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SHORT COMMUNICATION

Susceptibility of Polish *Bartonella henselae* Strains

EDYTA PODSIADŁY[1]*, DOROTA ŻABICKA[2], URSZULA DEMKOW[3], WALERIA HRYNIEWICZ[2] AND STANISŁAWA TYLEWSKA-WIERZBANOWSKA[1]

[1]National Institute of Public Health – National Institute of Hygiene, Warsaw, Poland
[2] National Medicines Institute, Warsaw, Poland, [3]Warsaw Medical University

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Abstract

Due to the fastidious nature of *B. henselae* and the limited number of available isolates worldwide, there are few data on its *in vitro* susceptibility to antibiotics. We determined the minimal inhibitory concentrations (MIC) of ten antimicrobial agents against 11 feline isolates of *B. henselae* by Etest method. The lowest MICs were obtained for rifampicin ≤0.002 mg/L. MICs of all isolates were <0.016 mg/L for ampicilin, amoxicillin/clavulanic acid, tetracycline and ranged from 0.016 to 0.032 mg/L for azithromycin. The MICs for two tested fluoroquinolones: ciprofloxacin and levofloxacin ranged from 0.016 to 0.125 mg/L. The highest MICs were obtained for gentamicin ranging from 0.025 to 2.0 mg/L. Sulphonamide resistance genes *sul 1*, *sul 2*, *sul 3* were not found in any of the tested isolates. Etest methodology seems to be a reliable method for determination of *B. henselae* susceptibility, however standardization is strongly desired.

Key words: *Bartonella henselae*, MIC, antibiotics

Over the last decade the number of *Bartonella* species has increased rapidly. Simultaneously some new species have been associated with clinical syndromes in humans (Chomel *et al*., 2006). Treatment of human bartonellosis depends on the clinical presentation of the disease. Recommendations for treatment are based on a few case reports and a few clinical studies (Rolain *et al*., 2004). Because of limited number of isolates, specially human isolates, also data regarding *in vitro* susceptibility are very limited. Isolation of *Bartonella* spp. from clinical specimens requires long incubation times, special growth conditions and is rarely possible. As feline strains are easier to culture, susceptibility testing can be done on these isolates. The aim of the present study was to determine the MICs of 10 antibiotics by Etest method for 11 *B. henselae* feline strains. Moreover, the presence of *sul* genes conferring sulphonamide resistance was determined in the examined isolates.

Eleven *B. henselae* strains were collected from healthy, stray cats in 2004–2005 in urban areas of Warsaw and surroundings. For susceptibility study the strains were recovered from frozen stocks onto chocolate blood agar supplemented with VITOX (Choc V, Oxoid) and cultured at 37°C in 5% CO_2 enriched atmosphere. Microorganisms were harvested, from second passage after refreezing, from logarithmic growth on chocolate agar (7–8 days), centrifuged and suspended in 2 ml of sterile 0.9% NaCl and adjusted to a McFarland standard of 3.0. The suspension was swabbed on the entire surface of a chocolate blood agar plate supplemented with VITOX (Choc V, Oxoid) 3 times, rotating the plate approximately 90 degrees each time. Excess moisture was allowed to absorb for about 10 to 15 minutes. Susceptibility testing was performed for: trimethoprim-sulphamethoxazole (0.002–32), ampicilin (0.016–256), amoxicillin/clavulanic acid (0.016–256), cefotaxime (0.016–256), azithromycin (0.016–256), ciprofloxacin (0.002–32), levofloxacin (0.002–32), tetracycline (0.016–256), gentamicin (0.016–256), clindamycin (0.016–256), rifampicin (0.002–32) using E-tests (bioMèrieux, former AB BIODISK, Sweden). Zones of inhibition were recorded on the 4th and 7th day of incubation as a point of intersection between the inhibition ellipse edge and Etest strip. A growth control plate was inoculated with each test run.

In order to check the performance of Etest CLSI reference strains: *Enterococcus faecalis* ATTC 29212 for gentamicin and clindamycin, *E. coli* ATCC 35218 for amoxicillin/clavulanic acid and *Haemophilus influenzae* ATCC 49247 for remaining tested antibiotics were used as the quality control.

