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Prevalence of extended-spectrum beta-lactamase-producing *Escherichia coli* isolates in faecal samples of broilers

Daniela Costa\textsuperscript{a,d}, Laura Vinué\textsuperscript{d}, Patricia Poeta\textsuperscript{a,c}, Ana Cláudia Coelho\textsuperscript{a}, Manuela Matos\textsuperscript{b}, Yolanda Sáenz\textsuperscript{d,e}, Sergio Somalo\textsuperscript{d}, Myriam Zarazaga\textsuperscript{d,e}, Jorge Rodrigues\textsuperscript{a,c}, and Carmen Torres\textsuperscript{d,e,*}

\textsuperscript{a}Universidade de Trás-os-Montes e Alto Douro, Departamento de Ciências Veterinárias; and \textsuperscript{b}Departamento de Genética e Biotecnología/Instituto de Biotecnologia e Bioengenharia, Vila Real, Portugal; \textsuperscript{c}Centre of Studies of Animal and Veterinary Sciences, Vila Real, Portugal; \textsuperscript{d}Área de Bioquímica y Biología Molecular, Universidad de La Rioja, Logroño, Spain.

\textbf{Running title:} *E. coli* harbouring ESBLs in faeces of poultry in Portugal

\textbf{Corresponding author.}

Carmen Torres

Area de Bioquímica y Biología Molecular

Universidad de La Rioja

Madre de Dios, 51

26006 Logroño

Spain

FAX: 34-941299721

Phone: 34-941299750

e-mail: carmen.torres@unirioja.es
Abstract

Seventy-six faecal samples were obtained from broilers at slaughterhouse level in Portugal. Samples were inoculated on cefotaxime-supplemented Levine agar plates. Cefotaxime-resistant *Escherichia coli* isolates were recovered from 32 samples (42.1%), obtaining a total of 34 *E. coli* isolates (one or two isolates per sample). Susceptibility to 16 antibiotics was studied by disk diffusion method, and 85% of the isolates presented a phenotype of multi-resistance that included antimicrobial agents of at least four different families. Extended-spectrum-beta-lactamases (ESBL) of the TEM and CTX-M groups were detected in 31 ESBL-positive *E. coli* isolates. Twenty-six isolates harbour the *bla*<sub>TEM-52</sub> gene and two of them also harboured *bla*<sub>TEM-1b</sub>. The *bla*<sub>CTX-M-14</sub> gene was identified in 3 isolates (in association with *bla*<sub>TEM-1b</sub> in one of them), and *bla*<sub>CTX-M-32</sub> was demonstrated in two additional isolates. Three of the 34 cefotaxime-resistant isolates (9%) did not produce ESBLs, and two of them presented mutations at positions -42 (C→T), -18 (G→A), -1 (C→T), and +58 (C→T) of the promoter/attenuator region of *ampC* gene. *tet*(A) and/or *tet*(B) genes were detected in all 34 tetracycline-resistant isolates, *aadA* in all 26 streptomycin-resistant isolates; *cmlA* in 3 of 6 chloramphenicol-resistant isolates, and *aac*(3)-II or *aac*(3)-I + *aac*(3)-IV genes in all 4 gentamicin-resistant isolates. Different combinations of *sul1*, *sul2* and *sul3* genes were demonstrated among the 22 trimethoprim-sulfamethoxazole-resistant isolates. Amino acid changes in GyrA and ParC proteins were identified in all 18 ciprofloxacin-resistant isolates. The results of this study indicate that the intestinal tract of healthy poultry is a reservoir of ESBL-positive *E. coli* isolates.

Keywords: TEM-52, CTX-M-14, CTX-M-32, broilers
1. Introduction

*Escherichia coli* is a common inhabitant of intestinal tract of humans and animals (Sørum and Sunde, 2001; Tannock, 1995), and can be easily disseminated in different ecosystems through the food chain and water (Skurnik et al., 2006). In addition, *E. coli* can become a human pathogen, mainly in immunocompromised patients, and beta-lactams are frequently used for treatment of infections caused by this microorganism.

