

IN-ST239-2 and 47 in IN-ST368-5, compared with 24 genes in IN-ST239-7) and the presence of plasmid *rep24*, which has been previously described in community-associated MRSA strains (pWBG745).¹⁵

To conclude, this study highlights the SCCmec region in the Indian ST239 clone as subject to recombination events that were evidenced by the formation of the largest SCCmec III composite element reported so far.

Genome accession numbers

The sequences of the five MRSA strains sequenced in this study were submitted to NCBI under BioProject accession number PRJNA316797. SCCmec STs and subtypes used for comparison (<http://www.sccmec.org/>) and downloaded from NCBI were: SCCmec I, AB033763.2; SCCmec IIa, D86934.2; SCCmec IIb, AB127982.1; SCCmec III, AB037671.1; SCCmec IVa, AB063172.2; SCCmec IVb, AB063173.1; SCCmec IVc, AB096217.1; SCCmec IVd, AB097677.1; SCCmec IVe, AJ810121.1; SCCmec IVg, DQ106887.1; SCCmec V, AB121219; SCCmec VT, AY894416.1; and SCCmec VI, AF411935.3.

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Transparency declarations

None to declare.

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Emergence of *Escherichia coli* ST131 H30/H30-Rx subclones in companion animals

Adriana Belas, Cátia Marques , Catarina Aboim and Constança Pomba *

Interdisciplinary Centre of Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Av. da Universidade Tecnica, 1300-477 Lisbon, Portugal

*Corresponding author. E-mail: cpomba@fmv.ulisboa.pt  orcid.org/0000-0002-0504-6820

Sir,

The *Escherichia coli* O25b-ST131, with its fluoroquinolone-resistant H30 subclone and its nested ESBL CTX-M-15-associated H30-Rx subclone, is the most disseminated MDR and virulent *E. coli* clonal group worldwide.¹ In previous work, we have reported the

Table 1. Characterization of *E. coli* O25b:H4-B2-ST131 H30 and H30-Rx subclones isolated from companion animals

Isolate	Year	Companion animal origin	Clonal group ST131	Subclone H30	Subclone H30-Rx	Antimicrobial resistance ^a	ESBL and/or pAmpC genes	Other resistance genes	PAIs	Virotype ^b	Virulence genes
FMV2358/03	2003	dog	yes	yes	no	AMP-TET-CIP-ENR	no	—	PAI _{I1536} -PAI _{I196} -PAI _{Iv536} -PAI _{IICFT073}	D	ecpA-hlyA-cnfl-sfaDE-papEF-papGIII-iucD
FMV5825/04	2004	dog	yes	yes	yes	AMP-AMC-CEF-CTX-CAZ-FOX-SXT-TET-CIP-ENR-GEN-AMK-TOB	bla _{CTX-M-15}	bla _{TEM} -bla _{OXA-1-sulII} -aac(6)-Ib-cr-qnrB2	PAI _{I1536} -PAI _{I196} -PAI _{IICFT073} -PAI _{Iv536} -PAI _{IICFT073}	D	ecpA-hlyA-cnfl-sfaDE-papEF-iucD
FMV2777/08	2008	cat	yes	yes	yes	AMP-AMC-CEF-CTX-CAZ-TET-CIP-ENR-GEN-TOB	bla _{CTX-M-15}	sac(6)-Ib-cr	PAI _{IICFT073} -PAI _{Iv536} -PAI _{IICFT073}	D	ecpA-hlyA-papEF-iucD
FMV6710/08	2008	dog	yes	yes	no	AMP-SXT-CIP-ENR	no	sul2	PAI _{IICFT073} -PAI _{Iv536} -PAI _{IICFT073}	D	ecpA-hlyA-papEF-iucD
FMV5695/09	2009	dog	yes	yes	no	AMP-TET-CIP-ENR	no	bla _{TEM} -oqxAB	PAI _{Iv536} -PAI _{IICFT073}	D	ecpA-papEF-iucD
FMV58/13	2013	cat	yes	yes	yes	AMP-CEF-CTX-SXT-CIP-ENR	bla _{CTX-M-1}	bla _{TEM} -dfrAI-sac(6)-Ib-cr	PAI _{IICFT073} -PAI _{Iv536} -PAI _{IICFT073}	D	ecpA-hlyA-sfaDE-papEF-iucD
FMV146/14	2014	dog	yes	yes	no	AMP-TET-CIP-ENR	no	sac(6)-Ib-cr	PAI _{I1536} -PAI _{IICFT073} -PAI _{Iv536}	D	ecpA-hlyA-papEF-afABC-iucD

AMC, amoxicillin/clavulanate; AMP, ampicillin; AMK, amikacin; CAZ, ceftazidime; CIP, ciprofloxacin; GEN, gentamicin; ENR, enrofloxacin; FOX, cefoxitin; SXT, trimethoprim/sulfamethoxazole; TET, tetracycline; TOB, tobramycin; CEF, cefalotin; pAmpC, plasmid-mediated AmpC.

