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Co-occurrence of aminoglycoside resistance gene *armA* in non-Typhi *Salmonella* isolates producing CTX-M-15 in Algeria

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Sir,

Aminoglycosides are clinically relevant antimicrobial agents often used in combination with β-lactams for the treatment of severe infections due to Gram-negative organisms. Resistance to aminoglycosides is due to numerous mechanisms. Among these, the production of 16S rRNA methylases corresponds to an emerging and threatening mechanism, since it leads to high-level resistance to almost all clinically relevant aminoglycosides. Seven types of 16S rRNA methylase genes (*armA*, *rmtA*, *rmtB*, *rmtC*, *rmtD*, *rmtE* and *npmA*) have been described so far, with *armA* as the most frequently identified methylase in Enterobacteriaceae.[1] The genes *armA* and *rmtC* have recently been detected among non-typhoidal *Salmonella* in several countries.[2–4]

The association of 16S rRNA methylases with the production of extended-spectrum β-lactamases (ESBLs), including the widespread CTX-M enzymes, has been reported often.[5]

The aim of our study was to investigate the prevalence of 16S rRNA methylase genes among ESBL-producing *Salmonella enterica* isolates that had been recovered in the region of Annaba, Algeria, from March 2008 to October 2009, and to evaluate the molecular epidemiology of these isolates.

During the 2 years, 67 non-duplicate *S. enterica* isolates recovered from community- and hospital-based patients with acute diarrhoea were collected from all three Annaba hospitals, and routinely analysed at the microbiology laboratory in Dorban hospital. All isolates were identified biochemically using the API-20E system (bioMérieux, Marcy-l'Étoile, France) and serotyped using the Kauffmann–White scheme. Antimicrobial susceptibility was assessed by disc diffusion, performed and interpreted according to CLSI guidelines.[6] Fifty out of the 67 strains were resistant to ampicillin, ticarcillin, piperacillin, cefalotin, third- and fourth-generation cephalosporins, trimethoprim, sulphonamides, and gentamicin. The 50 isolates remained susceptible to piperacillin/tazobactam, imipenem, both quinolones and fluoroquinolones, as well as colistin. Twenty-three of these 50 isolates were also resistant to kanamycin, 43 to tetracycline and 34 to chloramphenicol. The production of ESBLs was confirmed by the Etest® method using ceftazidime/ceftazidime plus clavulanate and cefotaxime/cefotaxime plus clavulanate strips (bioMérieux). In case of AmpC β-lactamase production, ESBL detection was performed by disc diffusion on Mueller–Hinton agar plates supplemented with 250 mg/L cloxacillin to inhibit cephalosporinase activity (AES-Chemunex, Bruz, France). This allowed the identification of 50 isolates expressing an ESBL phenotype and the *bla* genes were identified by PCR.

All of the 50 ESBL-producing *Salmonella* isolates were positive for the $bla_{\text{CTX-M}}$ gene. Among them, 43 and 35 isolates also harboured the bla_{CMY} and bla_{TEM} genes, respectively. Forty-two isolates belonged to the serotype Typhimurium, 4 isolates belonged to the serotype Enteritidis, and 4 isolates were monophasic or non-motile variants of serotype Typhimurium [*Salmonella* 4,12:-:1,2 (*n*=2), *Salmonella* 4,12:-:- (*n*=1), and *Salmonella* 4,12:i:- (*n*=1)].

Table 1. Phenotypic and genotypic characteristics of 18 *armA*-positive *S. enterica* isolates collected from all three Annaba hospitals in Algeria

