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Aminoglycoside Resistance in Clinical *Klebsiella pneumoniae* Isolates

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To analyze the *Klebsiella pneumoniae* (*K. pneumoniae*) susceptibility to aminoglycosides antibiotics in clinical isolates in Guangzhou, and to characterize the molecular resistance mechanisms of *K. pneumoniae* against aminoglycosides (AGs) agents. We examined 1495 strains clinical isolates of *Klebsiella pneumoniae* from Microbiology Laboratory of the Guangdong Provincial Traditional Chinese Medicine in China between 2008 and 2012 by MicroScan Wa1kAway-96. And detected Minimal inhibitory concentrations (MICs) of amikacin, gentamicin and tobramycin to *K. pneumoniae* by agar dilution methods. Isolates were analyzed by polymerase chain reaction (PCR) amplification techniques to determine whether six aminoglycoside modifying enzymes (AMEs) genes (aac(3)-a!, aac(6')-Ib, ant(3")-I, ant(2")-I, aac(3)-I, aac(6')-a!)and six 16S rRNA mehtylases genes (armA, rmtA, rmtB, rmtC, rmtD, npmA) were present. Additionally, 16S rRNA methylase gene positive isolates were chosen for a plasmid transfer test to explore whether the resistance marker was located on the transfer plasmid. Pulsed-field gel electrophoresis was used for genotyping 16S rRNA methylase gene positive isolates. A total of 265 isolates was identified as resistant to one or more aminoglycosides, with resistance rates to amikacin, tobramycin and gentamicin of 4.15%(62/1495), 9.50%(142/1495), and 16.05%(240/1495), respectively. Among the 265 resistant aminoglycoside isolates, 84.5% carried resistance genes (180 isolates carried AMEs and 44 isolates carried both AMEs and methylase genes). Aminoglycoside resistance in Guangzhou clinical isolates of *K. pneumoniae* is still low, and kept stable over the last 5 years; frequency of AMEs and 16S rRNA methylase in *K. pneumoniae* was low.

Keywords: *Klebsiella pneumoniae*; aminoglycosides; aminoglycoside modifying enzymes (AMES); 16S rRNA methylase; antimicrobial resistance; gene.

Klebsiella pneumoniae, the most common member of the *Enterobacteriaceae*, is a widely distributed opportunistic pathogen. In recent years, *K. pneumoniae* has become an important pathogen in nosocomial infection. The range of clinical diseases includes pneumonia, urinary tract infection, wound infection, osteomyelitis, meningitis, bacteremia, and septicemia. With the frequent use of antimicrobial agents, *K. pneumoniae* resistance to antimicrobial agents has increased seriously. Some reports state that â-lactam and fluoroquinolone antibiotics are helpful for the treatment of *K.*

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pneumoniae, however, aminoglycoside agents are primarily used in the treatment of infections caused by Gram-negative bacteria. Aminoglycoside agents have a strong therapeutic effect to most bacteria except for anaerobes. Due to the nature of the mechanism of uptake of aminoglycosides, which requires respiration, anaerobic bacteria are intrinsically resistant[1]. Aminoglycoside antibiotics are a complex family of compounds characterized for having an aminocyclitol nucleus linked to amino sugars through glycosidic bonds[2]. The mechanism of action for aminoglycosides (streptomycin, tobramycin, gentamicin) and semisynthetic aminoglycosides (amikacin, netilmicin, etimicin, isepamicin) is interference with bacterial cell protein synthesis by binding irreversibly to the 16S rRNA specific site of the 30S ribosomal subunit and thus effectively inhibiting bacterial growth[3,4].

