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ORIGINAL RESEARCH

In vitro activity of ceftazidime–avibactam, ceftolozane–tazobactam, and other comparable agents against clinically important Gram-negative bacilli: results from the 2017 Surveillance of Multicenter Antimicrobial Resistance in Taiwan (SMART)

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Objectives: We investigated the in vitro antimicrobial susceptibilities of clinically important Gram-negative bacteria (GNB) from 16 major teaching hospitals in Taiwan in 2017.

Materials and methods: *Escherichia coli* (n=686) and *Klebsiella pneumoniae* bloodstream isolates (n=673), non-typhoid *Salmonella* (NTS; n=221) from various sources, *Shigella* species (n=21) from fecal samples, and *Neisseria gonorrhoeae* (n=129) from the genitourinary tract were collected. Antibiotic minimum inhibitory concentrations (MICs) were determined using the broth microdilution method. Alleles encoding *K. pneumoniae* carbapenemases (KPCs), New Delhi metallo-β-lactamases (NDMs), Verona integron-encoded metallo-β-lactamase, imipenemase, OXA-48-like, and *mcr*-1-5 genes were detected by molecular methods in Enterobacteriaceae isolates.

Results: Five (0.7%) *E. coli* isolates harbored *mcr*-1 alleles. Twenty-four (3.6%), seven (1.0%), four (0.6%), and one (0.15%) *K. pneumoniae* isolates contained bla_{KPC}, $bla_{OXA\text{-}48\text{-}like}$, *mcr*-1, and bla_{NDM}, respectively. Three (1.4%) NTS and no *Shigella* isolates harbored *mcr*-1 genes. Seventy-one (10.5%) *K. pneumoniae* isolates displayed non-susceptibility (NS) to carbapenem agent(s). Phenotypically extended-spectrum β-lactamase (ESBL)-producing *K. pneumoniae* isolates showed significantly higher rates of ertapenem, tigecycline, and ceftolozane–tazobactam (CLZ–TAZ) NS (40.2%, 16.3%, and 71%–80%, respectively) than *E. coli* isolates exhibiting ESBL phenotypes (5.4%, 0.7%, and 18%–28%, respectively). All phenotypically ESBL-producing *E. coli* isolates were ceftazidime–avibactam (CAZ–AVB) susceptible. Two (8.3%) KPC-producing *K. pneumoniae* isolates showed CAZ–AVB NS. Hospital-acquired *K. pneumoniae* isolates were significantly less susceptible to ertapenem and CLZ–TAZ than hospital-acquired *E. coli* isolates.

Conclusion: Third-generation cephalosporins remain the optimal choice for treating NTS, *Shigella*, and gonococcal infections in Taiwan. Hospital-acquired and phenotypically ESBL-producing *K. pneumoniae* are a heavy resistance burden in Taiwan.

Keywords: Enterobacteriaceae, *Neisseria gonorrhoeae*, extended-spectrum β-lactamases, carbapenemase, ceftolozane–tazobactam, ceftazidime–avibactam

Introduction

Infections that become septicemic conditions, regardless of whether acquired from the community or hospitals, are typically associated with major fatalities and prolonged

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hospital stays,[1,2] particularly in the immunosuppressed[2] and elderly populations (≥60 years).[3,4] Over the last decade, infections caused by multidrug-resistant (MDR) Gram-negative enteric bacteria, including extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella pneumoniae* isolates, and carbapenemase-producing Enterobacteriaceae (CPE), have shown significantly higher case fatality rates than infections caused by susceptible bacteria.[5–8] Given the present antibiotic pipeline, it is necessary to prescribe the appropriate antimicrobials to combat difficult-to-treat pathogens and continuously monitor country-specific susceptibility profiles to determine the optimal treatment option, including antibiotics that will become available in a region in the future.

Ceftazidime–avibactam (CAZ–AVB) and ceftolozane–tazobactam (CLZ–TAZ), which are new second-generation β-lactam–β-lactamase inhibitors combinations, show good in vitro efficacy against *K. pneumoniae* carbapenemase (KPC)-producing Enterobacteriaceae and carbapenem non-susceptible (NS) *Pseudomonas aeruginosa* isolates, respectively.[9] These two novel drugs will become available in Taiwan in 2018. In addition, data regarding differences in the susceptibility of community- and hospital-acquired Gram-negative bacteria (GNB) to CAZ–AVB and CLZ–TAZ are lacking.

The Surveillance of Multicenter Antimicrobial Resistance in Taiwan (SMART) Program was initiated in 2002 to investigate the evolution of antibiotic susceptibility of a variety of clinically important pathogens collected from hospitals throughout Taiwan. Although the data related to CPE are important for providing real-time revision of infection control policies, little information was available regarding the prevalence and susceptibilities (particularly to new β-lactam combination agents) of diverse carbapenemases on clinical isolates of several important Enterobacteriaceae species in Taiwan until 2018.[10,11] This nationwide study was conducted, and isolates were collected in 2017 to survey susceptibility, including that of bloodstream isolates of *E. coli* and *K. pneumoniae*, isolates of non-typhoid *Salmonella* (NTS) regardless of sources, fecal *Shigella* species isolates, and genitourinary *Neisseria gonorrhoeae* isolates.