In the last years, the important increase of the prevalence of extended-spectrum beta-lactamases (ESBLs) among clinical *E. coli* isolates in human medicine is a cause of great concern, and this mechanism of resistance is implicated in failures in the treatment of infectious diseases (Paterson and Bonomo, 2005; Livermore, 2008; Cantón et al., 2006). ESBLs confer resistance to penicillins, first-, second- and third-generation cephalosporins, as well as to aztreonam, but not to cefoxitin or carbapenems. Different types of ESBLs have been reported, and those of the CTX-M type represent an emerging problem in human health, being considered as “the CTX-M pandemic” (Cantón et al., 2006; Rossolini et al., 2008).

Different reports have alerted in the last few years about the dissemination of ESBL-positive *E. coli* among the intestinal microbiota of healthy food-producing animals or food products in different countries of Europe (Aarestrup et al., 2006; Blanc et al., 2006; Briñas et al., 2003, 2005; Girlich et al., 2007; Smet et al., 2008), Asia (Duan et al., 2006; Kojima et al., 2005; Liu et al., 2007), or Africa (Jouini et al., 2007). These resistant bacteria could enter the food chain, representing a problem for food safety. Previous studies carried out in Portugal reported the dissemination of ESBL-containing *E. coli* isolates in the faecal microbiota of dogs and also of wild animals, specially in birds of prey (Costa et al., 2004, 2006), and very recently also in chickens and swine (Machado et al., 2008). The purpose of our work was to analyse the faecal carriage of ESBL-containing *E. coli* isolates in broilers at slaughterhouse level in Portugal, to identify the type of ESBLs, and to detect the presence of other antimicrobial resistance markers in these bacteria. This study provides more
information about the real problem of ESBL in food-producing animals, and valuable help
to control this emerging problem and to track its future evolution.

2. Material and methods

2.1. Bacteria included in this study

Seventy-six faecal samples from broilers were collected in a slaughterhouse located in
Northern Portugal, which received animals from different regions of the country. The
samples were obtained during 20 visits to the slaughterhouse from February to June 2004.
Faecal samples were obtained from different flocks that contained from 2,000 to 10,000
animals. Fresh dropping faecal samples were recovered from the crates, just before culling
the animals.

All the samples were seeded on Levine agar plates supplemented with 2 μg/ml of
cefotaxime and were incubated at 37 °C for 24 h. Two colonies per sample with typical
E. coli morphology were selected and identified by classical biochemical methods (Gram
staining, catalase, oxidase, indol, Methyl-Red-Voges-Proskauer, citrate and urease), and by
the API 20E system (BioMérieux, La Balme Les Grottes, France).

2.2 Antimicrobial susceptibility testing

Antimicrobial susceptibility was performed by the agar disk diffusion method
following the Clinical and Laboratory Standards Institute recommendations (CLSI, 2007),
and a total of 16 antimicrobial agents were tested: ampicillin, amoxicillin-clavulanic acid
(AMC), cefoxitin, cefotaxime (CTX), ceftazidime (CAZ), imipenem, aztreonam,
gentamicin, tobramycin, amikacin, streptomycin, tetracycline, trimethoprim-
sulfamethoxazole (SXT), nalidixic acid, ciprofloxacin and chloramphenicol. Broad-
spectrum cephalosporin-resistant isolates were selected and one isolate per sample was kept
for further studies (two isolates of the same sample were kept for further studies if they presented different antimicrobial resistance phenotypes). The double disk synergy test with cefotaxime or ceftazidime in the proximity to amoxicillin-clavulanic acid was used for the screening of ESBL.

2.3. Characterization of antimicrobial resistance genes

The presence of genes encoding TEM, SHV, OXA-1, CTX-M, and CMY beta-lactamases was studied by PCR in all broad-spectrum cephalosporin resistant isolates using primers and conditions previously reported (Briñás et al., 2003; Jouini et al., 2007). The obtained DNA amplicons were sequenced on both strands and sequences were compared with those included in the GeneBank database in order to identify the specific beta-lactamase gene. The promoter and attenuator region of the chromosomal ampC gene was also amplified by PCR (Briñas et al., 2003), sequenced, and compared with the same region of the E. coli K12 ampC gene, in order to analyse the mutations, associated to the overexpression of the ampC gene. In addition, the presence of tet(A), tet(B), tet(C), tet(D) and tet(E) genes was studied by PCR in tetracycline-resistant isolates (Sáenz et al., 2004). The following genes were also studied by PCR (Sáenz et al., 2004): aadA1 and aadA2 (in streptomycin-resistant isolates), aac(3)-I, aac(3)-II and aac(3)-IV (in gentamicin-resistant isolates), cmlA (in chloramphenicol-resistant isolates), sul1, sul2 and sul3 (in SXT-resistant isolates). Positive and negative PCR controls from the bacterial collection of the University of La Rioja, Spain, were used in all assays.