However, in clinical cases, *K. pneumoniae* resistance to aminoglycosides usually results from production of aminoglycoside-modifying enzymes (AMEs) such as acetyltransferases, phosphorylases, and adenyltransferases[5]. Shaw's[6] naming scheme is based on the type of AMEs (acetyltransferases (AACs), nucleotidyltranferases (ANTs), or phosphotransferases (APHs)) and their encoding gene; 2'0302"03"06'04'03'06 and 9 for the site of modification; aminoglycosides I, II, III, IV, V, etc., for modified enzyme substrate; and unique protein sequences (i.e., isoforms a, b, c, etc.) classified. Until now more than 50 kinds of AMEs have been identified, such as: AAC(3)-I 0AAC(3) - IAAC(3) -II AAC(6') - III AAC(6') -II0ANT(2")-I ANT(3") - II ANT(4) - II0ANT(4') -II ANT(4')-IIb APH(33) -I0APH(3")-II-APH(3') -VI, etc.[6,7], and their coding gene sequences have been identified. Modification enzyme recognition of the antibiotic site is different, so each modification enzyme has its own specificity of substrate, and generally only mediated resistance to one of or several structurally similar aminoglycosides[8], The genes encoding AMEs are often located on the plasmid, the plasmid can carry genes passed from cell to cell, and some genes are located on transposons and integrons which can cause rapid spread[5].

Yokoyama[9] found that *P. aeruginosa* isolates were highly resistant to aminoglycosides, and this resistance was mediated by one kind of 16S rRNA methylase *rmt*A, encoded by the plasmid *rmt*A gene, which could transform into *E. coli* and *K. pneumoniae* by conjugation, transformation, etc., resulting in high-level resistance to most aminoglycosides. In recent years, scholars around the world have discovered five other kinds of 16S rRNA methylase genes: *arm*A, *rmt*B, *rmt*C, *rmt*D and *npm*A[10-12]. The methylase modified a common locus of all aminoglycosides, so, in the event of modification, aminoglycosides would lose their antibacterial effect.

In our research, we aimed to characterize *K. pneumoniae* susceptibility to aminoglycosides and the positive rate of AMEs and 16S rRNA methylase in isolates from Guangzhou University of Traditional Chinese Medicine. Furthermore, we aimed to explore the molecular mechanism of aminoglycoside resistance and correlate drug-resistant phenotypes with genotypic characteristics.

MATERIALS AND METHODS

Reagents and Instruments

Amikacin (AMK), gentamicin (GEN), and tobramycin (TOB) were purchased from Sigma Chemical Co., St Louis, MO (USA). All primers of six AME genes and six 16S rRNA methylase gene alleles were synthesized by Boya Corp, Shanghai, China. The PCR kit was purchased from TAKARA Biotechnology Co., Dalian, China.

Strain Collection and Identification

Clinical isolates of *K. pneumoniae* were identified from the Microbiology Laboratory of the Guangdong Provincial Hospital of Traditional Chinese Medicine in between 2008 and 2012. Clinical specimens including sputum, ascites, blood, wound, and urine were collected from inpatients with suspected infections and only the first isolate from each patient was included. The organism was subcultured in 5% sheep blood agar, chocolate agar, and MacConkey agar and incubated at 35°C in 5% CO_2 for 24 hr or 48 hr, and isolates were identified by MicroScan Wa1kAway-96 with Neg Combo Panel Type 31 (Dade Behring, Inc. West Sacramento, CA USA).

Susceptibility testing

Antimicrobial susceptibility was determined by the broth microdilution method, The Microscan Gram-negative susceptibility card Neg Combo Panel Type 31 (NC31) evaluated a wide group of antibiotics: AMK, GEN, TOB, ampicillin,

cefotaxime, cefoxitin, cefpirome, cefpodoxime, ceftazidime, cefuroxime, cefalothin, trimethoprim/sulfamethoxazole, ciprofloxacin, mecillinam, nitrofurantoin, trimethoprim, amoxicillin/clavulanic acid, aztreonam, nalidixic acid, cefuroxime axetil, and piperacillin/tazobactam. Control strain *Escherichia coli* ATCC 25922 was included in every test run. The minimal inhibitory concentrations (MICs) of each antimicrobial agent was deûned according to the guideline of the Clinical and Laboratory Standards Institute (CLSI)[13]. From 2008 to 2012, a total of 1,495 isolates of *K. pneumoniae* were analyzed.

