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High Prevalence of the *aac(6′)-Ib-cr* Gene and Its Dissemination among *Enterobacteriaceae* Isolates by CTX-M-15 Plasmids in Bulgaria▽

Since 1998, three mechanisms of plasmid-mediated quinolone resistance (PMQR) have been reported: Qnr-mediated topoisomerase protection (6), enzymatic modification of ciprofloxacin and norfloxacin by the aminoglycoside acetyltransferase AAC(6′)-Ib-cr (10), and active efflux due to QepA (8). PMQR genes confer low-level quinolone resistance and are frequently cotransmitted with extended-spectrum β-lactamase (ESBL) genes (9).

We report the prevalence of *aac(6′)-Ib-cr* and its association with *qnr* genes in ESBL-producing *Enterobacteriaceae* isolates in a Bulgarian hospital.

A total of 163 ESBL-producing enterobacteria (4.6% of 3,516 consecutive isolates) were recovered among 10 species at increasing overall prevalence rates between 2000 and 2005 (Table 1). These increases reflected the increasing rates of ESBL production in *Escherichia coli* (from 1.2% in 2000 to 10.0% in 2005), whereas similar rates of ~7% in *Klebsiella pneumoniae* and the irregular appearance of ESBL production in other species have been observed during the study period. As shown in Table 1, using primers 5′-ACTGAGCATGACC TTGCGATGC-3′ and 5′-TTAGGCATCACTGCGTGTTCG-3′, *aac(6′)-Ib* was detected in 99 (60.7%) of the ESBL producers distributed among nine species. Of these, 52 (52.5%) were found to carry the *cr* variant by sequencing, including 2 *Citrobacter freundii* isolates; one isolate each of *Enterobacter aerogenes*, *Morganella morganii*, and *K. pneumoniae* (all from 2005); and 47 *E. coli* isolates recovered since 2002, increasing from 0% to 67% during that period. For seven *C. freundii* isolates, including the two *aac(6′)-Ib-cr*-positive isolates, PCR for *qnrA*, *qnrB*, and *qnrS* (11, 12) yielded amplicons only for *qnrB*, identified as *qnrB10* (GenBank accession number DQ631414), *qnrB13* (GenBank accession number EU273755), and *qnrB18* (GenBank accession number AM919399) by using sequencing (1). The overall prevalence of *qnrB* (7/163; 4.3%) was sevenfold lower than that of *aac(6′)-Ib-cr* (52/163, 31.9%), and the coexpression of QnrB and AAC(6′)-Ib-cr occurred only in two (1.4% of all) isolates.

The 52 *aac(6′)-Ib-cr*-positive isolates were characterized by antibiotic susceptibility testing and *bla* content determination as previously described (3, 13). Forty-three isolates were resistant to ciprofloxacin, 41 to gentamicin, and 25 to trimethoprim-sulfamethoxazole. All isolates were resistant to tobramycin and exhibited reduced susceptibility to amikacin, but worrisomely, 39 (75%) of the isolates were classified as amikacin susceptible according to CLSI breakpoints. Fifty isolates had both $bla_{\text{CTX-M-15}}$ and $bla_{\text{OXA-1}}$. Of these, 2 isolates also carried $bla_{\text{SHV-12}}$ and 32 carried $bla_{\text{TEM-1}}$. The remaining two *aac(6′)-Ib-cr*-positive isolates expressed ESBLs not of the TEM, SHV, CTX-M, VEB, PER, or GES type, associated with the TEM-1 enzyme.

Thirty different XbaI–pulsed-field gel electrophoresis patterns were observed among the 47 *E. coli* isolates (data not shown). These findings suggest that the high prevalence of *aac(6′)-Ib-cr* was not solely due to the spread of a specific *E. coli* clone. Transferability of AAC(6′)-Ib-cr determinant in broth and on filters was examined using rifampin-resistant recipient *E. coli* ML4909 (F^- *galK2 galT22 hsdR metB1 relA supE44* Rif^r) (4). Transconjugants were selected on bromothymol blue lactose agar containing rifampin (200 μg/ml) and kanamycin (25 μg/ml). Conjugative transfer of *aac(6′)-Ib-cr* was achieved for 42 of the 52 isolates, including one isolate each of *K. pneumoniae* and *M. morganii*, two *C. freundii* isolates, and 38 *E. coli* isolates. *aac(6′)-Ib-cr* was mostly cotransferred with $bla_{\text{CTX-M-15}}$ and $bla_{\text{OXA-1}}$, variably with $bla_{\text{TEM-1}}$, but not with $bla_{\text{SHV-12}}$ and *qnrB*.