2.4. Characterization of the mechanism of ciprofloxacin resistance

The quinolone-resistance-determining region (QRDR) of the gyrA gene, as well as the analogous region of the parC gene, was amplified by PCR in all ciprofloxacin-resistant E. coli isolates (Sáenz et al., 2003). Amplified fragments were purified (Qiagen), and both
strands were automatically sequenced by the Applied Biosystem 3730 sequencer (Genome Express, France), using the same set of primers as for the PCR reactions. Sequences obtained were compared with those previously reported for gyrA (GenBank accession number X06373) and parC genes (M58408 with the modification included in L22025).

2.5. REP-PCR clonal analysis and identification of phylogenetic groups.

A PCR-based DNA fingerprinting technique which uses primers based on the highly conserved repetitive extragenic palindromic (REP) repeated DNA element was used for typing 34 CTX-resistant E. coli isolates. This technique was preferentially used to assess the diversity of the isolates due to its easy methodology. REP-PCR was performed using Rep-F, IIIGCGCCGICATCAGGC and Rep-R, ACGTCTTATCAGGCCTAC primers under the following conditions: 30 cycles of 1 min at 94ºC, 1 min at 40ºC and 8 min at 65ºC, with a single final extension of 16 min at 65ºC. PCR end-products were separated on agarose 2% w/v gels and the gel images were analyzed visually comparing all the isolates.

The identification of the major phylogenetic groups of the isolates was determined by PCR (Clermont et al., 2000).

3. Results

Results showed that CTX-resistant E. coli isolates were present in 32 out of the 76 samples of our study, representing 42.1% of the total faecal samples. Two E. coli isolates per sample were recovered from these in 32 positive samples and the phenotype of antimicrobial resistance was analysed in all of them. An identical phenotype of resistance was demonstrated in the two CTX-resistant isolates obtained from 30 of these samples and one isolate from each one was kept for further studies. Nevertheless, a different phenotype of resistance was demonstrated in the two CTX-resistant isolates obtained from the remaining two faecal samples and both of them were included in this study. Consequently, a
total of 34 CTX-resistant *E. coli* isolates were included in this study for beta-lactamases characterization (Table 1).

### 3.1. Beta-lactamases detected in CTX-resistant *E. coli* isolates

The screening for ESBL production was performed in all 34 CTX-resistant *E. coli* isolates and results were positive in 31 of them, recovered from 29 of the 76 tested faecal samples (38.2%). The genes encoding ESBLs of the TEM and CTX-M groups were detected in these 31 ESBL-positive *E. coli* isolates. Twenty-six isolates harboured the *bla*$_{\text{TEM-52c}}$ gene, 3 isolates the *bla*$_{\text{CTX-M-14a}}$ gene, and 2 additional isolates the *bla*$_{\text{CTX-M-32}}$ gene. The *bla*$_{\text{TEM-1b}}$ gene was also detected in four of these 31 ESBL-positive isolates.

Three of our 34 CTX-resistant isolates presented a negative ESBL-screening test (9%) and they showed resistance to cefoxitin and amoxicillin-clavulanic acid. The promoter/attenuator region of the chromosomal *ampC* gene was amplified in these isolates and the amplicons were sequenced. Mutations at positions -42 (C→T), -18 (G→A), -1 (C→T), and +58 (C→T) were detected in two of these isolates (Table 2). The third CTX-resistant ESBL-negative *E. coli* isolate did not present important mutations in *ampC* gene (at positions −42 or −32), and all PCRs performed to detect acquired ESBLs or CMY-type beta-lactamase were negative (Table 1 and 2).

The presence of mutations in the promoter/attenuator region of the chromosomal *ampC* gene was also studied in four cefoxitin-resistant and ESBL-positive *E. coli* isolates (AV-48.1A, AV-65.1A, AV-5.1A and AV-8.1A) (Table 2). It is interesting to underline that the specific C→T mutation at position -42 was identified in two of the three TEM-52-containing isolates, but not in the CTX-M-14a-containing one.

