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# Letters to the Editor

## Plasmid-Mediated 16S rRNA Methylases among Extended-Spectrum β-Lactamase-Producing *Enterobacteriaceae* Isolates▽

High levels of resistance to aminoglycosides conferred by plasmid-mediated mechanisms of resistance to aminoglycosides corresponding to 16S rRNA methylases have been reported since 2003 (3, 11). To date, six enzymes (ArmA, RmtA, RmtB, RmtC, RmtD, and NpmA) have been described, with ArmA being the most frequently identified methylase in *Enterobacteriaceae* (2). Associations between 16S rRNA methylase- and extended-spectrum β-lactamase (ESBL)-encoding genes such as the $bla_{\text{CTX-M}}$ genes have been reported (1, 7, 8). The aim of this study was to evaluate the prevalence of 16S rRNA methylase genes among ESBL-producing *Enterobacteriaceae* isolates recovered in a French university hospital.

A total of 373 nonduplicate ESBL-producing enterobacterial clinical isolates, collected at the Bicêtre hospital between January 2005 and December 2007, have been studied. Among them, 19 isolates (5.1%) were resistant to all clinically used aminoglycosides. That collection was screened by PCR for detection of 16S rRNA methylase-encoding genes (*armA*, *rmtA*, *rmtB*, *rmtC*, *rmtD*, and *npmA*), as previously described (2). Four *armA*-positive isolates were detected in 2005 ($n = 2$) and in 2007 ($n = 2$), in addition to a single *rmtB*-positive isolate from 2006. No isolate was positive for the *rmtA*, *rmtC*, *rmtD*, and *npmA* genes. The *armA*-positive isolates produced the ESBL CTX-M-3 ($n = 3$) or CTX-M-14 ($n = 1$), whereas the single *rmtB*-positive isolate was a CTX-M-14 producer. (CTX-M enzymes were searched for as described previously [6].) The low prevalence rate of 16S rRNA methylase determinants among ESBL producers (1.3%) observed here is similar to that reported from Taiwan (1.1%) (8) and higher than those reported from Belgium (0.12%) and Japan (0.03%) (1, 10), but lower than that from Korea (3%) (7).

All 16S rRNA methylase and ESBL determinants were transferred by conjugation and transformation using *Escherichia coli* J53 and TOP10 recipient strains after selection on Trypticase soy agar containing amikacin (50 μg/ml) and/or cefotaxime (8 μg/ml) (6). The 16S rRNA methylase-positive transformants expressed a high level of resistance to aminoglycosides (Table 1). In three isolates, the *armA* and $bla_{\text{CTX-M-3}}$ genes were located on a same plasmid belonging to the IncL/M incompatibility group, as observed on the plasmid pCTX-M-3 (5), whereas the 16S rRNA methylase determinants were not physically linked to the ESBL genes for the two $bla_{\text{CTX-M-14}}$-positive remaining isolates. Sequencing of the 16S rRNA methylase-encoding genes showed a perfect identity with previously reported genes. The complete PCR-mapped region of 11.6 kb containing the *armA* gene was entirely sequenced for two isolates, showing its association with IS*CR1* inside a *sul1*-type integron structure, the same configuration observed in Tn*1548* (4). The *rmtB* gene was located on an 11.4-kb region bracketed by two IS*26* elements containing *tnpA*, $bla_{\text{TEM-1}}$, *intI1*/*groEL*, and *qepA* and followed by an IS*CR3* element, as observed on pHPA (9). Analysis of sequences flanking more closely the *armA* gene identified two novel insertion sequence elements, IS*Ec28* and IS*Ec29*, belonging to the IS*5* and IS*4* families, respectively (http://www-is.biotoul.fr). No direct repeat sequence bracketing each of those IS elements and the overall IS*Ec28*-*armA*-IS*Ec29* structure was identified.

This study underlines that the association on the same plas-

TABLE 1. MICs of antibiotics for clinical isolates producing 16S rRNA methylases, their corresponding *armA*- and *rmtB*-positive transformants, and *E. coli* TOP10[a]

