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Aminoglycoside Susceptibility Profiles of *Enterobacter cloacae* Isolates Harboring the *aac(6´)-Ib* Gene

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The aminoglycoside *6´-N*-acetyltransferases of type Ib *(aac(6´)-Ib)* gene confers resistance to amikacin, tobramycin, kanamycin, and netilmicin but not gentamicin. However, some isolates harboring this gene show reduced susceptibility to amikacin. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommends a revision of the phenotypic description for isolates harboring the *aac(6´)-Ib* gene. In this study, we determined the aminoglycoside susceptibility profiles of 58 AAC(6´)-Ib-producing *Enterobacter cloacae* isolates. On the basis of the CLSI and EUCAST breakpoints, a large proportion (84.5% and 55.2%, respectively) of these 58 isolates were found to be susceptible to amikacin. However, among the isolates that were shown to be anikacin-susceptible according to the CLSI and EUCAST breakpoints, only 30.6% and 18.8% isolates, respectively, could be considered to have intermediate resistance on the basis of the EUCAST expert rules. Further studies should be conducted to determine the aminoglycoside susceptibility profiles of *aac(6´)-Ib*-harboring isolates from various geographic regions and to monitor the therapeutic efficacy of amikacin in infections caused by these isolates.

Key Words: *aac(6´)-Ib,* Amikacin, *E. cloacae,* Breakpoint, Leu119Ser

Resistance to aminoglycosides is usually attributable to aminoglycoside-modifying enzymes. Among these, aminoglycoside *6´-N*-acetyltransferases of type Ib [AAC(6´)-Ib], is the most common cause of amikacin resistance among members of the family *Enterobacteriaceae* [1]. This enzyme can modify amikacin, tobramycin, kanamycin, and netilmicin but not gentamicin. Moreover, AAC(6´)-Ib often coexists with other antibiotic-inactivating enzymes such as β-lactamases, carbapenemases, and other aminoglycosidases; therefore, clinical practitioners should be aware of its significance [2, 3].

The expert rules laid down by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) suggest that if an isolate of the family *Enterobacteriaceae* is intermediate or resistant to tobramycin and susceptible to gentamicin and amikacin, then its amikacin susceptibility status should be revised from "susceptible" to "intermediate" because production of acquired AAC(6´)-I may not confer phenotypic amikacin resistance. In a previous study, we observed that over 40% of the *Enterobacter cloacae* isolates had the *aac(6´)-Ib* gene [4]; however, many of the isolates with this gene were susceptible to amikacin [4]. Therefore, in this study, we determined the aminoglycoside susceptibility profiles of *aac(6´)-Ib*-harboring *E. cloacae* isolates. Further, we investigated the *aac(6´)-Ib* mutations (Leu119Ser, Leu120Ser, Glu167Ala, Phe171Ala, and Tyr166Ala) that are known to be associated with the loss of amikacin resistance [5-7].

We had previously collected 178 consecutive, non-duplicate isolates of *E. cloacae* from specimens obtained at 12 clinical microbiology laboratories in Korea between March 2005 and July 2005. The *aac(6´)-Ib* gene was PCR amplified using 2 primers—5´-TTGCGATGCTCTATGGGCTA-´3 and 5´-CTCGAATGCCTGGCGTGTTT-´3—to obtain a 482-bp product [8]. In our previous study, all the 178 isolates were analyzed for the presence of *aac(6´)-Ib-cr* [4]. Of

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the 74 *E. cloacae* isolates that were found to be positive for *aac(6′)-Ib*, 58 were available for this study. For these 58 isolates, the minimum inhibitory concentrations (MICs) of amikacin (2-256 mg/L), kanamycin (2-256 mg/L), tobramycin (0.5-64 mg/L), and gentamicin (0.5-64 mg/L) were determined by the agar dilution method according to the CLSI guidelines [9].

To detect the mutations associated with the loss of amikacin resistance, the 482-bp PCR products were sequenced using a DNA Analyzer (Applied Biosystems, Foster City, CA, USA). To investigate the clonal relatedness of the isolates, pulsed-field gel electrophoresis (PFGE) was performed using a CHEF Mapper system (Bio-Rad Laboratories, Hercules, CA, USA). The whole-cell DNA was digested with *Xba*I, and the results were interpreted according to the criteria proposed by Tenover et al. [10].