In two of the tested faecal samples, different ESBLs were identified in the two *E. coli* isolates recovered from each of them. Two isolates from one faecal sample were positive for
CTX-M-32 and TEM-52, respectively. Two isolates of the other faecal sample contained CTX-M-14a and TEM-52, respectively.

3.2. Associated antimicrobial resistance markers in CTX-resistant isolates

Most of our CTX-resistant isolates (85%) presented a phenotype of multi-resistance that included antimicrobial agents belonging to at least four different families, and 10% of them showed resistance to 6 different antimicrobial families. A wide variety of resistance genes were detected among our isolates (Table 1). The tet(A) or tet(B) genes were detected in all 33 tetracycline-resistant isolates grouped as follows: tet(A) in 11 isolates, tet(B) in 12, and tet(A)+tet(B) in the remaining five isolates. The aadA gene was identified in all 26 streptomycin-resistant isolates; the cmlA gene in 3 of 6 chloramphenicol-resistant isolates, and the aac(3)-II or aac(3)-I + aac(3)-IV genes in all 4 gentamicin-resistant isolates. Different combinations of sul1, sul2 and sul3 genes were demonstrated among the 22 SXT-resistant isolates, and 50% of them harboured two of these genes.

3.3. Characterization of the mechanism of ciprofloxacin resistance

Eighteen of our ESBL-positive E. coli isolates exhibited ciprofloxacin-resistance and two amino acid changes were identified in GyrA protein (S83L+D87N in 16 isolates; S83L+D87G in 1 isolate; S83L+D87Y in 1 isolate), and one additional amino acid change in ParC protein (S80I in 18 isolates) (Table 1).

3.4. REP-PCR analysis and phylogenetic groups

The REP-PCR technique was applied in order to determine the clonal diversity of the 34 CTX-resistant E. coli isolates. Twenty-one different profiles were identified in this series (Table 1), and fifteen profiles were demonstrated among the 26 blaTEM-52-containing isolates, indicating that many different clones were disseminated in the poultry population.
Fifty-per cent of the 34 CTX-resistant isolates belonged to the B1 phylogenetic group, and none of them were included in the B2 group (Table 1). Figure 1 shows the number of ESBL-positive *E. coli* isolates distributed according to their phylogenetic group. Seventy-seven-per cent of TEM-52-containing isolates belonged to phylogenetic groups A or B1.

4. Discussion

Almost forty per cent of the faecal samples of broilers recovered in 2004 at slaughterhouse level in Portugal revealed ESBL-containing *E. coli* isolates. This result should arouse concern because these animals enter the food chain and they could transfer these resistant bacteria to humans. This percentage is higher than that recently found by Machado et al (2008) in faecal samples of healthy chickens (10%), but slightly lower than the prevalence found on chicken carcasses (60%). High prevalence of CTX-resistant or ESBL-producing *E. coli* isolates in faecal samples of food-producing animals has also been found in other European countries (Smet et al., 2008; Moreno et al., 2007; Blanc et al., 2006). The wide dissemination of ESBL-positive *E. coli* among faecal isolates of healthy food-producing animals is a problem of food safety and it is important to analyse the factors that could contribute to this situation. One possible origin of this problem might be the use of broad-spectrum cephalosporins in broiler production, although these antimicrobial agents are not widely used in this type of farming. Another possible reason would be the use in broilers of other antimicrobial agents (quinolones, tetracycline, sulfamides, trimethoprim, or aminoglycosides, among others) that could co-select ESBL-positive bacteria, that harbour their resistance genes in the same mobile structures. Other possible factor would be the high concentration of animals in flocks, which facilitates the transmission of resistant bacteria among animals. It is difficult to know the specific contribution of each factor to the global problem of the wide dissemination of ESBL in the animal ecosystem, and all these factors should be studied.
Recent reports in the last years also refer a relatively high frequency of the presence of ESBL-producing *E. coli* isolates in samples of healthy humans (Miró et al., 2005; Moubareck et al., 2005; Pallecchi et al., 2007; Valverde et al., 2004; Vinué et al., 2008), although the reported percentages are lower than those found in the poultry samples of this study. An important issue to be elucidated is whether the presence of ESBL-producing bacteria in the intestinal tract of healthy animals and humans has occurred in an independent way, or by contrast, both ecosystems interrelated in their evolution.

