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Risk Factors, Outcomes, and Mechanisms of Tigecycline-Nonsusceptible *Klebsiella pneumoniae* Bacteremia

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A rise in tigecycline resistance in *Klebsiella pneumoniae* has been reported recently worldwide. We sought to identify risk factors, outcomes, and mechanisms for adult patients with tigecycline-nonsusceptible *K. pneumoniae* bacteremia in Taiwan. We conducted a matched case-control study (ratio of 1:1) in a medical center in Taiwan from January 2011 through June 2015. The cases were patients with tigecycline-nonsusceptible *K. pneumoniae* bacteremia, and the controls were patients with tigecycline-susceptible *K. pneumoniae* bacteremia. Logistic regression was performed to evaluate the potential risk factors for tigecycline-nonsusceptible *K. pneumoniae* bacteremia. Quantitative reverse transcription-PCR was performed to analyze *acrA*, *oqxA*, *ramA*, *rarA*, and *kpgA* expression among these isolates. A total of 43 cases were matched with 43 controls. The 14-day mortality of patients with tigecycline-nonsusceptible *K. pneumoniae* bacteremia was 30.2%, and the 28-day mortality was 41.9%. The attributable mortalities of tigecycline-nonsusceptible *K. pneumoniae* at 14 and 28 days were 9.3 and 18.6%, respectively. Fluoroquinolone use within 30 days prior to bacteremia was the only independent risk factor for tigecycline-nonsusceptible *K. pneumoniae* bacteremia. The tigecycline-nonsusceptible *K. pneumoniae* bacteremia was mostly caused by overexpression of AcrAB and/or OqxAB efflux pumps, together with the upregulation of RamA and/or RarA, respectively. One isolate demonstrated isolated overexpression of *kpgA*. In conclusion, tigecycline-nonsusceptible *K. pneumoniae* bacteremia was associated with high mortality, and prior fluoroquinolone use was the independent risk factor for the acquisition of tigecycline-nonsusceptible *K. pneumoniae*. The overexpression of AcrAB and/or OqxAB contributes to tigecycline nonsusceptibility in *K. pneumoniae*.

Klebsiella pneumoniae is an important cause of bacteremia worldwide. In one population-based study of *K. pneumoniae* bacteremia, the case fatality rate was 20%, and the annual population mortality rate was 1.3 per 100,000 (1). The rise of multidrug resistance (MDR) in *K. pneumoniae* harboring extended-spectrum β-lactamases or AmpC β-lactamases has resulted in the widespread use of carbapenem antibiotics (2). The increased use of carbapenem antibiotics as the last resort for the treatment of MDR *K. pneumoniae* (MDRKP) has accelerated the emergence of carbapenem-resistant *K. pneumoniae* (CRKP) (3). Tigecycline and colistin were introduced to treat infections caused by CRKP (4).

Tigecycline, a minocycline derivative, has *in vitro* activity against most MDRKP or CRKP (5). Tigecycline nonsusceptibility among *K. pneumoniae* isolates, as reported in recent studies (6–8), is between 0 and 5.7% globally (9). Recently, we reported on the high mortality rate (28-day mortality: 38.9%) in patients with tigecycline-nonsusceptible *K. pneumoniae* bacteremia (8). Although tigecycline-nonsusceptible *K. pneumoniae* bacteremia has emerged as a critical problem, studies reporting the risk factors for such infections are limited (6, 10). Indeed, as far as we can tell, no studies focusing on the risk factors for tigecycline-nonsusceptible *K. pneumoniae* bacteremia have been published in the scientific literature.

Tigecycline resistance in *Enterobacteriaceae* is attributed to resistance/nodulation/cell division-type pumps and to other efflux pumps (11). The RamA and AcrAB pathway and RarA, together with OqxAB pathways are implicated in tigecycline resistance in *K. pneumoniae* (12). Studies of tigecycline resistance mechanisms in *K. pneumoniae* usually include only a small number of isolates in the literature (12–14). Moreover, studies addressing both the clinical features of bacteremia and the tigecycline resistance mechanism have never been reported.

In this study, we sought to identify the risk factors and outcomes for adult patients with tigecycline-nonsusceptible *K. pneumoniae* bacteremia in Taiwan using a matched case-control study. We further investigated the tigecycline resistance mechanisms among tigecycline-nonsusceptible *K. pneumoniae* clinical isolates.

