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ORIGINAL ARTICLE

In vitro activity of aminoglycosides against clinical isolates of *Acinetobacter baumannii* complex and other nonfermentative Gram-negative bacilli causing healthcare-associated bloodstream infections in Taiwan

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Abstract *Background/Purpose:* Aminoglycosides possess *in vitro* activity against aerobic and facultative Gram-negative bacilli. However, nationwide surveillance on susceptibility data of *Acinetobacter baumannii* complex and *Pseudomonas aeruginosa* to aminoglycosides was limited, and aminoglycoside resistance has emerged in the past decade. We study the *in vitro* susceptibility of *A. baumannii* complex and other nonfermentative Gram-negative bacilli (NFGNB) to aminoglycosides.
Methods: A total of 378 NFGNB blood isolates causing healthcare-associated bloodstream infections during 2008 and 2013 at four medical centers in Taiwan were tested for their susceptibilities to four aminoglycosides using the agar dilution method (gentamicin, amikacin, tobramycin, and isepamicin) and disc diffusion method (isepamicin).

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nonfermentative Gram-negative bacilli

Results: *A. baumannii* was highly resistant to all four aminoglycosides (range of susceptibility, 0–4%), whereas >80% of *Acinetobacter nosocomialis* and *Acinetobacter pittii* blood isolates were susceptible to amikacin (susceptibility: 96% and 91%, respectively), tobramycin (susceptibility: 92% and 80%, respectively), and isepamicin (susceptibility: 96% and 80%, respectively). All aminoglycosides except gentamicin possessed good *in vitro* activity (>94%) against *P. aeruginosa*. Amikacin has the best *in vitro* activity against *P. aeruginosa* (susceptibility, 98%), followed by *A. nosocomialis* (96%), and *A. pittii* (91%), whereas tobramycin and isepamicin were less potent against *A. pittii* (both 80%). Aminoglycoside resistances were prevalent in *Stenotrophomonas maltophilia* and *Burkholderia cepacia* complex blood isolates in Taiwan.

Conclusion: Genospecies among the *A. baumannii* complex had heterogeneous susceptibility profiles to aminoglycosides. Aminoglycosides, except gentamicin, remained good *in vitro* antimicrobial activity against *P. aeruginosa*. Further *in vivo* clinical data and continuous resistance monitoring are warranted for clinical practice guidance.

Introduction

Nonfermentative Gram-negative bacilli (NFGNB) are the leading pathogens that cause nosocomial bacteremia and infections.[1] They result in high fatality in critically ill patients and in patients with septic shock and bacteremia. The mortality rate attributed to NFGNB bacteremia could be as high as 25%.[2,3] Among NFGNB, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* complex, and *Stenotrophomonas maltophilia* were the most common pathogens causing healthcare-associated bloodstream infections.[3]

NFGNB are easily resistant to most of the beta-lactam antibiotics through beta-lactamase production, impermeability, and multidrug efflux pumps.[4] The emerging resistance of NFGNB to all commonly used antibiotics may lead to inappropriate administration of empirical antibiotics, which contributes to the high fatality rate of NFGNB bacteremia.[2,5,6] The limitation of the susceptible antibiotic spectrum also makes the clinical treatment more difficult. Therefore, periodic active surveillance for the epidemiology of NFGNB resistance is crucial for infection control and antibiotic stewardship.

Aminoglycosides possess *in vitro* activity against many aerobic and facultative Gram-negative bacilli, like *P. aeruginosa* and *Acinetobacter* spp., and they are intrinsically inactive against some NFGNB, such as *S. maltophilia* and *Burkholderia cepacia* complex. Therefore, the aminoglycosides are the drug of choice for antimicrobial combination for severe infections (e.g., bacteremia) caused by NFGNB.[7] However, resistance to aminoglycosides in *P. aeruginosa* and *Acinetobacter* spp. can develop by enzymatic modification, impermeability, or MexXY (also referred to as *Amr*AB) efflux pumps.[6,8] The susceptibility data of NFGNB to aminoglycosides were limited in Taiwan, and the previous study did not differentiate *A. baumannii* complex into the species level for investigation.[9] In this study, we aimed to compare the *in vitro* susceptibility of NFGNB blood isolates to aminoglycosides in patients with healthcare-associated bacteremia in Taiwan.