It is important to note that 84% of the ESBL-positive *E. coli* isolates recovered in this study harboured the TEM-52 enzyme and a wide diversity of clones were detected among them. A high prevalence of TEM-52 and a high heterogeneity of PFGE patterns were also reported in *E. coli* isolates recovered from healthy chickens, healthy swine and chicken meat products in Portugal (Machado et al., 2008).

Mutations in the promoter and attenuator regions of the chromosomal *ampC* gene of some *E. coli* isolates were found in our study, even associated to ESBL genes. The C→T mutation at position -42, associated with hyperproduction of AmpC beta-lactamase (Mulvey et al., 2005; Caroff et al., 2000), was found in the 57% of our cefoxitin-resistant isolates. The G→A mutation at position -18 was detected in 71% of cefoxitin-resistant isolates; some authors indicate that this mutation could play an important role in the expression of the chromosomal *ampC* gene (Caroff et al., 2000; Smet et al., 2008).

Most of the CTX-resistant *E. coli* isolates were also resistant to tetracycline and nalidixic acid (>90%), and a high percentage of them showed resistance to streptomycin (76%) and trimethoprim/sulfamethoxazole (65%). The association of beta-lactam resistance with tetracycline, nalidixic acid, streptomycin and trimethoprim resistance has also been previously reported (Smet et al., 2008). The presence of two amino acid changes in GyrA (S83L and D87N or D87Y or D87G) and one in ParC (S80I) were responsible for the ciprofloxacin resistance detected in eighteen isolates of this study. The substitutions S83L,
D87N and S80I were the most frequently detected (88.2%), and these results are similar to those of other reports (Kim et al., 2007).

In our study we have detected a high diversity of *E. coli* clones disseminated in the intestinal microbiota of poultry population. Future studies will be performed with the ESBL-positive isolates to find possible associations among serotypes, virulence factors, resistance mechanisms and phylogenetic groups that will be useful for epidemiological research.

As a conclusion, in this study a high prevalence of ESBLs has been detected in faecal samples of poultry collected at slaughterhouse level in Northern Portugal. TEM-52 beta-lactamase was specially frequent among these isolates. TEM-52 is as well the ESBL of the TEM class more frequently detected in human infections in Portugal (Machado et al., 2007). More studies should be carried out in the future in order to track the evolution of this type of resistance among faecal *E. coli* and to analyse the relationship between human and animal ESBL-containing *E. coli* isolates.

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**Transparency Declarations**

None to declare


spectrum β-lactamases of the CTX-M, TEM and SHV classes in faecal samples of wild

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Table 1. Characteristics of the 34 CTX-resistant *E. coli* isolates of poultry origin recovered from a slaughterhouse in Northern Portugal.