Studied strains of *B. henselae* were screened for *sul 1, sul 2* and *sul3* genes using specific oligonucleotide primers, as previously described (Kerrn *et al*., 2002; Perreten

* Corresponding author: E. Podsiadły, Laboratory of Rickettsiae, Chlamydiae and Spirochetes, National Institute of Public Health-National Institute of Hygiene, 24 Chocimska Street, 00-791 Warsaw; phone: +4822 4521250; **e-mail: epodsiadly@onet.pl**

and Boerlin, 2003). Total genomic DNA was extracted from *Bartonella* isolates, cultured for 7 days in conditions described above with QIAamp Tissue kit (Qiagen, Hilden, Germany) according to manufacturer instruction. The PCR conditions were as follows: initial denaturation 94°C for 5 min followed by 35 cycles: 94°C for 1 min, 60°C (*sul 1*, *sul 2*)/51°C (*sul 3*) 1 min, 72°C 1 min and final extension 72°C 10 min (Gradient Mastercycler, Eppendorf). Two *E. coli* strains susceptible and two resistant to trimethoprim-sulphamethoxazole in a standard disc diffusion method were run as controls.

Among 11 tested feline isolates of *B. henselae* by Etest method the lowest MICs were obtained for rifampicin ≤ 0.002 mg/L (Table I). All isolates were highly susceptible to tetracycline with MICs ≤ 0.016 mg/L. Also the macrolide – azithromycin was highly active with MICs ranging from 0.016 to 0.032 mg/L.

The tested *B. henselae* strains were highly susceptible to β-lactams. MICs of tested antibiotics from this group were as follows: ampicilin and amoxicillin/clavulanic acid < 0.016 mg/L; cefotaxime ranged from 0.016 mg/L to 0.047 mg/L. This fact however has limited clinical value as bactericidal effect of this group of antibiotics is restricted only to extracellular bacteria whereas intraerythrocytic *Bartonella* are protected from their activity.

The highest MICs were obtained for gentamicin, ranging from 0.25 mg/L to 2.0 mg/L. MICs determined for gentamycin were higher than for other antibiotics and ranged up to 2.0 mg/L. Among aminoglycosides netilmicin was reported to be the most active agent (Dorbecker *et al*., 2006).

For two tested fluoroquinolones: ciprofloxacin and levofloxacin MICs ranged from 0.016 to 0.125 mg/L. Eight of 11 (72.7%) tested strains had MIC for levofloxacin higher or equal to 0.094 mg/L whereas for ciprofloxacin for the majority of strains (63.3%, 7/11) values of MIC were 0.047 mg/L and lower. Comparing the obtained MICs for ciprofloxacin and levofloxacin it seems that ciprofloxacin is slightly more active against *Bartonella* spp. than levofloxacin however,a higher dosage is applied in case of the latter. Despite the fact that fluoroquinolones have the ability to achieve high intracellular concentrations, their potential role in the therapy of *Bartonella* infections remains unclear. Although successful treatment of *Bartonella* infections with fluoroquinolones has been reported, there have also been failures and relapses with these drugs (Wolfson *et al.*, 1996). Recently Angelakis *et al.* (2009) reported that *B. henselae* can easily become resistant to fluoroquinolones. A ciprofloxacin-resistant strain of *B. henselae* was obtained *in vitro* after only four passages, MIC for the strain increased from 0.38 to > 32 mg/L. *Bartonella* spp. present a natural Ser-83-Ala mutation (*E. coli* numbering) in the QRDR region of the DNA gyrase that is responsible for decreased susceptibility to fluoroquinolones. Additional mutation Asp-87-Gly in the gyrase A protein results in a high level of resistance to all quinolone compounds (Angelakis *et al.,* 2008). According to some authors, a second mutation may be obtained easily with consequent high level of resistance to fluoroquinolones. It is suggested that fluoroquinolone compounds should be avoided for treatment of bartonellosis. Bacterial resistance to fluoroquinolones developing during treatment is not restricted to *Bartonella* but is also observed in other bacteria species.

According to EUCAST (version 2.0, valid from January 1, 2012, www.eucast.org) for the non-species

Table I
MIC distribution (mg/L) determined by Etest method for 11 *B. henselae* isolates