MATERIALS AND METHODS

Study design and population. This retrospective matched case-control study was conducted at Taipei Veterans General Hospital (a 2,900-bed tertiary-care teaching hospital) in Taipei, Taiwan, from January 2011 through June 2015. The study protocol was approved by the Institutional Review Board at Taipei Veterans General Hospital.

We performed chart reviews for consecutive patients who had blood cultures positive for *K. pneumoniae*. The cases were patients with tigecycline-nonsusceptible *K. pneumoniae* bacteremia, and the controls were patients with tigecycline-susceptible *K. pneumoniae* bacteremia. Nonsusceptibility to tigecycline in a sample was defined by a MIC for tigecycline

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>2 mg/liter, according to the criteria from the U.S. Food and Drug Administration (15). Controls were matched to cases in a 1:1 ratio based on age, sex, and the at-risk period (the time from admission to the date when the culture was positive). The age and the at-risk period were matched as close as possible. We included the first blood cultures for patients with two or more positive blood cultures. Patients <20 years of age and those with incomplete medical records were excluded.

Data collection. Medical records were reviewed to obtain clinical information on the demographic characteristics, location at time of culture, the source of bacteremia, comorbidities, immunosuppression, surgeries, invasive procedures or devices, surgical drainage, mechanical ventilation, antimicrobial therapy, severity of illness, outcome, and mortality. Nosocomial infections were defined as *K. pneumoniae*-positive isolates identified in patients more than 48 h after admission to the hospital. Healthcare-associated infections were defined as patients meeting any of the following criteria: having received intravenous therapy at home or in an outpatient clinic during the previous 30 days, having received renal dialysis in a hospital or clinic during the previous 30 days, having hospitalization for 2 or more days during the previous 90 days, or having resided in a nursing home or long-term-care facility (8). Appropriate empirical antimicrobial therapy was defined as administration of at least one antimicrobial agent to which the causative pathogen was susceptible within 24 h after the onset of bacteremia, at the approved route and dosage for the affected target organ(s). Appropriate definite antimicrobial therapy was defined as administration of at least one antimicrobial agent to which the causative pathogen was susceptible after the report of antimicrobial susceptibility test, at the approved route and dosage for the affected target organ(s). Prior antibiotic exposure was defined as at least 2 days of therapy administered within 30 days prior to the bacteremia. The Pitt bacteremia score and the Acute Physiology and Chronic and Prevention Evaluation (APACHE) II score were calculated to determine the severity of illness within 24 h of the onset of bacteremia (2, 16).

Microbiological studies. Identification of *K. pneumoniae* was performed using the Vitek2 system (bioMérieux, Marcy l'Etoile, France) or matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (bioMérieux SA, Marcy l'Etoile, France). Antimicrobial susceptibility on this bacterium was performed using the Vitek2 system (bioMérieux) and interpreted according to the guidelines of the Clinical and Laboratory Standards Institute (17). Tigecycline-nonsusceptible *K. pneumoniae* (tigecycline MIC > 2 mg/liter) in an isolate was further confirmed by the Etest (bioMérieux) method according to the manufacturer's instructions. MDR in *K. pneumoniae*, as a phenotype, was defined in the isolates as nonsusceptibility to at least one agent in three or more antimicrobial categories (18).

Tigecycline resistance mechanism: overexpression of efflux pumps examined by qRT-PCR and detection of mutations in *ramR*, *acrR*, and *oqxR* and the 30S ribosomal protein S10 encoding region (*rpsJ*). All tigecycline-nonsusceptible *K. pneumoniae* and randomly selected tigecycline-susceptible *K. pneumoniae* isolates were examined by real-time quantitative reverse transcription-PCR (qRT-PCR) for the mRNA expression level of pump genes (*acrA*, *oqxA*, and *kpgA*), regulators of the *acrAB* operon (*ramA*), and transcriptional activator of the efflux pump OqxAB (*rarA*). The detailed methods were described previously (19). The relative expression of each target gene was then calibrated against the corresponding expression of a tigecycline-susceptible *K. pneumoniae* KP478, which served as reference strain (expression = 1; tigecycline MIC, 0.25 mg/liter). Specific primers for the aforementioned genes are listed in Table S1 in the supplemental material (20–22). The experiments were performed in triplicate. Data analysis used the cycle threshold ($\Delta\Delta C_T$) method, reported values were averaged across all experiments, and the standard errors of the means were calculated.

