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Original Article

Multicenter surveillance of antimicrobial susceptibilities and resistance mechanisms among *Enterobacterales* species and non-fermenting Gram-negative bacteria from different infection sources in Taiwan from 2016 to 2018

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KEYWORDS
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Pseudomonas aeruginosa;
Burkholderia cenocepacia complex;
Multidrug-resistant;
Extensively-drug-resistant;
Ceftolozane/tazobactam

Abstract *Objectives:* To explore the *in vitro* antimicrobial susceptibility among clinically important Gram-negative bacteria (GNB) in Taiwan.
Methods: From 2016 through 2018, a total of 5458 GNB isolates, including *Escherichia coli* ($n = 1545$), *Klebsiella pneumoniae* ($n = 1255$), *Enterobacter* species ($n = 259$), *Pseudomonas aeruginosa* ($n = 1127$), *Acinetobacter baumannii* complex ($n = 368$), and *Stenotrophomonas maltophilia* ($n = 179$), were collected. The susceptibility results were summarized by the breakpoints of minimum inhibitory concentration (MIC) of CLSI 2020, EUCAST 2020 (for colistin), or published articles (for ceftolozane/tazobactam). The resistance genes among multidrug-resistant (MDR) or extensively drug-resistant (XDR)-GNB were investigated by multiplex PCR.
Results: Significantly higher rates of non-susceptibility (NS) to ertapenem and carbapenemase production, predominantly KPC and OXA-48-like beta-lactamase, were observed in *Enterobacterales* isolates causing respiratory tract infection than those causing complicated urinary tract or intra-abdominal infection (12.7%/3.44% vs. 5.7%/0.76% or 7.7%/0.97%, respectively). Isolates of *Enterobacter* species showed higher rates of phenotypic extended-spectrum β-lactamase and NS to ertapenem than *E. coli* or *K. pneumoniae* isolates. Although moderate activity (54—83%) was observed against most potential AmpC-producing *Enterobacterales* isolates, ceftolozane/tazobactam exhibited poor *in vitro* (44.7—47.4%) activity against phenotypic AmpC *Enterobacter cloacae* isolates. Additionally, 251 (22.3%) *P. aeruginosa* isolates exhibited the carbapenem-NS phenotype, and their MDR and XDR rate was 63.3% and 33.5%, respectively. Fifteen (75%) of twenty *Burkholderia cenocepacia* complex isolates were inhibited by ceftolozane/tazobactam at MICs of $\leq$4 μg/mL.
Conclusions: With the increase in antibiotic resistance in Taiwan, it is imperative to periodically monitor the susceptibility profiles of clinically important GNB.

Introduction

Septicemia is usually associated with considerable morbidity and mortality, especially in debilitated patients and those with healthcare-associated (HA, i.e., collected $\geq$ 48 h after admission) infections due to Gram-negative bacteria (GNB).[1—7] Among them, the multidrug-resistant (MDR)- or extensively-drug-resistant (XDR)-GNB, including the producers of extended-spectrum β-lactamase (ESBL) and/or carbapenemase among *Enterobacterales* species, and isolates of non-susceptibility (NS) to carbapenems, and/or MDR/XDR-*Pseudomonas aeruginosa* as well as *Acinetobacter baumannii* complex, have become the difficult—to-treat pathogens in clinical settings.[8—10]

Initial empirical treatment with at least one *in vitro* active antibiotic against the causative pathogen(s) is essential for early control of severe infections. However, to prescribe optimal empirical antibiotic agents, primary care physicians should have the updated information of the distribution and antimicrobial susceptibility patterns of etiological pathogens causing various infection diseases.

The Study for Monitoring Antimicrobial Resistance Trends (SMART), conducted since 2002, has monitored the *in vitro* activities of antimicrobial agents against aerobic GNB causing respiratory tract infection (RTI), complicated intra-abdominal infections (cIAI), or complicated urinary tract infections (cUTI). Additionally, the *in vitro* susceptibility data of ceftolozane/tazobactam (C/T) against *Burkholderia cenocepacia* complex, a notoriously nosocomial pathogen mostly exhibiting XDR phenotypes,[11] are scarce. As the resistance burden becomes increasingly worse, this study aims to explore the evolutionary changes of *in vitro* susceptibility data as well as molecular characteristics of clinically important GNB collected from the above three infection sites between 2016 and 2018 in Taiwan. In this article, we also intensively compare the NS rates of available important antibiotics as well as molecular data relevant to resistance in Taiwanese GNB with those of other countries in recent years.

Material and methods

Bacterial isolates

Non-duplicate, clinically relevant Gram-negative aerobic and facultative bacteria were isolated from various clinical samples, including expectorated sputum or endotracheal aspirate, bronchoalveolar lavage fluid, bronchial brushing, and pleural fluid; peritoneal fluid, tissues of hepatic or pancreatic parenchyma, stomach, gallbladder, small intestine, appendix, colon, and intra-abdominal abscess; renal tissues, ureters, urinary bladder, urethra, and urine. The isolates were acquired as a part of the 2016—2018 SMART surveillance from eight hospitals throughout Taiwan. The species of all isolates were initially identified at each participating hospital and were then sent to the central laboratory (International Health Management Associates, Inc., Schaumburg, IL, USA) for further species identification using MALDI-TOF MS (Bruker Biotyper™, Bruker Daltonik GmbH, Bremen, Germany), and molecular analyses by multiplex PCR (see below). This study was approved by the

Institutional Review Board of National Taiwan University Hospital (Taipei, Taiwan) [NTUH 9561709108].

Antimicrobial susceptibility testing

MICs of the following antibiotics: colistin (CST), amikacin (AMK), aztreonam, C/T, piperacillin/tazobactam (TZP), ceftriaxone (CRO), ceftazidime (CAZ), cefepime (FEP), ertapenem (ETP), imipenem (IPM), meropenem (MEM), ciprofloxacin (CIP), and levofloxacin (LVX) were determined using the broth microdilution method. The custom-made dehydrated Trek Diagnostic Systems panel was purchased from Thermo Fisher Scientific (Independence, OH, USA). Tazobactam at a fixed concentration of 4 μg/mL in combination with two-fold dilutions of ceftolozane was used for susceptibility testing. Susceptibility was interpreted according to the MIC breakpoints recommended by the Clinical and Laboratory Standards Institute (CLSI) 2020.[12] Additionally, CST susceptibility was interpreted in accordance with the MIC breakpoint recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2021.[13] The MDR and XDR phenotypes were defined according to international recommendations.[14] *Escherichia coli* ATCC 25922, *E. coli* ATCC 35218, *P. aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 13883, and *K. pneumoniae* ATCC 700603 were used for CLSI and EUCAST-related quality control.

Definition of subgroups

Susceptibility analyses were performed according to the presence of carbapenem NS, phenotypic ESBL, culture sources (RTI, cIAI, or cUTI), patient location (emergency room, general ward, or intensive care unit), and recovery time. The carbapenem-NS phenotype among *Enterobacterales* isolates was defined as MIC of IPM or MEM >1 μg/mL, or ETP MIC of >0.5 μg/mL. In contrast, the carbapenem-NS phenotype among the isolates of *P. aeruginosa* or *A. baumannii* complex species was defined as MIC of IPM or MEM >2 μg/mL. The MIC breakpoints of C/T for non-fermenting GNB other than *P. aeruginosa* follow those used in previous studies (≤4/4, ≤2/4 and ≤ 4/4 μg/mL for *Stenotrophomonas maltophilia*, *A. baumannii* complex spp., and *B. cenocepacia* complex species, respectively).[15-17] Additionally, as the ETP-NS phenotype is a sensitive indicator for the initial screening for potential carbapenemase production among the *Enterobacterales* isolates,[18] we adopted this phenotypic criterion for analysis in this survey.

