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Table 1. Characteristics of the four ESBL-containing E. coli strains recovered from healthy pets in Portugal

<table>
<thead>
<tr>
<th>E. coli</th>
<th>Animal source</th>
<th>AMP</th>
<th>TIC</th>
<th>AMC</th>
<th>FOX</th>
<th>CTX</th>
<th>CAZ</th>
<th>ATM</th>
<th>IPM</th>
<th>Phenotype of resistance for non-β-lactams</th>
<th>ESBL screening test</th>
<th>ESBL encoding genes detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>E14</td>
<td>dog</td>
<td>&gt;256</td>
<td>&gt;128</td>
<td>16</td>
<td>4</td>
<td>32</td>
<td>16</td>
<td>4</td>
<td>0.06</td>
<td>STR–TET</td>
<td>+</td>
<td>bla&lt;sub&gt;TEM-52b&lt;/sub&gt;</td>
</tr>
<tr>
<td>E39</td>
<td>dog</td>
<td>&gt;256</td>
<td>&gt;128</td>
<td>16</td>
<td>8</td>
<td>16</td>
<td>16</td>
<td>4</td>
<td>0.06</td>
<td>STR</td>
<td>+</td>
<td>bla&lt;sub&gt;TEM-52b&lt;/sub&gt;</td>
</tr>
<tr>
<td>E55</td>
<td>dog</td>
<td>&gt;256</td>
<td>&gt;128</td>
<td>16</td>
<td>2</td>
<td>16</td>
<td>8</td>
<td>2</td>
<td>0.125</td>
<td>STR–TET</td>
<td>+</td>
<td>bla&lt;sub&gt;TEM-52b&lt;/sub&gt;</td>
</tr>
<tr>
<td>E42</td>
<td>dog</td>
<td>&gt;256</td>
<td>&gt;128</td>
<td>64</td>
<td>2</td>
<td>128</td>
<td>2</td>
<td>16</td>
<td>0.06</td>
<td>STR–TET–CHL–SUL</td>
<td>+</td>
<td>bla&lt;sub&gt;CTX-M-1&lt;/sub&gt;</td>
</tr>
</tbody>
</table>

AMP, ampicillin; TIC, ticarcillin; AMC, co-amoxiclav; FOX, cefoxitin; CTX, cefotaxime; CAZ, ceftazidime; ATM, aztreonam; IPM, imipenem; STR, streptomycin; TET, tetracycline; CHL, chloramphenicol; SUL, sulfamethoxazole.

*Non-β-lactam antibiotics tested: gentamicin, tobramycin, amikacin, nalidixic acid, ciprofloxacin, trimethoprim/sulfamethoxazole, sulfamethoxazole, chloramphenicol, streptomycin and tetracycline.

Mutations in the promoter–attenuator region of the chromosomal ampC gene were also studied by PCR and sequencing.¹

Broad-spectrum cephalosporin-resistant E. coli strains were detected in five of the 75 faecal samples analysed (6.6%, four dogs and one cat). Four of the five strains, obtained from four unrelated dogs, gave positive ESBL screening test results and were resistant to cefotaxime and/or ceftazidime. The bla<sub>TEM-52b</sub> gene was identified in three of these four strains and the bla<sub>CTX-M-1</sub> gene in the remaining one, these strains being negative for the other β-lactamases tested by PCR. The characteristics of these E. coli strains are included in Table 1. The three bla<sub>TEM-52b</sub>-containing strains were also resistant to streptomycin, and two of them also to tetracycline. The bla<sub>CTX-M-1</sub>-containing strain was resistant to streptomycin–tetracycline–sulfamethoxazole–chloramphenicol and also harboured the intI gene, associated with type I integrons (detected by PCR).

The remaining broad-spectrum cephalosporin-resistant E. coli strain that was recovered from a healthy cat, showed a negative ESBL screening test and all PCR assays for TEM, SHV, OXA, CTX-M, FOX and CMY β-lactamase-encoding genes were negative. Mutations at the −42, −18, −1 and +58 positions of the promoter–attenuator region of the chromosomal ampC gene were detected by PCR and sequencing. This strain showed a pattern of multi-resistance, which included nalidixic acid, ciprofloxacin, tetracycline, streptomycin, gentamicin, tobramycin, trimethoprim/sulfamethoxazole and chloramphenicol, and this strain also harboured the intI1 gene.