<table>
<thead>
<tr>
<th><em>E. coli</em> isolate</th>
<th>ESBL-test</th>
<th>ESBL detected</th>
<th>REP-PCR pattern&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Phylogenetic group</th>
<th>Phenotype of resistance to non-beta-lactams&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Antimicrobial resistance genes detected:</th>
<th>Amino acid changes in:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>GyrA</td>
<td>ParC</td>
</tr>
<tr>
<td>AV-7.1</td>
<td>+</td>
<td>CTX-M-14a</td>
<td>17</td>
<td>A</td>
<td>STR-TET-NAL</td>
<td>aadA, tet(B)</td>
<td></td>
</tr>
<tr>
<td>AV-13.1A</td>
<td>+</td>
<td>CTX-M-14a</td>
<td>20</td>
<td>B&lt;sub&gt;1&lt;/sub&gt;</td>
<td>STR-TET-NAL-CIP-SXT-CHL</td>
<td>aadA, tet(B), sul1</td>
<td>S83L+D87G</td>
</tr>
<tr>
<td>AV-8.1A</td>
<td>+</td>
<td>CTX-M-14a</td>
<td>ND</td>
<td>D</td>
<td>STR-TET-NAL-CIP-SXT-CHL-GEN-TOB</td>
<td>aadA, tet(A), sul1, sul2, cmlA, aac(3)-II, bla&lt;sub&gt;TEM-1b&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td>AV-11-1A</td>
<td>+</td>
<td>CTX-M-32</td>
<td>18</td>
<td>B&lt;sub&gt;1&lt;/sub&gt;</td>
<td>STR-TET-NAL-CIP-SXT</td>
<td>aadA, tet(B), sul2, sul3</td>
<td>S83L+D87N</td>
</tr>
<tr>
<td>AV-12-1B</td>
<td>+</td>
<td>CTX-M-32</td>
<td>19</td>
<td>B&lt;sub&gt;1&lt;/sub&gt;</td>
<td>STR-TET-NAL-CIP-SXT</td>
<td>aadA, tet(A), tet(B), sul2</td>
<td>S83L+D87N</td>
</tr>
<tr>
<td>AV-1.1A</td>
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<td>TEM-52</td>
<td>8</td>
<td>B&lt;sub&gt;1&lt;/sub&gt;</td>
<td>STR-TET-NAL-CIP-SXT-CHL</td>
<td>aadA, tet(A), sul1</td>
<td>S83L+D87N</td>
</tr>
<tr>
<td>AV-2.1A</td>
<td>+</td>
<td>TEM-52</td>
<td>9</td>
<td>A</td>
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<td>aadA, tet(A)</td>
<td></td>
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<tr>
<td>AV-3.1A</td>
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<td>A</td>
<td>STR-TET-NAL</td>
<td>aadA, tet(A)</td>
<td></td>
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<tr>
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<td>+</td>
<td>TEM-52</td>
<td>2</td>
<td>B&lt;sub&gt;1&lt;/sub&gt;</td>
<td>TET-NAL-CIP-SXT-CHL</td>
<td>tet(A), sul1, sul2, cmlA</td>
<td>S83L+D87N</td>
</tr>
<tr>
<td>AV-12.1A</td>
<td>+</td>
<td>TEM-52</td>
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<td>B&lt;sub&gt;1&lt;/sub&gt;</td>
<td>TET-NAL-CIP-SXT</td>
<td>tet(A), tet(B), sul2</td>
<td>S83L+D87N</td>
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<tr>
<td>AV-13.1B</td>
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<td>TEM-52</td>
<td>6</td>
<td>A</td>
<td>STR-TET-NAL-CIP</td>
<td>aadA, tet(B)</td>
<td>S83L+D87N</td>
</tr>
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<td>AV-14.1A</td>
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<td>TEM-52</td>
<td>1</td>
<td>B&lt;sub&gt;1&lt;/sub&gt;</td>
<td>STR-TET-NAL-CIP-SXT</td>
<td>aadA, tet(A), sul2, sul3</td>
<td>S83L+D87N</td>
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<tr>
<td>AV-15.1A</td>
<td>+</td>
<td>TEM-52</td>
<td>15</td>
<td>D</td>
<td>STR-TET-NAL-CIP-SXT</td>
<td>aadA, tet(B), sul2, bla&lt;sub&gt;TEM-1b&lt;/sub&gt;</td>
<td>S83L+D87N</td>
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<td>AV-18.1A</td>
<td>+</td>
<td>TEM-52</td>
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<td>STR-TET-NAL-CIP-SXT</td>
<td>aadA, tet(B), sul2, bla&lt;sub&gt;TEM-1b&lt;/sub&gt;</td>
<td>S83L+D87N</td>
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<tr>
<td>AV-16.1A</td>
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<td>B&lt;sub&gt;1&lt;/sub&gt;</td>
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<td>aadA, tet(A)</td>
<td>S83L+D87N</td>
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<td>AV-21.1A</td>
<td>+</td>
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<td>6</td>
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<td>TET-NAL-SXT</td>
<td>tet(A), tet(B), sul1, sul2</td>
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<td>Antibiotics</td>
<td>Days</td>
<td>Mode</td>
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<td>Other}</td>
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<td>13</td>
<td>D</td>
<td>STR-TET-NAL-SXT-GEN</td>
<td>tet(B), sul2, aac(3)-II</td>
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<td>AV-24.1A</td>
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<td>7</td>
<td>D</td>
<td>STR-TET-NAL-CIP-SXT</td>
<td>aadA, tet(B), sul2</td>
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<td>AV-52.1B</td>
<td>TEM-52</td>
<td>1</td>
<td>B₁</td>
<td>STR-TET-NAL-CIP-SXT</td>
<td>aadA, tet(B), sul2</td>
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<td>AV-36.1A</td>
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<td>12a</td>
<td>A</td>
<td>STR-TET-NAL-SXT-GEN-TOB-</td>
<td>aadA, tet(B), sul1, sul2, aac(3)-I, aac(3)-IV</td>
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<td>TEM-52</td>
<td>1</td>
<td>B₁</td>
<td>STR-TET-NAL-CIP-SXT</td>
<td>aadA, tet(A), tet(B), sul2</td>
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<tr>
<td>AV-44.1A</td>
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<td>ND</td>
<td>A</td>
<td>STR-TET-NAL-CIP-SXT</td>
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<td>AV-50.1A</td>
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<td>STR-TET-NAL</td>
<td>aadA, tet(A)</td>
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<td>TET</td>
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<td>B₁</td>
<td>STR-TET-NAL-SXT</td>
<td>aadA, tet(A), sul1, sul2</td>
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<td>TET-NAL-CIP</td>
<td>tet(B)</td>
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<td>AV-5.1A</td>
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<td>D</td>
<td>STR-TET-NAL-CIP-SXT-CHL</td>
<td>aadA, tet(B), sul2, sul3</td>
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<td>AV-65.1B</td>
<td>TEM-52</td>
<td>10</td>
<td>B₁</td>
<td>STR-TET-NAL-SXT</td>
<td>aadA, tet(A), tet(B), sul1, sul2</td>
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<td>AV-48.1A</td>
<td>TEM-52</td>
<td>11</td>
<td>B₁</td>
<td>NAL</td>
<td>blaTEM-1b</td>
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<tr>
<td>AV-68.1B</td>
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<td>16</td>
<td>B₁</td>
<td>STR-TET-NAL-SXT</td>
<td>tet(B), sul2, blaTEM-1b</td>
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<td>AV-75.1A</td>
<td>none</td>
<td>16</td>
<td>B₁</td>
<td>STR-TET-NAL-SXT</td>
<td>aadA, tet(B), sul1, sul2, blaTEM-1b</td>
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<td>AV-9.1A</td>
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<td>STR-TET-CHL-GEN-TOB</td>
<td>aadA, tet(B), cmlA, aac(3)-II</td>
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</tbody>
</table>

