(comprising 172 isolates) had peak profiles corresponding to \textit{bla\textsubscript{CTX-M-15}}-9 isolates representative of individual PFPs (comprising 78 isolates) had peak profiles corresponding to \textit{bla\textsubscript{CTX-M-14}}-2 isolates representative of individual PFPs (comprising 3 isolates) had peak profiles corresponding to \textit{bla\textsubscript{CTX-M-9}} and 1 isolate had a peak profile corresponding to \textit{bla\textsubscript{CTX-M-1}}.

The rapid dissemination of CTX-M-producing Enterobacteriaceae reported in a number of countries is a significant public health concern\textsuperscript{1,6,7}. In this study, members of CTX-M-group-1 and CTX-M-group-9-producing isolates were both common, accounting for 60% and 40% of isolates, respectively. This is in keeping with what has previously been observed in other European countries\textsuperscript{1,2,7}. CTX-M-14 accounts for most CTX-M-group-9 enzymes in Ireland. This is similar to the situation reported from Spain, where CTX-M-14 is the predominant group-9 CTX-M enzyme, but contrasts with the neighbouring UK where CTX-M-14 appears to be less common\textsuperscript{1,7}. CTX-M-15 is the predominant group 1 genotype, again in concordance with what has been observed in other European countries\textsuperscript{1,7}.

The dissemination of specific clones or clonal groups has been central to the dramatic increase in the prevalence of CTX-M \beta-lactamases in Europe and worldwide. In this study, six major clusters of related isolates comprising 50% of all CTX-M-producing \textit{E. coli} were defined by PFGE. Few healthcare institutions in Ireland have reported outbreaks of ESBL-PE transmission. These data show evidence of inter- and intra-hospital dissemination of clonal groups. We also identified the presence of the UK epidemic ‘strain A’, which has recently been confirmed as belonging to the internationally disseminated clone O25:H4-ST131\textsuperscript{5}.

The widespread dissemination of Enterobacteriaceae producing CTX-M-15 and CTX-M-14 in Ireland may reflect introduction via foreign travel, originally from areas where these genotypes are common, and subsequent significant local spread. Spread may be facilitated by levels of antimicrobial consumption. According to a recent European Surveillance of Antimicrobial Consumption report\textsuperscript{8}, the total outpatient use of antimicrobials in Ireland was 21.23 defined daily doses per 1000 inhabitants in 2006, representing mid- to high-range use compared with other European countries. Although there is recent evidence of increasing public policy emphasis on and public interest in the control of antimicrobial resistance, there is little evidence that measures taken to date have lead to improved control of the dissemination of ESBL-PE in the community and the principal routes of transmission in this setting have not been defined.

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Transparency declarations

None to declare.

Research letters

Supplementary data

Tables S1–S4 and Figure S1 are available as Supplementary data at JAC Online (http://jac.oxfordjournals.org).

References


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Plasmid-mediated 16S rRNA methylases among extended-spectrum-\beta-lactamase-producing \textit{Salmonella enterica} Senftenberg isolates from Algeria

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Keywords: ESBLs, nosocomial, CTX-M-3, ArmA

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Sir,

High levels of resistance to aminoglycosides conferred by plasmid-mediated mechanisms corresponding to 16S rRNA methylases have been reported since 2003. To date, six enzymes (ArmA, RmtA, RmtB, RmtC, RmtD and NpmA) are known, with ArmA being the most frequently identified methylase in Enterobacteriaceae. Associations between 16S rRNA methylase- and extended-spectrum β-lactamase (ESBL)-encoding genes such as the blaCTX-M genes have been reported. In 2004, an outbreak of CTX-M-producing Salmonella enterica serotype Senftenberg isolates associated with nosocomial diarrhea in newborns was described in the neonatology unit at the university hospital of Constantine, Algeria. These isolates remained susceptible to imipenem, ertapenem, cefoxitin, nalidixic acid, fluoroquinolones and colistin only.