To our knowledge, this is the first time that ESBL-encoding genes have been detected in healthy pets and also the first time that CTX-M β-lactamases have been detected in E. coli strains from animal origin in Portugal. More studies should be carried out in the future to track the evolution of this type of β-lactamase in different environments.

Acknowledgements

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References


Antibiotics in media for isolation of Campylobacter spp. do not enhance resistance

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Keywords: induction, gene expression, susceptibility

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Sir,

Campylobacter spp. have become the major cause of gastroenteritis in the Western world. However, isolation and culture of these bacteria from different source samples is difficult as they are fastidious and slow growing, and are thus out-competed by
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Table 1. Concentrations of agents in Campylobacter isolation media and MICs for C. jejuni NCTC 11168 (mg/L)

<table>
<thead>
<tr>
<th>Agent</th>
<th>Media</th>
<th>Concentration</th>
<th>MIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphotericin B</td>
<td>Blaser-Wang (Oxoid SR0098);</td>
<td>10</td>
<td>not done</td>
</tr>
<tr>
<td></td>
<td>CCDA (Oxoid SR0155)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cycloheximide</td>
<td>Preston (Oxoid SR0117)</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Cefalothin</td>
<td>Blaser-Wang</td>
<td>14</td>
<td>32</td>
</tr>
<tr>
<td>Cefoperazone</td>
<td>CCDA</td>
<td>16</td>
<td>64</td>
</tr>
<tr>
<td>Polymyxin</td>
<td>Blaser-Wang; Preston</td>
<td>1.25</td>
<td>16</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>Preston</td>
<td>10</td>
<td>32</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>Blaser-Wang; Preston</td>
<td>5</td>
<td>128</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>Blaser-Wang</td>
<td>10</td>
<td>64</td>
</tr>
</tbody>
</table>

other bacterial species and fungi. This has necessitated the use of laboratory media that contain various antibacterial and antifungal selective agents.1,2

Antibiotics have the tendency to select for resistant bacteria. For example, fluoroquinolones can select for mutants with gyrA mutations and/or that overexpress an efflux system.3 Therefore, there has been increasing concern that the use of antibiotics in Campylobacter isolation media may result in biased antibiotic susceptibility data sets.4 The antibiotics cefalothin, cefoperazone and rifampicin are incorporated in the currently used selective media. These agents are also substrates of CmeABC, an RND-type efflux system in Campylobacter, which can confer multiple antibiotic resistance.5,6 We hypothesized that as these agents are substrates of efflux pumps, these media could select for mutants that overexpress the cmeABC, cmeDEF and/or other efflux systems in Campylobacter jejuni NCTC 11168. Alternatively, induction of these pump genes could allow Campylobacter spp. to survive the presence of antibiotics in media.

C. jejuni NCTC 11168 was cultured on standard Mueller–Hinton (MH) agar and also on three commonly used Campylobacter selective media (Table 1).

The plates were incubated under microaerophilic conditions for 48 h. Colonies were resuspended in MH broth, MH broth plus Blaser-Wang supplement, MH broth plus CCDA supplement and MH broth plus Preston supplement and incubated overnight. The suspensions were used as inocula for MIC determination and gene expression analysis for cmeABC and cmeDEF, as described previously.4,6 The antibiotics tested were ampicillin, cefalothin, chloramphenicol, ciprofloxacin, erythromycin, kanamycin, rifampicin, tetracycline and trimethoprim.

Susceptibility testing revealed no differences in the MIC of the tested antibiotics between C. jejuni NCTC 11168 cultured in standard media versus selective media. Comparison of the concentrations of the antibiotics used in these media and their MICs for C. jejuni NCTC 11168 showed that the concentrations used in the media were substantially below the MIC values (Table 1). Gene expression analysis also revealed no differences in the expression of cmeABC or cmeDEF between C. jejuni NCTC 11168 cultured in MH media compared with any of the three selective media. Therefore, it is concluded that selective culture media used to isolate Campylobacter spp. inhibit competition by other bacteria and fungi, but do not induce overexpression of known efflux pump genes, for which some of these agents are substrates. Therefore, any antibiotic resistance observed in isolates is unlikely to be an artefact due to media composition as has been suggested previously.2

Acknowledgements

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Susceptibility testing Pasteurella multocida by BSAC standardized methodology


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