separated by 22 bp instead of 17 bp (see Figure S1, available as Supplementary data at JAC Online). An additional promoter was predicted by Softberry BPROM promoter prediction and consisted of the −35 box of IS18 and a −10 box separated by 13 bp (Figure S1, available as Supplementary data at JAC Online). Interestingly, the TTCAAT −35 box identical to that from Baz (blaOXA-228) was adjacent to the left inverted repeat.

OXA-228-like expression in isolate KH243 was compared with that in carbapenem-susceptible A. bereziniae isolate G3-59 by semi-quantitative RT–PCR (qRT–PCR) using rp08 as the reference gene. The primers used for qRT–PCR are shown in Table 1. Three independent experiments were performed using freshly prepared RNA and cDNA and revealed a 56-fold (±3.84) overexpression of blaOXA-228-like in isolate KH243 compared with that in G3-59. To investigate the potential to mediate carbapenem resistance, IS18:blaOXA-257 was cloned into the shuttle vector pWH1266, but we were unable to transfer this into A. bereziniae G3-59. However, the construct was successfully transferred into A. baumannii ATCC 17978 by electroporation, as previously described for Pseudomonas aeruginosa. Expression of blaOXA-257 in A. baumannii ATCC 17978 raised both imipenem and meropenem MICs from 0.25 to ≥32 mg/L, demonstrating that IS18:blaOXA-257 is able to confer carbapenem resistance.

In conclusion, this study has detected an IS upstream of the intrinsic blaOXA in A. bereziniae, a phenomenon that has not been described in this species so far. IS18 conferred overexpression of OXA-257, which mediated carbapenem resistance in A. bereziniae and A. baumannii. Moreover, because IS18 has previously been described adjacent to acquired blaOXA in A. baumannii, these data suggest a potential for dissemination of OXA-257 in the genus Acinetobacter.

Funding
This work was supported by grants from Bundesministerium für Bildung und Forschung (BMBF), Germany, Klinische Forschergruppe Infektiologie (grant number 01 KI 0771 to P. G. H and H. S.). E. Z. and H. S. were supported by Magic Bullet. Magic Bullet is a project funded by the European Union—Directorate General for Research and Innovation through the Seventh Framework Program for Research and Development (Grant Agreement 278232) and has been running since the 1 January 2012 (duration 36 months).

Transparency declarations
None to declare.

Supplementary data
Figure S1 is available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

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J Antimicrob Chemother 2014
doi:10.1093/jac/dkt343
Advance Access publication 10 September 2013

Within-lineage variability of ST131 Escherichia coli isolates from humans and companion animals in the south of Europe

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Keywords: pulsotypes, fluoroquinolone resistance, ESBLs, MLST, PFGE
Sir,

A multiresistant CTX-M-15-producing clonal group of Escherichia coli isolates belonging to phylogroup B2, namely O25b:H4/ST131, has recently emerged and spread across three continents; it predominantly causes community-onset infections in humans and to a lesser extent in dogs and cats.1–3 Some specific lineage variations within this clone, detected by PFGE, such as pulsotype 968, have been associated with clonal commonality across pets and humans.4,5 Important comparative studies to date have only included a small number of isolates from companion animals.

A total of 148 ST131 isolates, identified by screening unselected sequential isolates, were compiled in two different studies carried out in Lisbon (n = 59) and Seville (n = 89). The veterinary E. coli isolates (n = 31) were collected during 2004–09 from dogs and cats with a urinary tract infection (UTI). Portuguese human UTI clinical isolates (n = 28) were obtained during 2005–06 in one hospital and in a community diagnostic laboratory covering an area of 365,000 inhabitants in the Lisbon region. ST131 human isolates from Seville were collected from a systematic prospective study in which all E. coli isolates from two hospitals covering an area with 1 million inhabitants were screened for O25b serogroup during 30 weeks in 2010. Isolates from Seville were mainly recovered from UTI (98%).6

The ST131 clone was screened by PCR with specific primers for O25b rfb, allele 3 of the pabB gene and B2 genetic traits, and by multiplex PCR for phylogroup B2 typing using two different combinations of primers.7,8 Susceptibility testing was performed using commercial microdilution plates and the disc diffusion method for nalidixic acid, ciprofloxacin, amoxicillin/clavulanic acid, gentamicin, tobramycin, amikacin, trimethoprim/sulfamethoxazole and fosfomycin. Extended-spectrum β-lactamase (ESBL) production was screened for by the double-disc synergy test, and ESBL typing was determined by PCR and further sequencing.2 PFGE analysis was carried out according to the PulseNet protocol in both laboratories. Salmonella serotype Braenderup strain H9812 was used for normalization and dendrograms were created with Fingerprinting 3.0 software (Bio-Rad), using the Dice coefficient.