The aim of this study was to evaluate the prevalence of 16S rRNA methylase genes among representative ESBL-producing S. enterica Senftenberg isolates resistant to aminoglycosides recovered in the neonatology ward from January 1982 to December 2005. In total, 12 non-duplicate ESBL-producing S. enterica serotype Senftenberg clinical isolates, expressing variable levels of resistance to aminoglycosides, were studied. The isolates were screened by PCR for detection of 16S rRNA methylase-encoding genes (armA, rmtA, rmtB, rmtC, rmtD and npmA), as previously described. Among them, one isolate from 1998 was resistant to all clinically used aminoglycosides and was found to be positive for armA. The other isolates were all negative for all of the tested 16S RNA methylase genes and presented variable levels of resistance to the clinically used aminoglycosides. PCR experiments with primers for detection of the Ambler class A blaTEM, blaSHV, blaPER-1/2, blaVEB-1, blaGES-1 and blaCTX-M genes, followed by sequencing, identified two β-lactamases in the armA-positive isolate: TEM-1 and the ESBL CTX-M-3.

The 16S rRNA methylase and ESBL determinant were co-transferred by conjugation and transformation using Escherichia coli J53 and TOP10 recipient strains after selection on trypticase soy agar containing amikacin (50 mg/L) and/or ticarcillin (50 mg/L). The 16S rRNA methylase-positive transformants and transconjugants displayed the same antimicrobial resistance profile and expressed a high level of resistance to aminoglycosides (Table 1). The armA and blaCTX-M-3 genes were located on the same plasmid belonging to the IncL/M incompatibility group, as observed for plasmid pCTX-M-3. Sequencing of the 16S rRNA methylase-encoding genes showed perfect identity with previously reported genes. PCR mapping of the region containing the armA gene showed its association with ISCR1 inside a sulI-type integron structure, the same configuration as observed in Tn5486.

This is the first report of an Arm-type 16S rRNA methylase enzyme in Algeria and from S. enterica serotype Senftenberg. This study demonstrates that the association on the same plasmid of 16S rRNA methylase- and CTX-M-3-encoding genes can be found in Salmonella isolates as early as 1998, i.e. 3 years before its initial discovery. Their localization on the same conjugative plasmid is worrying since it has the potential to further expand the threat of multidrug-resistant Salmonella. Spread of these aminoglycoside resistance determinants at the same level as blaCTX-M genes in Enterobacteriaceae may seriously compromise the efficacy of aminoglycosides for treating Gram-negative infections. A more extensive study is now required to investigate the prevalence of these methylases in Salmonella isolates from Algeria. The prevalence rate of 16S RNA methylase determinants among ESBL producers is still generally low, ranging from 0.03% in Japan to 3% in Korea, but may be as high as 10% in China.

This work further underlines that Salmonella may be a reservoir for ESBL genes but also for 16S rRNA methylase genes in the community, and that these strains may then be responsible for hospital-acquired infections.

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Table 1. MICs of antibiotics for a clinical isolate producing 16S rRNA methylases, and its corresponding armA-positive transformant and E. coli

<table>
<thead>
<tr>
<th>Bacterial isolates</th>
<th>AMK</th>
<th>NET</th>
<th>GEN</th>
<th>TOB</th>
<th>CTX</th>
<th>CAZ</th>
<th>IMP</th>
<th>CIP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella Senftenberg 98</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;32</td>
<td>3</td>
<td>0.19</td>
<td>0.003</td>
</tr>
<tr>
<td>E. coli (pS98)</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>16</td>
<td>1</td>
<td>0.19</td>
<td>0.002</td>
</tr>
<tr>
<td>E. coli TOP10</td>
<td>1</td>
<td>0.25</td>
<td>0.25</td>
<td>0.38</td>
<td>0.05</td>
<td>0.38</td>
<td>0.19</td>
<td>0.002</td>
</tr>
</tbody>
</table>

AMK, amikacin; NET, netilmicin; GEN, gentamicin; TOB, tobramycin; CTX, cefotaxime; CAZ, ceftazidime; IMP, imipenem; CIP, ciprofloxacin.

MICs of antibiotics were determined using the Etest technique according to the manufacturer’s recommendations.

The clinical isolate was also resistant to sulphonamides and trimethoprim.

MICs for E. coli TOP10 transformants harbouring pS98 expressing ArmA and CTX-M-3.

MICs for E. coli TOP10.

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References


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Comparative in vitro activity of oritavancin against Staphylococcus aureus strains that are resistant, intermediate or heteroresistant to vancomycin

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Keywords: glycopeptide non-susceptible, MICs, MBCs

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Sir,

Widespread use of vancomycin has led to the emergence of isolates that show reduced susceptibility to vancomycin; vancomycin-resistant S. aureus (VISA), vancomycin-resistant S. aureus (VRSA) and heteroresistant VISA (hVISA) have been described.1 The emergence of such strains with concomitant vancomycin therapeutic failures2 has necessitated the development of other therapeutic agents.