| Clinical isolate or transformant[b] | 16S rRNA methylase or/and ESBLs | MIC (μg/ml) of[c]: | | | | | | | | | Plasmid size (kb) | Incompatibility group plasmid type |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | AMK | NET | GEN | TOB | CTX | CAZ | IMP | NOR | CIP | | |
| *E coli* VOG | *rmtB* and $bla_{\text{CTX-M-14}}$ | >256 | >256 | >256 | >256 | >32 | 6 | 0.19 | >256 | >32 | 80, 50 | |
| *E. coli* TOP10(pVOG1) | *rmtB*[d] | >256 | >256 | >256 | >256 | 0.047 | 0.38 | 0.19 | 0.032 | 0.003 | 80 | F |
| *E. coli* TOP10(pVOG2) | $bla_{\text{CTX-M-14}}$[e] | 1.5 | 1.5 | 0.25 | 3 | >32 | 2 | 0.19 | <0.016 | <0.002 | 50 | ND[f] |
| *E coli* COP | *armA and* $bla_{\text{CTX-M-14}}$ | >256 | >256 | >256 | >256 | >32 | 1 | 0.19 | >256 | >32 | 60, 38 | |
| *E. coli* TOP10(pCOP1) | *armA* | >256 | >256 | >256 | >256 | 0.047 | 0.38 | 0.19 | <0.016 | <0.002 | 60 | F |
| *E. coli* TOP10(pCOP2) | $bla_{\text{CTX-M-14}}$ | 1 | 0.125 | 0.125 | 0.19 | 8 | 1 | 0.19 | <0.016 | <0.002 | 38 | I1 |
| *E coli* MEZ | *armA* and $bla_{\text{CTX-M-3}}$ | >256 | >256 | >256 | >256 | >32 | 3 | 0.19 | >256 | >32 | 120 | |
| *E. coli* TOP10(pMEZ) | *armA* and $bla_{\text{CTX-M-3}}$ | >256 | >256 | >256 | >256 | >32 | 3 | 0.19 | 0.016 | <0.002 | 120 | L/M |
| *Enterobacter cloacae* BEH | *armA* and $bla_{\text{CTX-M-3}}$ | >256 | >256 | >256 | >256 | >32 | 16 | 0.19 | 1 | 0.19 | 130 | |
| *E. coli* TOP10(pBEH) | *armA* and $bla_{\text{CTX-M-3}}$ | >256 | >256 | >256 | >256 | >32 | 8 | 0.19 | 0.016 | <0.002 | 130 | L/M |
| *Klebsiella pneumoniae* DU | *armA* and $bla_{\text{CTX-M-3}}$ | >256 | >256 | >256 | >256 | >32 | 2 | 0.19 | 0.75 | 0.125 | 120 | |
| *E. coli* TOP10(pDU) | *armA* and $bla_{\text{CTX-M-3}}$ | >256 | >256 | >256 | >256 | >32 | 2 | 0.19 | 0.016 | <0.002 | 120 | L/M |
| *E. coli* TOP10 | | 1 | 0.25 | 0.25 | 0.38 | 0.047 | 0.38 | 0.19 | <0.016 | <0.002 | | |

[a] MICs of antibiotics were determined by using the Etest technique according to the manufacturer's recommendations. Analysis of plasmid content in the *armA*- and *rmtB*-positive isolates and their transformants was performed by the Kieser technique (7). The incompatibility group of plasmids was determined as previously described (2).
[b] All clinical isolates were also resistant to sulfonamides, trimethoprim, chloramphenicol, tetracycline, and rifampin.
[c] Antibiotic-resistant marker abbreviations: AMK, amikacin; NET, netilmicin; GEN, gentamicin; TOB, tobramycin; CTX, cefotaxime; CAZ, ceftazidime; IMP, imipenem; NOR, norfloxacin; and CIP, ciprofloxacin.
[d] MICs for *E. coli* TOP10 transformants harboring pVOG1 expressing RmtB, TEM-1, and QepA.
[e] MICs for *E. coli* TOP10 transformants harboring pVOG2 expressing CTX-M-14 and AAC(6′)-Ib.
[f] ND, not determined.

mid of 16S rRNA methylase- and ESBL-encoding genes remains rare. However, their localization on conjugative plasmids, and in some cases in association on the same plasmid, as observed here with CTX-M determinants, has the potential to further expand the threat of multidrug-resistant *Enterobacteriaceae*.

**Nucleotide sequence accession numbers.** The nucleotide sequences of the regions surrounding *qepA1* and *rmtB* have been submitted to the GenBank nucleotide sequence database under accession no. FJ183463 and FJ187822, respectively.

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**Béatrice Berçot**
*Service de Bactériologie-Virologie*
*Hôpital Lariboisière*
*Assistance Publique/Hôpitaux de Paris*
*Université Paris VII*
*Paris, France*

**Laurent Poirel**
**Patrice Nordmann***
*Service de Bactériologie-Virologie*
*Hôpital de Bicêtre*
*78 rue du Général Leclerc*
*Le Kremlin-Bicêtre 94275, France*

*Phone: 33-1-45-21-36-32
Fax: 33-1-45-21-63-40
E-mail: nordmann.patrice@bct.aphp.fr

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