For isolates with resistance to one or more aminoglycosides, MICs of AMK, GEN and TOB were further determined by broth microdilution according to the guidelines from the Clinical and Laboratory Standards Institute[13]. Briefly, bacterial strains were grown in cation-adjusted Mueller-Hinton broth for 18–20 hours at 35°C. A bacterial suspension was prepared and adjusted to 0.5 McFarland turbidity standard (about 1 to 2×10^8 CFU/mL).

Detection of genes for AMEs and 16S rRNA methylases

To detect six AME genes and six 16S rRNA methylase gene alleles, 265 *K. pneumoniae* isolates, which were resistant to GEN or AMK were included. According to AME and 16S rRNA methylase gene sequence information in the GenBank database, we designed two pairs of primers from GenBank accession numbers AB194779 and DQ914960 to amplify the *rmt*C and *rmt*D genes, respectively, the other part of the PCR primers and method used were as previously reported[12,13]. The primer sequences of the AME and 16S rRNA methylase genes are shown in Table 1. To prepare DNA templates, every isolate was grown on tryptose agar (Oxoid, London, England) plate at 35°C overnight. DNA templates were amplified in a total reaction volume of 50 µl containing 2.5 U of Taq polymerase, 0.025 to 0.125 µM of each primer, and 5 µl of 10×PCR buffer, 100 mM Tris-HCl, pH 8.3 at 25°C; 500 mM KCl; 15 mM $MgCl_2$; 0.01% gelatin (Sangon, Shanghai, China). Amplification was carried out with denaturation at 94°C for 5 min, followed by 30 cycles according to the following program: 94°C for 30 s, annealing temperature (Table 1) for 1 min, and 72°C for 1 min, plus a final extension for 10 min at 72°C to complete partial polymerizations. Amplification products were run on 1.5% agarose gels with 1×Tris-Acetate-EDTA (TAE) buffer, 10mM Tris-HCl 1mM EDTA PH=8.0 at 120V for 30 min^{-1} h, and bands visualized with ethidium bromide (Sigma). Gels were viewed and photographed with Biorad GelDoc XR (Biorad,CA,USA). Positive PCR products were purified and sequenced by Shanghai Invitrogen Biotechnology Co. (Shanghai, China), and the entire sequence of each gene was compared with the sequences in the GenBank nucleotide database at http//www.ncbi.nlm.nih.gov/. Sequencing was done with corresponding primers speciûc for AME and 16S rRNA methylase genes.

Conjugation experiments and plasmid analysis

The conjugal transfer of aminoglycoside resistance determinants was performed in broth culture with *E. coli* J53 as the recipient as described previously[10]. Transconjugants were selected on tryptose agar plates supplemented with 256 mg of AMK (Sigma Chemical Co.) per liter. Plasmids from transconjugants were extracted using a commercial kit (QIAprep spin miniprep kit; Qiagen, Hilden, Germany). We analyzed the restriction fragment length polymorphism of transferred plasmids using agarose gel electrophoresis of plasmid DNA samples treated with the restriction endonuclease EcoR I (New England Biolabs, Hertfordshire, United Kingdom).

Pulsed-field gel electrophoresis (PFGE) analysis

Molecular analysis using PFGE was performed on the *arm*A- and *rmt*B-positive isolates. DNAs were digested with *Xba* I (New England Biolabs, Hertfordshire, United Kingdom) and was separated on 1% agarose gels. PFGE was performed with a CHEF-DR III apparatus (Bio-Rad Laboratories, Hercules, CA) according to the instruction manual.

Statistical analysis

All statistical analyses were performed using Statistical Package for Social Sciences (SPSS) software (version 11.5) for Windows (χ2-test). P-values of <0.05 were considered significant.