^aSusceptibility was accessed according to CLSI M100-S27.

^bClassification of the virotype was in accordance with Nicolas-Chanoine *et al.*¹

first detection of this O25b-ST131 clone causing urinary tract infection (UTI) in a dog.² Additionally, later on, we found within-lineage variability of ST131 *E. coli* UTI isolates from humans and companion animals by PFGE analysis.³ The detection of human high-risk pandemic *E. coli* clones causing UTI in companion animals is a great public health concern.⁴

Between 1999 and 2015, 342 uropathogenic *E. coli* were isolated at the Laboratory of Antimicrobial Resistance, Faculty of Veterinary Medicine, University of Lisbon from companion animals (dogs and cats) with UTI. Significant bacteriuria was determined based on a quantitative urine culture according to the urine collection method used (puncture, catheter or free catch). Urine samples were collected from companion animals at the teaching hospital of the Faculty of Veterinary Medicine and at private veterinary hospitals in Lisbon, Portugal.

All B2 phylogroup isolates were studied by PCR for the ST131-associated SNP in the *mdh* and *gyrB* genes;⁵ 14.5% ($n = 25/172$) were the pandemic O25b:H4-B2-ST131 clone. The *E. coli* O25b:H4-B2-ST131 H30 and H30-Rx subclones were screened by PCR as previously described.⁶ Seven ($n = 7/25$; 28%) of the O25b:H4-B2-ST131 clone companion animal isolates were the H30 subclone and three out of these seven were the H30-Rx subclone (Table 1). To the best of our knowledge this is the first report of *E. coli* ST131 H30/H30-Rx subclones causing UTI in companion animals in Europe.

The presence of uropathogenic *E. coli* (UPEC) virotype markers was assessed, i.e. Pap fimbriae (*papEF* operon segment), Sfa fimbriae and Afa afimbrial adhesin (*sfa* and *afa* genes, respectively), the *hlyA* gene from the α -haemolysin operon, cytotoxic necrotizing factor 1 (*cnf1* gene), aerobactin siderophore (*iucD* gene), *E. coli* common pilus (*ecpA* gene) and the uropathogenic specific protein (*usp* gene).^{7,8} The distribution of alleles I, II and III of the P adhesin gene *papG* was studied.⁹

Additionally, pathogenicity-associated islands (PAIs) (PAI_{IV536}, PAI_{I536}, PAI_{II536}, PAI_{III536}, PAI_{IJ96}, PAI_{IIJ96}, PAI_{ICFT073}, PAI_{IICFT073}) were identified.¹⁰ The *E. coli* O25b:H4-B2-ST131-H30/H30-Rx most common pathogenicity and virulence-associated gene profiles were PAI_{ICFT073}-PAI_{IV536}-PAI_{IICFT073} (42.9%, $n = 3/7$) and *ecpA-hlyA-papEF-iucD* (28.6%, $n = 2/7$), respectively. The full pathogenicity and virulence genotype of *E. coli* O25b:H4-B2-ST131-H30/H30-Rx is shown in Table 1. All H30/H30-Rx subclones causing UTI in companion animals belonged to virotype D, which confirms their virulent characteristics.¹

Resistance to third-generation cephalosporins was detected only in the three isolates of the H30-Rx subclone. β -Lactamase genes were screened as reported elsewhere.² As expected from other studies, O25b:H4-B2-ST131 H30 and H30-Rx isolates were fluoroquinolone resistant,¹ yet one O25b:H4-B2-ST131-H30-Rx *E. coli* did not carry the ESBL *bla*_{CTX-M-15}, but instead carried the frequent *bla*_{CTX-M-1} gene, which is associated with *E. coli* isolates of farm animal origin and recently described in humans in Turkey with UTI.^{1,11} The mechanism of resistance to other antimicrobial classes was characterized by PCR and nucleotide sequencing for *sul1*, *sul2*, *sul3*, *dfra1*, *dfra12* and the plasmid-mediated quinolone resistance (PMQR) genes [*qnrA*, *qnrB*, *qnrS*, *qnrC*, *qnrD*, *qepA*, *aac(6')-Ib* and the MDR *oqxAB* genes of the efflux pump].¹² The *aac(6')-Ib-cr* gene was the most common PMQR gene detected (57.1%, $n = 4/7$) and was more frequently found in ST131 H30-Rx *E. coli* isolates than in ST131 H30 isolates. The *qnrB2* and *aac(6')*

