were necessary to develop resistance to either antibiotic (Table 1). Compared with the parental B. henselae strains, the pradofloxacin-resistant and enrofloxacin-resistant mutants had an amino acid change from serine to valine at the 83rd position (E. coli numbering), which is located in the quinolone resistance-determining region (‘QRDR’) of the DNA gyrase A protein. The Ser-83→Val mutation found in our study for pradofloxacin-resistant and enrofloxacin-resistant mutants has been reported previously by Tavío et al.\(^6\) in a fluoroquinolone-resistant E. coli isolate. In our study, no mutation was found in the gyrB, parC and parE genes for pradofloxacin-resistant and enrofloxacin-resistant mutants of B. henselae. These results also indicate a primary B. henselae target for quinolone antimicrobials. Because resistant mutants only showed changes in GyrA, the primary target is most likely DNA gyrase, rather than topoisomerase IV.

In conclusion, this is the first study to describe specific molecular mechanisms of azithromycin, pradofloxacin and enrofloxacin resistance for Bartonella henselae isolates obtained from cats and a human patient isolate. Our findings are clinically relevant and could explain relapses observed using azithromycin for the treatment of B. henselae infections. We believe that clinicians should be aware of these results when selecting azithromycin to treat diseases caused by B. henselae. Further work is required to define the frequency of administration and effectiveness of pradofloxacin and enrofloxacin for B. henselae infections in veterinary patients.

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Detection of pandemic B2-O25-ST131 Escherichia coli harbouring the CTX-M-9 extended-spectrum β-lactamase type in a feral urban brown rat (Rattus norvegicus)

Sebastian Guenther1*, Mirjam Grobbel1, Janine Beutlich2, Beatriz Guerra2, Rainer G. Ulrich3, Lothar H. Wieler1 and Christa Ewers1

1 Institut für Mikrobiologie und Tierseuchen, Fachbereich für Veterinärmedizin, Freie Universität Berlin, Philippstrasse 13, D-10115 Berlin, Germany; 2 Federal Institute for Risk Assessment (BfR), National Reference Laboratory for Antimicrobial Resistance (NRL-AR), Diedersdorfer Weg 1, D-12277 Berlin, Germany; 3 Federal Research Institute for Animal Health, Institute for Novel and Emerging Infectious Diseases, Süderhofer Damm 4, D-17503 Greifswald-Insel Riems, Germany

*Corresponding author. Tel: +49-30-2093-6028; Fax: +49-30-2093-6067; E-mail: guenther.sebastian@vetmed.fu-berlin.de

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Sir,

Apart from the increasing prevalence of extended-spectrum β-lactamases (ESBLs) in Escherichia coli isolates from human patients and diseased livestock their co-emergence in wildlife faecal samples has also been documented.\(^2\) As wildlife animals are known to disseminate bacteria of human and animal health concern and may carry, in particular, ESBL-producing E. coli, it is highly important to determine how widely these bacteria have spread into rural and urban ecosystems. Recently, broad geographical dissemination of an E. coli clone (B2-O25:H4-ST131) carrying the CTX-M-15 ESBL has been described for clinical and non-clinical settings in human medicine\(^3\) while only one very recent publication reports a case in one animal, namely a dog.\(^4\) The aim of this study was to determine the prevalence of E. coli harbouring genes of the CTX-M-ESBL type in a wildlife animal species with close contact with human settlements—the brown rat (Rattus norvegicus).
Knowledge of the prevalence of antimicrobial-resistant *E. coli* in the faeces of feral rat species remains scarce, although rats have been known as carriers of various pathogens for centuries. Within the network ‘Rodent-borne pathogens’, faecal samples from 66 feral urban brown rats were collected in the city area of Berlin (Germany) for our study. Rats were captured and euthanized by pest control technicians and brought to the laboratory on the same day. After taking cloacal swabs the animals were passed to the researchers, who determined species affiliation. At least four *E. coli* isolates were obtained from each faecal sample by using CHROM agar orientation (Chromagar, Paris, France). Duplicate clones were excluded by macrorestriction analysis. The resulting 211 unique isolates were further processed by agar disc diffusion testing as a preliminary screen for antimicrobial resistance. Of these, 69 (32.7%) exhibited resistance phenotypes against multiple antibiotics and were additionally tested by the broth microdilution method (Micronaut breakpoint plate ‘Kleintier’; Genzyme Diagnostics, Ru¨sselsheim, Germany) against 17 antimicrobials including β-lactams, aminoglycosides, tetracyclines, sulphonamides, chloramphenicol and fluoroquinolones according to CLSI standards. One (0.5%) of the isolates sampled in the city area of Berlin with a dense human population (13239 inhabitants/km²) showed a positive confirmatory test for ESBL production, as determined by the CLSI method and was therefore intensively characterized via a multifocus approach including phenotypic and genotypic methods. Multilocus sequence typing (http://mlst.ucc.ie/mlst/mlst/dbs/Ecoli/) and molecular determination of the *rfb25b* locus by PCR for resistance led to the detection of the previously reported sequence type ST131 and O antigen subtype O25b. Phenotypic antimicrobial resistance was detected for cefotaxime, cefalotin, cefovecin, ampicillin, oxacillin, ampicillin, oxacillin, ticarcillin, doxycycline, gentamicin, enrofloxacin, difloxacin, orbifloxacin and marbofloxacin (‘common veterinary antimicrobials for companion animals’). Isoelectric focusing (IEF) and PCR-based screening and sequencing of genetic determinants for resistance led to the detection of the β-lactamases type CTX-M-9 (pi=8.5) and TEM-1 (pi=5.4) and the non-β-lactamase genes tet(A), sul2, shA, blaTEM-1, and aac(3’)-IIV and aac(6’)-Ib-cr. Southern blotting and subsequent PCR-based replicon typing for characterization of the resistance-providing plasmids resulted in the identification of the respective gene on a plasmid (>100 kb) of the FIA/FIB replicon type. Self-transferability of the plasmid was confirmed by mating experiments, in liquid and filter, at 37°C and 22°C, using an azide-resistant *E. coli* strain as receptor. Finally, PFGE revealed a close genetic similarity to other O25b-ST131 *E. coli* strains of the human pandemic clonal group as depicted in Figure 1.