RESULTS

Prevalence of aminoglycoside resistance

Among the 1495 *K. pneumoniae* isolates collected from 2008 to 2012, there were 17.7% (265) of the isolates resistant to at least one

aminoglycoside. Of these 265 isolates, 135 were only resistant to one of the three aminoglycosides, 118 isolates (7.9%), 12 isolates (0.8%), and 5 isolates (0.3%) were resistant to GEN, TOB, and AMK, respectively; 73 isolates (4.9%) were resistant to GEN and TOB, 8 isolates (0.5%) were resistant to AMK and TOB, and 49 isolates (3.3%) were resistant to all AMK, GEN and TOB the three aminoglycosides. Resistance rates based on the entire *K. pneumoniae* population, which are reflected in Table 2, i.e., AMK, 0.0-0.6%, TOB, 0.4-1.3%, GEN, 7.2-8.7%, AMK+TOB,0.0-1.%, GEN+TOB,4.1-7.9%, and AMK+GEN+TOB,2.9-4.2% respectively, but we did not find AMK + GEN-resistant strains in the past five years (Table 2).

Overall, the aminoglycoside resistance rates of *K. pneumoniae* were stable from 2008 to 2012. Specifically, during the five years, AMK

Table 1. PCR primers sequence for AME and 16S rRNA methylase genes

Genes	Primers (5′ to 3′)	Expected amplicon size (bp)	Annealing temperature (°C)
aac(3)-I	P1:ACCTACTCCCAACATCAGCC P2:ATATAGATCTCACTACGCGC	169	52
aac(3)-II sequence	P1:ACTGTGATGGGATACGCGTC P2:CTCCGTCAGCGTTTCAGCTA	237	55
aac(6')-Ib	P1:ATGACTGAGCATGACCTTGC P2:TTAGGCATCACTGCGTGTTC	519	52
aac(6')-II	P1:TTCATGTCCGCGAGCACCCC P2:GACTCTTCCGCCATCGCTCT	178	56
ant(3")-I	P1:TGATTTGCTGGTTACGGTGAC P2:CGCTATGTTCTCTTGCTTTTG	284	55
ant(2")-I	P1:GAGCGAAATCTGCCGCTCTGG P2:CTGTTACAA CGGACTGGCCGC	320	58
*arm*A	P1:ATGGATAAGAATGATGTTGTTAAG P2:TTATTTCTGAAATCCACTAGTAATTA	774	55
*rmt*A	P1:CCTAGCGTCCATCCTTTCCTC P2:AGCGATATCCAACACACGATGG	315	56
*rmt*B	P1:ATGAACATCAACGATGCCCTC P2:TTATCCATTCTTTTTTATCAAGTATAT	756	55
*rmt*C	P1:ATGAAAACCAACGATAATTATC P2:TTACAATCTCGATACGATAAAATAC	846	50
*rmt*D	P1:ATGAGCGAACTGAAGGAAAAACTGCT P2:TCATTTTCGTTTCAGCA CGTAAAACAG	744	58
*npm*A	P1:TTGGGTACTGGAGACGGTAG P2:CAGCTTTGTATTGTTCGCTC	421	50

Table 2. The isolates of *K. pneumoniae* resistant to aminoglycosides isolates resistant to [a] (%)

0	isolates	AMK	TOB	GEN	AMK+GEN	AMK+TOB	GEN+TOB	AMK+GEN+TOB
2008	312	2 (0.6)	2 (0.6)	25 (8.0)	0 (0.0)	2 (0.6)	15 (4.8)	13 (4.2)
2009	306	1 (0.3)	3 (1.0)	22 (7.2)	0 (0.0)	1 (0.3)	13 (4.2)	10 (3.3)
2010	310	1 (0.3)	4 (1.3)	27 (8.7)	0 (0.0)	3 (1.0)	13 (4.2)	9 (2.9)
2011	339	1 (0.3)	2 (0.6)	26 (7.7)	0 (0.0)	2 (0.6)	14 (4.1)	10 (2.9)
2012	228	0 (0.0)	1 (0.4)	18 (7.9)	0 (0.0)	0 (0.0)	18 (7.9)	7 (3.1))
Total	1495	5(0.3)	12(0.8)	118(7.9)	0(0.0)	8(0.5)	73(4.9)	49(3.3)