Definition of phenotypic AmpC and phenotypic ESBL testing for *Enterobacterales* isolates, and PCR screening for various β-lactamase genes among *Enterobacterales* and *P. aeruginosa* isolates

Susceptibility to cefoxitin was not tested in this study. Thus, among the isolates of *Enterobacterales* species, we defined the potentially phenotypic AmpC producers as CRO- or CAZ-NS but FEP susceptibility were selected, as described elsewhere.[19,20] Additionally, the phenotypic ESBL confirmatory testing (i.e., MIC difference between CRO alone and CRO plus clavulanate) was performed against the enrolled *Enterobacterales* isolates that exhibited NS to CRO or CAZ. At least one half of isolates of phenotypic ESBL *Enterobacterales* species collected in this survey were chosen evenly from eight participating hospitals to elucidate the existence of genes encoding β-lactamases by PCR. Additionally, 230 IPM- or MEM-NS *P. aeruginosa* isolates were also elected randomly for elucidation of the genes encoding β-lactamases. Whole genomic DNA of the isolates was extracted using a QIAamp DNA minikit and QIAcube instrument (Qiagen, Valencia, CA, USA) from the colonies grown overnight on blood agar plate (Remel, Lenexa, KS, USA). Multiplex PCR examined five groups of β-lactamase genes: (1) bla_{TEM}, bla_{SHV}, bla_{VEB}, bla_{PER}, bla_{GES}; (2) $bla_{CTX\text{-}M\text{-}group\text{-}1}$, $bla_{CTX\text{-}M\text{-}group\text{-}2}$, $bla_{CTX\text{-}M\text{-}group\text{-}9}$; (3) $bla_{CTX\text{-}M\text{-}group\text{-}8}$, $bla_{CTX\text{-}M\text{-}group\text{-}25}$; (4) bla_{ACC}, bla_{CMY}, bla_{MOX}, bla_{ACT}, bla_{MIR}, bla_{DHA}, bla_{FOX}, bla_{PDC}; (5) bla_{KPC}, bla_{GIM}, bla_{SPM}, bla_{NDM}, bla_{IMP}, bla_{VIM}, and $bla_{OXA\text{-}48}$.[15,21] All detected genes encoding β-lactamases were amplified and sequenced as described previously.[15] In this survey, only ESBLs, rather than original spectrum β-lactamases, were subjected to full gene sequencing to validate the tested *Enterobacterales* isolates as ESBL producers.

Statistical analyses

Categorical variables were expressed as the percentages of the total number of isolates and analyzed by Fisher's exact test or chi-square test as appropriate. In addition, linear regression was used to analyze the trends in annual rates of (1) carbapenemase-encoding genes, (2) KPC production among all isolates of *Enterobacterales* species, and (3) carbapenem-NS among all *P. aeruginosa* isolates sampled between 2016 and 2018. Furthermore, the correlation between annual carbapenem (IPM or MEM)-NS rates and annual proportions in different infection sources among included *P. aeruginosa* isolates was calculated by the bivariate correlation analysis. All statistical calculations were two-tailed, and a *P*-value of <0.05 was considered statistically significant. All statistical analyses were performed using SPSS 17.0 (SPSS, Chicago, IL, USA).

Results

Rates of carbapenem resistance and phenotypic ESBL, distributions of various β-lactamases among isolates of important *Enterobacterales* species collected from different infection sources.

Between 2016 and 2018, a total of 5458 isolates were collected from hospitalized patients regardless of the hospitalization duration in Taiwan. Isolates of *E. coli* ($n = 1545$), *K. pneumoniae* ($n = 1255$), and *Enterobacter* species ($n = 259$) accounted for the majority (82.1%) of *Enterobacterales* isolates ($n = 3724$) enrolled. The ETP- and MEM-NS rate was 8.4% and 2.7%, respectively among all *Enterobacterales* isolates, while the IPM-NS rate was 5.8% among the *Enterobacterales* isolates excluding the genera of *Morganella*, *Proteus*, and *Providencia*. Concerning the *Enterobacterales* isolates from different infection sources, the rates of ETP-NS and carbapenemase production were highest in the RTI isolates (12.7% and 3.44%; $P < 0.001$), followed by cIAI (7.7% and 0.97%; $P < 0.001$) and cUTI (5.7% and 0.76%; $P < 0.001$) isolates. In addition, of the 1059 (28.4%) phenotypic ESBL *Enterobacterales* isolates, 553 (14.8%) isolates were submitted to PCR survey for detecting those harboring important β-

lactamase-encoding genes. The ACT/MIR-encoding genes were excluded in *Enterobacter* species due to intrinsic AmpC β-lactamases. The RTI group had a significantly higher rate of positive ESBL-encoding genes (12.7%) than the other two groups (6.5%; $P < 0.001$), as shown in Fig. 1. In contrast, the isolates of *P. aeruginosa* ($n = 1127$), *A. baumannii* complex species ($n = 368$), and *S. maltophilia* ($n = 179$) accounted for the majority (96.5%) of non-fermenting GNB ($n = 1734$) implicated in various infections.

Fig. 2 illustrates that the RTI isolates of *E. coli* and *Enterobacter* species had higher phenotypic ESBL rates than those of *K. pneumoniae* (*P* values, 0.021 and 0.083, respectively). Similar trends were also seen in the cIAI groups with more statistically significant differences (both of *P* values, 0.001). The cUTI isolates of *Enterobacter* species had a significantly higher phenotypic ESBL rate as compared to the other two species of cUTI groups ($P < 0.01$). Additionally, the isolates of *Enterobacter* species exhibited a significantly higher ETP-NS rate as compared to the other two species regardless of infection sources ($P < 0.05$).

Fig. 3 illustrates the rates of various β-lactamases (ESBL, plasmidic AmpC, and carbapenemases) among 553 tested phenotypic ESBL *Enterobacterales* isolates in different years. Various CMY types (with predominance of CMY-2) and DHA-1 accounted for most of the plasmidic AmpC enzymes in phenotypic ESBL *Enterobacterales* isolates. When calculated by linear regression, the annual rates of positive carbapenemase-encoding genes showed a rising trend ($r = 0.967$, $P = 0.164$). A similar trend was also observed in the positive bla_{KPC}-encoding gene rates ($r = 0.922$, $P = 0.253$). No New Delhi metallo-β-lactamase (NDM)-producing isolate was noted and only a few bla_{IMP}- or bla_{VIM}-harboring (Verona integron-mediated metallo-β-lactamase) isolates were detected during the study period. Additionally, among the 264 isolates of three major Enterobacteriaceae species (comprising *E. coli* [$n = 60$], *K. pneumoniae* [$n = 140$], and *Enterobacter* species [$n = 64$]) exhibiting the ETP-NS phenotype, various types of ESBL and plasmidic AmpC dominated in each species (as shown in Fig. 4). Moreover, 51 (19.3%) isolates were detected to have carbapenemase-encoding genes in above-mentioned ETP-NS isolates. *K. pneumoniae* carbapenemase (KPC; KPC-2 and KPC-17) and oxacillinase (OXA)-48-like enzymes accounted for the majority of carbapenemases (78.4% and 11.8%, respectively). No bla_{NDM}- and only few bla_{IMP-8} (imipenemase) or bla_{VIM-1}-harboring isolates of ETP-NS-*E. coli* (1.7%) and ETP-NS-*Enterobacter* species (4.7%) were detected. Furthermore, Fig. 5 illustrates the positive rates of genes encoding ESBL, plasmidic AmpC, and carbapenemases among the cIAI isolates of *Enterobacterales* species in different years. The rates of positive ESBL-, plasmidic AmpC (with CMY-2 and DHA-1 predominance)-, and carbapenemase-encoding genes ranged from 2.78.8%, 4.6—10.5%, and 0.5—1.5%, respectively, during the study period. Conversely, the proportions of non-carbapenemase producers among the ETP-NS *Enterobacterales* isolates were 73.8% (104/141), 87.3% (96/110), and 85.1% (57/67) in the RTI, cIAI, and cUTI, respectively.

