Molecular mechanisms of *Bartonella henselae* resistance to azithromycin, pradofloxacin and enrofloxacin

Silpak Biswas1, Ricardo G. Maggi1, Mark G. Papich2 and Edward B. Breitschwerdt1*

1Intracellular Pathogens Research Laboratory, Center for Comparative Medicine and Translational Research, College of Veterinary Medicine, North Carolina State University, Raleigh, NC 27606, USA; 2Department of Molecular Biomedical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC 27606, USA

*Corresponding author. Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, 4700 Hillsborough Street, Raleigh, NC 27606, USA. Tel: +1-919-513-8277; Fax: +1-919-513-6336; E-mail: ed_breitschwerdt@ncsu.edu

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Sir, *Bartonella henselae* are fastidious, facultative intracellular bacilli that can cause bacteraemia, endocarditis, cat-scratch disease in immunocompetent patients, and bacillary angiomatosis and peliosis hepatitis in immunocompromised patients.1,2 There are a limited number of studies that have addressed the antibiotic treatment of cats infected with *B. henselae*.3 Azithromycin, a macrolide compound, has seemingly become the drug of choice to treat cats and dogs for *B. henselae* infection. However, relapses after antibiotic withdrawal have been reported.4 Azithromycin, which is derived from erythromycin, binds to the 50S subunit of the bacterial ribosome and, thus, inhibits the translation of mRNA. Fluoroquinolone antibiotics exert their antibacterial effects by inhibiting certain bacterial topoisomerase enzymes. Pradofloxacin, a third-generation fluoroquinolone, is being exclusively developed for use in veterinary medicine. Enrofloxacin is a broad-spectrum antimicrobial agent with bactericidal activity against Gram-negative and Gram-positive bacteria, mycobacteria and rickettsia. As for *B. henselae*, potential mechanisms of resistance to azithromycin, pradofloxacin and enrofloxacin are not known. Therefore, the objective of this study was to select in vitro azithromycin-resistant, pradofloxacin-resistant and enrofloxacin-resistant mutants to determine the molecular mechanism of resistance.

Six *B. henselae* isolates (BhH1, Mina Mia, Stray7, Bh94FO73, BhFO1946 and Bh95FO101) were used in this study. Four of these isolates (Mina Mia, Stray7, Bh94FO73 and BhFO1946) were derived from cats from the USA. BhH1 is the ATCC type strain obtained by blood culture from a febrile, HIV-infected patient in Houston, Texas. Isolate Bh95FO101 was from a sick pet cat from Israel.

Pradofloxacin (5 μg) and enrofloxacin (5 μg) discs were purchased from AB Biodisk (Solna, Sweden) and supplied by Bayer HealthCare, Germany. Azithromycin (15 μg) discs were purchased from VWR International, USA. The selection of antibiotic-resistant mutants was performed by serial passages of each *B. henselae* isolate on blood agar plates containing an antibiotic disc. *Bartonella* strains were considered resistant when the inhibition zone was <6 mm.

The *B. henselae* azithromycin-susceptible strains and the azithromycin-resistant mutants were screened by PCR and sequencing using primers for 23S rRNA, gyrA, gyrB, parC and parE.

Six *B. henselae* azithromycin-resistant mutants were obtained after the second in vitro passage (Table 1). Compared with the parental strain, each *B. henselae* azithromycin-resistant mutant had a homogenous single nucleotide substitution at position 2058 (A2058G, *Escherichia coli* numbering) in the 23S rRNA gene. Mutations at A2058 for certain macrolides confer the highest levels of resistance.5 Many independent lines of evidence indicate that adenosine 2058 is the key nucleotide involved in macrolide interaction with the bacterial ribosome.5 An A2058 to G transition was the first RNA mutation shown to confer erythromycin resistance and is presently the most frequent substitution found in clinical isolates. Our in vitro results might explain relapses or treatment failures observed in vivo when using azithromycin as the sole antibiotic for treatment of *Bartonella*-related infections. We did not find any change in the L4 and L22 ribosomal proteins for the *B. henselae* azithromycin-resistant mutants.

All *B. henselae* isolates became resistant to pradofloxacin and enrofloxacin after differing numbers of subculture passages; however, in contrast to azithromycin, at least five passages...
were necessary to develop resistance to either antibiotic (Table 1). Compared with the parental \textit{B. henselae} strains, the pradofloxacin-resistant and enrofloxacin-resistant mutants had an amino acid change from serine to valine at the 83rd position (\textit{E. coli} numbering), which is located in the quinolone resistance-determining region (\textquoteleft QRDR\textquoteright) 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.\textsuperscript{5} in a fluoroquinolone-resistant \textit{E. coli} isolate. In our study, no mutation was found in the \textit{gyrB} and \textit{parE} genes for pradofloxacin-resistant and enrofloxacin-resistant mutants of \textit{B. henselae}. These results also indicate a primary \textit{B. henselae} target for quinolone antimicrobials. Because resistant mutants only showed changes in GyrA, the primary target is most likely DNA gyrase, rather than topo-isomerase IV.

In conclusion, this is the first study to describe specific molecular mechanisms of azithromycin, pradofloxacin and enrofloxacin resistance for \textit{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 \textit{B. henselae} infections. We believe that clinicians should be aware of these results when selecting azithromycin to treat diseases caused by \textit{B. henselae}. Further work is required to define the frequency of administration and effectiveness of pradofloxacin and enrofloxacin for \textit{B. henselae} infections in veterinary patients.

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**References**


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

Sebastian Guenther\*1, 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, Philippsstrasse 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, Südfuer 10, D-17493 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 \textit{Escherichia coli} isolates from human patients and diseased livestock\textsuperscript{1} their co-emergence in wildlife faecal samples has also been documented.\textsuperscript{2} As wildlife animals are known to disseminate bacteria of human and animal health concern and may carry, in particular, ESBL-producing \textit{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 \textit{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\textsuperscript{3} while only one very recent publication reports a case in one animal, namely a dog.\textsuperscript{4} The aim of this study was to determine the prevalence of \textit{E. coli} harbouring genes of the CTX-M-ESBL type in a wildlife animal species with close contact with human settlements—the brown rat (\textit{Rattus norvegicus}).