in 2007 are shown in Table 3. It is worth noting that a few carbapenem-nonsusceptible isolates were found in the ESBL and non-ESBL subgroups of the four species. The absolute number of carbapenem-nonsusceptible isolates in the respective non-ESBL subgroups exceeded that in the ESBL subgroup of the same species. However, there were no significant differences in the number of carbapenem-nonsusceptible isolates between the two subgroups of all four species. All of the *P. mirabilis* isolates were susceptible to ertapenem irrespective of ESBL production (MIC $\leq 0.06$ µg/mL; data not shown). Furthermore, a rightward shifting trend in imipenem MIC values was noted for *P. mirabilis* (regardless of ESBL production; Fig. 1A and B) and *E. cloacae* (ESBL subgroup, Fig. 1A) isolates. This trend was not noted for meropenem MIC levels (data not shown). Although the proportion of imipenem-nonsusceptible *P. mirabilis* isolates in the ESBL subgroup was significantly higher than that of imipenem-nonsusceptible *P. mirabilis* isolates in the non-ESBL-producing subgroup (50% vs. 32.8%, $p = 0.23$), the distribution of imipenem MIC values did not differ significantly between the two *P. mirabilis* subsets ($p = 0.96$). By contrast, the distributions of imipenem MICs among *P. mirabilis* isolates were significantly higher for both non-ESBL- and ESBL-producing subgroups when compared to the respective subgroups of the other three *Enterobacteriaceae* species (all $p < 0.05$). No such trend was found in distribution of meropenem MICs among the four species of the *Enterobacteriaceae* family.

**Discussion**

This surveillance study of antimicrobial resistance among important pathogens isolated from patients in the ICU reveals a number of important findings. First, colistin was demonstrated to have excellent *in vitro* activity against *A. baumannii*, which contrasted with moderate activity

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**Table 1** Susceptibility profile of isolates of *Enterobacteriaceae* species, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* against colistin in intensive care units in Taiwan in 2007

<table>
<thead>
<tr>
<th>Species (no.)</th>
<th>MIC (µg/mL)</th>
<th>Susceptibility</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC range</td>
<td>MIC$_{50}$</td>
</tr>
<tr>
<td><em>Enterobacteriaceae</em> spp. (n = 1088)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Escherichia coli (n = 344)</td>
<td>0.5–&gt;128</td>
<td>1</td>
</tr>
<tr>
<td>Klebsiella pneumoniae (n = 359)</td>
<td>0.5–16</td>
<td>1</td>
</tr>
<tr>
<td>Enterobacter cloacae (n = 103)</td>
<td>0.5–&gt;128</td>
<td>1</td>
</tr>
<tr>
<td>Citrobacter freundii (n = 36)</td>
<td>1–16</td>
<td>1</td>
</tr>
<tr>
<td>Serratia marcescens (n = 102)</td>
<td>1–&gt;128</td>
<td>&gt;128</td>
</tr>
<tr>
<td>Morganella morganii (n = 66)</td>
<td>1–&gt;128</td>
<td>&gt;128</td>
</tr>
<tr>
<td>Proteus mirabilis (n = 78)</td>
<td>16–&gt;128</td>
<td>&gt;128</td>
</tr>
<tr>
<td>Nonfermentative gram-negative bacteria (n = 503)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em> (n = 403)</td>
<td>2–8</td>
<td>2</td>
</tr>
<tr>
<td><em>Acinetobacter baumannii</em> (n = 100)</td>
<td>0.5–&gt;32</td>
<td>1</td>
</tr>
</tbody>
</table>

I = intermediate; MIC = minimum inhibitory concentration; R = resistant; S = susceptible.

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**Table 2** The percentages of isolates of *Pseudomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus* inhibited by different concentrations of ceftobiprole

<table>
<thead>
<tr>
<th>Species, and % inhibited by the concentration(s) of ceftobiprole</th>
<th>1 µg/mL</th>
<th>2 µg/mL</th>
<th>4 µg/mL</th>
<th>8 µg/mL</th>
<th>16 µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pseudomonas aeruginosa</em> (n = 403)</td>
<td>15.9%</td>
<td>56.1%</td>
<td>68.0%</td>
<td>81.1%</td>
<td>91.6%</td>
</tr>
<tr>
<td>MRSA (n = 141)</td>
<td>14.9%</td>
<td>88.7%</td>
<td>99.3%</td>
<td>99.3%</td>
<td>99.3%</td>
</tr>
</tbody>
</table>

MRSA = methicillin-resistant *Staphylococcus aureus*.
A search of the PubMed database revealed that infection caused by colistin-resistant Enterobacteriaceae species (5 K. pneumoniae, 2 S. marcescens) were successfully treated by various antimicrobial agents. More clinical evidence is needed to evaluate the outcome of patients with infection caused by colistin-resistant Enterobacteriaceae species.

