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 previously[6] 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 ISAba1-*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 This indicates that this segment has been replaced by a segment imported from another *A. baumannii* strain that included an ISAba1 upstream of the *ampC* gene. Hence, it appears that a DNA segment that included an ISAba1-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 ISAba1-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 cephalosporin resistance in *A. baumannii*.

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Transparency declarations

None to declare.

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Identification of fluoroquinolone-resistant extended-spectrum β-lactamase (CTX-M-8)-producing *Escherichia coli* ST224, ST2179 and ST2308 in buffalo (*Bubalus bubalis*)

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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.[1–10] 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 cephalosporin- 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

		Resistance profile[b]														
		MIC (mg/L)				Kirby–Bauer										
Strain	Year/state/farm[a]	ENR	CIP	EFT	CTX	AMP	AMC	CEF	FOX	TET	NAL	SXT	GyrA mutation[c]/PMQR	ESBL[d]	ERIC profile[e] (MLST)	Phylogroup
13B	2010/PR/F1	**>32**	**>32**	**>64**	**4**	**R**	S	**R**	S	**R**	**R**	S	Ser83Phe	CTX-M-8	A (ST224)	B1
14B	2010/PR/F1	**>32**	**>32**	<2	<1	S	S	S	S	**R**	**R**	S	Ser83Phe/QnrB		B	B1
15B	2010/PR/F1	**32**	**24**	<2	<1	**R**	S	S	S	S	**R**	S	Ser83Phe/QnrB		B	B1
16B	2010/PR/F1	**16**	**8**	<2	<1	**R**	S	S	S	**R**	**R**	**R**	Ser83Phe/QnrB		B	B1
18B	2010/PR/F1	**>32**	**>32**	<2	<1	S	S	S	S	**R**	**R**	S	Ser83Phe		D	B1
19B	2010/PR/F1	**8**	**4**	**>64**	**4**	**R**	S	**R**	S	S	**R**	S	Ser83Phe/QnrA	CTX-M-8	E (ST2179)	B1
20B	2010/PR/F1	**>32**	**>32**	<2	<1	**R**	S	S	S	**R**	**R**	**R**	Ser83Phe/QnrB		B	B1
21B	2010/PR/F1	**32**	**6**	**>64**	**4**	**R**	S	**R**	S	S	**R**	S	Ser83Phe	CTX-M-8	H (ST2308)	B1
22B	2010/PR/F1	**>32**	**8**	<2	<1	**R**	S	S	S	**R**	**R**	**R**	Ser83Phe/QnrB		B	B1
23B	2010/PR/F1	**>32**	**>32**	**4**	**3**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	Ser83Phe/QnrB	CMY-2	F (ST205)	B1
24B	2010/PR/F1	**>32**	**>32**	<2	<1	**R**	S	S	S	**R**	**R**	**R**	Ser83Phe/QnrB		B	B1
25B	2010/PR/F1	**16**	**8**	<2	<1	**R**	S	S	S	**R**	**R**	**R**	Ser83Phe/QnrB		B	B1
26B	2010/SP/F2	**32**	**32**	<2	<1	S	S	S	S	**R**	**R**	**R**	Ser83Phe		A	B1
28B	2010/SP/F3	**32**	**16**	<2	<1	S	S	S	S	**R**	**R**	S	Ser83Phe		C	B2
29B	2010/SP/F3	**32**	**16**	<2	<1	**R**	S	S	S	**R**	**R**	**R**	Ser83Phe		G	B1
31B	2010/SP/F3	**32**	**16**	<2	<1	**R**	S	S	S	S	**R**	**R**	Ser83Phe		G	B1
32B	2010/SP/F3	**32**	**16**	<2	<1	S	S	S	S	**R**	**R**	S	Ser83Phe		C	B2
34B	2010/SP/F3	**>32**	**16**	<2	<1	**R**	S	S	S	**R**	**R**	**R**	Ser83Phe		G	B1

[a]PR, Paraná State (Farm 1); SP, São Paulo State (Farms 2 and 3).
[b]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.
[c]Ser83Phe, substitution of serine for phenylalanine at codon 83 of the *gyrA* gene.
[d]GenBank accession numbers KF981450 ($bla_{CTX\text{-}M\text{-}8}$, *E. coli* strain 13B) and KF991585 ($bla_{CTX\text{-}M\text{-}8}$, *E. coli* strain 21B).
[e]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 (bioMeriéux, Marcy-l'Étoile, 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 genotypes 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 $bla_{\text{CTX-M-8}}$ and $bla_{\text{CMY-2}}$ 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*-like PMQR genes (Table 1). Genotyping by ERIC-PCR showed eight different genetic profiles (named A–H) among fluoroquinolone-resistant strains and phylogenetic investigation showed that the low-virulence phylogenetic group B1 (89%) was predominant (Table 1). Only two *E. coli* strains belonged to the high-virulence phylogenetic group B2, which were positive for *stx2* and *eae* 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 buffalo. Fluoroquinolone resistance was linked to a high MIC of ciprofloxacin ($MIC_{50}=16$ mg/L) and enrofloxacin ($MIC_{50}=32$ 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 $bla_{\text{CTX-M-8}}$ gene was described for the first time in 2000, in cefotaxime-resistant Enterobacteriaceae isolated in Brazil.[2] Since then, it is 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] Japan[9] 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.

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Isolation of ciprofloxacin-resistant *Legionella pneumophila* in a patient with severe pneumonia

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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[1] and is the cause of significant mortality. The mortality rate among patients with *L. pneumophila* infections continues to be high, up to 26%.[2] The antimicrobial agents most commonly used for treatment of LD are fluoroquinolones (e.g. ciprofloxacin or levofloxacin) and macrolides.[3] 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.[4]

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.[5] 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.[4]

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.[6] 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.[6,7] 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.[8] 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.[6,7] 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