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# Prevalence of *aac(6′)-Ib-cr* Encoding a Ciprofloxacin-Modifying Enzyme among *Enterobacteriaceae* Blood Isolates in Korea▽

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**The *aac(6′)-Ib* gene was detected in 86 of 555 (15.5%) *Enterobacteriaceae* isolates. Among these 86 *aac(6′)-Ib*-positive isolates, 19 (22.0%) were positive for *aac(6′)-Ib-cr*: 4 of 31 (12.9%) *Enterobacter* spp., 7 of 13 (53.8%) *Escherichia coli* isolates, and 8 of 42 (19.0%) *Klebsiella pneumoniae* isolates. There was a strong association between *aac(6′)-Ib-cr* and OXA-1 and CTX-M-1 group β-lactamase genes. One *aac(6′)-Ib*-positive *K. pneumoniae* isolate carried both *aac(6′)-Ib-cr* and *qnrS*.**

Plasmid-mediated quinolone resistance was first identified in a clinical isolate of *Klebsiella pneumoniae* (7, 12). Recently, a new mechanism of quinolone resistance was identified: transfer from species to species of a plasmid encoding *aac(6′)-Ib-cr*, a variant of aminoglycoside acetyltransferase that confers reduced susceptibility to ciprofloxacin and norfloxacin by N-acetylation of the amino nitrogen on its piperazinyl substituent (13). Genes responsible for plasmid-mediated quinolone resistance are thought to be linked to extended-spectrum β-lactamase genes (2, 6).

In Korea, Qnr determinants from *Enterobacteriaceae* have been reported (4, 8), but the presence of *aac(6′)-Ib-cr* has not been reported. We therefore assessed the prevalence of *aac(6′)-Ib-cr* genes among clinical isolates of *Enterobacteriaceae* in Korea.

During the period from January 2005 to December 2006, 555 nonduplicate enterobacterial isolates were collected from blood cultures at Asan Medical Center, a 2,200-bed tertiary care teaching hospital in Seoul, Korea. Screening for *aac(6′)-Ib* was carried out by PCR amplification with the specific primers 5′-TTGCGATGCTCTATGAGTGGCTA-3′ and 5′-CTCGAATGCCTGGCGTGTTT-3′, to produce a 482-bp product (9). Of the 555 *Enterobacteriaceae* clinical isolates, 86 (15.5%) were positive for the *aac(6′)-Ib* gene: 31 of 149 (20.8%) *Enterobacter* spp., 13 of 204 (6.4%) *Escherichia coli* isolates, and 42 of 202 (20.8%) *Klebsiella pneumoniae* isolates. Following digestion of the amplified products with BtsCI, we found that 19 of the 86 (22.0%) *aac(6′)-Ib*-positive isolates were positive for *aac(6′)-Ib-cr*: 4 of 31 (12.9%) *Enterobacter* spp. isolates, 7 of 13 (53.8%) *E. coli* isolates, and 8 of 42 (19.0%) *K. pneumoniae* isolates. The rate of *aac(6′)-Ib-cr* among *aac(6′)-Ib*-positive *E. coli* was higher than in *aac(6′)-Ib*-positive *Enterobacter* spp. and *K. pneumoniae* isolates. Random amplified polymorphic DNA analysis was performed by using a 254-decamer primer (5′-CCGCAGCCAA) to assess the clonal diversity (1). The 19 isolates gave 11 different patterns: 2 in *Enterobacter cloacae*, 3 in *E. coli*, and 6 in *K. pneumoniae* (Table 1).

