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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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Abstract *Background:* Data on susceptibility to ceftobiprole and colistin, and the complete evolutionary trends of minimum inhibitory concentrations (MICs) of important carbapenem agents among important pathogens collected in intensive care units (ICUs) in Taiwan are lacking.
Methods: We surveyed the MIC distribution patterns of ceftobiprole and colistin and susceptibility profiles of some important pathogens collected from patients hospitalized in intensive care units (ICUs) of major teaching hospitals throughout Taiwan in 2007. We also investigated the rates of nonsusceptibility to powerful carbapenems (imipenem, meropenem) among four important species of *Enterobacteriaceae* (*Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, and *Proteus mirabilis*) collected during the same period. MIC breakpoints recommended by the Clinical and Laboratory Standards Institute in 2014 were applied.
Results: Colistin showed excellent *in vitro* activity (susceptibility rate, 96%) against *Acinetobacter baumannii* isolates but moderate (73–77% susceptibility rate) activity against isolates of *Pseudomonas aeruginosa* and *E. cloacae*. The ceftobiprole MIC_{90} value was 4 μg/mL for methicillin-resistant *Staphylococcus aureus* and 16 μg/mL for *P. aeruginosa*. The phenotype of methicillin resistance did not markedly increase the MIC value of ceftobiprole among *S. aureus* isolates. Interestingly, the proportion of isolates that displayed nonsusceptibility to imipenem was significantly higher among *P. mirabilis* isolates than among isolates of the other three *Enterobacteriaceae* species, regardless of the production of extended-spectrum β-lactamase.
Conclusion: Continuous monitoring of susceptibility profiles of ICU pathogens to important antibiotics is warranted to provide appropriate antimicrobial regimens against infections in the ICU.

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.[1] 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[2,3] as well as high morbidity and mortality rates.[2,4] 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 methicillin-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.[5]

Ceftobiprole, a novel cephalosporin that is effective against MRSA[6] and many derepressed AmpC β-lactamase-producing enteric GNB species,[7] but not against extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae* spp.[6] has been shown to be as effective as cefepime against GNB, including *Pseudomonas aeruginosa* isolates.[6] In addition, ceftobiprole exerted less potential of selecting single-step *P. aeruginosa* mutants of AmpC hyperproducer than cefepime,[8] ceftobiprole has high binding affinity for penicillin-binding protein 2a (PBP2a) and PBP2x, which renders it highly active against penicillin- and ceftriaxone-resistant *Streptococcus pneumoniae*,[6,9] MRSA (regardless of the *mecA* expression level, and daptomycin susceptibility), and vancomycin-nonsusceptible *S. aureus* isolates.[10] 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 antibiotics against these clinical isolates collected in 2007 were determined by the broth microdilution method in accordance with CLSI guidelines. All isolates were stored at −70°C in trypticase soy broth (Difco Laboratories, Detroit, MI, USA) supplemented with 15% glycerol prior to testing. Isolates were then transported to the National Taiwan University Hospital, Taipei, Taiwan for further identification by the Phoenix PMIC/ID-30 identification system (Becton Dickinson Systems, Sparks, MD, USA). *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were used as quality control strains for each run of MIC tests. MIC testing was repeated if the results for ATCC strains were outside the expected range recommended by the CLSI.

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 cefepime 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,[13] was applied to detect ESBL production if isolates (*E. coli*, *K. pneumoniae*, *E. cloacae*, and *P. mirabilis*) had cefotaxime, ceftazidime, or cefepime 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, MIC_{50}, and MIC_{90} 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

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

Species (no.)	MIC (μg/mL)			Susceptibility		
	MIC range	MIC_{50}	MIC_{90}	S (%)	I (%)	R (%)
Enterobacteriaceae spp. ($n = 1088$)						
Escherichia coli ($n = 344$)	0.5—>128	1	1	334 (97.1)	—	10 (2.9)
Klebsiella pneumoniae ($n = 359$)	0.5—16	1	1	356 (99.2)	—	3 (0.8)
Enterobacter cloacae ($n = 103$)	0.5—>128	1	>128	75 (72.8)	—	28 (27.2)
Citrobacter freundii ($n = 36$)	1—16	1	8	30 (83.3)	—	6 (16.7)
Serratia marcescens ($n = 102$)	1—>128	>128	>128	9 (8.8)	—	93 (91.2)
Morganella morganii ($n = 66$)	1—>128	>128	>128	2 (3)	—	64 (97)
Proteus mirabilis ($n = 78$)	16—>128	>128	>128	0 (0)	—	78 (100)
Nonfermentative gram-negative bacteria ($n = 503$)						
Pseudomonas aeruginosa ($n = 403$)	2 - 8	2	4	309 (76.7)	61 (15.1)	33 (8.2)
Acinetobacter baumannii ($n = 100$)	0.5—>32	1	2	96 (96)	—	4 (4)