Materials and methods

Isolate collection

From January 2017 through December 2017, 16 major teaching hospitals in Taiwan, including eight, two, five, and one in the northern, central, southern, and eastern regions, respectively, participated in this nationwide resistance surveillance plan to examine clinically important pathogens. This survey was conducted by the Centers for Disease Control of Taiwan. In this survey, no less than four non-duplicate bloodstream isolates of *E. coli* and *K. pneumoniae* were required to be submitted by each medical center (n=11) every month, whereas at least two isolates were submitted by every district teaching hospital (n=5) each month. In addition, regardless of the source of the cultured isolate, at least one non-duplicate NTS and *N. gonorrhoeae* isolates and any available isolate of *Shigella* species were collected every month from all participating hospitals.

Compared to community-acquired isolates (defined as being collected ≤48 hours after admission to the hospital), isolates collected at >48 hours after admission as well as those collected from patients not displaying signs and symptoms of infections initially on admission were considered as hospital acquired. Moreover, hospital settings, such as the emergency department, outpatient clinic, general ward, and intensive care unit (ICU), and the outcomes of hospitalized patients from whom GNB isolates were collected were also recorded. The defined daily dose per 1,000 inhabitants per day in every hospital participating in this surveillance plan was also collected in 2017 to detect any gross changes in each antibiotic category. The institutional review board of the National Taiwan University Hospital (201609066RINB) approved this study and waived the written informed consent. Patient consent was waived because this in vitro antimicrobial susceptibility surveillance of research on bacterial isolates involved no more than a minimal risk to the subjects and the links to limited clinical information required for the subjects in this study were removed.

Antimicrobial susceptibility testing

In this survey, the broth microdilution method with Sensititre Gram-negative minimum inhibitory concentration (MIC) plates panels (Thermo Fisher Scientific, Waltham, MA, USA) was used to determine the MICs of the evaluated antibiotics for all included *Enterobacteriaceae* isolates. In addition, 1% GC agar with IsoVitaleX™ (Becton-Dickinson Microbiology Systems, Sparks, MD, USA) was used to determine the MICs of the evaluated antibiotics for the enrolled *N. gonorrhoeae* isolates. Previous studies[12,13] have described the phenotypic categories of Enterobacteriaceae isolates, and thus, the resistance mechanisms of the enrolled Enterobacteriaceae isolates were classified based on the results of susceptibility tests. The MIC breakpoints recommended by the Clinical and Laboratory Standards Institute (CLSI) in 2018,[14] European Committee on Antimicrobial Susceptibility Testing (EUCAST) in 2018,[15] and US Food and Drug Administration (FDA) were adopted to compare the differences in susceptibility rates

between the defined groups of enrolled GNB isolates. In addition, random amplification of polymorphic DNA was used to delineate the clonal relatedness of CPE isolates if necessary.

Determinations of key carbapenemase-encoding and *mcr* genes

The Xpert® Carba-R assay (Cepheid, Sunnyvale, CA, USA) was used to detect the carbapenemase-encoding alleles, including bla_{KPC}, bla_{NDM}, bla_{IMP}, bla_{VIM}, and $bla_{OXA\text{-}48\text{-}like}$, in Enterobacteriaceae isolates displaying in vitro NS to any carbapenem agents.[16] CPE isolates were defined as those harboring genes encoding any carbapenemase. PCR amplification of whole-cell DNA of the isolates showing colistin MICs of >2 mg/L was performed using previously described primers specific for *mcr-1*, *mcr-2*, *mcr-3*, *mcr-4*, and *mcr-5*, and the PCR products were sequenced[17] (Table S1).

Statistical analyses

Differences in group percentages were assessed using Pearson's chi-squared test or Fisher's exact test as appropriate. Two-tailed *P*-values of <0.05 were considered statistically significant. All statistical analyses were performed using the Statistical Package for Social Science Version 17 (SPSS Inc., Chicago, IL, USA).

Results

Profiles of overall susceptibility of all GNB isolates

The important MIC ranges and significant antibiotic susceptibility percentages of the clinical isolates of five different GNB species are illustrated in Table 1. There were notable differences in the susceptibility of *E. coli* and *K. pneumoniae* isolates to two anti-pseudomonal fluoroquinolones; NTS to levofloxacin; and *N. gonorrhoeae* to cefotaxime, cefixime, and azithromycin. In addition, no significant fluctuations were observed in daily dose per 1,000 inhabitants per day for all classes of antibiotics prescribed in the participating Taiwanese hospitals (data not shown). The complete MIC results, ranges, and susceptibility data for five GNB species in this study are shown in Table S2.