We also assessed whether these *Enterobacteriaceae* clinical isolates possessed the three *qnr* genes by PCR, as described previously (14). Ten *Enterobacter* spp. were positive for *qnrA*; 3 *Enterobacter* spp. and 8 *K. pneumoniae* isolates were positive for *qnrB*; and 1 *Enterobacter* spp., 1 *E. coli* isolate, and 4 *K. pneumoniae* isolates were positive for *qnrS*. One or more *qnr* genes were present in 27 of the 555 (4.9%) isolates: 14 of 149 *Enterobacter* spp. (9.4%), 1 of 204 *E. coli* isolates (0.5%), and 12 of 202 *K. pneumoniae* isolates (5.9%). The rate of *qnr* carriage among *Enterobacter* spp. was higher than in *E. coli* and *K. pneumoniae* isolates. One *K. pneumoniae* isolate (no. 135) contained both the *aac(6′)-Ib-cr* and *qnrS* genes; to our knowledge, this is the first such finding in a clinical isolate from Korea. The genetic structure between the *aac(6′)-Ib-cr* and *qnrS* genes in the *K. pneumoniae* no. 135 clinical isolate was determined by sequencing on plasmid DNA. Results showed that the β-lactamase-encoding genes $bla_{\text{OXA-1}}$, $bla_{\text{CTX-M-15}}$, and $bla_{\text{TEM-1}}$ were detected between the *aac(6′)-Ib-cr* and *qnrS* genes, along with other genes (Fig. 1).

In *Enterobacteriaceae* isolates, *aac(6′)-Ib-cr* is linked to the extended-spectrum β-lactamase genes (2, 6). Using PCR and DNA sequencing as described previously (3, 5, 10), we determined whether β-lactamase genes were present in our isolates and analyzed whether they were TEM, SHV, CTX-M, or OXA types (Table 1). All of the *aac(6′)-Ib-cr*-positive *E. cloacae* isolates produced CTX-M-3 and OXA-1. Most of the *aac(6′)-Ib-cr*-positive *E. coli* and *K. pneumoniae* isolates produced CTX-M-15 and OXA-1, except for *K. pneumoniae* no. 202, which produced CTX-M-3 and OXA-1. Some isolates also produced TEM-1 as well as CTX-M-15 and OXA-1. One *aac(6′)-Ib-cr*-positive *K. pneumoniae* isolate (no. 110) produced only OXA-1. These results showed that *aac(6′)-Ib-cr* is simultaneously associated with OXA-1 and CTX-M-1 (CTX-M-3 or CTX-M-15). No SHV-type gene was detected in any of the *aac(6′)-Ib-cr*-positive *E. cloacae* or *E. coli* isolates.

The transferability of the *aac(6′)-Ib-cr* gene was determined

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TABLE 1. Characteristics of *aac(6′)-Ib-cr-positive* isolates

| Species and isolate | CIP MIC (μg/ml) | Mutation(s) in QRDRs | | RAPD pattern[a] | β-Lactamases | Resistance to antibiotics[b] |
|---|---|---|---|---|---|---|
| | | *gyrA* | *parC* | | | |
| *E. cloacae* | | | | | | |
| 37 | 4 | Ser83Ile | Ser80Arg | I | CTX-M-3, OXA-1 | TOB, GEN, CTX, SXT |
| 101 | 8 | Ser83Ile | Ser80Ile | II | CTX-M-3, OXA-1 | TOB, GEN, CTX, CAZ, SXT |
| 153 | 8 | Ser83Ile | Ser80Ile | II | CTX-M-3, OXA-1 | TOB, GEN, CTX, CAZ, SXT |
| 165 | 8 | Ser83Ile | Ser80Ile | II | CTX-M-3, OXA-1 | CTX, CAZ, SXT |
| *E. coli* | | | | | | |
| 17 | >16 | Ser83Leu, Asp87Asn | Ser80Ile, Glu84Val | III | CTX-M-15, OXA-1, TEM-1 | TOB, GEN, CTX, CAZ, SXT |
| 24 | >16 | Ser83Leu, Asp87Asn | Ser80Ile, Glu84Val | III | CTX-M-15, OXA-1, TEM-1 | TOB, CTX, CAZ |
| 47 | >16 | Ser83Leu, Asp87Asn | Ser80Ile | IV | CTX-M-15, OXA-1 | TOB, GEN, CTX, CAZ, SXT |
| 48 | >16 | Ser83Leu, Asp87Asn | Ser80Ile | V | CTX-M-15, OXA-1 | TOB, CTX, CAZ |
| 125 | >16 | Ser83Leu, Asp87Asn | Ser80Ile, Glu84Val | III | CTX-M-15, OXA-1, TEM-1 | TOB, GEN, CTX, CAZ |
| 130 | >16 | Ser83Leu, Asp87Asn | Ser80Ile | IV | CTX-M-15, OXA-1 | TOB, CTX, CAZ |
| 189 | >16 | Ser83Leu, Asp87Asn | Ser80Ile, Glu84Val | III | CTX-M-15, OXA-1, TEM-1 | TOB, GEN, CTX, CAZ |
| *K. pneumoniae* | | | | | | |
| 78 | 1 | Ser83Tyr | | VI | CTX-M-15, OXA-1, SHV | TOB, GEN, CTX, CAZ, SXT |
| 80 | 1 | Ser83Tyr | | VI | CTX-M-15, OXA-1, SHV | TOB, GEN, CTX, CAZ, SXT |
| 110 | >16 | Ser83Ile | Ser80Ile | VII | OXA-1, SHV | TOB, GEN, CAZ, SXT |
| 132 | 0.125 | | | VIII | CTX-M-15, OXA-1, SHV | TOB, GEN, CTX, CAZ |
| 135 | >16 | Ser83Ile | Ser80Ile | IX | CTX-M-15, OXA-1, TEM-1, SHV | TOB, GEN, CTX, CAZ, SXT |
| 185 | 1 | Ser83Tyr | | X | CTX-M-15, OXA-1, SHV | TOB, GEN, CTX, SXT |
| 194 | 16 | Ser83Tyr | | VI | CTX-M-15, OXA-1, SHV | TOB, GEN, CTX, CAZ, SXT |
| 202 | >16 | Ser83Ile | Ser80Ile | XI | CTX-M-3, OXA-1, SHV | TOB, GEN, CTX, CAZ |