Antibiotic susceptibility of isolates of phenotypic AmpC *Enterobacterales* species

Table 1 illustrates the antibiotic susceptibility of phenotypic AmpC producers among the overall *Enterobacterales* isolates. Of all antibiotics evaluated, MEM and AMK exhibited best *in vitro* activities (susceptibility rates, 99.1% and 98.6%, respectively), followed by IPM (93.1%), CST (87%), ETP (85.2%), C/T (58.3%), and CIP (51.4%).

Antibiotic susceptibility data of cIAI-related *Enterobacterales* isolates

Table 2 shows the susceptibility data of antibiotics against GNB that resulted in cIAI. Against the cIAI-*E. coli* and *K. pneumoniae* isolates, AMK, CST, and three carbapenem agents showed >90% susceptibility rates, followed by C/T, FEP, and TZP exhibiting >80% susceptibility rates. In stark contrast, only MEM, IPM, and AMK were shown to have

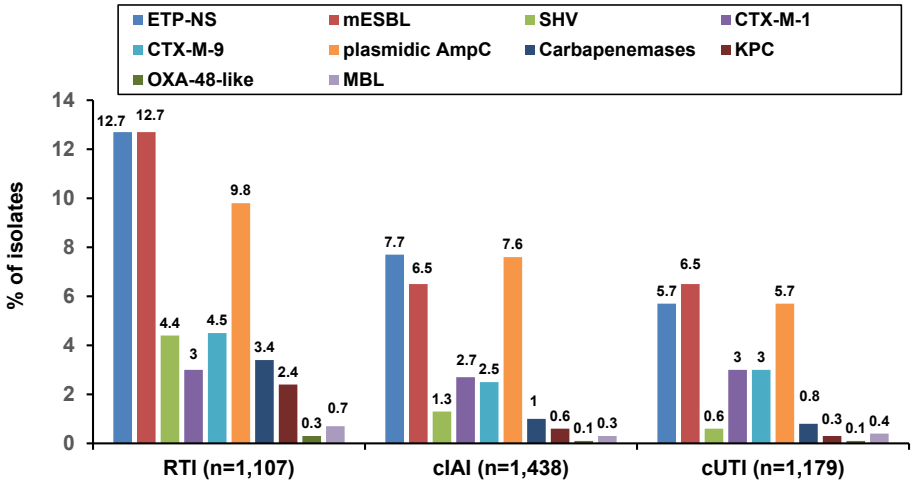

Figure 1. Rates of ETP-NS, mESBL, plasmidic AmpC, and carbapenemase-encoding genes among isolates of *Enterobacterales* species collected from respiratory tract infection, complicated intra-abdominal infection, or complicated urinary tract infection. ETP, ertapenem; NS, non-susceptibility; mESBL, molecular carriage of ESBL-encoding genes confirmed by PCR assay; KPC, *Klebsiella pneumoniae* carbapenemase; OXA, oxacillinase; MBL, metallo-β-lactamase.

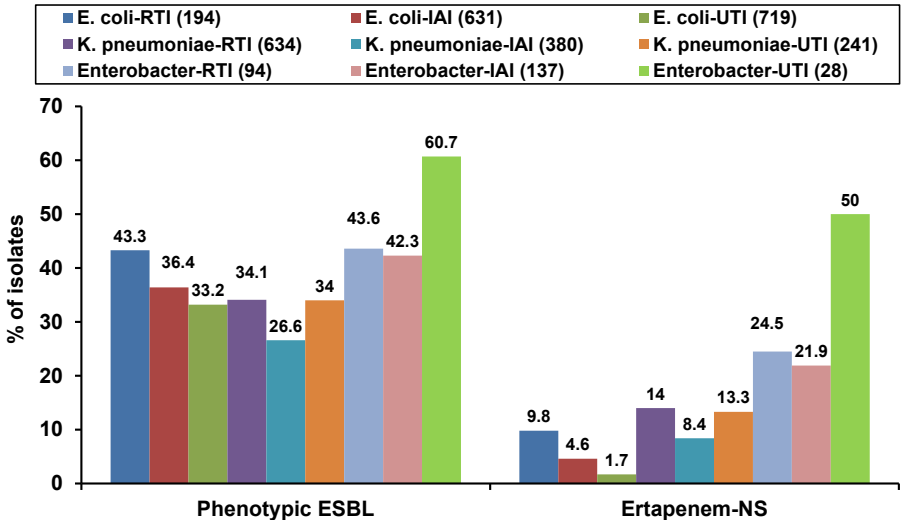

Figure 2. Rates of phenotypic extended-spectrum β-lactamase and ertapenem non-susceptibility among the isolates of three common Enterobacteriaceae species (i.e., *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterobacter* species) collected from respiratory tract infection, complicated intra-abdominal infection, or complicated urinary tract infection. NS, non-susceptibility; ESBL, extended-spectrum β-lactamase; RTI, respiratory tract infection; cIAI, complicated intra-abdominal infection; cUTI, complicated urinary tract infection.

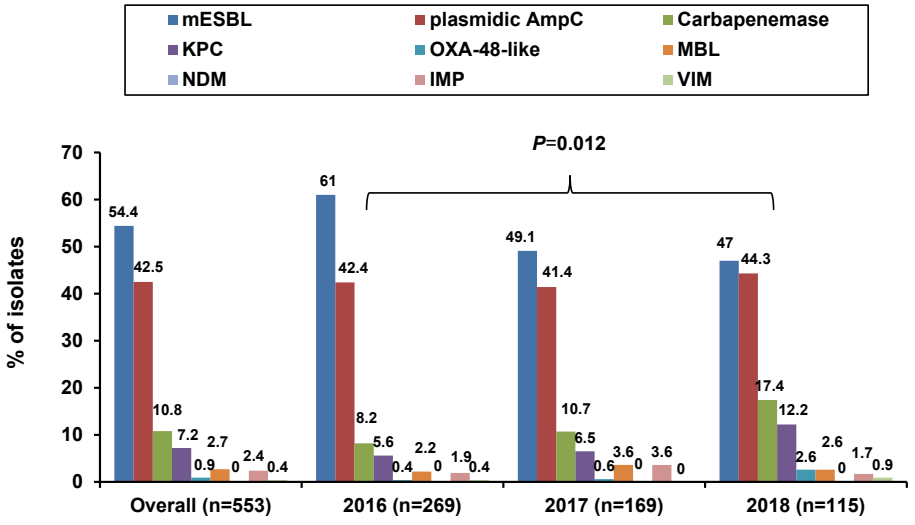

Figure 3. Rates of (1) molecularly-characterized extended-spectrum β-lactamase (mESBL), (2) plasmidic AmpC, and (3) carbapenemase-encoding genes among the 553 phenotypic ESBL isolates of *Enterobacterales* species from 2016 through 2018. KPC, *Klebsiella pneumoniae* carbapnemase; OXA, oxacillinase; MBL, metallo-β-lactamase; NDM, New Delhi metallo-β-lactamase; IMP, imipenemase; VIM, Verona integron-mediated metallo-β-lactamase.

>95% susceptibility rates (97.8%, 95.6% and 99.3%, respectively) against the isolates of *Enterobacter* species (of which *Enterobacter cloacae* complex accounted for 77.4%). Moreover, ETP and CIP were shown to have susceptibility rates of 98.6%/84.5% and 75.0%/75.0% against cIAI isolates of *Citrobacter* species (n = 71)/*Klebsiella aerogenes* (n = 40). Furthermore, C/T was shown to exhibit susceptibility rates of 65.7%, 77.5%, and 72.5% against the above-mentioned three species of potential AmpC producers (*E. cloacae* complex, *Citrobacter* species, and *K. aerogenes*, respectively). With exception of CST, IPM and LVX, the remaining antibiotics under evaluation were highly active against cIAI-*Proteus* isolates.