Methods

Bacterial isolates

Healthcare-associated bloodstream infections were defined as bacteremia occurring in patients admitted over 48 hours, in patients with recent hospitalization history within 90 days, in patients who were nursing home residents, and in patients who were frequent visitors of the hemodialysis facility or the hospital-based clinic. Blood isolates of NFGNB collected during 2008 to 2013 and preserved at four medical centers in Taiwan were retrospectively recruited using their collection numbers from the laboratory. The four hospitals included National Taiwan University Hospital (NTUH) and Taipei Veterans General Hospital (TVGH) located in northern Taiwan; China Medical University Hospital (CMUH) located in central Taiwan; and Kaohsiung Chang Gung Memorial Hospital (KCGMH) located in southern Taiwan. A total of 378 available NFGNB isolates (NTUH, 319 isolates; TVGH, 18 isolates; CMUH, 32 isolates; and KCGMH, 9 isolates) retrospectively identified from 2013 isolates were randomly selected by the collection numbers for aminoglycoside susceptibility testing. Those isolates included *P. aeruginosa* (100 isolates), *A. baumannii* (76 isolates), *Acinetobacter nosocomialis* (54 isolates), *Acinetobacter pittii* (45 isolates), *S. maltophilia* (50 isolates), and *B. cepacia* complex (53 isolates). None of the patients had duplicate bacterial isolates in this study.

Bacterial identification

The species of all isolates, including *A. baumannii* complex strains, were routinely identified using standard conventional microbiological methods or by the Vitek System (bioMérieux, Hazelwood, MO, USA) as required. The genospecies of *A. baumannii* strains were further identified according to the sequence of the 16S-23S rRNA gene intergenic spacer region as previously described.[10]

Antimicrobial susceptibility testing

Gentamicin supplied by Schering Plough (Bloomfield, NJ, USA), amikacin by Bristol-Myers Squibb (Princeton, NJ, USA), tobramycin by Eli Lilly (Indianapolis, IN, USA), and isepamicin by TTY Biopharm (Taipei, Taiwan) were used for susceptibility testing using the agar dilution method.

The minimal inhibitory concentration (MIC) for each aminoglycoside to the isolated bacteria was determined using the agar dilution method according to the Clinical and Laboratory Standards Institute (CLSI) standard procedure protocol. The susceptibility to gentamicin, amikacin, and tobramycin was also determined according to the MIC interpretive criteria suggested by CLSI.[11] Given that there is no consensus susceptibility criteria for isepamicin, the same MIC interpretive criteria for amikacin was applied to isepamicin as follows: susceptible, $\leq$16 μg/mL; intermediate, 32 μg/mL; resistant, $\geq$64 μg/mL. Because there is no consensus clinical breakpoint for *S. maltophilia* and *B. cepacia* complex either, the same susceptibility criteria for *P. aeruginosa* was applied for these two clinical species as follows: (for gentamicin, tobramycin) susceptible, $\leq$4 μg/mL; intermediate, 8 μg/mL; resistant, $\geq$16 μg/mL; (for amikacin) susceptible, $\leq$16 μg/mL; intermediate, 32 μg/mL; resistant, $\geq$64 μg/mL. The disk diffusion test to isepamicin for the isolated bacteria was also performed according to CLSI standard protocol[12] The zone diameter interpretive criteria for amikacin were applied for the isepamicin because of the same reason: susceptible, $\geq$17 mm; intermediate, 15–16 mm; resistant, $\leq$14 mm. *Escherichia coli* American Type Culture Collection (ATCC; Rockville, MD, USA) 25922 and *P. aeruginosa* ATCC 27853 were used for daily quality control testing as recommended by the CLSI.

Statistical analysis

Susceptibility data were classified as dichotomous outcomes, which were compared using chi-square statistics, and $p < 0.05$ was defined as statistically significant. For comparisons between multiple groups, the logistic regression model was used. Data were analyzed using Stata software, version 12 (StataCorp, College Station, TX, USA).