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

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 of ≤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 ≤ 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

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

Species, and % inhibited by the concentration(s) of ceftobiprole	1 μg/mL	2 μg/mL	4 μg/mL	8 μg/mL	16 μg/mL
Pseudomonas aeruginosa ($n = 403$)	15.9%	56.1%	68.0%	81.1%	91.6%
MRSA ($n = 141$)	14.9%	88.7%	99.3%	99.3%	99.3%

MRSA = methicillin-resistant *Staphylococcus aureus*.

Table 3 Minimum inhibitory concentration values of two carbapenem agents (imipenem, meropenem) against the species of *Enterobacteriaceae* family (extended-spectrum β-lactamase vs. nonextended-spectrum β-lactamase producing isolates) collected in 2007 and the rates of susceptibility evaluated by the minimum inhibitory concentration breakpoints of the Clinical and Laboratory Standards Institute, 2014

Species and antibiotic evaluated	MIC (μg/mL)			Susceptibility		
	Range	MIC_{50}	MIC_{90}	S (%)	I (%)	R (%)
Escherichia coli (ESBL) $n = 67$						
IMI	0.06–1	0.25	0.25	67 (100)	0 (0)	0 (0)
MEM	0.03–0.06	0.03	0.06	67 (100)	0 (0)	0 (0)
E. coli (non-ESBL), $n = 277$						
IMI	0.06–16	0.12	0.25	273 (98.6)	0 (0)	4 (1.4)
MEM	0.03–4	0.03	0.03	273 (98.6)	1 (0.4)	3 (1.1)
Klebsiella pneumoniae (ESBL) $n = 75$						
IMI	0.12–>128	0.25	0.5	70 (93.3)	1 (1.3)	4 (5.3)
MEM	0.03–>64	0.03	0.12	70 (93.3)	1 (1.3)	4 (5.3)
K. pneumoniae (non-ESBL), $n = 284$						
IMI	0.06–8	0.25	0.5	273 (96.1)	2 (0.7)	9 (3.2)
MEM	0.03–64	0.03	0.06	276 (97.2)	3 (1.1)	5 (1.8)
Enterobacter cloacae (ESBL), $n = 19$						
IMI	0.25–8	0.25	1	18 (94.7)	0 (0)	1 (5.3)
MEM	0.03–8	0.06	1	18 (94.7)	0 (0)	1 (5.3)
E. cloacae (non-ESBL), $n = 84$						
IMI	0.12–64	0.5	1	80 (95.2)	1 (1.2)	3 (3.6)
MEM	0.03–8	0.06	0.25	81 (96.4)	1 (1.2)	2 (2.4)
Proteus mirabilis (ESBL), $n = 14$						
IMI	0.25–4	1	4	7 (50)	3 (21.4)	4 (28.6)
MEM	0.03–0.25	0.12	0.25	14 (100)	0 (0)	0 (0)
P. mirabilis (non-ESBL), $n = 64$						
IMI	0.06–8	1	2	43 (67.2)	16 (25)	5 (7.8)
MEM	0.03–4	0.06	0.25	63 (98.4)	0 (0)	1 (1.6)

ESBL, extended-spectrum β-lactamase; IMI = imipenem; MEM = meropenem; MIC = minimum inhibitory concentration.

against the isolates of *P. aeruginosa* and some important species of *Enterobacteriaceae* in 2007. Second, MICs of ceftobiprole were very low for *P. aeruginosa* and MRSA isolates, indicating that ceftobiprole is an attractive choice for empirical management of infections in the ICU setting. Third, we found that imipenem-nonsusceptible *P. mirabilis* was present in ICUs of Taiwanese hospitals in 2007.