MDR, XDR rates and susceptibility data among *P. aeruginosa* isolates collected from different infection sources, and isolates of *A. baumannii*, *S. maltophilia*, and *B. cenocepacia* complex species

Against overall *P. aeruginosa* isolates, the MDR and XDR rate was 26.0% and 8.0%, respectively; CST, AMK and C/T were the

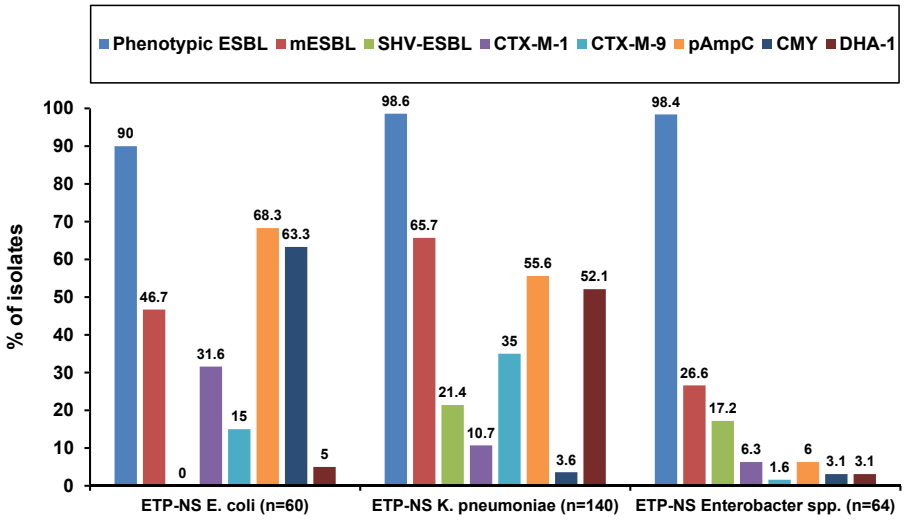

Figure 4. Rates of ertapenem non-susceptibility and carriage of ESBL/AmpC β-lactamase-encoding genes among the isolates of three major Enterobacteriaceae species (*Escherichia coli*, *Klebsiella pneumoniae*, and *Enterobacter* species) from 2016 through 2018. ESBL, extended-spectrum β-lactamase; ETP, ertapenem; NS, non-susceptibility; mESBL, molecular carriage of ESBL-encoding genes confirmed by multiplex PCR; pAmpC, carriage of plasmidic AmpC-encoding genes confirmed by multiplex PCR.

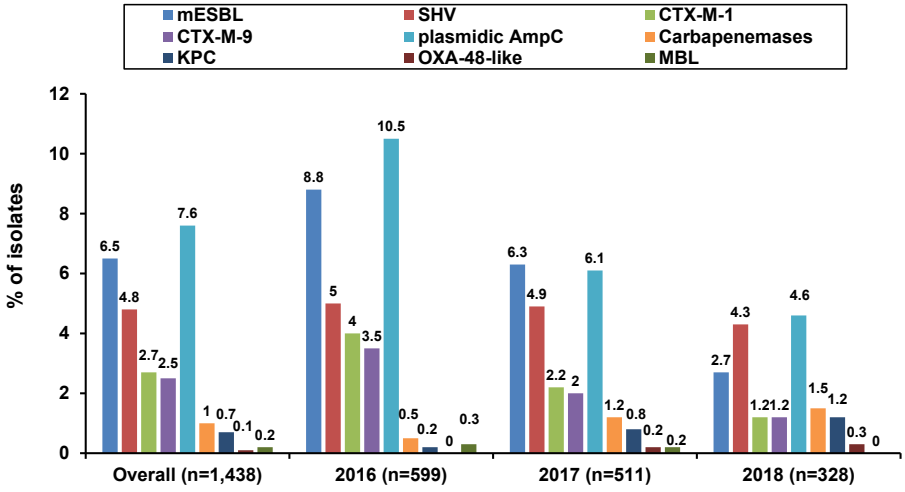

Figure 5. Rates of molecular carriage of the genes encoding ESBL, plasmidic AmpC, or carbapenemases among the isolates of *Enterobacterales* species collected from complicated intra-abdominal infections from 2016 through 2018. mESBL, molecular carriage of ESBL-encoding genes confirmed by multiplex PCR; KPC, *Klebsiella pneumoniae* carbapenemase; OXA, oxacillinase; MBL, metallo-β-lactamase.

most effective antibiotics (susceptibility rates > 97%), followed by CIP, FEP, MEM, and IPM (susceptibility rates > 75%). A similar result was observed among IPM or MEM-NS *P. aeruginosa* isolates. Moreover, the susceptibility rates of CST, AMK, C/T, CAZ, FEP, and CIP against 90 XDR-*P. aeruginosa* isolates (HA infection rate, 78.9%) were 98.9%, 90%, 76.7%, 22.2%, 8.9%, and 10%, respectively. In contrast, *A. baumannii* isolates (HA infection rate, 79.3%) showed high (ranging 55—85%) *in vitro* NS rates to most antibiotics except CST (NS rate, 5.2%). The rate of NS (MIC > 2/4 μg/mL) to C/T for the isolates of *A. baumannii* isolates was 66%.

The MDR/XDR rates for *P. aeruginosa* subsets of RTI, cIAI, and cUTI were 29.4%/9.1%, 22.6%/7%, and 15.6%/4.5%, respectively. Of 1127 *P. aeruginosa* isolates, 251 (22.3%) exhibited NS to either IPM or MEM, 80.5% were judged as hospital acquisition, and the MDR/XDR rate was 63.3%/33.5% in IPM- or MEM-NS *P. aeruginosa* isolates. Furthermore, apart from CST, AMK and C/T were shown to have significantly lower NS rates in carbapenem-NS *P. aeruginosa* isolates (2.3—4.3% and 4.3—11.4%, respectively) than the other evaluated antibiotics exhibiting 35%—69% NS rates regardless of infection sources. During the study period, two *P. aeruginosa* isolates (one from RTI, and the other from cIAI) were shown to harbor carbapenemase-encoding genes (bla_{VIM-2} and bla_{KPC-3}, respectively). Nevertheless, the annual carbapenem-NS rates among *P. aeruginosa*

Table 1 Susceptibility data of the isolates of overall *Enterobacterales* species with AmpC-producing phenotype,[22,24,25] but without genes encoding ESBL and carbapenemase (validated by multiplex PCR) from hospitalized patients in Taiwan from 2016 to 2018.

Pathogens (no. of isolates)	Susceptibility rates, % (no. of isolates)								
	CST	AMK	C/T	TZP	ETP	IPM	MEM	CIP	LVX
Escherichia coli (67)	97.0 (65)	100 (67)	53.7 (36)	55.2 (37)	86.6 (58)	97.0 (65)	98.5 (66)	23.9 (16)	1.5 (1)
ETP-S (58)	96.6 (56)	100 (58)	48.3 (28)	51.7 (30)	–	100 (58)	100 (58)	20.7 (12)	1.7 (1)
Enterobacter cloacae (47)	83.0 (39)	100 (47)	44.7 (21)	38.3 (18)	80.9 (38)	97.9 (46)	100 (47)	68.1 (32)	17.0 (8)
ETP-S (38)	81.6 (31)	100 (38)	47.4 (18)	39.5 (15)	–	100 (38)	100 (38)	71.1 (27)	10.5 (4)
Citrobacter spp. (41)	100 (41)	92.7 (38)	58.5 (24)	51.2 (21)	100 (41)	100 (41)	100 (41)	65.9 (27)	7.3 (3)
Klebsiella aerogenes (37)	97.3 (36)	100 (37)	67.6 (25)	35.1 (13)	78.4 (29)	94.6 (35)	100 (37)	67.6 (25)	21.6 (8)
ETP-S (29)	96.6 (28)	100 (29)	75.9 (22)	41.4 (12)	–	93.1 (27)	100 (29)	69 (20)	24.1 (7)
Aeromonas spp. (7)	71.4 (5)	100 (7)	100 (7)	28.6 (2)	57.1 (4)	71.4 (5)	85.7 (6)	71.4 (5)	28.6 (2)
ETP-S (4)	100 (4)	100 (4)	100 (4)	25 (1)	–	100 (4)	100 (4)	50 (2)	50 (2)
Serratia marcescens (11)	18.2 (2)	100 (11)	72.7 (8)	63.6 (7)	72.7 (8)	63.6 (7)	100 (11)	27.3 (3)	9.1 (1)
ETP-S (8)	12.5 (1)	100 (8)	75 (6)	75 (6)	–	62.5 (5)	100 (8)	25 (2)	12.5 (1)
Morganella morganii (6)	0 (0)	100 (6)	83.3 (5)	83.3 (5)	100 (6)	33.3 (2)	100 (6)	50 (3)	16.7 (1)
Overall (216)	87 (188)	98.6 (213)	58.3 (126)	47.7 (103)	85.2 (184)	93.1 (201)	99.1 (214)	51.4 (111)	11.1 (24)
ETP-S (184)	87.5 (161)	98.4 (181)	58.2 (107)	48.9 (90)	–	95.1 (175)	100 (184)	50.5 (93)	10.3 (19)