Results

In vitro susceptibility of NFGNB to aminoglycosides using the agar dilution method

In vitro susceptibilities of the 378 blood isolates of NFGNB to aminoglycosides were determined according to MIC using the agar dilution method (Table 1). Three species in *A. baumannii* complex have different susceptibility profiles to aminoglycosides. *A. baumannii* was highly resistant to all four aminoglycosides. However, >80% of *A. nosocomialis* isolates and *A. pittii* isolates were susceptible to amikacin, tobramycin, and isepamicin, but not to gentamicin. Among the tested aminoglycosides, amikacin possessed the best *in vitro* activity against both *A. nosocomialis* (susceptibility: 96%, MIC_{90}: 8 μg/mL) and *A. pittii* (susceptibility: 91%, MIC_{90}: 16 μg/mL). Tobramycin had similar high activity to isepamicin against *A. nosocomialis* (susceptibility: 92% vs. 96%, respectively; and MIC_{90}: 4 μg/mL and 8 μg/mL, respectively), however, their activities against *A. pittii* were moderate (susceptibility: 80% for both; and MIC_{90}: 8 μg/mL and 32 μg/mL, respectively). Gentamicin had poor activity against the current *A. baumannii* complex. The differences in susceptibility rates to the four aminoglycosides between *A. baumannii and A. nosocomialis*, *A. baumannii* and *A. pittii*, and among the three *A. baumannii* complex strains were all statistically significant ($p < 0.001$).

All four aminoglycosides still had good activity against current *P. aeruginosa* blood isolates from patients with healthcare-associated bloodstream infections in Taiwan. The susceptibility rates of the *P. aeruginosa* isolates to isepamicin, amikacin, tobramycin, and gentamicin were 99%, 98%, 98%, and 94%, respectively. The MIC_{90} values of isepamicin, amikacin, tobramycin, and gentamicin for the *P. aeruginosa* isolates were 8 μg/mL, 4 μg/mL, 1 μg/mL, and 4 μg/mL, respectively.

Both the *S. maltophilia and B. cepacia* complex isolates were intrinsically resistant to all four aminoglycosides. The susceptible rates of *S. maltophilia* isolates to aminoglycosides varied from 4% to 10% with all the MIC_{90} values being >128 μg/mL. The susceptible rates of *B. cepacia* complex isolates varied from 4% to 8% with all the MIC_{90} values being >128 μg/mL.

Comparing the activities of the four aminoglycosides against the 378 NFGNB isolates from patients with healthcare-associated bloodstream infections in Taiwan, amikacin possessed the greatest activity against *P. aeruginosa* (susceptibility: 98%), *A. nosocomialis* (susceptibility: 96%), and *A. pittii* (susceptibility: 91%), but poor activity against *A. baumannii* (susceptible: 4%), *S. maltophilia* (susceptible: 4%), and *B. cepacia* complex (susceptible: 6%); tobramycin and isepamicin had lesser activity against *A. pittii* (susceptibility: 80% for both aminoglycosides) compared to amikacin. Among all NFGNB isolates in this study, only *P. aeruginosa* was still highly susceptible to all four aminoglycosides.

In vitro susceptibility of NFGNB to isepamicin by the disc diffusion method

Zone diameter results and the susceptibility rates of NFGNB isolates to isepamicin using the disc diffusion method are listed in Table 2. Isepamicin possessed good activity against *A. nosocomialis* (susceptibility: 94%) and *P. aeruginosa* (susceptibility: 99%), but had poor activity against *A. baumannii* (susceptible: 4%), *S. maltophilia* (susceptible: 26%), and *B. cepacia* complex (susceptible: 17%); these results were similar to those by the agar dilution method. However, the susceptibility rate of *A. pittii* to isepamicin was better using the disc diffusion method than by using the agar dilution method (the susceptibility was 91% in the disk diffusion method and 80% in the agar dilution method). The differences in the susceptibility rates to isepamicin using the disc diffusion method between *A. baumannii* and *A. nosocomialis*, *A. baumannii* and *A. pittii*, and among the three *A. baumannii* complex strains were all statistically significant ($p < 0.001$). Except for *S. maltophilia*

Table 1 *In vitro* susceptibility of blood isolates of nonfermentative Gram-negative bacilli (NFGNB) to aminoglycosides using the agar dilution method.