The presence of mutations in the *ramR* (23), *oqxR* (24), and *rpsJ* (25) were compared to the reference tigecycline-susceptible *K. pneumoniae* strain MGH78578 (CP000647) (23). The primers were described in Table S1 in the supplemental material.

Statistical analyses. We used chi-square and Fisher exact test to analyze categorical variables. We used the Student *t* test and Mann-Whitney rank sum test (Wilcoxon rank-sum test) to analyze continuous variables. To identify independent predictors of tigecycline-nonsusceptible *K. pneumoniae* bacteremia, we used logistic regression. All biologically possible variables with *P* values of < 0.1 in univariate analyses were incorporated into a model using a backward approach. The mortality attributable to tigecycline nonsusceptibility was calculated by subtracting the mortality rate of patients with tigecycline-susceptible *K. pneumoniae* bacteremia from the mortality rate of patients with tigecycline-nonsusceptible *K. pneumoniae* bacteremia (26).

For all calculations, a two-tailed *P* value of <0.05 was considered statistically significant. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software, version 17.0 (SPSS, Inc., Chicago, IL).

RESULTS

During the 54-month study period, 43 cases with tigecycline-nonsusceptible *K. pneumoniae* bacteremia and 43 matched controls with tigecycline-susceptible *K. pneumoniae* bacteremia were identified. The risk factors of tigecycline-nonsusceptible *K. pneumoniae* bacteremia are listed in Table 1. Tigecycline-nonsusceptible *K. pneumoniae* bacteremia was more likely to be associated with any antibiotic exposure (72.1% versus 41.9%, $P = 0.005$). Prior use of fluoroquinolone (25.6% versus 2.3%, $P = 0.003$), third- or fourth-generation cephalosporin (20.9% versus 2.3%, $P = 0.015$), and metronidazole (18.6% versus 2.3%, $P = 0.03$) were significantly more common in tigecycline-nonsusceptible *K. pneumoniae* bacteremia than that in tigecycline-susceptible *K. pneumoniae* bacteremia. Table 2 showed clinical outcomes of patients in case and control group. Patients in case group were more likely to receive inappropriate empirical antimicrobial therapy than the control group (53.5% versus 9.3%, $P < 0.001$). The 14-day mortality for tigecycline-nonsusceptible *K. pneumoniae* was 30.2%, and the 28-day mortality of tigecycline-nonsusceptible *K. pneumoniae* was 41.9%. The 14- and 28-day mortality attributable to tigecycline nonsusceptibility was 9.3 and 18.6%, respectively.

The antimicrobial susceptibilities of the *K. pneumoniae* clinical isolates from this study showed that tigecycline-nonsusceptible *K. pneumoniae* exhibited a higher rate of MDRKP than the tigecycline-susceptible ones (88.4% versus 30.2%, $P < 0.001$). The resistant rates for gentamicin, ceftriaxone, ceftazidime, cefepime, ciprofloxacin, and levofloxacin were statistically higher in tigecycline-nonsusceptible *K. pneumoniae* isolates than those of the tigecycline-susceptible *K. pneumoniae* ones (Table 3). We also found less than one-fifth of isolates were resistant to carbapenem.

All plausible biological risk factors for tigecycline-nonsusceptible *K. pneumoniae* bacteremia analyzed in the univariate analyses with *P* values of <0.1 were further analyzed in the multivariate analyses. Fluoroquinolone use within 30 days prior to bacteremia (odds ratio, 10.81; 95% confidence interval, 1.26 to 93.13; $P = 0.030$) was the only independent risk factor for tigecycline-nonsusceptible *K. pneumoniae* bacteremia (Table 4).