[a] Resistance rates were calculated accordingly when the studies indicated rates of susceptibility.
AMK, amikacin; GEN, gentamicin; TOB, tobramycin

resistance rate in all K. pneumoniae isolates fluctuated between 3.07% to 5.45%, TOB resistance rate fluctuated between 8.26% to 11.4%, and GEN resistance rate fluctuated between 14.71% to 18.86% respectively (Fig. 1). Among the 265 aminoglycoside-resistant strains of *K. pneumoniae*, 90.1% (240/265) showed resistance to GEN, 53.6% (142/265) were resistant to TOB, and only 23.4% (62/265) were resistant to AMK (Table 3).

Co-resistance to other classes of antibiotics

The results from the Microscan Gram-negative susceptibility card Neg Combo Panel Type 31 (NC31) were used to evaluate the level of resistance to other classes of antibiotics. A total of 90 (34%) of the 265 aminoglycoside-resistant isolates showed a phenotypic ESBL profile while 143 (54%) were resistant to ciprofloxacin and 175 (66%) were resistant to trimethoprim/ sulfamethoxazole.

Table 3. The susceptibility of aminoglycosides to 265 isolates *K. pneumoniae*

Antimicrobial	Resistance		Intermediate		Susceptibility		MIC_{50}	MIC_{90}	MIC_{range}
agents	isolates	RR(%)	isolates	IR(%)	isolates	SR(%)	(μg/mL)	(μg/mL)	(μg/mL)
Amikacin	62	23.4	3	1.3	200	75.5	4	512	0.25-512
Gentamicin	240	90.6	0	0.0	25	9.4	2	≥512	0.5-≥512
Tobramycin	142	53.6	1	0.4	122	46.0	2	512	0.25-512

RR: Resistant rate; IR: Intermediate rate; SR: Susceptible rate

Table 4. Condition of 224 *Klebsiella pneumoniae* isolates harbouring AMEs genes and 16S rRNA methylases genes

Genes	isolates	Constituent ratio(%)
aac(3)-II	55	24.6
aac(6')-Ib	34	15.2
ant(3")-I	19	8.5
ant(2")-I	7	3.1
*arm*A+*aac*(3)-II	15	6.7
*rmt*B+*aac*(3)-II	8	3.6
*arm*A+*ant*(3")-I	5	2.2
aac(3)-a!+*aac*(6')-Ib	26	11.6
aac(3)-a!+*ant*(3")-I	14	6.3
aac(6')-Ib+*ant*(3")-I	13	5.8
aac(6')-Ib+*ant*(2")-I	10	4.5
*arm*A+*aac*(3)-a!+*aac*(6')-Ib	6	2.6
*rmt*B+*aac*(3)-II+*aac*(6')-Ib	5	2.2
*arm*A+*aac*(3)-II+*ant*(3")-I	4	1.8
aac(6')-Ib+*ant*(3")-I+*ant*(2")-I	2	0.9
*arm*A +*aac*(3)-II+*aac*(6')-Ib+*ant*(3")-I	1	0.4
Total	224	100.0

Among the 265 strains aminoglycosides resistance *K. pneumoniae*, the aac (3)-I, aac(6')-Ib, ant(3")-I, ant(2")-I gene positive strains were 134(50.6%),101(38.1%), 59(22.3%), 22(8.3%) strains, while armA, rmtB were 31(11.7%), 13(4.9%) respectively. 44(16.6%) isolates detected the 16S rRNA gene armA or/and rmt B, however, there was no rmtA, rmtC, rmtD, npmA, aac(3)-I, and aac(6')-, gene positive strains. Furthermore?the Electrophoregram result confirned that aac(3)-II aac(6')-Ib, ant(3")-I, ant(2")-I, armA, rmtB gene is the same with the PCR results. The nucleotide sequence of the gene are homology of 99% or 100% with them registered in GenBank nucleotide sequence.