Susceptibility of bloodstream *E. coli* isolates

Among the 686 bloodstream *E. coli* isolates, 14 (2.0%) isolates showed NS to ertapenem. A total of 148 (21.6%) isolates exhibited ESBL phenotypes not associated with KPC or New Delhi metallo-β-lactamase (NDM) production (NS rates for piperacillin–tazobactam, ceftazidime, and cefepime were 18.2%, 75.0%, and 99.3%, respectively). Among these 148 isolates, 43 (29.1%) isolates were hospital acquired. Figure 1A illustrates the NS to some important antibiotics of hospital-acquired (n=106) and community-acquired (n=580) *E. coli* isolates. Significantly higher NS rates ($P<0.05$) to ertapenem (4.7%) and CLZ–TAZ (13.2%–17.9%) were found among hospital-acquired *K. pneumoniae* isolates compared to community-acquired isolates (1.6% vs 4.3%–8.3%, respectively). All phenotypically ESBL-producing *E. coli* isolates were susceptible to CAZ–AVB. In contrast, a trend toward a significant difference in the NS rate ($P=0.053$) to CLZ–TAZ was observed when comparing the MIC breakpoints of the CLSI 2018 (18.2%) and EUCAST 2018 (27.7%). In addition, high NS rates (up to 75%) to ciprofloxacin and levofloxacin were found among phenotypically ESBL-producing *E. coli* isolates. When in vitro susceptibility of the phenotypically ESBL-producing *E. coli* isolates was further assessed, 78 (11.4%) isolates were judged to be likely producers of ESBL alone. All 78 isolates were sensitive in vitro to CAZ–AVB and tigecycline, while less than 10% (5.1% based on the CLSI 2018 criteria and 9.0% based on the EUCAST 2018 criteria) were NS to CLZ–TAZ.

Moreover, five (0.7%) *E. coli* isolates harbored the *mcr*-1 allele, while the bla_{KPC} and $bla_{NDM\text{-}1}$ alleles were each detected in one *E. coli* isolate. All five *mcr*-1-harboring *E. coli* isolates were susceptible to all carbapenem agents, tigecycline, CLZ–TAZ, and CAZ–AVB, while three displayed ESBL phenotypes.

Susceptibility of bloodstream *K. pneumoniae* isolates

Figure 1B illustrates the NS to important antibiotics of hospital-acquired (n=212) and community-acquired (n=461) *K. pneumoniae* isolates. There were significantly higher NS rates ($P<0.05$) to ertapenem (23.6%), CLZ–TAZ (31%–38%), tigecycline (10.4%), and colistin (4.2%) among hospital-acquired *K. pneumoniae* isolates compared to those in community-acquired (n=461) *K. pneumoniae* isolates (4.6%, 8.9%–10.6%, 10.4%, and 1.5%, respectively).

Of the 673 bloodstream *K. pneumoniae* isolates, 71 (10.5%) displayed NS to at least one carbapenem agent. A total of 92 (13.8%) *K. pneumoniae* isolates exhibited ESBL phenotypes not associated with KPC and/or NDM production (NS rates of piperacillin–tazobactam, ceftazidime, and cefepime were 92.4%, 100%, and 73.9%, respectively). Among the 92 phenotypically ESBL-producing *K. pneumoniae* isolates, 57 (61.0%) isolates were hospital acquired, three (3.3%)

Table 1 In vitro susceptibilities (evaluated based on the criteria of the Clinical and Laboratory Standards Institute 2018 and European Committee on Antimicrobial Susceptibility Testing 2018[a]) of bloodstream isolates of *Escherichia coli*, *Klebsiella pneumoniae*, and other Gram-negative bacteria, including isolates of non-typhoid *Salmonella* species from any site, fecal *Shigella* species, and *Neisseria gonorrhoeae* from the genitourinary system, collected from patients treated at 16 major teaching hospitals across Taiwan in 2017 to key antimicrobial agents tested