Table S2 in the supplemental material detailed the resistance mechanisms of 43 tigecycline-nonsusceptible *K. pneumoniae* isolates. Twenty-three (TNSKP1 to -23) isolates had elevated expression levels of *acrA* (>2-fold) but not of *rarA* and *oqxA* (<4-fold). Most of them (TNSKP5 to -22) had elevated expression levels of both *ramA* and *acrA* (>2-fold), but 4 isolates (TNSKP1 to -4) had a baseline expression level of *ramA* (<2-fold). Five isolates (TNSKP24 to -28) had elevated expression levels of *oqxA*

TABLE 1 Bivariate analyses of risk factors for tigecycline-nonsusceptible *K. pneumoniae* bacteremia[a]

Variable	Tigecycline nonsusceptible ($n = 43$)	Tigecycline susceptible ($n = 43$)	*P*
Demographics			
Mean age (yrs) ± SD	70.81 ± 13.87	70.79 ± 13.90	0.997
No. of male patients	30 (69.8)	30 (69.8)	1.000
Median days (IQR) of hospitalization prior to culture	14.0 (1.0–35.0)	7.0 (1.0–16.0)	0.078
Location at time of culture			
Medical ward	19 (44.2)	22 (51.2)	0.517
Surgical ward	5 (11.6)	7 (16.3)	0.534
Intensive care unit	8 (18.6)	3 (7.0)	0.195
Emergency department	11 (25.6)	11 (25.6)	1.000
Nosocomial infection	28 (65.1)	26 (60.5)	0.655
Healthcare-associated infection	9 (20.9)	13 (30.2)	0.323
Community-acquired infection	6 (14.0)	4 (9.3)	0.738
Infection source			
Respiratory tract	12 (27.9)	9 (20.9)	0.451
Urinary tract	4 (9.3)	3 (7.0)	1.000
Intra-abdomen	6 (14.0)	10 (23.3)	0.268
Skin and soft tissue	2 (4.7)	1 (2.3)	1.000
Intravenous device	1 (2.3)	1 (2.3)	1.000
Biliary tract	7 (16.3)	5 (11.6)	0.534
Primary bacteremia	11 (25.6)	14 (32.6)	0.476
Comorbidity			
Malignancy type			
Hematological	8 (18.6)	8 (18.6)	1.000
Solid	15 (34.9)	20 (46.5)	0.272
Diabetes mellitus	18 (41.9)	11 (25.6)	0.110
Chronic kidney disease	18 (41.9)	13 (30.2)	0.261
Hemodialysis	6 (14.0)	4 (9.3)	0.738
Congestive heart failure	3 (7.0)	2 (4.7)	1.000
Liver cirrhosis	3 (7.0)	2 (4.7)	1.000
Cerebral vascular disease	7 (16.3)	6 (14.0)	0.763
Dementia	4 (9.3)	3 (7.0)	1.000
Chronic obstructive lung disease	6 (14.0)	3 (7.0)	0.483
Collagen vascular disease	1 (2.3)	1 (2.3)	1.000
Transplantation	3 (7.0)	0 (0.0)	0.241
Immunosuppression[b]	15 (34.9)	17 (39.5)	0.655
Surgery within 2 weeks	4 (9.3)	5 (11.6)	1.000
Invasive procedures and devices			
Central venous catheter	13 (30.2)	8 (18.6)	0.209
Nasogastric/nasojejunal tube	7 (16.3)	10 (23.3)	0.417
Urinary catheter	15 (34.9)	7 (16.3)	0.048
Endotracheal tube	2 (4.7)	2 (4.7)	1.000
Tracheostomy	3 (7.0)	3 (7.0)	1.000
Surgical drainage	5 (11.6)	4 (9.3)	1.000
Mechanical ventilation	4 (9.3)	2 (4.7)	0.676
Prior antibiotic exposure			
First- or second-generation cephalosporin[c]	8 (18.6)	5 (11.6)	0.366
Third- or fourth-generation cephalosporin[d]	9 (20.9)	1 (2.3)	0.015
β-Lactam and β-lactamase inhibitor[e]	12 (27.9)	10 (23.3)	0.621
Carbapenem[f]	7 (16.3)	4 (9.3)	0.520
Fluoroquinolone[g]	11 (25.6)	1 (2.3)	0.003
Aminoglycoside[h]	2 (4.7)	1 (2.3)	1.000
Tigecycline	5 (11.6)	3 (7.0)	0.713

(Continued on following page)

TABLE 2 (Continued)