CST, colistin; AMK, amikacin; C/T, ceftolozane/tazobactam; TZP, piperacillin/tazobactam; ETP, ertapenem; IPM, imipenem; MEM, meropenem; CIP, ciprofloxacin; LVX, levofloxacin; S, susceptible; No., number. –, not applicable.

isolates showed a trend towards prominent escalation (95% confidence interval: 0.545–0.936; $P = 0.016$), and tended to correspond with the annual proportions of RTI-causing *P. aeruginosa* isolates ($r = 0.980$, $P = 0.127$).

Among 179 *S. maltophilia* isolates, the NS rate to C/T (defined as MIC $>$ 4/4 μg/mL), CAZ, and LVX was 66.5%, 65.9%, and 41.9%, respectively. Moreover, among 20 isolates of *B. cenocepacia* complex species (all from RTI), the susceptibility rate to C/T (defined as MIC $\leq$ 4/4 μg/mL), TZP, CAZ, MEM, and LVX was 75%, 5%, 75%, 95%, and 20%, respectively.

Discussion

In the 2016–2018 SMART survey in Taiwan, we clearly characterize the prevalence rates of *Enterobacterales* isolates harboring a variety of carbapenemase- and ESBL-encoding genes and those displaying the ETP-NS phenotype in different infection sources. Although we did not accurately elucidate the resistance mechanisms of drug-resistant GNB in detail, this *in vitro* susceptibility investigation provides a clear scope in guiding the prescription of antibiotics against clinically important GNB. Moreover, the rates of carbapenem-NS and MDR (26% and 22.3%, respectively) among overall *P. aeruginosa* isolates were lower than those of two reports in China (40% and 88%, respectively).[21,22] Compared to our study, the above-mentioned two studies in China disclosed that 8.5% of carbapenem-resistant *P. aeruginosa* isolates harbored metallo-β-lactamase-encoding genes (predominantly VIM-2/9).[21,22] The MDR rate (63.3%) in the Taiwanese IPM- or MEM-NS *P. aeruginosa* isolates was close to that reported in the 2015 US study.[23] However, the MDR rate (29.4%) among Taiwanese RTI-causing *P. aeruginosa* isolates was higher than that (24.7%) reported in the 2015–2016 US study.[24] As shown in other surveys,[15,25] C/T was shown to have an excellent susceptibility rate (90%) close to that of AMK against Taiwanese IPM- or MEM-NS *P. aeruginosa* isolates, including those likely having an efflux pump. The other pathogen worth noting is MDR and XDR-*A. baumannii* complex because they frequently cause high case-fatality rates and pose difficulty in prescribing a single *in vitro* effective antibiotic for treatment.[26,27] Spreads of clonal MDR-*A. baumannii* complex strain have also been reported in many countries.[9,28,29]

As shown in Fig. 1, similarities in rates of ETP-NS and molecularly characterized ESBL production in *Enterobacterales* isolates regardless of infection sources reflect that complex resistance mechanisms regarding NS to ETP involve diverse β-lactamases (not only carbapenemase), porin change and emergence of efflux pump among enteric GNB.[30] Besides, approximately 1.0% of the Taiwanese cIAI *Enterobacterales* isolates harbored carbapenemase genes. This proportion was higher than that (0.44%) of the previous Asia–Pacific cIAI 2008–2014 study.[18] Additionally, notably higher rates of carbapenem-NS Enterobacteriaceae among the overall (12.7%) and HA-RTI *Enterobacterales* groups (14.7% vs. 11.9% of HA-cIAI subset, and 12.1% of HA-cUTI subset, respectively, not shown in Results) but a similar ESBL rate (12.7%) were noted in our study, as compared to those (3.8% and 6.1–11.4%, respectively) in the investigation at the US medical centers.[31] Furthermore, the phenotypic ESBL rates among isolates of three major Enterobacteriaceae species of the RTI group in the present study were high, ranging from 34% to 44% (Fig. 2), similar to

Table 2 Susceptibility data of important Gram-negative bacterial isolates ([A] overall [B] carbapenem non-susceptible, and [C] ertapenem-susceptible but positive ESBL-encoding genes) causing complicated intra-abdominal infections among hospitalized patients in Taiwan from 2016 to 2018.