Bacterial species (isolate no.) and antimicrobial agent tested	MIC (mg/L)			% of indicated susceptibility		
	Range	MIC_{50}	MIC_{90}	S	I	R
Escherichia coli (686)						
Ceftolozane–tazobactam	0.12–>64	0.25	1	94.3	2.2	3.5
Ceftazidime–avibactam	≤0.06–>64	0.12	0.25	99.9[a]	NA	0.1[a]
Ceftazidime	≤0.12–>256	0.25	32	74.5	5.1	20.4
Cefepime	≤0.12–>64	≤0.12	64	78.0	7.0	15.0
Ertapenem	≤0.06–>64	≤0.06	0.12	98.0	0.7	1.3
Ciprofloxacin	≤0.06–>64	0.25	64	68.2/59.9[a]	1.3/6.7[a]	30.5/33.4[a]
Levofloxacin	≤0.06–>64	0.25	16	69.2/63.8[a]	0.6/4.4[a]	30.2/31.8[a]
Colistin	≤0.12–8	0.25	0.25	98.8	NA	1.2
Klebsiella pneumoniae (673)						
Ceftolozane–tazobactam	≤0.06–>64	0.5	64	84.1/80.7[a]	1.2/NA	14.7/19.1[a]
Ceftazidime–avibactam	≤0.06–>64	0.12	1	99.1[a]	NA	0.9[a]
Ceftazidime	≤0.12–>256	0.25	256	73.7	3.4	22.9
Cefepime	≤0.12–>64	≤0.12	32	82.8	2.5	14.7
Ertapenem	≤0.06–>64	≤0.06	1	89.5	3.6	7.0
Meropenem	≤0.06–>64	≤0.06	0.12	94.5	0.6	4.9
Ciprofloxacin	≤0.06–>64	≤0.06	64	79.5/66.4[a]	1.8/6.7[a]	18.7/26.9[a]
Levofloxacin	≤0.06–>64	≤0.06	32	81.0/69.7[a]	2.2/9.5[a]	16.8/20.8[a]
Colistin	≤0.12–>64	0.25	0.5	97.6	NA	2.4
Non-typhoid *Salmonella* spp. (221)						
Ampicillin	0.5–>64	4	>64	51.6	1.8	46.6
Cefixime	≤0.12–>64	≤0.12	>64	87.3	0.5	12.2
Ceftriaxone	≤0.12–64	≤0.12	1	92.3	1.8	5.9
Ertapenem	≤0.06–0.25	≤0.06	≤0.06	100	0	0
Ciprofloxacin	≤0.06–16	≤0.06	0.5	78.7/78.7[a]	18.1/NA[a]	3.2/21.3[a]
Levofloxacin	≤0.06–16	≤0.06	1	78.7/89.6[a]	10.9/8.6[a]	10.4/1.8[a]
Moxifloxacin	≤0.06–16	0.12	2	79.2[a]	NA[a]	20.8[a]
Gentamicin	≤0.12–>64	0.5	1	96.4	0.5	3.2
Trimethoprim–sulfamethoxazole	≤0.12–>32	≤0.12	>32	74.2	NA	25.8
Colistin	0.25–16	2	4	53.8	NA	46.2
***Shigella* spp.** (21)						
Ampicillin	1–>64	>64	>64	38.1	0	61.9
Ceftriaxone	≤0.12	≤0.12	≤0.12	100	0	0
Ertapenem	≤0.06	≤0.06	≤0.06	100	0	0
Ciprofloxacin	≤0.06–16	≤0.06	16	61.9	0	38.1
Levofloxacin	≤0.06–8	≤0.06	8	61.9	19.0	19.0
Trimethoprim–sulfamethoxazole	≤0.12–>32	1	>32	61.9	NA	38.1
Azithromycin–*Shigella flexneri* (17)	1–>64	4	>64	70.6	NA	29.4
Azithromycin–*Shigella sonnei* (4)	8–128	8	128	75	NA	25
N. gonorrhoeae (129)						
Cefotaxime	≤0.03–0.5	0.06	0.25	100/89.1[a]	NA/NA[a]	NA/10.9[a]
Cefixime	≤0.03–0.5	0.06	0.12	96.1/89.1[a]	NA/NA[a]	NA/10.9[a]
Cefpodoxime	≤0.03–2	0.25	0.5	89.9	NA	NA
Ciprofloxacin	≤0.008–128	4	16	2.3	0	97.7
Doxycycline	0.25–32	8	16	NA	NA	NA
Azithromycin	≤0.03–>32	0.25	0.5	96.9/60.5[a]	NA/31.0[a]	3.1/8.5[a]

Note: [a]Denotes a significantly statistical difference ($P<0.05$) between different susceptibility subgroups.
Abbreviations: I, intermediate; MIC, minimum inhibitory concentration; NA, non-applicable; R, resistant; S, susceptible.

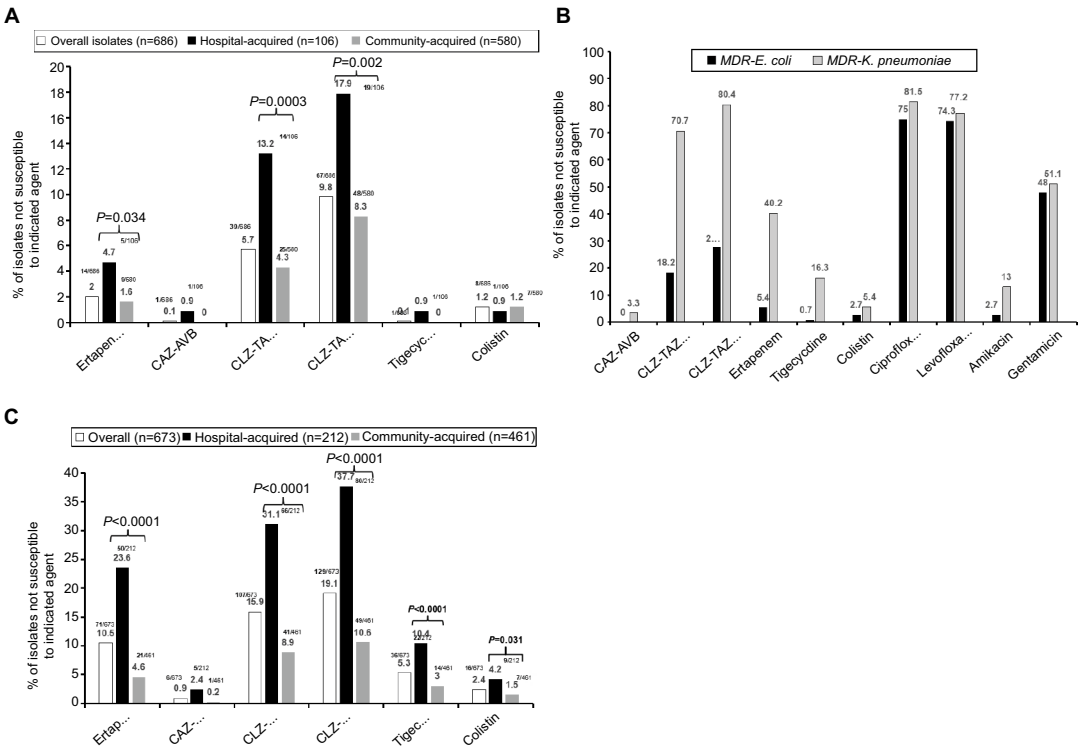

Figure 1 Comparison of (**A**) in vitro non-susceptibility to important antibiotics among overall, hospital-acquired, and community-acquired bloodstream *Escherichia coli* isolates, (**B**) *Klebsiella pneumoniae* isolates collected from 16 major teaching hospitals across Taiwan in 2017, and (**C**) in vitro non-susceptibility to important antibiotics between bloodstream *Escherichia coli* (n=148) and *K. pneumoniae* isolates (n=92) exhibiting the phenotype of extended-spectrum β-lactamase (unrelated to carbapenemase) production (ie, MDR phenotype).

