number CU459141) and in the draft genomes of WM98, 3208 and D2 differed from one another by fewer than five single nucleotide differences, but only the WM98 sequence was not interrupted by large insertions or deletions (positions of insertions/deletions are indicated in Figure 1). AB307-0294 (GenBank accession number CP001172) was also identical over most of this span, but contained patches that differed and lacked a large span. The AB0057 sequence (GenBank accession number CP001182), for which the ampC gene was previously corrected, differed at 33 more positions (single base substitutions or additions or deletions; mainly the absence of an A or a T in a run of As or Ts) and many of these differences may be errors caused by the sequencing technology used.

Contigs containing ampC and its surrounds were retrieved from the whole genome sequence of G7 reported previously⁶ and joined using the manually determined sequence described above. Comparison with the WM98 sequence revealed a segment of 31.8 kb, defined as between the first and last base differences surrounding the ISAb1-ampC in G7, which differed from the corresponding region in WM98 by 2.2% (Figure 1). This indicates that this segment has been replaced by a segment imported from another A. baumannii strain that included an ISAb1 upstream of the ampC gene. Hence, it appears that a DNA segment that included an ISAb1-activated ampC gene was introduced into an isolate belonging to the GC1 clonal complex, possibly by conjugation, and that homologous recombination incorporated it into the chromosome displacing the resident copy. Examination of the regions on either side of the 31.8 kb diverged segment revealed the presence of two smaller replaced patches of 4.7 and 2.8 kb in G7, which differed from the corresponding regions in WM98 by 4.5% and 2.1%, respectively (Figure 1). Outside these patches, WM98 and G7 differed by only 3 bp.

This is the first study providing evidence for horizontal transfer of a DNA segment that contains an ISAb1-activated ampC gene between two A. baumannii strains. The findings highlight the significance of the horizontal transfer of chromosomal DNA segments in the generation of cefalosporin resistance in A. baumannii.

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Transparency declarations
None to declare.

References

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Identification of fluoroquinolone-resistant extended-spectrum β-lactamase (CTX-M-8)-producing Escherichia coli

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Keywords: E. coli, multidrug resistant, ESBLs, PMQR, pAmpC, CMY-2, MLST, Brazil

Sir,

The epidemiology of extended-spectrum β-lactamases (ESBLs) has changed worldwide over recent years and nowadays ESBLs of the CTX-M-8 group are being increasingly detected in Escherichia coli from humans, animals and food.¹–⁵ In this regard, a recent report published in JAC suggested the possibility of transmission of CTX-M-8-positive strains by travellers or contaminated food.¹ In this study, we report for the first time the isolation of CTX-M-8-producing E. coli in dairy buffalo, highlighting a new reservoir of ESBL producers.

From March 2010 to June 2011, during a local surveillance study established to monitor the occurrence of broad-spectrum cefalosporin- and/or fluoroquinolone-resistant Enterobacteriaceae in food-producing animals, 18 enrofloxacin-resistant E. coli isolates
Table 1. Characteristics of fluoroquinolone- and third-generation cephalosporin-resistant E. coli isolated from buffalo in dairy farms in south-east Brazil