*TET, tetracycline; NAL, nalidixic acid; CIP, ciprofloxacin; STR, streptomycin; SXT, trimethoprim/sulfamethoxazole; GEN, gentamicin; TOB, tobramycin; CHL, chloramphenicol; ND: no determined.*
Table 2. Mutations in promoter/attenuator region of *ampC* gene in the 7 cefoxitine- and amoxicillin-clavulanic acid-resistant *E. coli* isolates detected in this study.

<table>
<thead>
<tr>
<th><em>E. coli</em></th>
<th>ESBL gene</th>
<th>Mutations in <em>ampC</em> promoter region</th>
</tr>
</thead>
<tbody>
<tr>
<td>AV-68.1B</td>
<td>none</td>
<td>-42 (C→T), -18 (G→A), -1 (C→T), +58 (C→T)</td>
</tr>
<tr>
<td>AV-75.1A</td>
<td>none</td>
<td>-42 (C→T), -18 (G→A), -1 (C→T), +58 (C→T)</td>
</tr>
<tr>
<td>AV-9.1A</td>
<td>none</td>
<td>-18 (G→A), -1 (C→T), and +58 (C→T)</td>
</tr>
<tr>
<td>AV-48.1A</td>
<td>TEM-52</td>
<td>-42 (C→T), -18 (G→A), -1 (C→T), +58 (C→T)</td>
</tr>
<tr>
<td>AV-65.1A</td>
<td>TEM-52</td>
<td>-42 (C→T), -18 (G→A), -1 (C→T), +58 (C→T)</td>
</tr>
<tr>
<td>AV-5.1A</td>
<td>TEM-52</td>
<td>No mutations</td>
</tr>
<tr>
<td>AV-8.1A</td>
<td>CTX-M-14a</td>
<td>No mutations</td>
</tr>
</tbody>
</table>

*a* The CMY encoding gene was negative by PCR in all these isolates.
Caption

Figure 1. Phylogenetic groups identified in the *E. coli* isolates harbouring different ESBLs
Figure 1

Phylogenetic group: □ A □ B1 □ D