[a] RAPD, randomly amplified polymorphic DNA.
[b] CIP, ciprofloxacin; TOB, tobramycin; GEN, gentamicin; CTX, cefotaxime; CAZ, ceftazidime; SXT, trimethoprim-sulfamethoxazole.

by conjugation experiments using azide-resistant *E. coli* J53 Azi$^r$ as the recipient. The *aac(6′)-Ib-cr* gene was successfully transferred from eight isolates, and its presence was confirmed in all eight transconjugants by PCR (Table 2). Most of the *aac(6′)-Ib-cr*-positive isolates were resistant to gentamicin, tobramycin, and nalidixic acid, as well as ciprofloxacin. The MICs of ciprofloxacin and norfloxacin against transconjugants were two- to fourfold higher than for the recipient *E. coli* J53, indicating that *aac(6′)-Ib-cr* contributed to the decrease in ciprofloxacin susceptibility. The transconjugant for the *aac(6′)-Ib-cr*-positive *K. pneumoniae* isolate 135, which carried both *aac(6′)-Ib-cr* and *qnrS*, showed a 32-fold increase in MIC for ciprofloxacin (1 μg/ml), the clinical breakpoint for susceptibility, compared with the MIC shown by the recipient *E. coli* J53. Thus, when both the *aac(6′)-Ib-cr* and *qnr* genes are present in the same cells, the level of resistance is much higher than that conferred by *aac(6′)-Ib-cr* alone.

Almost all *aac(6′)-Ib-cr*-positive isolates contained a CTX-M-1 group β-lactamase gene, except for *K. pneumoniae* no. 110, which expressed only OXA-1. The cefotaxime MICs for such CTX-M-1-producing isolates were higher than those of ceftazidime, and this result was also found in their transconjugants (Table 2). The MICs of ceftazidime and cefotaxime for isolates producing CTX-M-3 were lower than those for isolates producing CTX-M-15 (Table 2). One amino acid difference at position 240 in CTX-M-15 was found to confer increased catalytic activity compared to that of CTX-M-3 (11).

The MICs of ciprofloxacin in *aac(6′)-Ib-cr*-positive isolates were much higher than those for the corresponding transconjugants, with MICs of 1 to ≥ 32 μg/ml, except for *K. pneumoniae* no. 110 (0.125 μg/ml). To determine if any target modification occurred in *aac(6′)-Ib-cr*-positive isolates, their quinolone resistance-determining regions (QRDRs) were sequenced. All *aac(6′)-Ib-cr*-positive isolates had point mutations in the QRDRs of the *gyrA* gene, at codon 83 and/or codon 87, except for *K. pneumoniae* no. 110, which did not have mutations in the QRDRs of the *gyrA* or *parC* genes. All *aac(6′)-Ib-cr*-positive *E. cloacae* and *E. coli* and three *aac(6′)-Ib-cr*-positive *K. pneumoniae* isolates had mutations in the QRDRs of the *parC* gene, at codon 80 and/or codon 84.