<table>
<thead>
<tr>
<th>Strain</th>
<th>Year/state/farm</th>
<th>Resistance profile</th>
<th>MIC (mg/L)</th>
<th>Kirby–Bauer</th>
<th>GyrA mutation&lt;sup&gt;3&lt;/sup&gt;/PMQR</th>
<th>ESBL&lt;sup&gt;4&lt;/sup&gt;</th>
<th>ERIC profile&lt;sup&gt;5&lt;/sup&gt; (MLST)</th>
<th>Phylogroup</th>
</tr>
</thead>
<tbody>
<tr>
<td>13B</td>
<td>2010/PR/F1</td>
<td>&gt;32 &gt;32 &gt;64 4</td>
<td>R S R S R R S</td>
<td>Ser83Phe</td>
<td>CTX-M-8</td>
<td>A (ST224)</td>
<td>B1</td>
<td></td>
</tr>
<tr>
<td>14B</td>
<td>2010/PR/F1</td>
<td>&gt;32 &gt;32 &lt;2 &lt;1</td>
<td>S S S S R R S</td>
<td>Ser83Phe/QnrB</td>
<td></td>
<td></td>
<td>B1</td>
<td></td>
</tr>
<tr>
<td>15B</td>
<td>2010/PR/F1</td>
<td>32 24 &lt;2 &lt;1</td>
<td>R S S S R R S</td>
<td>Ser83Phe/QnrB</td>
<td></td>
<td></td>
<td>B1</td>
<td></td>
</tr>
<tr>
<td>16B</td>
<td>2010/PR/F1</td>
<td>16 8 &lt;2 &lt;1</td>
<td>R S S S R R R</td>
<td>Ser83Phe/QnrB</td>
<td></td>
<td></td>
<td>B1</td>
<td></td>
</tr>
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<td>Ser83Phe</td>
<td></td>
<td></td>
<td>D1</td>
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</tr>
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<td>8 4 &gt;64 4</td>
<td>R S R S S R R</td>
<td>Ser83Phe/QnrA</td>
<td>CTX-M-8</td>
<td>E (ST2179)</td>
<td>B1</td>
<td></td>
</tr>
<tr>
<td>20B</td>
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<td>&gt;32 &gt;32 &lt;2 &lt;1</td>
<td>R S S S R R R</td>
<td>Ser83Phe/QnrB</td>
<td></td>
<td></td>
<td>B1</td>
<td></td>
</tr>
<tr>
<td>21B</td>
<td>2010/PR/F1</td>
<td>32 6 &gt;64 4</td>
<td>R S R S S R R</td>
<td>Ser83Phe</td>
<td>CTX-M-8</td>
<td>H (ST2308)</td>
<td>B1</td>
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<tr>
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<td></td>
<td>B1</td>
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<td>&gt;32 &gt;32 4 3</td>
<td>R R R R R R R</td>
<td>Ser83Phe/QnrB</td>
<td>CMY-2</td>
<td>F (ST205)</td>
<td>B1</td>
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</tr>
<tr>
<td>24B</td>
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<td>Ser83Phe/QnrB</td>
<td></td>
<td></td>
<td>B1</td>
<td></td>
</tr>
<tr>
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<td>Ser83Phe/QnrB</td>
<td></td>
<td></td>
<td>B1</td>
<td></td>
</tr>
<tr>
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<td></td>
<td>A1</td>
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</tr>
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<td>Ser83Phe</td>
<td></td>
<td></td>
<td>C1</td>
<td></td>
</tr>
<tr>
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<td>32 16 &lt;2 &lt;1</td>
<td>S S S S R R R</td>
<td>Ser83Phe</td>
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<td>G1</td>
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</tr>
<tr>
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<td>S S S S R R R</td>
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<tr>
<td>32B</td>
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<td>32 16 &lt;2 &lt;1</td>
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<td>R S S S R R R</td>
<td>Ser83Phe</td>
<td></td>
<td></td>
<td>G1</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup>P, Parana State (Farm 1); SP, Sao Paulo State (Farms 2 and 3).
<sup>2</sup>MICs were determined by the Etest or agar dilution method; resistance is indicated in bold. 11,12 ENR, enrofloxacin; CIP, ciprofloxacin; EFT, ceftiofur; CTX, cefotaxime; AMP, ampicillin; AMC, amoxicillin/clavulanate; CEF, cefalotin; FOX, cefoxitin; TET, tetracycline; NAL, nalidixic acid; SXT, trimethoprim/sulfamethoxazole.
<sup>3</sup>Ser83Phe, substitution of serine for phenylalanine at codon 83 of the gyrA gene.
<sup>4</sup>GenBank accession numbers KF981450 (bla<sub>CTX-M-8</sub>, E. coli strain 13B) and KF991585 (bla<sub>CTX-M-8</sub>, E. coli strain 21B).
<sup>5</sup>ERIC patterns were analysed using the Dice similarity coefficient and the unweighted pair group method using the average linkage cluster method (BioNumerics software; Applied Maths, Kortrijk, Belgium). Clusters A–H were assigned based on <90% similarity of banding patterns.
were recovered from faecal swab samples collected from 46 buffalo calves (up to 6 months of age) raised semi-extensively for milk production on six dairy farms in the states of São Paulo and Paraná, south-eastern Brazil. Non-repetitive faecal sampling included 13 samples from Farm 1, 7 from Farm 2, 6 from Farm 3, 2 from Farm 4, 6 from Farm 5 and 12 from Farm 6.