Although colistin was administered clinically for only a short period of time in Taiwan prior to 2007, the rates of nonsusceptibility among isolates of *P. aeruginosa* and *A. baumannii* collected from ICUs in Taiwan in 2007 were relatively higher (23.3% and 4%, respectively) than those collected in Turkey during 2011–2012.[15] Similarly, the rate of susceptibility to colistin (76.7%) among *P. aeruginosa* isolates obtained in 2007 from ICUs in Taiwan was significantly lower than that among *P. aeruginosa* isolates obtained from ICUs in the United States during 2009–2011 (99.4%).[16] A search of the PubMed database revealed a single small case series that documented the outcome of patients with infections due to colistin-resistant enteric GNB. In that study from Saudi Arabia, five of seven critically ill patients with colistin-resistant *Enterobacteriaceae* species (5 *K. pneumoniae*, 2 *S. marcescens*) were successfully treated by various antimicrobial agents.[17] 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 [a peak serum concentration of 35.5 μg/mL and a high free-drug concentration (plasma protein-bound degree, 16%)[18]] and a good pharmacodynamic profile (the concentration above MIC is >50% of the total dosing interval for MRSA isolates with vancomycin MICs < 4 μg/mL) when the drug is administered at a dose of 500 mg every 8 hours with a 2-hour intravenous infusion.[7] In this survey, the ceftobiprole MIC_{90} value was 4 μg/mL against MRSA isolates obtained from ICUs in Taiwan, which is twofold higher than that reported in other surveys.[6,10,19] It is noteworthy that MRSA isolates with oxacillin MICs > 128 μg/mL largely have ceftobiprole MIC values ranging from 2 μg/mL to 4 μg/mL. This suggests that elevated *mecA* expression levels in MRSA have little effect on the MICs of ceftobiprole, as reported by Fritsche et al.[20] In contrast to ceftaroline fosamil, another anti-MRSA cephalosporin agent with minimal *in vitro* activity against nonfermentative GNB (especially *P. aeruginosa* isolates),[21] ceftobiprole was verified to have a MIC_{90} value of 16 μg/mL against ICU isolates of *P. aeruginosa*, a finding similar to that reported in other surveys.[19,22] Using Monte Carlo simulation, Lodise et al[7] 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

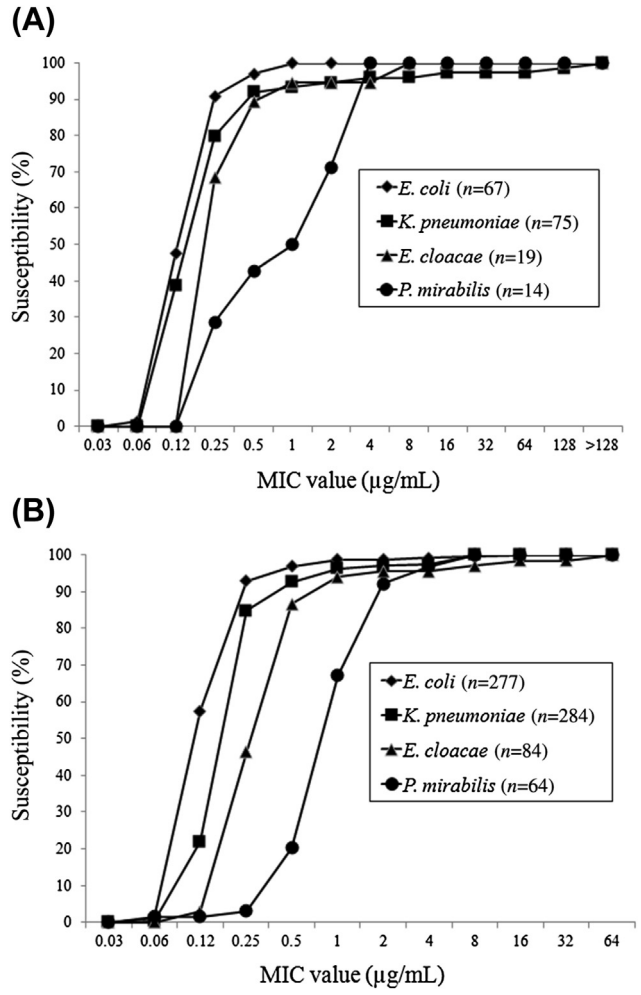

Figure 1. Cumulative minimum inhibitory concentration (MIC) distribution curves for imipenem against (A) ESBL-producing and (B) non-ESBL-producing *Enterobacteriaceae* species (*Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, and *Proteus mirabilis*) collected from intensive care units in 2007 as part of the SMART study. ESBL = extended-spectrum β-lactamase.

intravenously every 8 hours with a 2-hour infusion duration.[7] 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.[23–25] 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.[26–28] 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,[29] 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.[30] Mehtar et al[31] 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,[32] 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,[31] have diminished PBP1a expression, or reduced imipenem binding to PBP2.[33]

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 SCC*mec* 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.

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