Variable	Tigecycline nonsusceptible ($n = 43$)	Tigecycline susceptible ($n = 43$)	*P*
Glycopeptide[i]	8 (18.6)	6 (14.0)	0.559
Metronidazole	8 (18.6)	1 (2.3)	0.030
Median Pitt bacteremia score (IQR)	3.0 (0.0–5.0)	3.0 (2.0–5.0)	0.372
Pitt bacteremia score ≥4	15 (34.9)	15 (34.9)	1.000
Median APACHE II score (IQR)	22.0 (17.0–29.0)	18.0 (13.0–23.0)	0.059

[a] Data are presented as the number (%) of patients, unless stated otherwise in column 1. SD, standard deviation; IQR, interquartile range; APACHE, Acute Physiology and Chronic Health Evaluation.
[b] Immunosuppression was defined as meeting one of the following criteria: neutropenia, use of corticosteroids, or receiving chemotherapy.
[c] Includes cefazolin and cefuroxime.
[d] Includes cefoperazone, ceftriaxone, cefotaxime, cefepime, and cefpirome.
[e] Includes amoxicillin-clavulanate, ampicillin-sulbactam, piperacillin-tazobactam, and ticarcillin-clavulanate.
[f] Includes ertapenem, imipenem, meropenem, and doripenem.
[g] Includes ciprofloxacin, levofloxacin, and moxifloxacin.
[h] Includes amikacin, gentamicin, and isepamicin.
[i] Includes vancomycin and teicoplanin.

(>4-fold), but not of *ramA* and *acrA* (<2-fold). Thirteen isolates (TNSKP29 to -41) had higher expression levels of both *acrA* and *oqxA*. To verify the role of efflux pumps in tigecycline resistance, we randomly selected 7 tigecycline-susceptible *K. pneumoniae* isolates from the control group for the same experiments, and only one of them (tigecycline MIC = 1 mg/liter) showed elevated expression level of both *acrA* and *oqxA* compared to the reference KP478 strain. These data indicate that tigecycline nonsusceptibility in most of these isolates was caused by the overexpression of the AcrAB and/or OqxAB efflux pumps. The upregulation of RamA and/or RarA contributed to the majorities of efflux pumps overexpression. Interestingly, we found 16 isolates had overexpression of *kpgA* (>100-fold), in addition to the overexpression of the AcrAB and/or OqxAB efflux pumps. One isolate (TNSKP42, MIC = 4 mg/liter) showed overexpression of *kpgA* but baseline expression of these 4 efflux-related genes. Finally, we found the remaining 1 TNSKP isolate (TNSKP43, MIC = 3 mg/liter) exhibited baseline expression of these 5 efflux-related genes. No mutation in *rpsJ*, coding for ribosomal protein S10, was detected in all the 43 isolates.

DISCUSSION

This study investigated the risk factors for tigecycline-nonsusceptible *K. pneumoniae* bacteremia using a matched case-control study, and elucidated possible mechanisms for tigecycline nonsusceptibility among tigecycline-nonsusceptible *K. pneumoniae* isolates. We found that fluoroquinolone use in the previous 30 days was the only independent risk factor for tigecycline-nonsusceptible *K. pneumoniae* bacteremia. Tigecycline-nonsusceptible *K. pneumoniae* bacteremia was associated with high 14- and 28-day mortality in the patients. The 14- and 28-day mortality attributable to tigecycline nonsusceptibility was 9.3 and 18.6%, respectively.

Despite the growing clinical concerns about the increasing prevalence of tigecycline-nonsusceptible *K. pneumoniae* isolates, the impact and clinical characteristics of patients with tigecycline-nonsusceptible *K. pneumoniae* infections are rarely reported in the scientific literature (8). In our previous study, we reported the high 28-day mortality among patients with tigecycline-nonsusceptible *K. pneumoniae* bacteremia (8). Here, we further compared tigecycline-nonsusceptible *K. pneumoniae* and tigecycline-susceptible *K. pneumoniae* bacteremia and demonstrated the mortality attributable to tigecycline nonsusceptibility. Given the high mortality of tigecycline-nonsusceptible *K. pneumoniae* bacteremia, better control measures and efficient surveillance are necessary.

