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 amikacin-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')-Ib 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'-TTTGTGATCTCTATGCGGCTA-3' and 5'-CTCGTATGCGCTGGGATCT-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
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 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. Findings of PFGE showed 10 different clones and no clonal relatedness among isolates from different clinical microenvironments.

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

| Pheno-type | Amikacin N (%) | Kanamycin N (%) | Tobramycin N (%) | Gentamicin N (%) |
|------------|----------------|----------------|-----------------|-----------------|
| S          | 49 (84.5)      | 2 (3.4)        | 2 (3.4)         | 17 (29.3)       |
| I          | 1 (1.7)        | 3 (5.2)        | 0               | 7 (12.1)        |
| R          | 8 (13.8)       | 53 (91.4)      | 56 (96.6)       | 34 (58.6)       |

*CLSI-recommended MIC breakpoints for amikacin and kanamycin (S ≤ /I/R ≥ ) are 16/32/64, 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.

![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.](http://dx.doi.org/10.3343/kjlm.2011.31.4.279)  
**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.  
*CLSI-recommended MIC breakpoints for amikacin and kanamycin (S ≤ /I/R ≥ ) are 16/32/64, and the corresponding EUCAST breakpoints for amikacin (S ≤ /I/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.](http://dx.doi.org/10.3343/kjlm.2011.31.4.279)  
**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 gentamicin (S ≤ /I/R ≥ ) are 4/8/16, and the corresponding EUCAST breakpoints (S ≤ /I/R ≥ ) are 2/4. Abbreviation: EUCAST; European Committee on Antimicrobial Susceptibility Testing.
Amikacin Susceptibility of AAC(6\')-Ib Producers

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REFERENCES

1. Neonakis I, Gikas A, Scoulia E, Manios A, Georgiladakis A, Tselenitis Y. Evolution of aminoglycoside resistance phenotypes of four gram-negative bacteria: a 8-year survey in a university hospital in Greece. Int J Antimicrob Agents 2003;22:526-31.

2. Kim JY, Park YJ, Kwon HJ, Han K, Kang MW, Woo GJ. Occurrence and mechanisms of amikacin resistance and its association with \(\beta\)-lactamases in Pseudomonas aeruginosa: a Korean nationwide study. J Antimicrob Chemother 2008;62:479-83.

3. Sabitcheva S, Kaku M, Saga T, Ishii Y, Kantardjiev T. High prevalence of the aac(6\')-Ib-cr gene and its dissemination among Enterobacteriaceae isolates by CTX-M-15 plasmids in Bulgaria. Antimicrob Agents Chemother 2009;53:335-6.

4. Kim SY, Park YJ, Yu JK, Kim YS, Han K. Prevalence and characteristics of aac(6\')-Ib-cr in AmpC-producing Enterobacter cloacae, Citrobacter freundii, and Serratia marcescens: a multicenter study from Korea. Diagn Microbiol Infect Dis 2009;63:314-8.

5. Casin I, Bordon F, Bertin P, Coutrot A, Podglajan I, Brasseur R, et al. Aminoglycoside 6\'-N-acetyltransferase variants of the Ib type with altered substrate profile in clinical isolates of Enterobacter cloacae and Citrobacter freundii. Antimicrob Agents Chemother 1998;42:209-15.

6. Rather PN, Munayyer H, Mann PA, Hare RS, Miller GH, Shaw KJ. Genetic analysis of bacterial acetyltransferases: identification of amino acids determining the specificities of the aminoglycoside 6\'-N-acetyltransferase Ib and Ia proteins. J Bacteriol 1992;174:3196-203.

7. Shimara A, Weisnetel N, Dery KJ, Chavideh R, Tolmasky ME. Systematic analysis of a conserved region of the aminoglycoside 6\'-N-acetyltransferase type Ib. Antimicrob Agents Chemother 2001;45:3287-92.

8. Park CH, Robicsek A, Jacoby GA, Sahm D, Hooper DC. Prevalence in the United States of aac(6\')-Ib-cr encoding a ciprofloxacin-modifying enzyme. Antimicrob Agents Chemother 2006;50:3953-5.

9. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; Nineteenth informational supplement, M100-S19. Wayne, PA: Clinical and Laboratory Standards Institute, 2009.

10. Tenover FC, Arbet RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol 1995;33:2233-9.

11. Caulin E, Coutrot A, Carbon C, Collatz E. Resistance to amikacin and isepamicin in rabbits with experimental endocarditis of an aac(6\')-Ib-bearing strain of Klebsiella pneumoniae susceptible in vitro. Antimicrob Agents Chemother 1996;40:2848-53.