No strain	16S rRNA type	MLVA profile[1]	Am	XL	CT	AZ	CI	LE	TC	TS	GM	CM	RI
3	II	9, 14, 5, 1, 2	0.016	0.016	0.016	0.016	0.047	0.125	0.016	nd	0.094	0,25	0.003
12	II	11, 20, 10, 1, 2	0.016	0.016	0.016	0.016	0.047	0.094	0.016	nd	0.5	>256	0.002
13	I	12, 24, 2, 5, 3	<0.016	<0.016	0.016	0.016	0.094	0.125	0.016	nd	0.25	0.75	0.004
28	II	10, 14, 2, 2, 1	0.016	<0.016	0.032	0.032	0.125	0.064	0.016	nd	1.0	>256	0.003
30	II	14, 24, 10, 7, 3	0.016	0.016	0.016	0.016	0.047	0.094	0.016	nd	0.5	>256	0.002
129	II	9, 15, 18, 1,1	0.016	0.016	0.016	0.016	0.047	0.032	0.016	nd	1.0	0.064	0.002
130	II	9, 15, 18, 1, 1	0.16	0.016	0.016	0.016	0.064	0.125	0.016	nd	0.38	0.25	0.003
150	II	9, 15, 2, 2, 1	0.016	0.016	0.016	0.023	0.125	0.094	0.016	nd	1.0	>256	0.004
154	II	12, 15, 2, 2, 4	0.016	0.016	0.016	0.016	0.047	0.064	0.016	nd	0.75	>256	0.002
159	II	9, 15, 2, 1, 1	<0.016	<0.016	0.047	0.032	0.016	0.125	0.016	nd	2.0	3	0.030
163	II	12,23,18,5,3	0.016	0.016	0.016	0.016	0.047	0.125	0.016	nd	0.5	6	0.002

[1]profiles shown as numbers (alleles) corresponding in order with the loci BHV-A, BHV-B, BHV-C, BHV-D, BHV-E; nd – not detectable; Am-ampicillin, XL-amoxicillin/clavulanic acid, CT-cefotaxim, AZ-azithromycin, CI-ciprofloxacin, LE-levofloxacin, TC-tetracycline, TC-trimetoprim/sulfamethoxazole (1/19), GM-gentamicin, CM-clindamycin, RI-rifampicin

related breakpoints based on standard dosages and pharmacokinetic and pharmacodynamic properties of an antibiotic all analyzed drugs were in the range of susceptible values. However the gentamicin MIC = 2.0 mg/L obtained for one strain in our study is the highest possible value for susceptible strains.

Reading and interpretation zones of inhibition for sulphonamides and trimethoprim was not possible because the point of complete inhibition was not distinguishable at the edge of E-test. PCR testing for the presence of sulphonamide resistance genes was applied. In none of our tested strains sulphonamide resistance genes *sul 1*, *sul 2*, *sul 3* were found. Sulfonamides, especially in the combination as trimethoprim-sulfamethoxazole, are widely used to treat bacterial and protozoal infections (Perreten and Boerlin, 2003). These agents are also effective in the treatment of CSD (Rolain *et al*., 2004). Sulfonamides alone are widely used to prevent and treat diarrhea and other infectious diseases in intensive animal husbandry. That can have an impact on the spread of sulphonamide resistance genes, which are associated with class 1 integrons residing in plasmids or the bacterial chromosome. Especially that persistent asymptomatic bacteriemia in cats is reported to last up to 2 years (Chomel *et al.*, 2006). This is to our knowledge the first study searching for the presence of sulfonamide resistance genes in *Bartonella.*

The susceptibility of the strains was set against previous molecular characteristics of the strains (Podsiadly *et al.*, 2012). No significant variation in susceptibility with genotype (16S rRNA type) was detected. However, three already described profiles for BHV A-B-C-D-E (9-15-2-1-1, 10-14-2-2-1 and 9-15-2-2-1) corresponding to three common European VNTR profile for feline isolates, presented four dilutions higher MIC for gentamicin and six dilutions higher MICs for ciprofloxacin (Table I).

Etest methodology seems to be a reliable method for determination of *B. henselae*. susceptibility. Our results correlate with MICs reported by Pendle *et al.* (2006) and Angelakis *et al*. (2009). MICs obtained for fluoroquinolones and gentamicin are compatible with the results of testing other *Bartonella* spp. isolates (Tsuneoka *et al*., 2010). However, the differences in MICs between studies are also visible. For example in the study by Dorbecker *et al*. (2006) MICs for gentamicin were up to 8 mg/L whereas in our study only in 1 of 11 strains MIC equaled 2 and in 3 strains MIC = 1.0 mg/L. A similar situation was found for the MICs of ciprofloxacin and levofloxacin for which recorded MICs were 4 times higher than in our study. The discrepancies in MIC values might be influenced by many factors such as: medium, inoculum and others. Standardization should be proposed to monitor the development of resistance in isolates and to enable comparison of results reported in the different studies.

Routine susceptibility testing of *B. henselae* isolates is impossible, therefore the surveillance studies made by specialized reference centres are important to review treatment recommendations, specially of persistent bartonella infections and for monitoring the emergence of resistance in this group of bacteria.

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