Nigo et al. performed a nested case-control study on the emergence of tigecycline-resistant MDRKP isolates among patients who initially presented with a tigecycline-susceptible MDRKP iso-

TABLE 2 Clinical outcomes of patients with *K. pneumoniae* bacteremia[a]

Variable	Tigecycline nonsusceptible ($n = 43$)	Tigecycline susceptible ($n = 43$)	*P*
Appropriate empirical antimicrobial therapy	20 (46.5)	39 (90.7)	<0.001
Appropriate definite antimicrobial therapy	35 (81.4)	39 (90.7)	0.351
Median length of stay in days after bacteremia (IQR)	13.0 (8.0–25.0)	14.0 (6.0–23.0)	0.983
Septic shock when bacteremia	23 (53.5)	31 (72.1)	0.074
Mortality			
In-hospital mortality	18 (41.9)	12 (27.9)	0.175
14-day mortality	13 (30.2)	9 (20.9)	0.323
28-day mortality	18 (41.9)	10 (23.3)	0.066

[a] Data are presented as the number (%) of patients, unless stated otherwise in column 1. IQR, interquartile range.

TABLE 3 Antimicrobial susceptibilities of clinical isolates of *K. pneumoniae*[a]

Antimicrobial agent	Tigecycline nonsusceptible ($n = 43$)	Tigecycline susceptible ($n = 43$)	*P*
Amikacin	3 (7.0)	2 (4.7)	1.000
Gentamicin	19 (44.2)	8 (18.6)	0.011
Ceftriaxone	21 (48.8)	9 (20.9)	0.007
Ceftazidime	24 (55.8)	10 (23.3)	0.002
Cefepime	18 (41.9)	7 (16.3)	0.009
Ciprofloxacin	30 (69.6)	7 (16.3)	<0.001
Levofloxacin	30 (69.6)	7 (16.3)	<0.001
Ertapenem	8 (18.6)	5 (11.6)	0.366
Imipenem	2 (4.7)	4 (9.3)	0.397

[a] Data are presented as the number (%) of isolates resistant to the antibiotic indicated.

TABLE 4 Multivariate analyses of risk factors for tigecycline-nonsusceptible *K. pneumoniae* bacteremia

Variable	OR (95% CI)[a]	*P*
Urinary catheter	2.29 (0.72–7.32)	0.163
Prior exposure to third- or fourth-generation cephalosporins[b]	7.36 (0.81–67.14)	0.077
Prior exposure to fluoroquinolone[c]	10.81 (1.26–93.13)	0.030
Prior exposure to metronidazole	5.60 (0.58–53.9)	0.136
Septic shock when bacteremia	0.47 (0.17–1.28)	0.139

[a] OR, odds ratio; CI, confidence interval.
[b] Includes cefoperazone, ceftriaxone, cefotaxime, cefepime, and cefpirome.
[c] Includes ciprofloxacin, levofloxacin, and moxifloxacin.

late (10). They found that receipt of tigecycline was the only independent predictor of subsequent isolation of a tigecycline-resistant strain in the study (10). van Duin et al. described that tigecycline use was independently associated with the development of subsequent tigecycline resistance in the same patient with CRKP bacteriuria (6). In the present study, we used a different study design to those of Nigo et al. and van Duin et al. (6, 10). The previous two studies focused on patients who initially presented with tigecycline-nonsusceptible *K. pneumoniae* in their urine (6) or other sites (10), regardless of colonization or infection. Our inclusion of patients with *K. pneumoniae* bacteremia was not limited to those who initially presented with tigecycline-susceptible *K. pneumoniae* isolates. We found that most tigecycline-nonsusceptible *K. pneumoniae* isolates from blood were susceptible to carbapenems, which was consistent to our previous study (8). The present study showed that only 5 cases had prior use of tigecycline. One study from India also showed that tigecycline nonsusceptibility was not observed among CRKP isolates, and the increase of MIC values of tigecycline was not associated with the use of tigecycline (27). The geographic differences might be responsible for the different resistance phenotype of tigecycline-nonsusceptible *K. pneumoniae* between Asia and western countries.