Table 5. MIC50 and MIC90 of the isolates harboring only AME genes versus those harboring both AME and 16S rRNA methylase genes

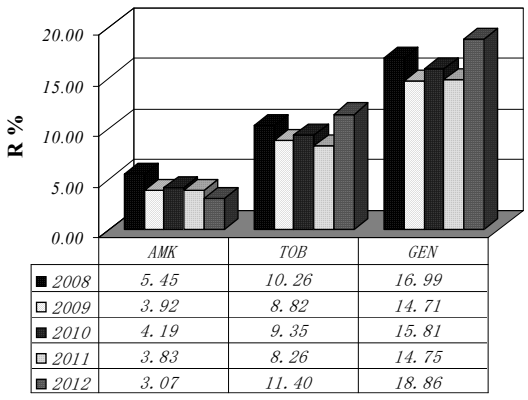
Antimicrobial agents	AMEs genes only (n=34)		Both AMEs genes and 16S rRNA methylase genes (n=28)	
	MIC_{50}(μg/ml)	MIC_{90}(μg/ml)	MIC_{50}(μg/ml)	MIC_{90}(μg/ml)
Amikacin	16	32	256	512
Gentamicin	64	256	256	≥512
Tobramycin	64	256	256	512

Prevalence of aminoglycoside modifying enzymes (AMEs) and 16S rRNA methylases

We detected 6 kinds of AMEs and 6 kinds of 16S rRNA methylation enzyme gene for the 265 strains *K. pneumoniae*, showed that 224 strains carried one or more resistant genes, accounting for 84.5%. Among them, there were 11509101701 strains carrying 1020304 kinds of above-mentioned resistance genes strains, respectively. The specific situation of carrying resistance genes are shown in Table 3. 124 strains carried AMEs gene only, 44 strains carried both AMEs and 16SRNA Methylase gene, however, no strain only exist 16SRNA Methylase gene, and the results are shown in Table 4.

Comparison of genetype and resistant to aminoglycosides phenotype

The comparison of genotypes and phenotypic resistance to aminoglycosides showed 224 genotype-positive strains with resistance to at least one aminoglycoside. There were 44 16S rRNA methylation enzyme gene-positive strains that displayed high-level to all three aminoglycosides and trimethoprim/ sulfamethoxazole (MICs > 256 mg/l), but were susceptible to imipenem (MICs ≤ 1 mg/l). Of the 31 *arm*A-positive isolates, 24 (77.4%), 19 (61.3%) and 10 (32.3%) isolates were resistant to chloramphenicol (MICs > 256 mg/l), tetracycline (MICs > 256 mg/l) and ciprofloxacin (MICs > 4 mg/ l), respectively, and all 13 (100%) *rmtB*-positive isolates were resistant to all of these three drugs. The MIC_{50} and MIC_{90} of AMK, GEN, and TOB in Table 5 show that *K. pneumoniae* strains carrying both AME and 16S rRNA methylase genes are higher than those that only carried AME genes. The other 41 strains with no resistance gene showed varying degrees of aminoglycoside resistance.



Note: Resistance rates were calculated accordingly when the studies indicated rates of susceptibility. AMK, amikacin; GEN, gentamicin; TOB, tobramycin

Fig. 1. Aminoglycoside-resistant isolates of *K. pneumoniae*

Plasmid transfer studies

In the experiment of plasmid transfer, among the 44 strains 16S rRNA methylation enzyme gene-positive strains, 32 strains obtained the zygote through the AMK and sodium azide screening plates; 23 strains were *arm*A positive, 9 strains were *rmt*B positive and 12 strains had no zygote. The zygote antimicrobial susceptibility test showed all 32 strains had resistance to TOB, GEN, and AMK.