Table 1. Aminoglycoside susceptibilities of the 58 *aac(6′)-Ib*-harboring *Enterobacter cloacae* isolates

Phenotype*	Amikacin N (%)		Kanamycin† N (%)	Tobramycin N (%)		Gentamicin N (%)	
	CLSI	EUCAST	CLSI	CLSI	EUCAST	CLSI	EUCAST
S	49 (84.5)	32 (55.2)	2 (3.4)	2 (3.4)	2 (3.4)	17 (29.3)	11 (19)
I	1 (1.7)	17 (29.3)	3 (5.2)	2 (3.4)	0	7 (12.1)	6 (10.3)
R	8 (13.8)	9 (15.5)	53 (91.4)	54 (93.1)	56 (96.6)	34 (58.6)	41 (70.1)

*CLSI-recommended minimum inhibitory concentration breakpoints for amikacin and kanamycin (S ≤ /I/R ≥) are 16/32/64, and those for tobramycin and gentamicin are 4/8/16; the corresponding EUCAST breakpoint for amikacin (S ≤ /R >) are 8/16, and those for tobramycin and gentamicin are 2/4; †MIC breakpoint for kanamycin was not recommended by the EUCAST.

Abbreviations: S, susceptible; I, intermediate; R, resistant; EUCAST, European Committee on Antimicrobial Susceptibility Testing.

Table 1 shows the antibiotic susceptibilities for the isolates. Of the 58 isolates harboring the *aac(6′)-Ib* gene, 49 (84.5%) were susceptible to amikacin (≤ 16 mg/L); 2 (3.4%), to kanamycin (≤ 16 mg/L); 2 (3.4%), to tobramycin (≤ 4 mg/L); and 17 (29.3%), to gentamicin (≤ 4 mg/L) according to the CLSI breakpoints. According to the EUCAST breakpoints, 32 (52.2%) isolates were susceptible to amikacin (≤ 8 mg/L); 2 (3.4%), to tobramycin (≤ 2 mg/L); and 11 (19%), to gentamicin (≤ 2 mg/L). The distributions of the MICs of different aminoglycosides for the 58 *E. cloacae* isolates are shown in Fig. 1 and 2. Of the 49 amikacin-susceptible isolates (MIC ≤ 16 mg/L), only 2 isolates—KN7 and SO15—had the Leu119Ser mutation in the *aac(6′)-Ib* gene. The KN7 isolate had 1 more amino acid change (Arg173Lys) in this gene. Findings of PFGE showed 10 different clones and no clonal relatedness among isolates from different clinical mi-

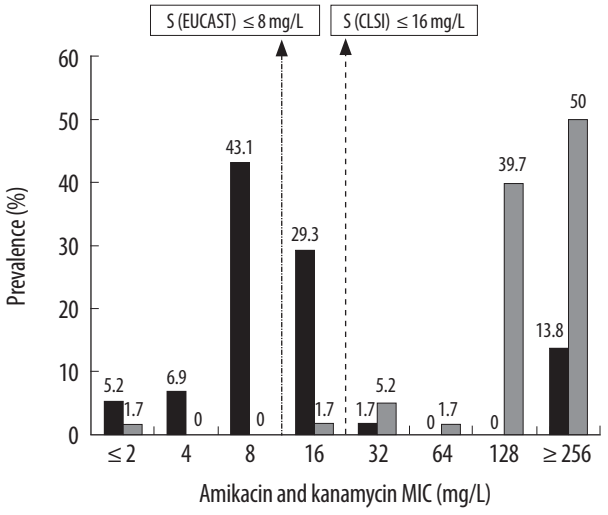

Fig. 1. Distribution of the minimum inhibitory concentrations (MICs) of amikacin and kanamycin for the 58 *Enterobacter cloacae* isolates harboring the *aac(6′)-Ib* gene*.

Black bars, amikacin; hatched bars, kanamycin.

*C1LSI-recommended MIC breakpoints for amikacin and kanamycin (S ≤ /I/R ≥) are 16/32/64, and the corresponding EUCAST breakpoints for amikacin (S ≤ /R >) are 8/16.