Pathogens (number of tested isolates)	Susceptibility rates, % (isolate number)												
	CST	AMK	ATM	C/T	TZP	CRO	CAZ	FEP	ETP	IPM	MEM	CIP	LVX
Escherichia coli (631)	97.6 (616)	99.5 (628)	67.7 (427)	92.6 (584)	89.5 (565)	67.2 (424)	67.2 (424)	80.3 (507)	95.4 (602)	99.2 (626)	99.4 (627)	57.4 (362)	14.4 (91)
MIC_{50}/MIC_{90}, μg/mL	1/1	4/4	1/>16	0.25/2	2/32	1/>32	1/32	1/>16	0.06/0.25	0.5/0.5	0.12/0.12	0.25/>2	1/>4
ETP-NS (29)	96.6 (28)	93.1 (27)	13.8 (4)	27.6 (8)	24.1 (7)	13.8 (4)	6.9 (2)	27.6 (8)	—	86.2 (25)	86.2 (25)	37.9 (11)	6.9 (2)
ETP-susceptible, (+) ESBL-encoding gene (34)	91.2 (31)	97.1 (33)	8.8 (3)	79.4 (27)	79.4 (27)	0 (0)	23.5 (8)	8.8 (3)	100 (34)	100 (34)	100 (34)	17.6 (6)	0 (0)
Klebsiella pneumoniae (380)	98.2 (373)	96.8 (368)	82.6 (314)	87.9 (334)	84.5 (321)	84.2 (320)	75.0 (285)	86.3 (328)	91.6 (348)	93.2 (354)	95.0 (361)	67.9 (258)	16.8 (64)
MIC_{50}/MIC_{90}, μg/mL	1/1	4/4	1/>16	0.25/>8	4/>64	1/>32	1/32	1/16	0.06/0.5	0.5/1	0.12/0.12	0.25/>2	1/>4
ETP-NS (32)	78.1 (25)	78.1 (25)	6.3 (2)	18.8 (6)	12.5 (4)	15.6 (5)	3.1 (1)	31.3 (10)	—	37.5 (12)	46.9 (15)	3.1 (1)	0 (0)
ETP-susceptible, (+) ESBL-encoding gene (16)	100 (16)	75.0 (12)	12.5 (2)	31.3 (5)	31.3 (5)	0 (0)	18.8 (3)	12.5 (2)	100 (16)	93.8 (15)	100 (16)	6.3 (1)	0 (0)
Pseudomonas aeruginosa (199)	100 (199)	99.5 (198)	71.9 (143)	97.0 (193)	73.9 (147)	—	79.4 (158)	83.9 (167)	—	79.4 (158)	82.4 (164)	84.9 (169)	76.9 (153)
MIC_{50}/MIC_{90}, μg/mL	1/1	4/4	4/>16	0.5/4	8/>64	—	4/>32	2/16	—	1/16	0.5/8	0.25/2	1/4
IPM- or MEM-NS (44)	100 (44)	97.7 (43)	38.6 (17)	93.2 (41)	43.2 (19)	—	56.8 (25)	59.1 (26)	—	6.8 (3)	22.7 (10)	50 (22)	63.6 (28)
Enterobacter spp. (137)	81.0 (111)	99.3 (136)	57.7 (79)	65.7 (90)	68.6 (94)	59.1 (81)	53.3 (73)	74.5 (102)	78.1 (107)	95.6 (131)	97.8 (134)	77.4 (106)	13.1 (18)
MIC_{50}/MIC_{90}, μg/mL	1/>4	4/4	2/>16	0.5/>32	4/>64	2/>32	2/>32	1/16	0.12/2	0.5/1	0.12/0.25	0.25/>2	1/4
ETP-NS (30)	93.3 (28)	100 (30)	0 (0)	6.7 (2)	13.3 (4)	3.3 (1)	3.3 (1)	23.3 (7)	—	90.0 (27)	90.0 (27)	53.3 (16)	6.7 (2)
ETP-susceptible, (+) ESBL-encoding gene (1)	100 (1)	0 (0)	0 (0)	0 (0)	100 (1)	0 (0)	0 (0)	0 (0)	100 (1)	100 (1)	100 (1)	0 (0)	0 (0)
Citrobacter spp. (71)	100 (71)	97.2 (69)	59.2 (42)	77.5 (55)	76.1 (54)	59.2 (42)	56.3 (40)	95.8 (68)	98.6 (70)	95.8 (68)	98.6 (70)	84.5 (60)	8.5 (6)
MIC_{50}/MIC_{90}, μg/mL	1/1	4/4	1/>16	0.5/>8	4/64	1/>32	1/>32	1/2	0.06/0.25	0.5/1	0.12/0.12	0.25/1	1/1
ETP-NS (1)	100 (1)	100 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	—	0 (0)	0 (0)	0 (0)	0 (0)
Acinetobacter spp. (53)	98.1 (52)	58.5 (31)	—	34.0[a] (18)	30.2 (16)	13.2 (7)	41.5 (22)	39.6 (21)	—	39.6 (21)	35.8 (19)	39.6 (21)	47.2 (25)
MIC_{50}/MIC_{90}, μg/mL	1/1	4/>32	>16/>16	16/>32	>64/>64	>32/>32	>32/>32	>32/>32	—	32/>32	32/>32	>2/>2	2/>4
IPM- or MEM-NS (32)	96.9 (31)	31.3 (10)	—	—	0 (0)	0 (0)	15.6 (5)	9.4 (3)	—	—	—	9.4 (3)	15.6 (5)
Proteus spp. (48)	1 (2.1)	100 (48)	97.9 (47)	97.9 (47)	97.9 (47)	89.6 (43)	97.9 (47)	97.9 (47)	100 (48)	41.7 (20)	100 (48)	85.4 (41)	20.8 (10)
MIC_{50}/MIC_{90}, μg/mL	>4/>4	4/8	1/2	0.5/2	2/2	1/2	1/1	1/1	0.06/0.06	2/4	0.12/0.12	0.25/>2	1/2
Stenotrophomonas maltophilia (43)	—	—	—	41.9[b] (18)	—	—	46.5 (20)	—	—	—	—	—	58.1 (25)
MIC_{50}/MIC_{90}, μg/mL	—	—	—	16/>32	—	—	16/>32	—	—	—	—	—	1/>4
Klebsiella aerogenes (40)	97.5 (39)	100 (40)	47.5 (19)	72.5 (29)	62.5 (25)	60 (24)	42.5 (17)	82.5 (33)	75.0 (30)	80.0 (32)	92.5 (37)	75.0 (30)	17.5 (7)
MIC_{50}/MIC_{90}, μg/mL	1/1	4/4	8/>16	1/8	16/64	8/32	16/>32	1/8	0.12/4	1/2	0.12/0.5	0.25/>2	1/>4

ETP-NS (10)	100 (10)	100 (10)	10 (1)	20 (2)	20 (2)	10 (1)	100 (10)	50 (5)	–	60 (6)	70 (7)	50 (5)	10 (1)
Aeromonas spp. (34)	64.7 (22)	97.1 (33)	82.4 (28)	88.2 (30)	70.6 (24)	64.7 (22)	76.5 (26)	85.3 (29)	67.6 (23)	67.6 (23)	76.5 (26)	79.4 (27)	23.5 (8)
MIC_{50}/MIC_{90}, μg/mL	1/>4	4/16	4/16	0.5/16	2/>64	1/>32	1/>32	1/4	0.06/>4	0.5/32	0.12/16	0.25/1	1/1
Morganella morganii (29)	0 (0)	100 (29)	96.6 (28)	96.6 (28)	96.6 (28)	96.6 (28)	89.7 (26)	96.6 (28)	100 (29)	13.8 (4)	100 (29)	65.5 (19)	24.1 (7)
MIC_{50}/MIC_{90}, μg/mL	1/1	4/4	1/2	0.25/0.5	2/2	1/2	1/8	1/1	0.06/0.06	2/4	0.12/0.25	0.25/2	1/2
Klebsiella oxytoca (26)	100 (26)	100 (26)	92.3 (24)	96.2 (25)	92.3 (24)	92.3 (24)	96.2 (25)	96.2 (25)	100 (26)	100 (26)	100 (26)	96.2 (25)	19.2 (5)
MIC_{50}/MIC_{90}, μg/mL	1/1	4/4	1/2	0.25/0.5	2/4	1/1	1/1	1/1	0.06/0.06	0.5/0.5	0.12/0.12	0.25/0.25	1/1

[a] C/T MIC >2/4 μg/mL was set as the NS breakpoint.[15]
[b] C/T MIC >4/4 μg/mL was set as the NS breakpoint.[15,16]
NS, non-susceptibility. spp., species. CST, colistin. AMK, amikacin. ATM, aztreonam. C/T, ceftolozane/tazobactam. TZP, piperacillin/tazobactam. CRO, ceftriaxone. CAZ, ceftazidime. FEP, cefepime. ETP, ertapenem. IPM, imipenem. MEM, meropenem. CIP, ciprofloxacin. LVX, levofloxacin. MIC, minimum inhibitory concentration. –, not applicable (+), positive.

some studies from other Asian and Arabic countries.[27,32,33] Nevertheless, of the RTI-causing *Enterobacterales* isolates in Taiwan, the isolates harboring bla_{ESBL} validated by multiplex PCR accounted for 12.7% (Fig. 1), lower than that (20.1%) of the 2015–2017 investigation at the US medical centers ($P < 0.001$).[31] Furthermore, the isolates of *Enterobacter* species from the different infection groups displayed a high rate of ETP-NS phenotype (22%–50%) (Fig. 2), corresponding with those of a prior Taiwan survey.[34]

In similarity to two prior Asia–Pacific cIAI *Enterobacterales* studies addressing that CTX-M-15 (CTX-M-1 group) was the main ESBL,[35,36] the predominant ESBL type among our ETP-NS *E. coli* isolates was also the CTX-M-1 group (Fig. 4). The distribution of dominant CTX-M-type ESBL in *E. coli* isolates enrolled in this Taiwanese survey, however, was different from those of the other studies in China where CTX-M-9 group accounted for the most abundant (38.2–46.8%) ESBL type.[37,38]

Contrary to the 2013 Spanish survey demonstrating 87.2% susceptibility of overall *Enterobacter* isolates to C/T,[39] C/T exhibited moderate *in vitro* activity (susceptibility rate, 65.7%) against our cIAI isolates of *Enterobacter* species. In spite of being markedly different from one US survey,[31] as shown in the other study,[40] C/T was more active against the ESBL alone producers of cIAI-related *E. coli* than *K. pneumoniae* (79.4% vs. 31.3%; $P = 0.002$). Nevertheless, these susceptibility rates of C/T against ESBL producers of this study were significantly lower than those addressed by Livermore et al. in the UK study.[41]

The survey conducted by Robin et al. observed that the potential AmpC producers of *E. cloacae* complex isolates exhibited a high NS rate of C/T (76.5%).[42] Such a finding was consistent with that (44.7%) of our and other studies.[40,41,43,44] Moreover, we observed that the susceptibility rate of C/T was significantly lower than that of ETP against the potentially phenotypic AmpC producers in overall *Enterobacterales* species (58.3% vs. 85.2%; $P < 0.01$, Table 1). The susceptibility rate of C/T did not vary considerably for the ETP-susceptible phenotypic AmpC isolates. Except for *Morganella morganii* and *Aeromondales* species, C/T was shown to exhibit variable *in vitro* activity (54–73%) against other phenotypic AmpC producers of *Enterobacterales* species. Consequently, C/T could not be recommended as an empiric treatment option against most chromosomally-mediated AmpC-producing *Enterobacterales* species in Taiwan.