Newer drugs of the glycopeptide class, such as oritavancin, with activity against isolates with reduced susceptibility to vancomycin are currently in development. Additional mechanisms exhibited by oritavancin help explain its rapid, concentration-dependent bactericidal activity in vitro3 and distinguish it from vancomycin.

In surveillance studies, oritavancin activity was demonstrated against vancomycin-susceptible S. aureus isolates that are susceptible and resistant to methicillin.4 This study examined the in vitro activity of oritavancin against clinical isolates of VRSA, VISA and hVISA using recently updated broth microdilution methodology.5 MICs and MBCs of oritavancin were determined for these groups of organisms and compared with those of seven clinically used antimicrobial agents.

MICs of antimicrobial agents were determined by broth microdilution according to CLSI (formerly NCCLS) guidelines.5 Polysorbate-80 was included when testing oritavancin, so as to limit losses of oritavancin to the surfaces of test vessels as recommended in CLSI guidelines.5

MBCs were also determined according to CLSI guidelines.6 Aliquots (50 μL) were plated onto brain heart infusion agar (BHIA) plates for the VISA and hVISA isolates, or BHIA plates containing 4 mg/L vancomycin for the VRSA isolates to maintain selection for vancomycin, and incubated at 35°C for 24 h. The MBC was defined as the lowest concentration killing >99.9% of the original inoculum.6

Oritavancin showed good in vitro activity against the hVISA isolates (MIC range of 0.12–2 mg/L and MIC90 of 1 mg/L; Table 1). The oritavancin MIC90 was identical to those of linezolid and quinupristin/dalfopristin, and one doubling dilution higher than that of tigecycline. The oritavancin MIC90 was 2- to 8-fold lower than those of the rest of the comparators. The oritavancin MBC range and MBC90 against hVISA were 0.25–2 and 1 mg/L, respectively. The oritavancin MBC90 against the hVISA isolates was identical to that of tigecycline and 2- to 64-fold lower than those of the rest of the comparators. The oritavancin MBC90/MIC90 ratio of 1 indicates that oritavancin is bactericidal against hVISA. The oritavancin MBC90/MIC90 ratio was identical to that of daptomycin and 2- to 16-fold lower than those of the rest of the comparators.

Oritavancin showed good in vitro activity against the VISA isolates (MIC range of 0.5–4 mg/L and MIC90 of 2 mg/L; Table 1). The MIC90 was identical to that of quinupristin/dalfopristin and was two doubling dilutions higher than that of tigecycline. The oritavancin MIC90 was 2- to 8-fold lower than those of the rest of the comparators. The oritavancin MBC range and MBC90 against VISA were 0.5–8 and 4 mg/L, respectively. The oritavancin MBC90 for the VISA isolates was 4-fold higher than that of tigecycline and 2- to 8-fold lower than those of the rest of the comparators. The oritavancin MBC90/MIC90 ratio of 2 indicates that oritavancin is bactericidal against VISA. The oritavancin MBC90/MIC90 ratio was identical to, or within a dilution of, those of the comparators.

Oritavancin showed good in vitro activity against the VRSA isolates (MIC range of 0.12–1 mg/L and MIC90 of 0.5 mg/L; Table 1). The MIC90 was identical to that of daptomycin and was a doubling dilution higher than that of tigecycline. The oritavancin MIC90 was 2- to >1024-fold lower than those of the rest of the comparators. The oritavancin MBC range and MBC90 against VRSAs were 0.25–1 and 1 mg/L, respectively. The oritavancin MBC90 for the VRSA isolates was 4-fold higher than that of tigecycline and 2- to 8-fold lower than those of the rest of the comparators. The oritavancin MBC90/MIC90 ratio of 2 indicates that oritavancin is bactericidal against VRSA. The oritavancin MBC90/MIC90 ratio was at least 2-fold lower than those of the comparators.

Against the 35 isolates of S. aureus tested in this study, the oritavancin MIC range and MIC90 were 0.12–4 and 1 mg/L, respectively (Table 1). The oritavancin MIC90 was identical to that of quinupristin/dalfopristin and was a doubling dilution...