Abbreviations: CAZ–AVB, ceftazidime–avibactam; CLSI, Clinical and Laboratory Standards Institute 2018; CLZ–TAZ, ceftolozane–tazobactam; EUCAST, European Committee on Antimicrobial Susceptibility Testing; MDR, multidrug resistance.

isolates were NS to CAZ–AVB, and five (5.4%) were NS to colistin. Furthermore, compared to the 148 phenotypically ESBL-producing *E. coli* isolates, these isolates showed significantly higher NS rates to ertapenem (40.2%), tigecycline (7.6% based on the US FDA criteria and 16.3% based on the EUCAST 2018 criteria), CLZ–TAZ (70.7% based on the CLSI 2018 criteria and 80.4% based on the EUCAST 2018 criteria), and amikacin (13.0%), as shown in Figure 1C. Careful evaluation of antibiotic susceptibility of 92 phenotypically ESBL-producing *K. pneumoniae* isolates revealed that the in vitro phenotypes of 31 (4.6%) *K. pneumoniae* isolates were consistent with those producing ESBL alone. Evaluation based on either the CLSI 2018 or the EUCAST 2018 criteria showed that these ESBL producers still had a high rate of NS to CLZ–TAZ (38.7% based on the CLSI 2018 criteria and 54.8% based on the EUCAST 2018 criteria), but fewer isolates were NS to tigecycline (9.7% based on the US FDA criteria and 16.1% based on the EUCAST 2018 criteria) and only one isolate was NS to colistin.

Notably, among the bloodstream *K. pneumoniae* isolates verified as CPE, 24 (3.6%) harbored bla_{KPC} alleles, with 16 (66.7%) acquired in hospital settings (one of which was susceptible to all carbapenems), seven (1.0%) harbored $bla_{OXA\text{-}48\text{-}like}$ alleles (six exhibited ESBL phenotypes), and one (0.15%) and four (0.6%) isolates harbored bla_{NDM} and *mcr*-1 alleles, respectively. Moreover, two (8.3%) KPC-producing isolates were NS to CAZ–AVB in contrast to 22 (91.7%) isolates, which were NS to CLZ–TAZ ($P<0.001$). In addition, five (20.8%) KPC-producing *K. pneumoniae* isolates were NS to colistin and four (16.7%) were NS to tigecycline based on the EUCAST 2018 criteria. Notably, the MICs for CAZ–AVB/CLZ–TAZ/tigecycline/colistin for one *E. coli* and one *K. pneumoniae* isolates harboring the bla_{NDM} allele were >64/>64/0.06/0.25 and >64/>64/0.25/0.25 mg/L, respectively.

Susceptibility of *E. coli* and *K. pneumoniae* isolates carrying *mcr*-1 genes

Table 2 shows the MIC levels against important antibiotics for five *E. coli* and four *K. pneumoniae* isolates harboring the *mcr*-1 gene. Compared to *mcr*-1-harboring *E. coli* isolates, grossly lower susceptibility rates to cefoxitin (0%),

Table 2 MIC values of some key antibiotics against isolates of *mcr*-1-harboring *Escherichia coli* (n=5) and *Klebsiella pneumoniae* (n=4) collected from 16 major teaching hospitals throughout Taiwan in 2017

Bacterial species and isolates	MIC (mg/L)								
	E. coli					*K. pneumoniae*			
	1	2	3	4	5	1	2[a]	3	4[b]
Amoxicillin–clavulanate	32	8	8	64	8	32	128	64	128
Cefoxitin	128	8	4	128	2	32	128	128	128
Ceftazidime	16	0.5	0.5	32	0.0625	1	512	512	512
Ceftriaxone	16	128	128	128	0.0625	0.25	128	16	128
Cefepime	0.25	4	4	8	0.0625	0.25	128	0.25	128
Piperacillin–tazobactam	4	2	2	8	2	256	256	256	256
Ceftazidime–avibactam	0.12	0.12	0.03	0.25	0.03	0.50	4	2	128
Ceftolozane–tazobactam	1	0.25	0.25	2	0.50	1	64	32	128
Ertapenem	0.03	0.03	0.03	0.03	0.03	0.03	128	1	128
Imipenem	0.25	0.12	0.12	0.25	0.12	0.03	64	0.50	64
Meropenem	0.03	0.03	0.03	0.03	0.03	0.03	128	0.12	128
Ciprofloxacin	128	0.03	0.03	128	8	0.25	128	8	64
Amikacin	2	2	2	2	1	2	1	2	128
Tigecycline	0.25	0.50	0.25	0.50	0.25	1	2	1	0.50