This is the first report of *qnrB* and *aac(6′)-Ib-cr* in clinical *Enterobacteriaceae* isolates from a Bulgarian hospital. The *aac(6′)-Ib* and its *cr* variant were highly prevalent in ESBL-producing *E. coli*. CTX-M-15 plasmid-mediated dissemination of *aac(6′)-Ib-cr* among *Enterobacteriaceae* isolates was particularly observed, as has been found in other countries (2, 5). In this work, *qnrB* had a low prevalence and was not cotransferred with the *aac(6′)-Ib-cr* gene. This result supports previous findings suggesting that *aac(6′)-Ib-cr* might already be widespread

TABLE 1. Distribution of *aac(6′)-Ib-cr* and *qnrB* genes among 163 ESBL-producing enterobacterial isolates at the National Oncology Center, Sofia, Bulgaria, from 2000 to 2005 and the respective ESBL prevalence rates

Species	No. of isolates with indicated *aac(6′)-Ib* variant and *qnrB*/total no. of ESBL-positive isolates (%)[a]													
	2000		2001		2002			2003		2004		2005		
	cr	Any	*cr*	Any	*cr*	Any	*qnrB*[c]	*cr*	Any	*cr*	Any	*cr*	Any	*qnrB*[d]
E. coli	0/3	2/3 (67)	0/8	5/8 (63)	3/10 (30)	7/10 (70)		9/16 (56)	14/16 (88)	16/24 (67)	16/24 (67)	19/33 (58)	21/33 (64)	
K. pneumoniae	0/4	1/4 (25)	0/5	4/5 (80)	0/2	0/2		0/18	14/18 (78)	0/5	1/5 (20)	1/6 (17)	1/6 (17)	
C. freundii	0/0	0/0	0/0	0/0	0/5	5/5	5/5 (100)	0/0	0/0	0/1	0/1	2/4 (50)	2/4 (50)	2/4 (50)
M. morganii	0/0	0/0	0/0	0/0	0/0	0/0		0/0	0/0	0/0	0/0	1/1 (100)	1/1 (100)	
E. aerogenes	0/0	0/0	0/0	0/0	0/0	0/0		0/0	0/0	0/0	0/0	1/1 (100)	1/1 (100)	
Other[b]	0/1	1/1 (100)	0/1	0/1	0/2	2/2 (100)		0/0	0/0	0/3	0/3	0/10	1/10 (10)	
Total	0/8	4/8 (50)	0/14	9/14 (64)	3/19 (16)	14/19 (74)		9/34 (26)	28/34 (82)	16/33 (48)	17/33 (52)	24/55 (44)	27/55 (49)	

[a] The numbers of ESBL-positive isolates/total numbers of isolates are as follows: for 2000, 8/461 (1.7%); for 2001, 14/553 (2.5%); for 2002, 19/646 (2.9%); for 2003, 34/546 (6.2%); for 2004, 33/674 (4.9%); and for 2005, 55/636 (8.6%).

[b] This category included one isolate each of *Providencia rettgeri* (recovered in 2000) and *Escherichia hermannii* (recovered in 2002), two *Klebsiella oxytoca* isolates (recovered in 2002 and 2005), six *Serratia marcescens* isolates (recovered in 2001, 2003, and 2005), and seven *Enterobacter cloacae* isolates (recovered in 2005). The four *aac(6′)-Ib*-positive isolates included one each of *P. rettgeri*, *E. hermannii*, *K. oxytoca*, and *E. cloacae*.

[c] Existence of *qnrB10* (two isolates) and *qnrB18* (three isolates) in *C. freundii*.

[d] Coexistence of *qnrB13* or *qnrB18* with *aac(6′)-Ib-cr* in *C. freundii* isolates.

and substantially more prevalent than *qnr* genes (7, 10). Most of the isolates carrying the *aac(6′)-Ib-cr* variant were resistant to ciprofloxacin, probably reflecting its ability to promote higher-level quinolone resistance mutations (10).