In this collection of ST131 isolates from the B2 phylogenetic group, the overall prevalence of ESBL producers was 41% (n = 60). Fifty-six (93%) harboured CTX-M-15, three (5%) CTX-M-32 and one (2%) CTX-M-14. Higher rates of ESBL production were observed in the human-derived isolates than in the pet-derived isolates: 57 ESBL-producing human isolates out of 117 (49%) versus three ESBL-producing animal isolates out of 31 (10%). Human ST131 isolates were found to be more resistant to ciprofloxacin, trimethoprim/sulfamethoxazole and tobramycin than isolates from pets (P < 0.001). With respect to the ESBL-producing isolates examined

Figure 1. Dendogram showing the six ST131 E. coli clusters based on the XbaI-generated profile (Dice similarity value ≥ 94%, black line), which included pulsotypes common between Spain and Portugal, common between human and pets, and matching with international pulsotypes. Grey squares, positive trait; white squares, negative trait.
in this study, most harboured CTX-M-15, as was universally observed in previous surveys of humans and companion animals.\(^5\)\(^,\)\(^6\)\(^,\)\(^9\) Fosfomycin resistance was higher in Portugal than in Seville among human-derived isolates (\(P<0.001\)) and, in contrast with previous reports from Spain,\(^1\)\(^0\) this resistance trait has arisen independently in different human pulsotypes.

A total of 95 pulsotypes were distinguished (see Figure S1, available as Supplementary data at JAC Online). Three clusters (7% of human isolates) included isolates from Seville and Lisbon. Only one cluster containing one human and one dog isolate was detected. Seven (6%) human isolates exhibited a close genetic relationship with international PFGE profiles: four isolates from Seville matched type 903, and a cluster containing three human isolates from Lisbon and one from Seville matched type 968 (Figure 1). An additional Spanish isolate showed a similar profile to pulsotype 800. Isolates clustering with pulsotype 968 were all resistant to ciprofloxacin, three out of four were intermediately resistant or resistant to fosfomycin and one was a CTX-M-15 producer.

This study confirmed that some predominant lineages within the ST131 clone, mainly pulsotype 968, are present and have spread in Portugal and Spain. However, common patterns between these two countries accounted for less than 10% of all isolates, probably because of the different time frame of the collections from Portugal (2004–09) and Spain (2010). This is the first report of pulsotype 968 in Spain. The 968 pulsotype had been previously detected among human ST131 isolates in Portugal.\(^6\)\(^,\)\(^7\) In our survey, this transboundary 968 type was exclusively observed among human infection isolates, in contrast with previous studies where it was associated with pets.\(^4\)\(^,\)\(^1\(^1\)\) The 968 type belongs to the fimH30 type, which was significantly associated with fluoroquinolone resistance.\(^4\)\(^,\)\(^1\(^2\)\)

Only 3% of the Portuguese group contained pulsotypes that were common between humans and pets. Our results agree with the conclusions of a large international comparison of isolates carried out by Johnson et al.,\(^4\)\(^,\)\(^1\(^1\)\) which argued against pet–human commonality for ST131 isolates. None of the animal isolates could be associated with the prevalent pulsotypes. Isolates from companion animals were also less clustered and represented single pulsotypes, which may suggest less dissemination among pets.

The present work demonstrates the presence of high-prevalence international pulsotypes in two different countries in the south of Europe. However, the study has some limitations, in terms of the small number of cases in some groups and the differences in time frame between the areas. Nonetheless, variability among E. coli ST131 isolates at the pulsotype level was country-specific with little exchange between Seville and Lisbon and between humans and companion animals. In this study, the E. coli ST131 within-lineage genetic variation that was found argues in favour of a rapid host species adaptation and an ongoing dissemination of this antimicrobial drug-resistant pathogen.

Acknowledgements
We thank James R. Johnson for kindly providing 15 isolates representing high-prevalence ST131 pulsotypes.

Funding
This work was co-funded by FEDER funds through the Programa Operacional Factores de Competitividade – COMPETE and by National funds through the FCT – Fundação para a Ciência e a Tecnologia, Project PEst-OE/AGR/UI0276/2011, the Ministerio de Ciencia e Innovacion (Instituto de Salud Carlos III, PI10/01955), Junta de Andalucia (PI0034–2009, P09-CTS 5259) and co-financed by the European Development Regional Fund ‘A way to achieve Europe’ ERDF, Spanish Network for Research in Infectious Diseases (REIPI RD12/0015).

Transparency declarations
None to declare.

Supplementary data
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