In conclusion, the ESBL-producing *E. coli* isolate found in a brown rat from an urban region with a high human population density belongs to the currently spreading B2-O25b-ST131 clone. Although a direct clonal relatedness of this novel strain to human isolates was demonstrated via PFGE, its ESBL type was determined to be the more unusual CTX-M-9. This ESBL type has been recently found in a single Spanish clinical O25b-ST131 isolate. To our knowledge this is the first description of the B2-O25b-ST131 pandemic ESBL-producing *E. coli* strain in the brown rat. In addition to the long known threat of urban rats as a potential risk factor for human health, our finding provides the first evidence of a possible role of these urban rodents in the spread of CTX-M-type ESBL-producing *E. coli*.

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**Transparency declarations**

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**References**

Hong-Ning Wang and Yohei Doi*

Escherichia coli producing multilocus sequence typing types, including ST10 complex/A, ST23

1Division of Infectious Diseases, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania, USA; 2Animal Disease Prevention and Food Safety Key Laboratory of Sichuan Province, School of Life Sciences, Sichuan University, Chengdu, China; 3Philippine General Hospital, Manila, Philippines; 4National Institutes of Health, University of the Philippines, Manila, Philippines

*Corresponding author. Division of Infectious Diseases, University of Pittsburgh Medical Center, Scaife Hall S829, 3550 Terrace Street, Pittsburgh, PA 15261, USA. Tel: +1-412-648-9445; Fax: +1-412-648-8521; E-mail: yod4@pitt.edu

Keywords: ESBLs, plasmid-mediated AmpC β-lactamases, plasmid-mediated quinolone resistance, 16S rRNA methylase

Sir,
The Philippines, an archipelago in the Western Pacific region with a population of ~89 million, has a tradition of robust emigration and thus large traffic in international travel with countries such as China, India and the USA. However, data on the epidemiology of antimicrobial resistance genes among Enterobacteriaceae in the Philippines are limited. Here we report an analysis of plasmid-mediated antimicrobial resistance determinants present among extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae identified at a tertiary hospital in Manila, Philippines.

Three hundred non-duplicate Enterobacteriaceae that were identified from clinical specimens at the Central Microbiology Laboratory of the Philippine General Hospital were randomly collected between September and December 2007. Clinical isolates that were suspected to produce ESBL based on the disc diffusion method1 were subjected to Etest ESBL (bioMérieux, Durham, NC, USA) for confirmatory testing. A reduction in the MIC of ceftazidime of at least three dilutions in the presence of clavulanate was interpreted as a positive test. As a result, a total of 39 ESBL-producing isolates were identified. The species were then further verified with the API20E system (bioMérieux). They included 15 Escherichia coli, 15 Klebsiella pneumoniae, 5 Enterobacter cloacae, 3 Citrobacter freundii and 1 Proteus mirabilis. PFGE was performed for all K. pneumoniae and E. coli isolates using XbaI as the restriction enzyme (New England Biolabs, Ipswich, MA, USA). For E. coli, phylogenetic typing and PCR analysis for identification of the sequence type (ST) 131 international epidemic clone were also performed.2,3 PCR analyses were performed to identify various resistance genes in all study isolates. They included: β-lactamase genes blaTEM, blaSHV and blaCTX-M (including blaCTX-M-1, blaCTX-M-2 and blaCTX-M-9 groups),4 plasmid-mediated AmpC β-lactamase genes blacMY-1, blacMY-2, blahav, blasco, blaACT and blaoxy,5 16S rRNA methylase genes armA, mttB and mttC,6 pentapeptide repeat protein genes qnrA, qnrB, qnrC and qnrS; the fluoroquinolone-modifying aminoglycoside acetyltransferase gene aac(6′)-Ib-cr; and the plasmid-mediated fluoroquinolone efflux pump gene qepA.7 PCR products were sequenced on both strands using an ABI 3100 instrument (Applied Biosystems, Foster City, CA, USA).

Of the 39 study isolates, 30 (77%), 39 (100%), 19 (49%) and 34 (87%) were non-susceptible to ceftazidime, cefotaxime, cefepime and aztreonam, respectively. All were susceptible to ertapenem. As with non-β-lactam agents, non-susceptibility to ciprofloxacin and gentamicin was very common [37 isolates (95%) for both], whereas susceptibility to amikacin was better maintained [7 isolates (18%) non-susceptible].

No or little clonal relationship was detected among the 15 E. coli and 15 K. pneumoniae isolates. Of the E. coli isolates, three, five, one and six belonged to phylogenetic groups A, B1, B2 and D, respectively. The only isolate belonging to phylogenetic group B2 was identified as ST131, which represents the international epidemic clone.

Table 1 summarizes various resistance genes present in the study isolates. An ESBL gene was identified in all 39 isolates. Thirty-seven (95%) possessed blaoCTX-M. Of them, the blaoCTX-M-1 group was the most common, followed by the blaoCTX-M-9 group.