Bacterium (no. of isolates tested)	Antimicrobial agents	MIC (mg/L)			Susceptibility (no. of isolates tested)			*p*
		Range	MIC_{50}	MIC_{90}	S	I	R	
Acinetobacter baumannii (76)	Gentamicin	64—>128	>128	>128	0 (0)	0 (0)	100 (76)	< 0.001[a]
	Amikacin	4—>128	>128	>128	4 (3)	0 (0)	96 (73)	< 0.001[a]
	Tobramycin	2—>128	>128	>128	5 (4)	1 (1)	94 (71)	< 0.001[a]
	Isepamicin	2—>128	>128	>128	4 (3)	0 (0)	96 (73)	< 0.001[a]
Acinetobacter nosocomialis (54)	Gentamicin	1—>128	8	16	30 (16)	48 (26)	22 (12)	< 0.001[b]
	Amikacin	4—>128	8	8	96 (52)	0 (0)	4 (2)	< 0.001[b]
	Tobramycin	1—>128	2	4	92 (50)	4 (2)	4 (2)	< 0.001[b]
	Isepamicin	2—>128	4	8	96 (52)	0 (0)	4 (2)	< 0.001[b]
Acinetobacter pittii (45)	Gentamicin	1—>128	8	32	44 (20)	38 (17)	18 (8)	< 0.001[c]
	Amikacin	2—>128	4	16	91 (41)	4 (2)	4 (2)	< 0.001[c]
	Tobramycin	0.5—>128	2	8	80 (36)	16 (7)	4 (2)	< 0.001[c]
	Isepamicin	2—>128	8	32	80 (36)	13 (6)	7 (3)	< 0.001[c]
Stenotrophomonas maltophilia (50)	Gentamicin	2—>128	128	>128	4 (2)	6 (3)	90 (45)	
	Amikacin	4—>128	128	>128	4 (2)	4 (2)	92 (46)	
	Tobramycin	2—>128	128	>128	4 (2)	2 (1)	94 (47)	
	Isepamicin	4—>128	128	>128	10 (5)	2 (1)	88 (44)	
Burkholderia cepacia complex (53)	Gentamicin	0.5—>128	>128	>128	4 (2)	0 (0)	96 (51)	
	Amikacin	2—>128	>128	>128	6 (3)	11 (6)	83 (44)	
	Tobramycin	2—>128	128	>128	4 (2)	0 (0)	96 (51)	
	Isepamicin	1—>128	128	>128	8 (4)	8 (4)	84 (45)	
Pseudomonas aeruginosa (100)	Gentamicin	1—>128	2	4	94 (94)	2 (2)	3 (3)	
	Amikacin	5—32	4	4	98 (98)	2 (2)	0 (0)	
	Tobramycin	0.25—>128	1	1	98 (98)	0 (0)	2 (2)	
	Isepamicin	1—64	4	8	99 (99)	0 (0)	1 (1)	

[a] Comparison of *in vitro* susceptibility of all three *Acinetobacter baumannii* complex species.
[b] Comparison of *in vitro* susceptibility of *Acinetobacter baumannii* and *Acinetobacter nosocomialis*.
[c] Comparison of *in vitro* susceptibility of *Acinetobacter baumannii* and *Acinetobacter pittii*.
I = intermediate; MIC = minimal inhibitory concentration; MIC_{50} = minimum concentration inhibiting 50% of isolates; MIC_{90} = minimum concentration inhibiting 90% of isolates; R = resistant; S = susceptible.

Table 2 *In vitro* susceptibility of blood isolates of nonfermentative Gram-negative bacilli (NFGNB) to isepamicin using the disc diffusion method and the agar dilution method.

Bacterium (no. of isolates tested)	Zone diameter (nearest whole mm)	Susceptibility (no. of isolates tested) by disc diffusion method			Susceptibility (no. of isolates tested) by agar dilution method			*p*[a]
	Range	S	I	R	S	I	R	
Acinetobacter baumannii (75)	6—21	4 (3)	0 (0)	96 (72)	4 (3)	0 (0)	96 (73)	1.000
Acinetobacter nosocomialis (54)	3—24	94 (51)	0 (0)	6 (3)	96 (52)	0 (0)	4 (2)	0.647
Acinetobacter pittii (45)	6—26	91 (41)	0 (0)	9 (4)	80 (36)	13 (6)	7 (3)	0.134
Stenotrophomonas maltophilia (50)	6—25	26 (13)	18 (9)	56 (28)	10 (5)	2 (1)	88 (44)	0.037
Burkholderia cepacia complex (53)	6—33	17 (9)	4 (2)	79 (42)	8 (4)	8 (4)	84 (45)	0.139
Pseudomonas aeruginosa (100)	13—31	99 (99)	0 (0)	1 (1)	99 (99)	0 (0)	1 (1)	1.000

[a] Comparison of *in vitro* susceptibility by the disc diffusion method and the agar dilution method.
I = intermediate; R = resistant; S = susceptible.