The isolates were identified by conventional techniques (i.e. Vitek 2, API and biochemical tests) and the antimicrobial susceptibility was evaluated by disc diffusion assay.11,12 MIC determinations were performed by the agar dilution or Etest methods, according to CLSI standards.11,12 and ESBL production was investigated by using a double-disc synergy test, Etest ESBL strips (bioMérieux, Marcy-l’Etoile, France) and CHROMagar® ESBL (Paris, France). The presence of ESBL- and plasmid-mediated AmpC (pAmpC) β-lactamase genes was confirmed by PCR and sequencing. Further screening of plasmid-mediated quinolone resistance (PMQR) genes was performed by PCR and chromosomal mutations in the quinolone resistance-determining region of the gyrA gene were investigated by restriction fragment length polymorphism analysis. Next, phylogenetic grouping and virulence gene patterns were screened by PCR and the clonal relatedness was investigated by enterobacterial repetitive intergenic consensus (ERIC)-PCR and multilocus sequence typing (MLST) (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli).

Forty-five buffalo had faecal cultures positive for E. coli, of which 18 (40%) were resistant to fluoroquinolones (Table 1). Among fluoroquinolone-resistant strains, 22% (n = 4) exhibited further resistance to human and veterinary broad-spectrum cephalosporins (i.e. ceftiofur and cefotaxime). In this regard, three E. coli strains showed a positive ESBL phenotype, being susceptible to cefoxitin, whereas the fourth E. coli strain displayed resistance to cefoxitin, being suspected of harbouring a pAmpC enzyme. In fact, the presence of the blaCTX-M-8 and blaCMY-2 genes was confirmed by PCR and sequencing in cefoxitin-susceptible and cefoxitin-resistant E. coli strains, respectively. On the other hand, all fluoroquinolone-resistant strains had an identical mutation in gyrA (Ser83Phe) and nine of them carried qnr genes, characterizing them as Shiga toxin-producing E. coli. Finally, while CTX-M-8-producing E. coli strains belonged to sequence types ST224, ST2179 and ST2308, the CMY-2-producing E. coli belonged to ST205.

In this study, we report the occurrence of fluoroquinolone-resistant and ESBL-producing E. coli in commercial dairy buffaloes. Fluoroquinolone resistance was linked to a high MIC of ciprofloxacin (MICCIP = 16 mg/L) and enrofloxacin (MICEN = 32 mg/L) and the predominant genetic basis for this resistance was mutations in gyrA, whereas resistance to broad-spectrum cephalosporins was associated with CTX-M-8 and CMY-2 enzymes and as such is a cause of clinical concern.

In Brazil, buffalo are generally raised without a national buffalo breeding improvement programme and thus there is no available report concerning the use of antimicrobial drugs. However, in this study, all farms reported previous use of enrofloxacin for the empirical treatment of infectious diarrhoea in dairy calves.

The correlation of antimicrobial resistance in E. coli isolates with the low-virulence phylogenetic group B1 suggests that selection of silent carriers of acquired resistance genes, among commensal E. coli, in buffalo is ongoing. In this regard, it has been proposed that commensal microflora could represent a long-term reservoir of resistance genes that could be transferred horizontally to other bacteria. In fact, the blaCTX-M-8 gene was described for the first time in 2000, in cefotaxime-resistant Enterobacteriaceae isolated in Brazil.2 Since then, it has been widely identified in E. coli isolates from South America (Uruguay, Argentina and French Guiana),1,7,8 North America (the USA and Canada),1,3 Africa (Kenya and Tunisia),10 Asia (Japan)9 and Europe (Spain, the UK and Germany).1,4,5 Of particular interest has been the description of CTX-M-8-producing E. coli in chicken meat in the UK,5 Sweden,6 Japan9 and Tunisia, mostly in imported meat from South America, which suggests the possibility of transmission of CTX-M-8-positive strains by contaminated food, highlighting the potential for CTX-M-8 to become endemic worldwide.