Notes: [a]This isolate also harbors bla_{KPC}- and $bla_{OXA\text{-}48\text{-}like}$ alleles. [b]This isolate also harbors the bla_{KPC} allele.
Abbreviation: MIC, minimum inhibitory concentration.

ceftazidime (25%), piperacillin–tazobactam (0%), and all carbapenem agents (25%, 50%, and 50% to ertapenem, imipenem, and meropenem, respectively) were observed for *mcr-1*-harboring *K. pneumoniae* isolates. Among the four *mcr*-1-harboring *K. pneumoniae* isolates, one isolate concomitantly carried the bla_{KPC} and $bla_{OXA\text{-}48\text{-}like}$ alleles, while the other co-harbored the bla_{KPC} allele.

Susceptibility of NTS, *Shigella*, and *N. gonorrhoeae* isolates

A total of 221 NTS isolates were collected in 2017; none displayed an ESBL phenotype, while six (2.7%) were considered as likely AmpC producers. Modest susceptibilities to ciprofloxacin, levofloxacin (78.7%), trimethoprim–sulfamethoxazole (TMP–SMX; 74.2%), and amoxicillin–clavulanate (81.0%) were observed. Approximately 24.0% of NTS isolates were NS to ampicillin plus TMP–SMX. Notably, 46.2% of these NTS isolates were NS to colistin. Based on the PCR results, however, only three (1.4%) NTS isolates harbored the *mcr*-1 alleles, while all NTS isolates were susceptible to ceftazidime and ceftriaxone. In addition, among the 26 (11.8%) hospital-acquired NTS isolates, half belonged to serogroup D. Among the 26 patients suffering from hospital-acquired NTS infections (case fatality rate, 27.2%), most (88.5%) NTS isolates were collected at general wards. Of 21 *Shigella* isolates (17 *S. flexneri* and 4 *S. sonnei* isolates) collected from fecal specimens, modest susceptibilities to two anti-pseudomonal fluoroquinolones (61.9%), azithromycin (71.4%), and TMP–SMX (61.9%) were detected.

Among the 129 urinary *N. gonorrhoeae* isolates collected in 2017, most (≥89%) were susceptible in vitro to spectinomycin and third-generation cephalosporins, including ceftriaxone, cefotaxime, cefixime, and cefpodoxime, based on the CLSI 2018 and EUCAST 2018 criteria. However, a significant discrepancy in the susceptibilities to azithromycin was found in evaluations based on the CLSI 2018 and EUCAST 2018 criteria (96.9% vs 60.5%, $P<0.001$). In addition, an extremely low susceptibility rate (2.3%) against ciprofloxacin was found for these gonococcal isolates in Taiwan.

Susceptibility of hospital-acquired *E. coli* and *K. pneumoniae* isolates and fatality rates of infected hospitalized patients

The case fatality rates for the patient subsets who experienced hospital-acquired bloodstream infections with *E. coli* (n=106) and *K. pneumoniae* (n=212) were 31.0% and 36.5%, respectively. As shown in Figure 2, significantly higher NS rates ($P<0.001$) were observed for hospital-acquired *K. pneumoniae* than for *E. coli* isolates against ertapenem (23.6% vs 4.7%), colistin (4.2% vs 0.9%), tigecycline (10.4% vs 0.9% based on the EUCAST 2018 criteria), and CLZ–TAZ (31.1% vs 13.2% based on the CLSI 2018 criteria and 37.7% vs 17.9% based on the EUCAST 2018 criteria) but not against CAZ–AVB (2.4% vs 0.9%, $P=0.38$), ceftazidime (45.3% vs 45.3%), and cefepime (34.4% vs 41.5%) by chi-squared