Abbreviation: EUCAST; European Committee on Antimicrobial Susceptibility Testing.

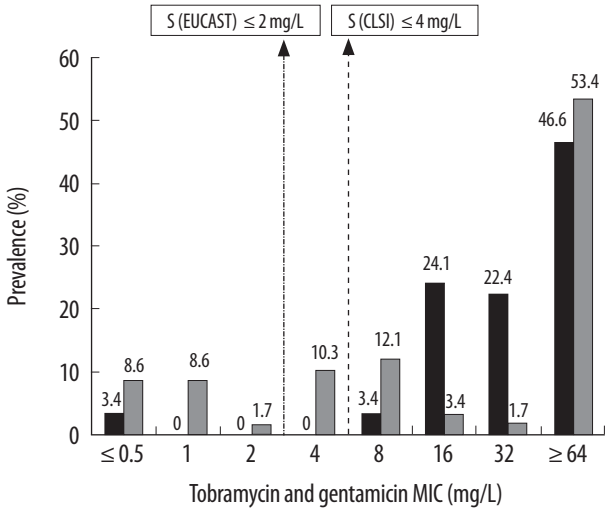

Fig. 2. Distribution of the minimum inhibitory concentrations (MICs) of tobramycin and gentamicin for the 58 *Enterobacter cloacae* isolates harboring the *aac(6′)-Ib* gene*.

Black bars, tobramycin; hatched bars, gentamicin.

*CLSI-recommended MIC breakpoints for tobramycin and gentamycin (S ≤ /I/R ≥) are 4/8/16, and the corresponding EUCAST breakpoints (S ≤ /R >) are 2/4.

Abbreviation: EUCAST; European Committee on Antimicrobial Susceptibility Testing.

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crobiology laboratories. However, clonal relatedness was observed among isolates from same hospitals: 2 out of 8, 2 out of 4, 2 out of 6, 3 and 2 out of 7, 2 out of 6, 2 and 2 out of 8, 4 out of 5, and 2 out of 2 isolates collected from 8 clinical microbiology laboratories. The isolates belonging to the same clone revealed identical PFGE pattern, but one isolate from hospital GM was related. We observed that 19 isolates belonging to 8 different clones were susceptible to amikacin (MIC ≤ 16 mg/L) and had different MICs for other aminoglycosides. However, 4 isolates belonging to the remaining 2 clones were resistant to all the tested aminoglycosides (MIC for amikacin and kanamycin, ≥256 mg/L; MIC for tobramycin and gentamicin, ≥64 mg/L) (data not shown).

A very high percentage of the *aac(6´)-Ib*-harboring isolates were susceptible to amikacin (84.5% and 55.2% according to the CLSI and EUCAST breakpoints, respectively). However, among the isolates that were shown to be amikacin-susceptible according to the CLSI and EUCAST criteria, the phenotypes of only 30.6% (15/49) and 18.8% (6/32) isolates, respectively, could be revised according to the EUCAST expert rules because many of these amikacin-susceptible isolates were resistant to gentamicin. The gentamicin resistance may be because of gentamicin-modifying enzymes such as AAC(3)-I, AAC(3)-VI, AAC(2´)-I, AAC(3)-IV, Ant (2´)-I, and ACC(3)-II or impermeability. Since only 2 isolates had mutations associated with the loss of amikacin resistance, we think that the remaining isolates might have produced low-levels of AAC(6´)-Ib. Low levels of AAC(6´)-Ib could not confer resistance to amikacin and isepamicin in vitro but were able to efficiently modify the small amounts of drug entering the bacterial cell and thereby conferred resistance in vivo. Therefore, a previous study did not report any noticeable difference in the in vivo efficacies of amikacin or isepamicin for low- and high-level AAC(6´)-Ib-producing organisms [11].

Our results show that the MICs of amikacin for many AAC(6´)-Ib-producing isolates were below the susceptibility breakpoints recommended by the CLSI or EUCAST and that most of these susceptible isolates could not be considered to have intermediate amikacin resistance according to the above-mentioned EUCAST expert rules. Data on the epidemiology and optimal therapy of infections caused by these strains are scarce; therefore, further studies are needed to characterize these infections.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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