After extensive use of LVX in clinical treatment, the susceptibility rate (58.1%) of LVX against *S. maltophilia* isolates was similar to that (50.6%) in China[45] but was significantly lower than that (77%) of an earlier ICU survey in Taiwan in 2016 ($P = 0.009$).[46] Despite a poor *in vitro* activity of C/T against *S. maltophilia* isolates, the susceptibility rate (MIC ≤ 4/4 μg/mL) of C/T against *B. cenocepacia* complex isolates (75.0%) in the present study was close to that of the UK study (80.3%).[41]

There are some limitations in this study. First, the susceptibility of some antibiotics, including trimethoprim-sulfamethoxazole, tetracyclines, macrolides, were not tested against GNB isolates. Second, some MDR genes might not be detected and the clonal spread of XDR-GNB isolates (e.g., sequence type 131-*E. coli*) was not evaluated.

In conclusion, the percentages of ETP-NS phenotype and positive carbapenemase (KPC, OXA-48-like enzymes mainly)-encoding gene were more common among the RTI group of *Enterobacterales* isolates than those of the cIAI and cUTI groups in Taiwan. The prevalence rates of CR- and MDR-*P. aeruginosa* are on the rise and need close monitor. In contrast to moderate activity (54—100%) against most potential AmpC-producing enteric GNB, C/T exhibited poor (45—47%) *in vitro* activity against AmpC-producing *E. cloacae* complex isolates, regardless of ETP susceptibility. Nevertheless, the *in vitro* activity of C/T against isolates of carbapenem-NS, MDR/XDR-*P. aeruginosa* and *B. cenocepacia* complex was high (>88%) and moderate (75%), respectively. The *in vitro* susceptibility rate of LVX against Taiwanese *S. maltophilia* isolates notably decreased. The increasing burden of antimicrobial resistance has become a serious worldwide health issue. Consequently, it is imperative to perform periodical monitoring of the susceptibility profile of clinically important GNB for effective treatment of bacterial infections.

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Declaration of competing interest

The authors declare no conflict of interest.

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References

1. Chandorkar G, Huntington JA, Gotfried MH, Rodvold KA, Umeh O. Intrapulmonary penetration of ceftolozane/tazobactam and piperacillin/tazobactam in healthy adult subjects. *J Antimicrob Chemother* 2012;**67**:2463—9.
2. Liu WD, Shih MC, Chuang YC, Wang JT, Sheng WH. Comparative efficacy of doripenem versus meropenem for hospital-acquired and ventilator-associated pneumonia. *J Microbiol Immunol Infect* 2019;**52**:788—95.
3. Chusri S, Chongsuvivatwong V, Silpapojakul K, Singkhamanan K, Hortiwakul T, Charernmak B, et al. Clinical characteristics and outcomes of community and hospital-acquired *Acinetobacter baumannii* bacteremia. *J Microbiol Immunol Infect* 2019;**52**:796—806.
4. Oh DH, Kim MH, Jeong WY, Kim YC, Kim EJ, Song JE, et al. Risk factors for mortality in patients with low lactate level and septic shock. *J Microbiol Immunol Infect* 2019;**52**:418—25.
5. Erlanger D, Assous MV, Wiener-Well Y, Yinnon AM, Ben-Chetrit E. Clinical manifestations, risk factors and prognosis of patients with *Morganella morganii* sepsis. *J Microbiol Immunol Infect* 2019;**52**:443—8.
6. Akatsuka M, Tatsumi H, Sonoda T, Masuda Y. Low immunoglobulin G level is associated with poor outcomes in patients with sepsis and septic shock. *J Microbiol Immunol Infect* 2020 Aug 19. https://doi.org/10.1016/j.jmii.2020.08.013. S1684-S1182(20)30211-5; [Online ahead of print].
7. Leung CH, Liu CP. Diabetic status and the relationship of blood glucose to mortality in adults with carbapenem-resistant *Acinetobacter baumannii* complex bacteremia. *J Microbiol Immunol Infect* 2019;**52**:654—62.
8. Liu LH, Wang NY, Wu AY, Lin CC, Lee CM, Liu CP. *Citrobacter freundii* bacteremia: risk factors of mortality and prevalence of resistance genes. *J Microbiol Immunol Infect* 2018;**51**:565—72.
9. Jean SS, Lee WS, Lam C, Hsu CW, Chen RJ, Hsueh PR. Carbapenemase-producing Gram-negative bacteria: current epidemics, antimicrobial susceptibility and treatment options. *Future Microbiol* 2015;**10**:407—25.
10. Jean SS, Chang YC, Lin WC, Lee WS, Hsueh PR, Hsu CW. Epidemiology, treatment, and prevention of nosocomial bacterial pneumonia. *J Clin Med* 2020;**9**:275.
11. Rhodes KA, Schweizer HP. Antibiotic resistance in *Burkholderia* species. *Drug Resist Updates* 2016;**28**:82—90.
12. Clinical and Laboratory Standards Institute (CLSI). *Performance standards for antimicrobial susceptibility testing: 30th informational supplement. CLSI document M100-S30*. Wayne, PA, USA: CLSI; 2020.
13. European Committee on Antimicrobial Susceptibility Testing. *Breakpoint tables for interpretation of MICs and zone diameters*. Version 11.0. 2021. https://www.eucast.org.
14. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect* 2012;**18**:268—81.
15. Kuo SC, Liu CE, Lu PL, Chen YS, Lu MC, Ko WC, et al. Activity of ceftolozane-tazobactam against Gram-negative pathogens isolated from lower respiratory tract infections in the Asia-Pacific region: SMART 2015-2016. *Int J Antimicrob Agents* 2020;**55**:105883.
16. Grohs P, Taieb G, Morand P, Kaibi I, Podglajen I, Lavollay M, et al. *In vitro* activity of ceftolozane-tazobactam against multidrug-resistant nonfermenting Gram-negative bacilli isolated from patients with cystic fibrosis. *Antimicrob Agents Chemother* 2017;**61**:e02688. 16.
17. Van Dalem A, Herpol M, Echahidi F, Peeters C, Wybo I, De Wachter E, et al. *In vitro* susceptibility of *Burkholderia cepacia* complex isolated from cystic fibrosis patients to ceftazidime-avibactam and ceftolozane-tazobactam. *Antimicrob Agents Chemother* 2018;**62**:e00590. 18.
18. Jean SS, Lee WS, Hsueh PR. Ertapenem non-susceptibility and independent predictors of the carbapenemase production among the *Enterobacteriaceae* isolates causing intra-abdominal infections in the Asia-Pacific region: results from the Study for Monitoring Antimicrobial Resistance Trends (SMART). *Infect Drug Resist* 2018;**11**:1881—91.
19. Mushtaq S, Warner M, Ge Y, Kaniga K, Livermore DM. *In vitro* activity of ceftaroline (PPI-0903M, T-91825) against bacteria with defined resistance mechanisms and phenotypes. *J Antimicrob Chemother* 2007;**60**:300—11.
20. Jacoby GA. AmpC β-lactamases. *Clin Microbiol Rev* 2009;**22**:161—82.