($p = 0.037$), there were no discrepancies of antimicrobial susceptibilities of aminoglycosides against other NFGNB blood isolates between the agar dilution method and the disc diffusion method (all $p > 0.05$). There was high categorical agreement between the susceptibility rates determined using the disc diffusion method and the agar dilution method (Pearson correlation coefficient $r = 0.9887$, $p = 0.0002$).

Discussion

Our study demonstrated different *in vitro* susceptibilities to aminoglycosides among genospecies of *A. baumannii* complex. In addition, *P. aeruginosa* isolates from nosocomial bacteremia in Taiwan remained highly susceptible to aminoglycosides. Finally, amikacin possessed the widest spectrum coverage among the four aminoglycosides against NFGNB, including *P. aeruginosa*, *A. nosocomialis*, and *A. pittii*.

A. baumannii complex comprises Acinetobacter genospecies 1 (*A. calcoaceticus*), genospecies 2 (*A. baumannii*), genospecies 3 (*A. pittii*), and genospecies 13TU (*A. nosocomialis*). They are phenotypically similar and the commercial identification system has limited capacity to differentiate between them.[13,14] Compared to *A. nosocomialis* and *A. pittii*, *A. baumannii* had higher carbapenem resistance and corresponded to higher attributable mortality of the patients with *A. baumannii* bacteremia.[14] Previous studies showed different resistant mechanisms of *A. baumannii* from *A. nosocomialis* and *A. pittii*, and the results of the susceptibility to aminoglycosides varied.[15–17] Most of the studies showed high resistant rates of *A. baumannii* to gentamicin, amikacin, tobramycin, and isepamicin.[14,15,17–19] However, susceptibilities of *A. nosocomialis* and *A. pittii* to aminoglycosides were not consistent between studies, with the susceptibility rates varied from 22% to 80.6% for *A. nosocomialis* and 25% to 66.7% for *A. pittii*.[14,15] The sample sizes of the blood isolates of *A. nosocomialis* and *A. pittii* in those studies were limited, especially in data from Taiwan.[9,14–18] None of the previous studies provided the susceptibility of *A. nosocomialis* and *A. pittii* to isepamicin. Our study revealed that both isepamicin and amikacin produced similar activities against *A. baumannii* complex. Consistent with these studies,[9,14–18] our results showed high resistance of *A. baumannii* to all four aminoglycosides in patients with nosocomial *A. baumannii* bacteremia in Taiwan. Nevertheless, aminoglycosides, except gentamicin, still possessed good activity against *A. nosocomialis* and *A. pittii* in nosocomial bacteremic patients (>80% isolates were susceptible), especially for amikacin (96% of the *A. nosocomialis* isolates and 91% of the *A. pittii* isolates were susceptible to amikacin). These results implied the importance of genospecies differentiation of the *A. baumannii* complex for clinical treatment guidance.

P. aeruginosa was the leading NFGNB that caused nosocomial NFGNB bacteremia.[3,20] Increasing prevalence of multidrug resistant *P. aeruginosa* even worsened the clinical treatment, with increased mortality rate in bacteremic patients.[4,8,21–24] Chromosomally encoding both *Rmt*D methylase and metallo-β-lactamases genes have also been found leading to pan-drug resistant *P. aeruginosa*.[25,26] Few antibiotics were available and were susceptible to *P. aeruginosa* when it was resistant to beta-lactam antibiotics due to cross-resistance. Compared to carbapenems and quinolones, aminoglycosides have the lowest cross-resistance and coresistance rates for piperacillin-resistant *P.* aeruginosa.[5,21] Our current study showed high susceptibility of *P. aeruginosa* to all aminoglycosides in nosocomial bacteremic patients in Taiwan. However, several studies have shown increasing resistance of gentamicin and tobramycin with resistant rates around 15–30% in *P. aeruginosa* isolates in Taiwan.[9,25] Overall, amikacin and probably isepamicin, remained active for *P. aeruginosa*, however, emerging multidrug resistance to aminoglycosides should be monitored regularly and intensively.

All four aminoglycosides had poor activity against *S. maltophilia* and *B. cepacia* complex isolates in our study, which was consistent with the previous study and our knowledge.[9] In addition, most of the NFGNB from patients with nosocomial NFGNB bacteremia in Taiwan were resistant to gentamicin. Given that other studies revealed increasing resistance to gentamicin in *P. aeruginosa* isolates, gentamicin should not be administered alone empirically for NFGNB infection in Taiwan before the drug susceptibility is available. Among the four aminoglycosides we tested in our study, amikacin had the broadest spectrum coverage against NFGNB, including *P. aeruginosa* (susceptibility: 98%), *A. nosocomialis* (susceptibility: 96%), and *A. pittii* (susceptibility: 91%).

