a 552 bp amplicon. Then, primers CTX-M-F2, 5'-CCAGAATAAGGAATCCCATG-3' and CTX-M-R2, 5'-GCCGTCTAAGGC-GATAAAC-3', were used to amplify the entire allele (876 bp) for sequencing. This revealed that *S. enterica* serovar Senftenberg strain 9-4 produced CTX-M-3, which was first identified in Poland.4 Southern hybridization using the whole *bla* _CTX-M_ gene as a probe showed that the gene was located on a plasmid of <38 kb in size. Attempts to transfer this plasmid to *E. coli* HB101 were unsuccessful. The *bla* _CTX-M_ gene was cloned into pBluescript II SK(–) and expressed in *E. coli* XL1-Blue cells. The MICs for both *S. enterica* serovar Senftenberg 9-4 and *E. coli* XL1-Blue transformants are shown in Table 1.

Detection of CTX-M-3 in *S. enterica* serovar Senftenberg may have public health implications. Previously, the ESBL SHV-5 was detected in clinical isolates of this serovar causing an outbreak in burn wounds in a hospital in Delhi, India.5 Our results show that non-clinical isolates can constitute a reservoir for *bla* _CTX-M_ alleles, and can facilitate the spread of cephalosporinases to clinical isolates. In support of this hypothesis, the metallo-β-lactamase Sfh-I was recently detected in bacteria not of clinical origin.6 Therefore, researchers working on ESBLs especially in endemic areas should also direct their attention to non-clinical isolates found in these regions.

Nucleotide sequence accession number

The nucleotide sequence of the CTX-M-3 gene of *S. enterica* serovar Senftenberg has been assigned the DDBJ/GenBank/EMBL accession no. AB168117.

**Table 1.** MICs of extended-spectrum β-lactams for *S. enterica* serovar Senftenberg 9-4 and *E. coli* XL1-Blue transformants

<table>
<thead>
<tr>
<th>β-Lactam(s)</th>
<th>S. senftenberg 9-4</th>
<th>Transformant 1 (cloned CTX-M-3)</th>
<th>Transformant 2 (empty pBluescript)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefotaxime</td>
<td>128</td>
<td>64</td>
<td>0.125</td>
</tr>
<tr>
<td>Cefotaxime+CLA</td>
<td>0.5</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>64</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Ceftriaxone+CLA</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Cepodoxime</td>
<td>128</td>
<td>64</td>
<td>0.25</td>
</tr>
<tr>
<td>Cepodoxime+CLA</td>
<td>0.5</td>
<td>0.5</td>
<td>0.25</td>
</tr>
<tr>
<td>Cefazidime</td>
<td>4</td>
<td>4</td>
<td>0.125</td>
</tr>
<tr>
<td>Cefazidime+CLA</td>
<td>0.5</td>
<td>0.5</td>
<td>0.125</td>
</tr>
</tbody>
</table>

*CLA, clavulanic acid at a fixed concentration of 4 mg/L.

**References**


**Correspondence**

Journal of Antimicrobial Chemotherapy
DOI: 10.1093/jac/dkh307
Advance Access publication 9 June 2004

**Genetic relatedness between human and animal polymorphic bla*TEM* genes strengthens zoonotic potential among uropathogenic *Escherichia coli* strains**

Manuela Caniça¹*, Ricardo Dias¹ and José Duarte Correia²Constança Pomba².

¹Antibiotic Resistance Unit, Centre of Bacteriology, National Institute of Health Dr. Ricardo Jorge, Av. Padre
Sir,

TEM β-lactamases are encoded by genes which have various coding region frameworks and types of promoter. Six \textit{bla}_{TEM} genes coding for TEM-1 β-lactamase (\textit{bla}_{TEM-1A}, \textit{bla}_{TEM-1B}, \textit{bla}_{TEM-1C}, \textit{bla}_{TEM-1D}, \textit{bla}_{TEM-1E} and \textit{bla}_{TEM-1F}) and the \textit{bla}_{TEM-2} gene coding for TEM-2 β-lactamase have been found in isolates from humans.\textsuperscript{1–3} Recently, we reported a new sequence framework \textit{bla}_{TEM-1G} in an \textit{Escherichia coli} strain of animal origin.\textsuperscript{4} These genes differ by silent mutations at any of 10 positions in their nucleotide sequences (32, 162, 175, 226, 346, 436, 604, 682, 913 and 925). The amino acid sequences of TEM-1 and TEM-2 β-lactamases differ at one position (Gln-39 → Lys, respectively) due to a single base difference (C317A).\textsuperscript{1} We investigated the molecular diversity of \textit{bla}_{TEM} genes encoding TEM-1 enzymes (\textit{pI} of 5.4) produced by amoxicillin-resistant uropathogenic \textit{E. coli} strains isolated from dogs.