Studies have shown that ceftobiprole has a good pharmacokinetic profile [peak serum concentration of 35.5 \mu g/mL and a high free-drug concentration (plasma protein-bound degree, 16%)] and a good pharmacodynamic profile (the concentration above MIC is >50% of the total dosing interval for MRSA isolates with vancomycin MICs <4 \mu g/mL) when the drug is administered at a dose of 500 mg every 8 hours with a 2-hour intravenous infusion. In this survey, the ceftobiprole MIC\textsubscript{90} value was 4 \mu g/mL against MRSA isolates obtained from ICUs in Taiwan, which is twofold higher than that reported in other surveys. It is noteworthy that MRSA isolates with oxacillin MICs >128 \mu g/mL largely have ceftobiprole MIC values ranging from 2 \mu g/mL to 4 \mu g/mL. This suggests that elevated mecA expression levels in MRSA have little effect on the MICs of ceftobiprole, as reported by Fritsche et al. In contrast to ceftaroline fosamil, another anti-MRSA cephalosporin agent with minimal in vitro activity against nonfermentative GNB (especially P. aeruginosa isolates), ceftobiprole was verified to have a MIC\textsubscript{90} value of 16 \mu g/mL against ICU isolates of P. aeruginosa, a finding similar to that reported in other surveys. Using Monte Carlo simulation, Lodise et al. found that a fair probability (62.0%) of target attainment for maximal bactericidal performance (requiring at least 60% of a total dosing interval for a ceftobiprole concentration being above its MIC of the GNB organism) against P. aeruginosa isolates could be achieved when 500 mg is administered.
intravenously every 8 hours with a 2-hour infusion duration. Therefore, ceftobiprole is a suitable monotherapy for empirical management of many health care-acquired infections in Taiwan.

Class A carbapenemase [especially, *K. pneumoniae* carbapenemase (KPC)] was not detected in the species of *Enterobacteriaceae* collected in ICU settings in Taiwan before 2011. Therefore, porin deficiency (impermeability) or efflux pumps in combination with ESBL and/or AmpC enzymes were most likely the major mechanisms conferring carbapenem resistance among *Enterobacteriaceae* in Taiwan, as seen in other countries. In this SMART study conducted in 2007, when KPC (especially KPC-2) did not prevail in ICU settings in Taiwan, we clearly demonstrated that only a low (<5.0%) percentage of important enteric GNB species, with the exception of *P. mirabilis*, displayed nonsusceptibility to imipenem. In contrast to the data reported in the SMART 2005 study, we found that the ESBL and non-ESBL subgroups of *P. mirabilis* isolates obtained from ICUs were also significantly more nonsusceptible to imipenem than to meropenem and ertapenem (50%/32.8% vs. 0%/1.6% and 0%/0%) based on CLSI 2014 criteria, a finding consistent with that in a recent survey on GNB species responsible for intra-abdominal infections in China. Mehtar et al. reported that clinical *P. mirabilis* isolates with imipenem MICs > 4 μg/mL predicted treatment failure of imipenem. In this survey, only two (2/14, 14.3%) ESBL-producing *P. mirabilis* isolates and none (0/64, 0%) of the non-ESBL-producing *P. mirabilis* isolates had imipenem MICs > 4 μg/mL. Therefore, the effect of a MIC limit of 4 μg/mL on *P. mirabilis* analyzed in our ICU study was trivial. In addition, a recent study by Tsai et al., who investigated 47 patients with bacteremia caused by ESBL-producing *P. mirabilis* isolates, reported that none of the three patients with infections due to ESBL-producing *P. mirabilis* isolates exhibiting imipenem MIC values ≥ 2 μg/mL who were treated with imipenem died within 28 days of diagnosis. Further investigations are needed to determine whether the imipenem MIC breakpoint for *P. mirabilis* isolates should be adjusted. In our study, only three (14.2%) of 21 imipenem-nonsusceptible *P. mirabilis* isolates were nonsusceptible to cefotaxime and ceftazidime (data not shown). The reason for that finding is probably because most of these imipenem-nonsusceptible *P. mirabilis* isolates lack imipenem-specific outer membrane proteins, have diminished PBP1a expression, or reduced imipenem binding to PBP2.

There are a few limitations in this study. First, the existence of clonal dissemination could not be completely excluded. Second, we did not characterize the molecular type of SCCmec among MRSA isolates.

In summary, based on 2014 MIC breakpoints recommended by the CLSI, colistin showed only moderate *in vitro* activity against *P. aeruginosa* and *E. cloacae* isolates obtained from ICUs in Taiwan in 2007. Ceftobiprole showed excellent bactericidal activity against MRSA isolates regardless of their oxacillin MIC levels. These two agents exhibited acceptable *in vitro* activities against *P. aeruginosa* isolates. The clinical importance of high imipenem nonsusceptibility among *P. mirabilis* isolates based on CLSI 2014 criteria remains debatable. Uninterrupted monitoring of susceptibility profiles of some important ICU pathogens to important antibiotics is still warranted.

Conflicts of interest

None declared.

Acknowledgments

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References