There are limitations to the current study. Firstly, we identified patients with healthcare-associated bloodstream infections and randomly selected from the available preserved blood isolates for aminoglycoside susceptibility testing due to the limitation of faculties. The true disease burden of each NFGNB therefore could not be assessed. Secondly, we only did the susceptibility testing to aminoglycosides without investigating the susceptibility profiles of other antibiotics and the possible resistant mechanisms to aminoglycosides. Therefore, the relationships of cross-resistance or coresistance of aminoglycosides with other antimicrobial agents were unknown. Finally, no clinical or treatment data of patients were extracted in our study. The *in vitro* susceptibility to aminoglycosides may not be fully translated into the clinical treatment response *in vivo*.

In summary, genospecies of *A. baumannii* complex possessed heterogeneous susceptibility to aminoglycosides. Amikacin had the greatest *in vitro* activity against *P. aeruginosa*, *A. nosocomialis*, and *A. pittii*, whereas tobramycin and isepamicin were less potent against *A. pittii*. Further *in vivo* clinical data and continuous resistance monitoring will be warranted to establish clinical recommendations for aminoglycosides treatment against NFGNB in patients with healthcare-associated bloodstream infections in Taiwan.

Conflicts of interest

All contributing authors declare no conflicts of interest.

References

1. Chang TY, Lee CH, Liu JW. Clinical characteristics and risk factors for fatality in patients with bloodstream infections