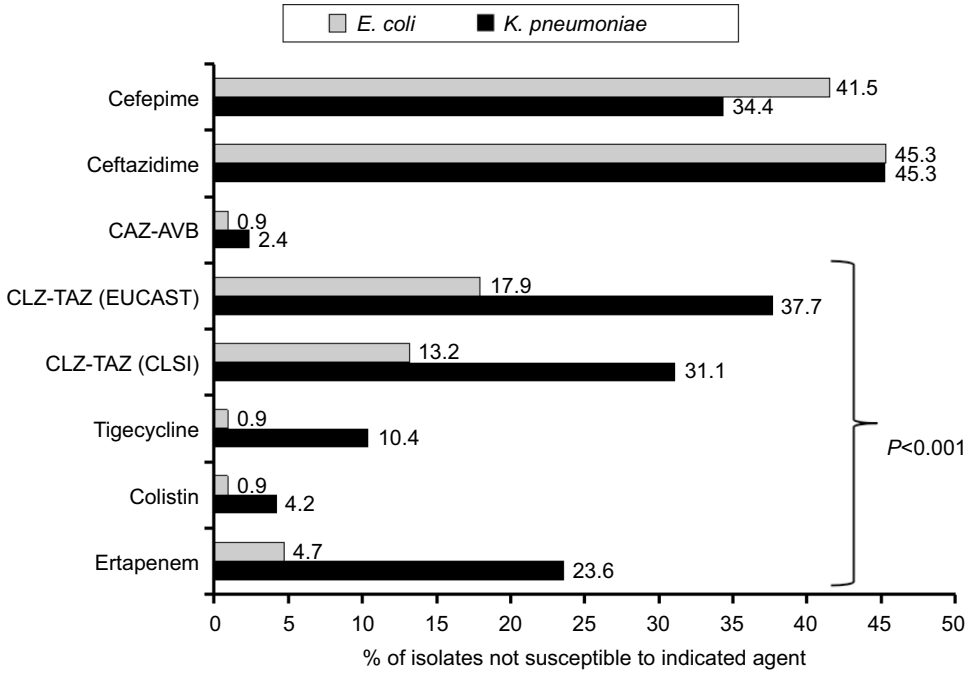

Figure 2 Comparison of in vitro non-susceptibility to important antibiotics of hospital-acquired *Escherichia coli* (n=106) and *Klebsiella pneumoniae* bloodstream isolates (n=212) collected from 16 major teaching hospitals across Taiwan in 2017.
Abbreviations: CAZ–AVB, ceftazidime–avibactam; CLSI, Clinical and Laboratory Standards Institute 2018; CLZ–TAZ, ceftolozane–tazobactam; EUCAST, European Committee on Antimicrobial Susceptibility Testing 2018.

analysis. Furthermore, 10 *E. coli* (none were CPE) and 44 *K. pneumoniae* (nine KPC producers and two harbored $bla_{\text{OXA-48-like}}$ alleles) isolates were collected from patients hospitalized in the ICU.

Discussion

No comparative studies have addressed the distinctions in NS rates against CAZ–AVB and CLZ–TAZ between hospital-acquired and community-acquired bloodstream *E. coli* and *K. pneumoniae* isolates. As expected, significantly higher NS rates of hospital-acquired isolates than those of community-acquired bacteremic *E. coli* and *K. pneumoniae* isolates to these two new β-lactam combination agents were found, although these two agents have not been launched in Taiwan. Previously, one Taiwanese multicenter survey detected four *K. pneumoniae* isolates harboring $bla_{\text{OXA-48-like}}$ allele among the 760 carbapenem-NS *K. pneumoniae* isolates collected between 2012 and 2014.[18] In contrast, seven *K. pneumoniae* isolates harbored plasmidic $bla_{\text{OXA-48-like}}$ (three of which were community acquired), and a significantly higher ertapenem NS rate (40.2%) was observed among phenotypically ESBL-producing *K. pneumoniae* isolates than that (5.4%) of ESBL-producing *E. coli* isolates in 2017, raising concerns about *K. pneumoniae* displaying NS to carbapenem agent(s) in Taiwan. In addition, although the bla_{NDM}-harboring Enterobacteriaceae isolates have high potential for dissemination (from the Indian subcontinent to Vietnam and the Philippines, identified among the abdominal isolates since 2011),[19] the prevalence rates of bloodstream bla_{NDM}-harboring *E. coli* and *K. pneumoniae* isolates were low (both were 0.15%) in Taiwan in 2017.

A review of the PubMed database on ESBL rates of clinical Taiwanese *E. coli* and *K. pneumoniae* isolates revealed that one survey focusing on ICU *Enterobacteriaceae* isolates in 2005 found that 14% of *E. coli* and 26% of *K. pneumoniae* were ESBL producers.[20] Another survey on bloodstream *E. coli* and *K. pneumoniae* isolates cultured from patients with hematological malignancies in 2008–2013 showed that 33.2% of *E. coli* and 12.6% of *K. pneumoniae* isolates were ESBL producers.[4] Therefore, the ESBL rates in our study (21.6% of *E. coli* and 13.8% of *K. pneumoniae*) were more similar to the results of the latter survey. Moreover, 45.3% of Taiwanese hospital-acquired *K. pneumoniae* isolates had a ceftazidime-NS phenotype, which was only slightly lower than those (58.1%) collected from patients with hematologic malignancies in Italy.[3] This result raised concerns regarding the future role of ceftazidime for treating hospital-acquired *K. pneumoniae* septicemia in Taiwan.

The prevalence rate of KPC positivity (3.6%) among Taiwanese bloodstream *K. pneumoniae* isolates in 2017 was much lower than that in Italy (42%).[21] In addition, among the ertapenem-NS *E. coli* (n=14) and *K. pneumoniae* (n=71) isolates in this study, two *E coli* isolates, of which one (7.1%) was a KPC producer, and 27 *K. pneumoniae* isolates, of which 23 (32.4%) were KPC producers, were determined as CPE. The KPC-producing *Enterobacteriaceae* isolates also have a high potential for spreading in clinical setting,[5] an important monitor focus in infection control. Regardless of the isolate source, one Taiwanese multicenter study investigating 247 *K. pneumoniae* isolates with NS to imipenem or meropenem in 2012 showed that the KPC production rate was 16.6%.[10] In contrast, another Taiwanese multicenter study (2010–2012) focusing on 1,135 *Enterobacteriaceae* isolates with NS to any carbapenem agent revealed that 2.8% of *K. pneumoniae* isolates produced KPC.[11] Therefore, among the ertapenem-NS bloodstream *K. pneumoniae* isolates collected in 2017, a strikingly higher rate of KPC production was observed than that previously observed in Taiwan ($P<0.001$). However, in contrast to carbapenemase production, a Taiwanese survey investigating the main resistance mechanisms of carbapenem-NS *E. coli* isolates found that plasmidic AmpC (mainly CMY-2) in combination with changes in outer membrane porins (OmpC/F) predominantly conferred resistance to carbapenems.[22] This result agrees with our findings of the low carbapenemase rate among ertapenem-NS *E. coli* isolates. Notably, five (0.7%) *E. coli* and four (0.6%) *K. pneumoniae* bloodstream isolates collected in 2017 harbored plasmidic *mcr*-1 alleles (all of which were clonally unrelated, data not shown). The low *mcr*-1 prevalence was similar to those in China from 2013 through 2014.[23] In addition, of the Taiwanese isolates of *mcr*-1-harboring *E. coli* and *K. pneumoniae*, the former species were less likely to co-harbor other carbapenemase(s)-encoding alleles than the latter, resulting in more significant drug-resistant phenotypes. Furthermore, all four *mcr-1*-harboring *E. coli* isolates showed susceptibility to all carbapenem agents, which agrees with previous results.[24]