The present study showed that prior fluoroquinolone exposure was the only independent risk factor for tigecycline-nonsusceptible *K. pneumoniae* bacteremia. Overexpression of the AcrAB multidrug efflux system has been reported to contribute to fluoroquinolone resistance in *Klebsiella* spp. (28). Cross-resistance to olaquindox, chloramphenicol, and the quinolones due to elevated expression of the chromosomal *oqxAB* operon in *K. pneumoniae* has been reported recently (29). Recent studies have established that overexpression of AcrAB efflux pump plays a major role in tigecycline resistance in *K. pneumoniae* (23, 30). Overexpression of OqxAB has contributed to tigecycline resistance in one study (12). The previous use of fluoroquinolones that are also effluxed through the AcrAB or OqxAB pump might result in overexpression of these efflux pumps and subsequently reduce susceptibility to tigecycline. In one recent study, Roy et al. reported that the increment of MIC values of tigecycline in *K. pneumoniae* might be related to the use of other antibiotics that are also the substrates the AcrAB pump because of the restricted use of tigecycline in their unit (27). Furthermore, our recent study regarding *in vivo* evolution of tigecycline resistance in patients demonstrated the cases infected with tigecycline-nonsusceptible strains derived from their isogenic tigecycline-susceptible ones. We found two cases had been exposed to fluoroquinolones before the appearance of the tigecycline-nonsusceptible strains (19). The results from the above-mentioned studies might support our important finding that tigecycline-nonsusceptible *K. pneumoniae* bacteremia can occur among patients recently treated with fluoroquinolones. The present study provides insight on the issue about antibiotic exposure and resistance.

Regarding mechanisms for tigecycline resistance, most studies have emphasized that the efflux pump AcrAB, which can be upregulated by *ramR* mutations and subsequent *ramA* activation, contributed to reduced susceptibility to tigecycline in *K. pneumoniae* clinical isolates (12–14, 24, 31). However, the role of OqxAB pump in tigecycline resistance was either undefined (14, 24) or not described (31) in some studies. In the present study, most tigecycline-nonsusceptible *K. pneumoniae* isolates had elevated expression levels of *ramA* and *acrA*. Several tigecycline-nonsusceptible *K. pneumoniae* isolates had markedly elevated expression levels of *rarA* and *oqxA*, but baseline expression level of *acrA*. The results presented here support a previous report that *rarA* is one of the regulator pathways that controls the expression of *oqxA* in *K. pneumoniae* and contributes to tigecycline nonsusceptibility (21). OqxR has been suggested as a repressor not only of the *oqxAB* efflux operon, but also of *rarA* (24). Interestingly, we observed higher expression of *oqxA* and *rarA* in several clinical isolates with a silence mutation of *oqxR* (see Table S2 in the supplemental material). These isolates could harbor additional genetic alterations responsible for the overexpression of *oqxA* or *rarA*. Among tigecycline-susceptible *K. pneumoniae* isolates, we only found one isolate had higher expression of *acrA* and *oqxA*, which further supported the role of the above-mentioned efflux pumps in tigecycline resistance.

Interestingly, we found one strain (TNSKP42) with the isolated overexpression of *kpgA* leading to tigecycline nonsusceptibility. Our findings might suggest *kpgABC* play some role in tigecycline resistance. Finally, the reported regulatory pathways of tigecycline resistance were completely absent in one tigecycline-nonsusceptible *K. pneumoniae* isolate, which indicated that alternative regulatory pathways may exist in tigecycline resistance mechanisms.

Our study was limited by several factors. The case numbers in this study were relatively small. Although there were limited numbers of tigecycline-nonsusceptible *K. pneumoniae* bacteremia clinically, the present study still represents the largest collection in the literature. Being retrospective in nature, there might be selection bias in this study. The matched case-control study design helped limit this type of bias. Another limitation of this study is that data on prior antibiotic usage were evaluated only for the 1-month period prior to the bacteremia. This may have resulted in an underestimation of antibiotic usage. Finally, because the case and control groups were from a single medical center, it is possible that the results are not generalizable.

In conclusion, patients with tigecycline-nonsusceptible *K. pneumoniae* bacteremia were associated with high mortality. Our matched case-control study has shown that fluoroquinolone use within 30 days prior to bacteremia in patients was the only independent risk factor for tigecycline-nonsusceptible *K. pneumoniae* bacteremia. This study further underscores the role of AcrAB and OqxAB in tigecycline-nonsusceptible *K. pneumoniae*.

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