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Antibiotic resistance in *Bartonella bacilliformis* clinical isolates from an endemic area of Peru

Sir,

Bartonella bacilliformis is a facultative, intracellular, aerobic, Gram-negative coccobacillus causing the so-called Carrión's disease, a human infection endemic to specific areas mainly inhabited by low-income communities of Peru but also present in other Andean communities. It is considered a truly neglected tropical disease and is transmitted through the bite of female sandflies of the genus *Lutzomyia* [1].

Carrión's disease has two different clinical presentations; an initial febrile and haemolytic anaemia phase, known as Oroya fever, which has a mortality rate ranging from 44% to 88% in untreated patients; and a second phase characterised by the development of dermal eruptions known as Peruvian wart [1,2]. The recommended treatment schedule for the acute phase includes ciprofloxacin as first-line therapy in adults or children aged >14 years, whilst chloramphenicol, trimethoprim/sulfamethoxazole, amoxicillin/clavulanic acid and ceftriaxone are used second-line or in children and pregnant women. Meanwhile, rifampicin, streptomycin or ciprofloxacin are used in the treatment of Peruvian warts [2]. Despite antibiotic treatment usually resulting in fever reduction and clearance of blood micro-organisms within 24 h of starting treatment, both treatment failures and persistent asymptomatic bacteraemia have been reported. In fact, Peruvian guidelines for diagnosis and treatment are supported by few studies. It has been showed that *B. bacilliformis* is intrinsically resistant to nalidixic acid owing to the presence of Ala in positions 91 and 85 of GyrA and ParC, respectively [2].

Moreover, in vitro-selected ciprofloxacin-resistant *B. bacilliformis* mutants that possess the amino acid changes D90G or D95N in GyrA have been reported [3]. The aim of this study was to evaluate the antibiotic susceptibility of *B. bacilliformis* strains isolated from patients diagnosed with Oroya fever and to analyse the quinolone resistance-determining regions (QRDR) of GyrA and ParC. Blood samples ($n = 21$) collected during March 2009 from patients from Cajamarca Region clinically diagnosed with Oroya fever were evaluated. The presence of *B. bacilliformis* was confirmed by amplification of the 16S rRNA gene [4]. Samples were cultured as previously described [4].

Antimicrobial susceptibility testing was performed by the disk diffusion method. The antimicrobial agents tested were amoxicillin/clavulanic acid (20/10 μg), ceftazidime (30 μg), gentamicin (10 μg), amoxicillin (20 μg), tetracycline (30 μg), chloramphenicol (30 μg), trimethoprim/sulfamethoxazole (1.25/23.75 μg), ciprofloxacin (5 μg) and azithromycin (15 μg). Following DNA extraction from a 200 μL blood sample using a commercial extraction kit (High Pure Template Preparation Kit; Roche Applied Science, Mannheim, Germany), a 233-bp fragment of the *gyrA* gene and a 349-bp fragment of the *parC* gene were amplified as described previously [2].

A total of three *B. bacilliformis* isolates (3/21; 14.3%) were recovered from blood samples. In all cases the isolates were susceptible to all of tested antimicrobial agents except gentamicin. In the case of gentamicin, two of the isolates (EC1 and EC2) exhibited a reduced inhibitory halo (13 mm), which in the absence of specific breakpoints might be considered intermediate or resistant (Table 1). Analysis of the QRDRs did not show any alterations (wild-type).

Bartonella bacilliformis is a fastidious micro-organism that is difficult to culture. This fact has results in the presence of scarce data regarding antibiotic susceptibility and mechanisms of resistance. The high levels of antibiotic susceptibility of the analysed isolates are in agreement with that previously described by Sobraquès et al. [5] who analysed four isolates (two recent clinical isolates and two ATCC strains). Similarly to ours, the resistance levels to aminoglycosides were relatively higher than those of other antimicrobial agents, with minimum inhibitory concentrations (MICs) of 2 mg/L in the case of gentamicin and 8 mg/L in the case of amikacin [5]. Moreover, the MICs of the two clinical isolates analysed by Sobraques et al. tended to be slightly higher than those of the collection strains, suggesting a possible influence of antimicrobial use in the area [5].

Regarding molecular mechanisms of resistance, similar results has been described when clinical or collection isolates have been studied. However, in vitro studies have shown the possibility of the selection of amino acid changes in GyrA that result in high levels of fluoroquinolone resistance [2]. Moreover, selection of isolates exhibiting resistance to others antibiotics has been achieved in vitro, highlighting the risk of in vivo selection and the spread of antibiotic-resistant isolates. The sample size might be considered a limitation, however it is necessary to highlight that data on antibiotic resistance levels in clinical isolates are very scarce. In summary, the present report shows the general antibiotic susceptibility levels of *B. bacilliformis*, but it is necessary to monitor in a continuous manner the possible and predicted development of antibiotic resistance in order to minimise its effect, which might be

dramatic considering the high levels of lethality exhibited by *B. bacilliformis* in the pre-antibiotic era.

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Table 1 Antibiotic susceptibility of three *Bartonella bacilliformis* isolates

Antibiotic	Inhibition zone (mm)		
	EC1	EC2	EC8
Ciprofloxacin	31	29	>40
Azithromycin	30	21	>40
Chloramphenicol	57	49	>40
Amoxicillin/clavulanic acid	23	29	31
Sulfamethoxazole	18	21	>40
Ceftazidime	24	21	34
Tetracycline	26	21	21
Amoxicillin	21	31	28
Gentamicin	13	13	23