21. Wang J, Zhou JY, Qu TT, Shen P, Wei ZQ, Yu YS, et al. Molecular epidemiology and mechanisms of carbapenem resistance in *Pseudomonas aeruginosa* isolates from Chinese hospitals. *Int J Antimicrob Agents* 2010;**35**:486—91.
22. Zhang JF, Chen BL, Xin XY, Zhao HB, Wang HY, Song H, et al. Carbapenem resistance mechanism and risk factors of *Pseudomonas aeruginosa* clinical isolates from a University Hospital in Xi'an, China. *Microb Drug Resist* 2009;**15**:41—5.
23. Walters MS, Grass JE, Bulens SN, Hancock EB, Phipps EC, Muleta D, et al. Carbapenem-resistant Pseudomonas aeruginosa at US emerging infections program sites, 2015. *Emerg Infect Dis* 2019;**25**:1281—8.
24. Sader HS, Castanheira M, Duncan LR, Flamm RK. Antimicrobial susceptibility of *Enterobacteriaceae* and *Pseudomonas aeruginosa* isolates from United States medical centers stratified by infection type: results from the International Network for Optimal Resistance Monitoring (INFORM) surveillance program, 2015-2016. *Diagn Microbiol Infect Dis* 2018;**92**:69—74.
25. van Duin D, Bonomo RA. Ceftazidime/avibactam and ceftolozane/tazobactam: second-generation β-lactam/β-lactamase inhibitor combinations. *Clin Infect Dis* 2016;**63**:234—41.
26. Lee YC, Huang YT, Tan CK, Kuo YW, Liao CH, Lee PI, et al. *Acinetobacter baumannii* and *Acinetobacter* genospecies 13TU and 3 bacteraemia: comparison of clinical features, prognostic factors and outcomes. *J Antimicrob Chemother* 2011;**66**: 1839—46.
27. Jean SS, Hsueh PR. High burden of antimicrobial resistance in Asia. *Int J Antimicrob Agents* 2011;**37**:291—5.
28. Chang KC, Lin MF, Lin NT, Wu WJ, Kuo HY, Lin TY, et al. Clonal spread of multidrug-resistant *Acinetobacter baumannii* in eastern Taiwan. *J Microbiol Immunol Infect* 2012;**45**:37—42.
29. Royer S, Faria ALS, Seki LM, Chagas TPG, de Campos PA, Batistão DWDF, et al. Spread of multidrug-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa* clones in patients with ventilator-associated pneumonia in an adult intensive care unit at a university hospital. *Braz J Infect Dis* 2015;**19**:350—7.
30. Wang Y, Wang J, Wang R, Cai Y. Resistance to ceftazidime-avibactam and underlying mechanisms. *J Glob Antimicrob Resist* 2020;**22**:18—27.
31. Carvalhaes CG, Castanheira M, Sader HS, Flamm RK, Shortridge D. Antimicrobial activity of ceftolozane-tazobactam tested against gram-negative contemporary (2015-2017) isolates from hospitalized patients with pneumonia in US medical centers. *Diagn Microbiol Infect Dis* 2019;**94**:93—102.
32. Lai CC, Lee K, Xiao Y, Ahmad N, Veeraraghavan B, Thamlikitkul V, et al. High burden of antimicrobial drug resistance in Asia. *J Glob Antimicrob Resist* 2014;**2**:141—7.
33. Kurup A, Liau KH, Ren J, Lu MC, Navarro NS, Farooka MW, et al. Antibiotic management of complicated intra-abdominal infections in adults: the Asian perspective. *Ann Med Surg (Lond)* 2014;**3**:85—91.
34. Jean SS, Lee WS, Bai KJ, Yu KW, Hsu CW, Yu KW, et al. Carbapenem susceptibility among *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterobacter cloacae* isolates obtained from patients in intensive care units in Taiwan in 2005, 2007, and 2009. *Diagn Microbiol Infect Dis* 2015;**81**:290—5.
35. Jean SS, Hsueh PR. Distribution of ESBLs, AmpC β-lactamases and carbapenemases among Enterobacteriaceae isolates causing intra-abdominal and urinary tract infections in the Asia-Pacific region during 2008-14: results from the Study for Monitoring Antimicrobial Resistance Trends (SMART). *J Antimicrob Chemother* 2017;**72**:166—71.
36. Sheng WH, Badal RE, Hsueh PR. Distribution of extended-spectrum β-lactamases, AmpC β-lactamases, and carbapenemases among Enterobacteriaceae isolates causing intra-abdominal infections in the Asia-Pacific region: results of the study for Monitoring Antimicrobial Resistance Trends (SMART). *Antimicrob Agents Chemother* 2013;**57**:2981—8.
37. Xia S, Fan X, Huang Z, Xia L, Xiao M, Chen R, et al. Dominance of CTX-M-type extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* isolated from patients with community-onset and hospital-onset infection in China. *PloS One* 2014;**9**: e100707.
38. Zhang J, Zheng BW, Zhao L, Wei Z, Ji J, Li L, et al. Nationwide high prevalence of CTX-M and an increase of CTX-M-55 in *Escherichia coli* isolated from patients with community-onset infections in Chinese county hospitals. *BMC Infect Dis* 2014; **14**:659.
39. Tato M, García-Castillo M, Bofarull AM, Cantón R. *In vitro* activity of ceftolozane/tazobactam against clinical isolates of *Pseudomonas aeruginosa* and *Enterobacteriaceae* recovered in Spanish medical centres: results of the CENIT study. *Int J Antimicrob Agents* 2015;**46**:502—10.
40. Farrell DJ, Flamm RK, Sader HS, Jones RN. Antimicrobial activity of ceftolozane-tazobactam tested against *Enterobacteriaceae* and *Pseudomonas aeruginosa* with various resistance patterns isolated in U.S. Hospitals (2011-2012). *Antimicrob Agents Chemother* 2013;**57**:6305—10.
41. Livermore DM, Mushtaq S, Meunier D, Hopkins KL, Hill R, Adkin R, et al. Activity of ceftolozane/tazobactam against surveillance and 'problem' *Enterobacteriaceae*, *Pseudomonas aeruginosa* and non-fermenters from the British Isles. *J Antimicrob Chemother* 2017;**72**:2278—89.
42. Robin F, Auzou M, Bonnet R, Lebreuilly R, Isnard C, Cattoir V, et al. *In vitro* activity of ceftolozane-tazobactam against *Enterobacter cloacae* Complex clinical isolates with different β-Lactam resistance phenotypes. *Antimicrob Agents Chemother* 2018;**62**:e00675. 18.
43. Hawkey PM, Warren RE, Livermore DM, McNulty CAM, Enoch DA, Otter JA, et al. Treatment of infections caused by multidrug-resistant gram-negative bacteria: report of the British society for antimicrobial chemotherapy/healthcare infection society/British infection association joint working party. *J Antimicrob Chemother* 2018;**73**:iii2—78.
44. Sader HS, Castanheira M, Mendes RE, Flamm RK. Frequency and antimicrobial susceptibility of Gram-negative bacteria isolated from patients with pneumonia hospitalized in ICUs of US medical centres (2015-17). *J Antimicrob Chemother* 2018; **73**:3053—9.
45. Hu LF, Gao LP, Ye Y, Chen X, Zhou XT, Yang HF, et al. Susceptibility of *Stenotrophomonas maltophilia* clinical strains in China to antimicrobial combinations. *J Chemother* 2014;**26**: 282—6.
46. Lai CC, Chen YS, Lee NY, Tang HJ, Lee SJ, Lin CF, et al. Susceptibility rates of clinically important bacteria collected from intensive care units against colistin, carbapenems, and other comparative agents: results from Surveillance of Multicenter Antimicrobial Resistance in Taiwan (SMART). *Infect Drug Resist* 2019;**12**:627—40.