In conclusion, *aac(6′)-Ib-cr* was detected in three genera of *Enterobacteriaceae* (*E. cloacae* [four isolates], *E. coli* [seven

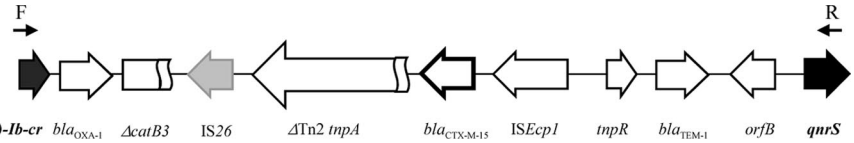


FIG. 1. Genetic organization of the 13-kb region between the *aac(6′)-Ib-cr* and *qnrS* genes in the *K. pneumoniae* no. 135 clinical isolate. The genes and their transcription orientations are represented by horizontal arrows. For sequencing of plasmid DNA, PCR was done with primers F (5′-TTGCGATGCTCTATGAGTGGCTA-3′) and R (5′-TAAATTGGCACCCTGTAGGC-3′). Both strands of the PCR product were used for DNA sequencing.

TABLE 2. Susceptibility profiles of *aac(6′)-Ib-cr-positive* isolates and their transconjugants

| Isolate[a] | MIC (μg/ml)[b] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | CIP | NOR | MXF | NAL | TOB | GEN | CAZ | CTX |
| *E. cloacae* 37 | 4 | >16 | 2 | >256 | >16 | >32 | 8 | 128 |
| Tc Ecl 37 | 0.06 | 0.25 | 0.06 | 8 | >16 | >32 | 2 | 64 |
| *E. cloacae* 101 | 8 | >16 | 4 | >256 | >16 | >32 | >32 | 256 |
| Tc Ecl 101 | 0.06 | 0.25 | 0.06 | 8 | 2 | 0.25 | 2 | 32 |
| *E. cloacae* 153 | 8 | >16 | 4 | >256 | >16 | >32 | >32 | 256 |
| Tc Ecl 153 | 0.06 | 0.25 | 0.06 | 8 | >16 | >32 | 1 | 32 |
| *E. coli* 17 | >16 | >16 | >16 | >256 | >16 | >32 | 32 | 256 |
| Tc Eco 17 | 0.06 | 0.25 | 0.06 | 8 | 16 | 32 | 16 | 256 |
| *E. coli* 48 | >16 | >16 | 16 | >256 | 16 | 0.25 | >32 | >256 |
| Tc Eco 48 | 0.06 | 0.25 | 0.06 | 8 | 8 | 0.25 | >32 | >256 |
| *K. pneumoniae* 132 | 0.125 | 0.5 | 0.125 | 8 | >16 | >32 | 32 | >256 |
| Tc Kp 132 | 0.03 | 0.125 | 0.06 | 8 | 16 | 32 | 16 | >256 |
| *K. pneumoniae* 135 | >16 | >16 | >16 | >256 | >16 | >32 | >32 | 256 |
| Tc Kp 135 | 1 | 8 | 1 | 32 | >16 | >32 | >32 | 256 |
| *K. pneumoniae* 202 | >16 | >16 | 16 | >256 | >16 | >32 | >32 | >256 |
| Tc Kp 202 | 0.03 | 0.125 | 0.06 | 8 | >16 | >32 | 1 | 16 |
| J53, recipient | 0.016 | 0.06 | 0.06 | 8 | 0.25 | 0.25 | 0.25 | 0.06 |

[a] Tc, transconjugant.
[b] CIP, ciprofloxacin; NOR, norfloxacin; MXF, moxifloxacin; NAL, nalidixic acid; TOB, tobramycin; GEN, gentamicin; CAZ, ceftazidime; CTX, cefotaxime.

isolates], and *K. pneumoniae* [eight isolates]), indicating horizontal transfer among the *Enterobacteriaceae*. The *aac(6′)-Ib-cr* gene showed a high association with β-lactamase genes, including OXA-1, CTX-M-3 or -15, and TEM-1, in isolates from Korea.

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