Seventeen amoxicillin-resistant uropathogenic \textit{E. coli} strains (named in this study from Fmv1 to Fmv17) were randomly chosen from the collection of the Clinical Pathology Laboratory, Faculty of Veterinary Medicine, Portugal. The MICs of various β-lactams were determined (Table 1) using a microdilution method with an inoculum of 5×10^5 cfu/mL. DNA amplification by PCR, analysis of restriction-fragment length polymorphism (RFLP) and nucleotide sequencing were used for genotyping as previously described.\textsuperscript{2} PCR-RFLP identifies all the mutations cited above, except that at position 913 (Table 1). PCR DNA fragments of 1092 bp from five of the 17 \textit{bla}_{TEM} genes were purified with Qiaquick spin columns (Qiagen, Hilden, Germany), and then sequenced on both strands with the primer pair

<table>
<thead>
<tr>
<th>Nucleotide (aa)</th>
<th>promoter region</th>
<th>coding region</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>32 162 175</td>
<td>226 317 (39)</td>
</tr>
<tr>
<td></td>
<td>346 436 604</td>
<td>682 913 925</td>
</tr>
</tbody>
</table>

**Table 1.** Nucleotides at selected positions in parental \textit{bla}_{TEM-1A}, \textit{bla}_{TEM-1B}, \textit{bla}_{TEM-1C}, \textit{bla}_{TEM-2} genes and the 17 \textit{bla}_{TEM} genes in animal isolates, as determined by PCR-RFLP and nucleotide sequencing

AMX, amoxicillin; AMC, co-amoxiclav; TIC, ticarcillin; MEC, mecillinam; CEF, cefalothin; CAZ, ceftazidime; CXM, cefuroxime. ND, not determined.

\textsuperscript{a} Data from Ref. 1.

\textsuperscript{b} Data from Ref. 3.

\textsuperscript{c} Linkage groups according to polymorphic sequence variations in \textit{bla}_{TEM} genes of animal isolates.

\textsuperscript{d} Sequenced genes (1092 bp).

\textsuperscript{e} Resistance to CAZ and CXM due to the production of an AmpC enzyme.
Correspondence

Journal of Antimicrobial Chemotherapy
DOI: 10.1093/jac/dkh273
Advance Access publication 2 June 2004

Subinhibitory concentrations of florfenicol enhance the adherence of florfenicol-susceptible and florfenicol-resistant Staphylococcus aureus

Maren Blickwede¹, Peter Valentín-Weigand² and Stefan Schwarz¹*

¹Institut für Tierzucht, Bundesforschungsanstalt für Landwirtschaft (FAL), Holtzstrasse 10, 31535 Neustadt-Mariensee; ²Institut für Mikrobiologie, Zentrum für Infektionsmedizin, Tierärztliche Hochschule Hannover, Bischofsholer Damm 15, 30173 Hannover, Germany

Keywords: invasion, fibronectin, surface hydrophobicity

*Corresponding author. Tel: +49-5034-871-241; Fax: +49-5034-871-246; E-mail: stefan.schwarz@fal.de

Sir,

The ability of Staphylococcus aureus to adhere to surfaces of host tissues is thought to be essential for colonization and establishment of infections. There is increasing evidence that subinhibitory concentrations of antibiotics interfere with microbial adherence to host cells. In this study, the effect of subinhibitory concentrations of the fluorinated chloramphenicol derivative florfenicol on adherence properties of florfenicol-susceptible and florfenicol-resistant S. aureus was investigated.

The florfenicol-susceptible strain S. aureus Newman (ATCC 25904) and its florfenicol-resistant derivative carrying the cfr-erm(33)-spp multiresistance plasmid pSCFS1 from Staphylococcus sciuri were cultivated for 20 h at 37°C in brain heart infusion broth (BHI, Oxoid, Wesel, Germany) and in BHI supplemented with either 0.2 mg/L florfenicol (Essex Tierarznei, Munich, Germany) or 2 mg/L florfenicol (=1/20 and 1/2 MIC for S. aureus Newman, respectively). In addition, S. aureus Newman:pSCFS1 was cultivated in BHI supplemented with 64 mg/L florfenicol (=1/2 MIC for S. aureus Newman:pSCFS1). MICs of florfenicol were determined by broth microdilution according to the NCCLS guideline M31-A2. Adherence and invasion assays using 5 × 10^6 bacteria per experiment and confluent monolayers of HEP-2 cells (≈3 × 10^5 cells per well) were carried out as described by Dziewanowska et al. The results were recorded as percentage of cfu of adherent or intracellular bacteria. For microscopic confirmation, adherence assays were carried out on culture slides containing ~2 × 10^5 HEP-2 cells. Washed monolayers with adherent bacteria were fixed with 0.37% formaldehyde (Sigma, Taufkirchen, Germany) at 4°C, washed with PBS and covered with blocking buffer [10% fetal calf serum (Sigma) in PBS] for 1 h. Adherent staphylococci were detected by use of a rabbit-anti-staphylococcal antibody, an FITC-conjugated goat-anti-rabbit IgG antibody (Dianova, Hamburg, Germany) and subsequent immunofluorescence microscopy. Bacteria-per-epithelial cell ratios were determined by counting manually the number of bacteria adherent to

Acknowledgements

This work was supported by POCTI/CVT/36253/99 (FEDER) grant from Fundação para a Ciência e a Tecnologia, Lisbon, Portugal.

References