Ib-cr genes were detected in the FMV5825/04 dog isolate as expected from previous data (Table 1).²

In Europe, carbadox and olaquinox, which are quinoxaline derivatives with antibacterial properties, were used for the prevention of dysentery and as growth promoters in pigs since 1974 and 1976, respectively. Fortunately, their use has been banned in farm animals in Europe for decades, but not in China.¹³ The efflux pump OqxAB also extrudes antibiotics such as chloramphenicol and fluoroquinolones. Isolate FMV5695/09 O25b:H4-B2-ST131-H30 harboured both *oqxA* and *oqxB* efflux pump genes, which could potentially be related to the reduced fluoroquinolone susceptibility. To the best of our knowledge, this is the first description of an ST131 *E. coli* harbouring the *oqxAB* efflux pump. Further studies will be necessary to elucidate the importance of this resistance mechanism in the ST131 pandemic *E. coli* clone.

This study reports the detection and frequency of the *E. coli* O25b:H4-B2-ST131 H30/H30-Rx subclones in companion animals with UTI in Portugal and, to the best of our knowledge, in Europe. In conclusion, the findings presented in this study are relevant and, to the best of our knowledge, represent the first detection of ST131 H30/H30-Rx *E. coli* isolates associated with UTI in companion animals in Europe. To the best of our knowledge, this is the first report in Europe of the disseminated *E. coli* O25b:H4-B2-ST131-H30/H30-Rx, MDR, fluoroquinolone-resistant human high-risk clone and its CTX-M-15-H30-Rx and CTX-M-1-H30-Rx subsets in companion animals with UTI. Studies of ST131 H30/H30-Rx in humans in Portugal are scarce, yet H30/H30-Rx subclones have been described in faecal samples in healthy humans.¹⁴ These results raise public health concerns since these subclones may have an impact on human health through the close and direct contact between companion animals and owners. Moreover, the close contact between companion animals and humans creates opportunities for interspecies transmission of resistant bacteria.

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Mechanisms of high-level ceftolozane/tazobactam resistance in *Pseudomonas aeruginosa* from a severely neutropenic patient and treatment success from synergy with tobramycin

Wonhee So^{1*}, James Shurko^{2,3}, Ralph Galega^{2,3}, Rod Quilitz¹, John N. Greene¹ and Grace C. Lee^{2,3}

¹Moffitt Cancer Center, 12902 USF Magnolia Drive, Tampa, FL 33612, USA; ²College of Pharmacy, The University of Texas at Austin, Austin, TX, USA; ³School of Medicine, The University of Texas Health San Antonio, San Antonio, TX, USA

*Corresponding author. Tel: +1-813-745-8529; Fax: +1-813-449-8900; E-mail: Wonhee.so@moffitt.org

Sir,
We read the article by Fraile-Ribot *et al.*¹ with great interest and herein report another case of ceftolozane/tazobactam-resistant *Pseudomonas aeruginosa* bacteraemia that developed after 5 weeks of exposure to ceftolozane/tazobactam. A retrospective review of the medical records of a single patient case does not mandate review by the University of South Florida College of Medicine Institutional Review Board (IRB), thus informed consent was not required.

The patient had AML and was induced with cladribine, cytarabine, filgrastim plus mitoxantrone on days 1–6 then reinduced with 5 days of cladribine plus cytarabine on days 15–19. The patient had a long history of hidradenitis suppurativa with draining sinus tracts in the left axilla and perianal area at presentation. Upon neutropenic fever, oral ciprofloxacin was escalated to antipseudomonal β-lactams (Table 1). The first bacteraemia developed on day 25, despite being on 500 mg of meropenem every 6 h since day 7, then cleared on day 29 while on a combination of 500 mg of meropenem every 6 h, 750 mg of ciprofloxacin every 12 h and 7 mg/kg tobramycin every 24 h, which was later adjusted to 3 g of ceftolozane/tazobactam every 8 h plus 7 mg/kg tobramycin every 24 h when the susceptibility was finalized on day 30. The patient was discharged on day 47 with a plan to continue 4.5 g of ceftolozane/tazobactam over 24 h as a continuous infusion until absolute neutrophil count (ANC) >500 cells/mm³ while the patient's ANC remained <100 cells/mm³ until day 75. In the meantime, the patient was readmitted with neutropenic fever on day 59 and the second bacteraemia occurred on day 65 while on 1.5 g of ceftolozane/tazobactam every 8 h. With a combination of 3 g of ceftolozane/tazobactam every 8 h, given over 4 h, plus 7 mg/kg tobramycin every 24 h, bacteraemia was