Plasmid profile analysis and PFGE result

The plasmid profile analysis of *arm*A- and *rmt*B-positive strains showed that all *arm*A- and *rmt*B-positive strains have several kind plasmids, however, the result is different among the *arm*A- and *rmt*B-positive strains. To look for clusters that could suggest a dissemination of a successful clone, PFGE banding patterns were obtained for 31 *arm*A-positive isolates and 13 *rmt*B-positive isolates.

DISCUSSION

Although aminoglycosides represent a small fraction of antibiotic consumption, it is still an important group of routine, primary antibiotics in treating serious Gram-negative bacterial infections according to CLSI. Monitoring the level of aminoglycoside resistance is therefore an important task. The main mechanism causing aminoglycoside resistance is the presence of aminoglycoside modifying enzymes (AMEs), which catalyze the modification at different -OH or -NH_2 groups in the antibiotic molecule, changing its structure, leading to loss of the ability to bind to the target site, and subsequent aminoglycoside resistance[7]. Furthermore, high level and broad-spectrum aminoglycoside-resistant *K. pneumoniae* isolates were often associated with 16S rRNA methylases[7,8]. The 16S rRNA methylase acts mainly through methylation of a site of one or a few bases on the bacterial 30S ribosomal subunit 16S rRNA, causing high level resistance to aminoglycosides[18]. In addition, efflux pump mechanisms and impermeability of the cell membrane may also cause reduced susceptibility to aminoglycosides.

In our study, we collected 1495 isolates *K. pneumoniae* from Guangdong Provincial Hospital of Traditional Chinese Medicine in China, and identified 265 (17.7%) isolates resistant to at least one aminoglycoside. Resistance to aminoglycosides appear quite stable over the 5-year period, but the resistance rates for AMK, GEN, and TOB were different, which was highest for GEN(over 10%) and lowest for AMK(about 5%), and the rate of resistance to TOB follows the same trend as for GEN with higher prevalence of resistance compared to AMK. Our data suggest that AMK should be the aminoglycoside of choice for treatment of infections with *K. pneumoniae.*

Furthermore, the analysis of the six AME genes and six 16S rRNA methylase genes of these 265 resistant strains of *K. pneumoniae*, showed that 84.5% of the isolates carried one of these resistance genes. Specifically, 180 strains carried only an AME gene while 44 isolates carried both AME and 16S rRNA methylase genes; no strains carried only a 16S rRNA methylase gene. While the majority of aminoglycoside-resistant *K. pneumoniae* in our hospital were found to harbor the resistance genes studied, the 41 remaining aminoglycoside-resistant strains may either have produced other unusual AMEs or some other genotypic factor, e.g., change of cell membrane permeability and/or activity of efflux pumps[7-9].

Results suggest that 16S rRNA methylase *arm*A and *rmt*B gene caused the prevalence of *K. pneumoniae* aminoglycoside resistance. It is of interest to identify whether there are other 16S rRNA methylase genes involved and associated prevalence in Guangzhou; therefore, we should select more samples to confirm the phenomenon.

Our work confirmed that 16S rRNA methylase and AME genes are mostly located on transposons within transferable plasmids, which provides them with the potential to spread horizontally and may explain the already worldwide distribution of this resistance mechanism[11-13], The encoding gene of AMEs, 16S rRNA methylases and extended-spectrum β-lactamase (ESBLs) may exist in the same mobile genetic elements such as plasmids, transposons or integrons. Therefore, the such mobile resistance genes can be horizontal shift with these componentstransferred between the same or different bacteria, causing multidrug-resistance[12] and sSome of these organisms have been found to coproduce extended-spectrum β-lactamasesESBLs, contributing to multidrug-resistant phenotypes[13]. Moreover, encoding genes of AMEs or 16S rRNA methylases and ESBLs can co-exist in the same transferable plasmid leading to multidrug resistance[10-13], and thus should warrant clinical attention.

In all, the level of aminoglycoside resistance in Guangzhou clinical isolates of *K. pneumoniae* is still low, aminoglycosides still can act as the basis for anti-Klebsiella pneumoniae antibiotics.

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