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REFERENCES

1. **Jacoby, G., V. Cattoir, D. Hooper, L. Martínez-Martínez, P. Nordmann, A. Pascual, L. Poirel, and M. Wang.** 2008. *qnr* gene nomenclature. Antimicrob. Agents Chemother. **52:**2297–2299.
2. **Karisik, E., M. J. Ellington, R. Pike, R. E. Warren, D. M. Livermore, and N. Woodford.** 2006. Molecular characterization of plasmids encoding CTX-M-15 β-lactamases from *Escherichia coli* strains in the United Kingdom. J. Antimicrob. Chemother. **58:**665–668.
3. **Lee, S., Y. Park, M. Kim, H. K. Lee, K. Han, C. S. Kang, and M. W. Kang.** 2005. Prevalence of Ambler class A and D β-lactamases among clinical isolates of *Pseudomonas aeruginosa* in Korea. J. Antimicrob. Chemother. **56:**122–127.
4. **Ma, L., Y. Ishii, M. Ishiguro, H. Matsuzawa, and K. Yamaguchi.** 1998. Cloning and sequencing of the gene encoding Toho-2, a class A β-lactamase preferentially inhibited by tazobactam. Antimicrob. Agents Chemother. **42:** 1181–1186.
5. **Machado, E., T. M. Coque, R. Cantón, F. Baquero, J. C. Sousa, L. Peixe, and the Portuguese Resistance Study Group.** 2006. Dissemination in Portugal of CTX-M-15-, OXA-1-, and TEM-1-producing *Enterobacteriaceae* strains containing the *aac(6′)-Ib-cr* gene, which encodes an aminoglycoside- and fluoroquinolone-modifying enzyme. Antimicrob. Agents Chemother. **50:**3220–3221.
6. **Martinez-Martinez, L., A. Pascual, and G. A. Jacoby.** 1998. Quinolone resistance from a transferable plasmid. Lancet **351:**797–799.
7. **Park, C. H., A. Robicsek, G. A. Jacoby, D. Sahm, and D. C. Hooper.** 2006. Prevalence in the United States of *aac(6′)-Ib-cr* encoding a ciprofloxacin-modifying enzyme. Antimicrob. Agents Chemother. **50:**3953–3955.
8. **Périchon, B., P. Courvalin, and M. Galimand.** 2007. Transferable resistance to aminoglycosides by methylation of G1405 in 16S rRNA and to hydrophilic fluoroquinolones by QepA-mediated efflux in *Escherichia coli*. Antimicrob. Agents Chemother. **51:**2464–2469.
9. **Robicsek, A., G. A. Jacoby, and D. C. Hooper.** 2006. The worldwide emergence of plasmid-mediated quinolone resistance. Lancet Infect. Dis. **6:**629–640.
10. **Robicsek, A., J. Strahilevitz, G. A. Jacoby, M. Macielag, D. Abbanat, K. Bush, and D. C. Hooper.** 2006. Fluoroquinolone modifying enzyme: a novel adaptation of a common aminoglycoside acetyltransferase. Nat. Med. **12:**83–88.
11. **Robicsek, A., J. Strahilevitz, D. F. Sahm, G. A. Jacoby, and D. C. Hooper.** 2006. *qnr* prevalence in ceftazidime-resistant *Enterobacteriaceae* isolates from the United States. Antimicrob. Agents Chemother. **50:**2872–2874.
12. **Saga, T., T. Akasaka, H. Takase, M. Tanaka, K. Sato, and M. Kaku.** 2007. First detection of the plasmid-mediated quinolone resistance determinant *qnrA* in *Enterobacteriaceae* clinical isolates in Japan. Int. J. Antimicrob. Agents **29:**738–739.
13. **Saladin, M., V. T. Cao, T. Lambert, J. L. Donay, J. L. Herrmann, Z. Ould-Hocine, C. Verdet, F. Delisle, A. Philippon, and G. Arlet.** 2002. Diversity of CTX-M β-lactamases and their promoter regions from *Enterobacteriaceae* isolated in three Parisian hospitals. FEMS Microbiol. Lett. **209:**161–168.

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