Number of isolates	Identification	Resistance pattern	β-Lactamases	PFGE type
12	*Salmonella* Typhimurium	AMP AMC CEF FOX CTX CAZ FEP STR KAN GEN CHL SSS TMP TET	CTX-M-15+CMY-2+TEM	XB0077
1	*Salmonella* Typhimurium	AMP CEF FOX CTX CAZ FEP SSS TMP STR KAN GEN	CTX-M-15	XB0151
1	*Salmonella* 4,12:-:1,2	AMP AMC CEF FOX CTX CAZ FEP STR KAN GEN CHL SSS TMP TET	CTX-M-15+CMY-2+TEM	XB0077
2	*Salmonella* Enteritidis	AMP CEF FOX CTX CAZ FEP KAN GEN SSS TMP	CTX-M-15	XB0047
1	*Salmonella* Enteritidis	AMP CEF FOX CTX CAZ FEP KAN GEN SSS TMP	CTX-M-15	XB0033
1	*Salmonella* Enteritidis	AMP CEF FOX CTX CAZ FEP KAN GEN SSS TMP	CTX-M-15	XB0046

AMP, ampicillin; AMC, amoxicillin/clavulanate; CEF, cefalotin; FOX, cefoxitin; CTX, cefotaxime; CAZ, ceftazidime; FEP, cefepime; STR, streptomycin; KAN, kanamycin; GEN, gentamicin; CHL, chloramphenicol; SSS, sulphonamides; TMP, trimethoprim; TET, tetracycline.

Twenty-two out of these 50 ESBL-producing *Salmonella* isolates showed variable levels of resistance to the aminoglycosides tested (gentamicin, kanamycin and streptomycin) and were screened for detection of the *armA* methylase gene by PCR, as described by Gonzalez-Zorn *et al.*[7] The *armA* gene was detected in the isolates showing high-level resistance (no inhibition zone) to all of the aminoglycosides tested: *Salmonella* Typhimurium ($n=13$); and *Salmonella* 4,12:-:1,2 ($n=1$). The *armA* gene was also detected in four isolates of *Salmonella* Enteritidis displaying high-level resistance to gentamicin and kanamycin but susceptibility to streptomycin. No *armA* gene was detected among the four isolates displaying high-level resistance to gentamicin and streptomycin but susceptibility to kanamycin. Sequencing of the $bla_{CTX\text{-}M}$ genes from the 18 *armA*-positive isolates allowed identification of the CTX-M-15 determinant. Moreover, 13 of them co-produced CMY-2 (Table 1).

The conjugal transfer of the 16S rRNA methylase and ESBL determinants was performed with *Escherichia coli* J53 and nalidixic acid-resistant *E. coli* JM109 as recipient strains. Selection of the transconjugants was performed on agar plates containing 100 mg/L sodium azide supplemented with 50 mg/L amoxicillin, and on agar plates supplemented with 30 mg/L amikacin and 30 mg/L gentamicin. In each experiment, no strain highly resistant to aminoglycosides and to β-lactams was recovered after this selection.

The genetic diversity of the *armA*-positive isolates was analysed by PFGE using the standardized PulseNet protocol.[8] PFGE analysis of 12 *armA*-positive isolates of *Salmonella* Typhimurium revealed an identical pattern, suggesting the spread of an epidemic clone. The four *armA*-positive *Salmonella* Enteritidis isolates displayed three distinct PFGE patterns, most likely indicating horizontal spread of the resistance determinants (Table 1).

So far, enterobacterial isolates co-harbouring the $bla_{CTX\text{-}M\text{-}15}$ and *armA* genes have been identified as *Klebsiella pneumoniae*, *Klebsiella oxytoca* and *E. coli*.[5,9,10] Interestingly, in the study by Bogaerts *et al.*,[5] the patients seemed to have been transferred from Algeria. We report here the concomitant occurrence of these two genes among ESBL-producing *S. enterica* isolates that have been recovered in Algeria. In that country, the *armA* gene had been previously identified among CTX-M-3-producing *S. enterica* serotype Senftenberg isolates.[4]

The emergence of the *armA* methylase gene among CTX-M-15 ESBL-harbouring strains of *S. enterica* is a public health concern in Algeria. It certainly requires the implementation of a strict hospital infection control policy as well as the promotion of epidemiological surveys in the community in order to avoid the dissemination of such multiresistant strains.

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Transparency declarations

None to declare.

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In vitro susceptibility of *Actinobaculum schaalii* to mecillinam

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