Additional studies should be performed in the future to track changes in the epidemiology and evolution of ESBLs and their frequencies in different ecosystems and animal hosts. Moreover, management practices focusing on eliminating resistance development in food-producing animals could reduce the burden of antibiotic resistance on farms. In summary, we showed that dairy buffalo can represent a new reservoir of genes conferring resistance to critically important antibiotics used in human and veterinary medicine.

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Transparency declarations

None to declare.

References

6 Egervárn M, Börjesson S, Byfors S et al. Escherichia coli with extended-spectrum β-lactamases or transferable AmpC β-lactamases and
Isolation of ciprofloxacin-resistant 
Legionella pneumophila in a patient 
with severe pneumonia

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Keywords: L. pneumophila, Legionnaires’ disease, susceptibility

Sir,

Legionella species are responsible for 1%–5% of cases of community-acquired pneumonia. Legionella pneumophila serogroup 1 (SG1) accounts for >90% of Legionnaires’ disease (LD) in North America and Europe and is the cause of significant mortality. The mortality rate among patients with L. pneumophila infections continues to be high, up to 26%. The antimicrobial agents most commonly used for treatment of LD are fluoroquinolones (e.g. ciprofloxacin or levofloxacin) and macrolides. In recent studies, we established wild-type distributions and determined the epidemiological cut-off values (ECOFFs) in clinical L. pneumophila SG1 isolates for 10 antimicrobials commonly used for the treatment of Legionella infections.

A patient sought care at his general practitioner after several days of falling and body pains. On examination, the patient appeared ill and was sent to the emergency department of a nearby hospital. The initial chest radiograph demonstrated an infiltrate of the left lower lung field. The patient was admitted to the intensive care unit. Blood cultures were taken and antibiotic treatment was started with cefazolin and gentamicin. Urine was examined for the presence of Legionella antigens and when this test was reported positive, treatment was switched to 400 mg of ciprofloxacin intravenously twice daily. After initial improvement the clinical condition of the patient deteriorated, leading to intubation and mechanical ventilation. A new chest radiograph revealed a diffuse interstitial pneumonia. Bronchoalveolar lavage (BAL) was performed 4 days after treatment with ciprofloxacin was started and the patient slowly recovered. Eventually, culture of the BAL grew L. pneumophila SG1 after 4 days of ciprofloxacin treatment. After 10 days, the patient could be transferred to the ward. Therapy was then switched to 500 mg of clarithromycin orally twice daily. The patient’s further recovery was uncomplicated.

The L. pneumophila SG1 strain was sent, as part of a national Legionella outbreak detection programme, to the reference laboratory for Legionella in Haarlem, The Netherlands. Susceptibility testing for ciprofloxacin was performed with Etest and an MIC value of ciprofloxacin of 2 mg/L was found. This value is outside the wild-type distribution range ECOFF = 1 mg/L as previously described and therefore potentially resistant.

For sequencing of gyrA and gyrB (DNA gyrase) and parC and parE (topoisomerase IV) genes, extraction of L. pneumophila DNA was performed. The DNA extraction was performed by use of Qiagen’s BioRobot EZ1 (Hilden, Germany) according to the manufacturer’s instructions. The sequencing reaction was performed twice by using primer systems previously described for the L. pneumophila SG1 strain Paris. A comparative analysis of the obtained sequences was done using the published L. pneumophila genomes and data from the literature describing mutations in the quinolone resistance-determining region (QRDR) of type II topoisomerase of L. pneumophila by using DNASTar (WI, USA) software and the NCBI database. For control experiments, the wild-type strain MTZ OLDA and a spontaneous quinolone-resistant mutant of this strain were used. MTZ OLDA is an environmental isolate (L. pneumophila SG1), isolated from the water supply of a large building. A point mutation in the QRDR of the gyrA gene was identified and this mutation led to an amino acid exchange at position 83 (Escherichia coli numbering system). The result of this amino acid exchange is a change in ciprofloxacin susceptibility. Mutation at the same position (amino acid 83) has also been reported for other spontaneous quinolone-resistant mutants (Table 1).

It is known that, in general, pathogens can become resistant during the course of a patient’s therapy and also induction of resistance upon exposure to antibiotics has been described. The origin of resistance in the clinical isolate is as yet unclear. There are two possibilities. The first is that the patient contracted an L. pneumophila SG1 strain with this point mutation from the environment. Alternatively, the mutation occurred during the