One review article addressed that KPC producers are likely to co-harbor ESBL alleles to a considerable degree.[5] As stated by van Duin et al[9] and Tato et al,[25] our survey also revealed that CAZ–AVB had much better in vitro efficacy against ESBL-producing *K. pneumoniae* isolates (regardless of whether co-producing carbapenemase) than CLZ–TAZ.

Based on the susceptibilities observed in this study, third-generation cephalosporins are considered the mainstay for treating NTS, shigellosis, and gonococcal urinary tract infections (UTIs). Compared to the NS rates (1999–2003) of Taiwanese NTS isolates, in which *Salmonella enterica* serovar Choleraesuis accounted for most MDR-NTS isolates according to the study by Su et al,[26] an increasing trend in the ciprofloxacin NS rate (5.0% vs 21.3%) was found among the Taiwanese NTS isolates in 2017. An alarmingly high rate (>45%) of NS to colistin was detected in Taiwanese NTS isolates in 2017, contrasting the only three NTS isolates harboring *mcr*-1 alleles. The high colistin-NS rate was notably different from the rate of 17% calculated in an India-Arabian study investigating human NTS isolates.[27] However, a high *mcr*-1-independent colistin-NS rate among NTS isolates was found to be related to intrinsic *Pmr*D overexpression.[28,29] In contrast, nanoarchitectural changes in capsular polysaccharides that increase capsule thickness and hardness following colistin exposure were observed for only a few *K. pneumoniae* isolates also displaying *mcr*-1-independent colistin NS.[29,30]

Apart from the modest (28.6%) NS rate to azithromycin, approximately 40% of Taiwanese *Shigella* isolates showed NS to fluoroquinolones, a rate similar to the norfloxacin resistance rate (36.8%) of clinical *S. flexneri* isolates collected from China.[31] Because >20% of NTS and fecal *Shigella* isolates among Taiwanese isolates were NS to two anti-pseudomonal fluoroquinolones in 2017, these agents should be cautiously prescribed when treating foodborne enteropathogens.

Ison et al[32] suggested that azithromycin (<5% with MIC ≥1 mg/L) can be combined with cefixime to treat gonococcal infections in the UK. In accordance with pharmacokinetic data on azithromycin determined by Ballow et al,[33] its urinary excretion was low (7.0%). A high percentage (40%) of *N. gonorrhoeae* isolates in this study had an azithromycin MIC of >0.25 mg/L, which is the NS MIC breakpoint of EUCAST 2018. Furthermore, among the 21 gonococcal isolates exhibiting NS to any third-generation cephalosporin agent, 13 (61.9%) had azithromycin MICs of >0.25 mg/L. Therefore, azithromycin is possibly positioned as a supplement to enhance the success of treating gonococcal UTI in Taiwan. By contrast, as suggested by Cunha[34] and Agwuh and MacGowan,[35] the pharmacokinetic data on doxycycline revealed a urinary excretion rate of 35%–60%, with a high urinary concentration (>150 mg/L). The use of doxycycline to treat gonococcal UTI in Taiwan is likely plausible.

There were some limitations to this survey. First, information on the clinical sources of bloodstream *E. coli* and *K. pneumoniae* isolates was lacking, precluding further analysis of differences in NSs between isolates of various origins. Second, the presence of the sequence type 131 *E. coli* clone

among the collected *E. coli* isolates was not excluded. Third, we did not delineate the existence of membrane impermeability on carbapenem-resistant *Enterobacteriaceae* isolates.

Conclusion

Significantly higher ertapenem- and CLZ–TAZ-NS rates were found among the phenotypically ESBL-producing *K. pneumoniae* bloodstream isolates in Taiwan. In contrast, CAZ–AVB showed excellent in vitro efficacy against all GNB under survey, regardless of where the infection was acquired. Third-generation cephalosporins remain a reliable mainstay for treating NTS infection, fecal shigellosis, and gonococcal infections. Regular monitoring of local antimicrobial resistance profiles of clinically important pathogens is crucial for guiding the prescription of effective antibiotics and for early initiation of control measures to stop the spread of GNB with high resistance levels.

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Disclosure

The authors report no conflicts of interest in this work.

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