caused by glucose non-fermenting gram-negative Bacilli. *J Microbiol Immunol Infect* 2010;**43**:233—9.
2. Chiu CW, Li MC, Ko WC, Li CW, Chen PL, Chang CM, et al. Clinical impact of Gram-negative nonfermenters on adults with community-onset bacteremia in the emergency department. *J Microbiol Immunol Infect* 2015;**48**:92—100.
3. Liu KS, Wang YT, Lai YC, Yu SF, Huang SJ, Huang HJ, et al. Antimicrobial resistance of bacterial isolates from respiratory care wards in Taiwan: a horizontal surveillance study comparison of the characteristics of nosocomial infection and antimicrobial-resistant bacteria in adult Intensive Care Units and two respiratory care facilities for mechanically ventilated patients at a tertiary care centre in Taiwan. *Int J Antimicrob Agents* 2011;**37**:10—5.
4. Enoch DA, Birkett CI, Ludlam HA. Non-fermentative Gram-negative bacteria. *Int J Antimicrob Agents* 2007;**29**:S33—41.
5. McGowan Jr JE. Resistance in nonfermenting gram-negative bacteria: multidrug resistance to the maximum. *Am J Infect Control* 2006;**34**:S29—37.
6. Potron A, Poirel L, Nordmann P. Emerging broad-spectrum resistance in *Pseudomonas aeruginosa* and *Acinetobacter baumannii*: mechanisms and epidemiology. *Int J Antimicrob Agents* 2015;**45**:568—85.
7. Lee HS, Loh YX, Lee JJ, Liu CS, Chu C. Antimicrobial consumption and resistance in five Gram-negative bacterial species in a hospital from 2003 to 2011. *J Microbiol Immunol Infect* 2015;**48**:647—54.
8. Poole K. Aminoglycoside resistance in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 2005;**49**:479—87.
9. Tsai TY, Chang SC, Hsueh PR, Feng NH, Wang JT. *In vitro* activity of isepamicin and other aminoglycosides against clinical isolates of Gram-negative bacteria causing nosocomial bloodstream infections. *J Microbiol Immunol Infect* 2007;**40**:481—6.
10. Lin YC, Sheng WH, Chang SC, Wang JT, Chen YC, Wu RJ, et al. Application of a microsphere-based array for rapid identification of *Acinetobacter* spp. with distinct antimicrobial susceptibilities. *J Clin Microbiol* 2008;**46**:612—7.
11. CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Ninth Edition*. CLSI document M07-A9. Wayne, PA: Clinical and Laboratory Standards Institute; 2012.
12. CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Twelfth Edition*. CLSI document M02-A11. Wayne, PA: Clinical and Laboratory Standards Institute; 2015.
13. Gerner-Smidt P, Tjernberg I, Ursing J. Reliability of phenotypic tests for identification of *Acinetobacter* species. *J Clin Microbiol* 1991;**29**:277—82.
14. Chuang YC, Sheng WH, Li SY, Lin YC, Wang JT, Chen YC, et al. Influence of genospecies of *Acinetobacter baumannii* complex on clinical outcomes of patients with *Acinetobacter* bacteremia. *Clin Infect Dis* 2011;**52**:352—60.
15. Lim YM, Shin KS, Kim J. Distinct antimicrobial resistance patterns and antimicrobial resistance-harboring genes according to genomic species of *Acinetobacter* isolates. *J Clin Microbiol* 2007;**45**:902—5.
16. Ribera A, Fernandez-Cuenca F, Beceiro A, Bou G, Martinez-Martinez L, Pascual A, et al. Antimicrobial susceptibility and mechanisms of resistance to quinolones and β-lactams in *Acinetobacter* genospecies 3. *Antimicrob Agents Chemother* 2004;**48**:1430—2.
17. Chuang YC, Sheng WH, Lauderdale TL, Li SY, Wang JT, Chen YC, et al. Molecular epidemiology, antimicrobial susceptibility and carbapenemase resistance determinants among *Acinetobacter baumannii* clinical isolates in Taiwan. *J Microbiol Immunol Infect* 2014;**47**:324—32.
18. Samonis G, Maraki S, Vouloumanou EK, Georgantzi GG, Kofteridis DP, Falagas ME. Antimicrobial susceptibility of non-fermenting Gram-negative isolates to isepamicin in a region with high antibiotic resistance. *Eur J Clin Microbiol Infect Dis* 2012;**31**:3191—8.
19. Rattanaumpawan P, Ussavasodhi P, Kiratisin P, Aswapokee N. Epidemiology of bacteremia caused by uncommon non-fermentative gram-negative bacteria. *BMC Infect Dis* 2013;**13**:167.
20. Jones RN, Sader HS, Beach ML. Contemporary *in vitro* spectrum of activity summary for antimicrobial agents tested against 18569 strains non-fermentative Gram-negative bacilli isolated in the SENTRY Antimicrobial Surveillance Program (1997—2001). *Int J Antimicrob Agents* 2003;**22**:551—6.
21. Karlowsky JA, Jones ME, Thornsberry C, Evangelista AT, Yee YC, Sahm DF. Stable antimicrobial susceptibility rates for clinical isolates of *Pseudomonas aeruginosa* from the 2001-2003 tracking resistance in the United States today surveillance studies. *Clin Infect Dis* 2005;**40**:S89—98.
22. Morata L, Cobos-Trigueros N, Martinez JA, Soriano A, Almela M, Marco F, et al. Influence of multidrug resistance and appropriate empirical therapy on the 30-day mortality rate of *Pseudomonas aeruginosa* bacteremia. *Antimicrob Agents Chemother* 2012;**56**:4833—7.
23. Lockhart SR, Abramson MA, Beekmann SE, Gallagher G, Riedel S, Diekema DJ, et al. Antimicrobial resistance among Gram-negative bacilli causing infections in intensive care unit patients in the United States between 1993 and 2004. *J Clin Microbiol* 2007;**45**:3352—9.
24. Lauderdale TL, Clifford McDonald L, Shiau YR, Chen PC, Wang HY, Lai JF, et al. The status of antimicrobial resistance in Taiwan among Gram-negative pathogens: the Taiwan surveillance of antimicrobial resistance (TSAR) program, 2000. *Diagn Microbiol Infect Dis* 2004;**48**:211—9.
25. Doi Y, Ghilardi AC, Adams J, de Oliveira Garcia D, Paterson DL. High prevalence of metallo-β-lactamase and 16S rRNA methylase coproduction among imipenem-resistant *Pseudomonas aeruginosa* isolates in Brazil. *Antimicrob Agents Chemother* 2007;**51**:3388—90.
26. Gurung M, Moon DC, Tamang MD, Kim J, Lee YC, Seol SY, et al. Emergence of 16S rRNA methylase gene *arm*A and cocarriage of bla_{IMP-1} in *Pseudomonas aeruginosa* isolates from South Korea. *Diagn Microbiol Infect Dis* 2010;**68**:468—70.