human-to-animal transfer through close contacts. In our study, the OXA-48-positive animal was living in France, where OXA-48 is not endemic in humans.[6] This suggests that OXA-48 producers in animals might be underreported in OXA-48-endemic countries. The origin of the OXA-48 *E. coli* in this dog could not be traced. No $bla_{CTX\text{-}M\text{-}15}$ gene was identified along with $bla_{OXA\text{-}48}$, a combination that is, on the contrary, rather common in human isolates. Also, carbapenem-susceptible ST372 *E. coli* isolates have already been associated with human and canine infections.[7]

In conclusion, even though resistance to carbapenems is uncommon in animals, carbapenemase genes are associated with a high potential for dissemination and their prevalence in non-human sources may be underestimated, potentially even in countries or continents where CPE are not highly prevalent in humans. CPE in animals should be more thoroughly monitored worldwide in order to clarify the role of non-human settings as possible reservoirs of carbapenemase genes.

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Transparency declarations

None to declare.

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Finafloxacin overcomes *Burkholderia pseudomallei* efflux-mediated fluoroquinolone resistance

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Sir,

Burkholderia pseudomallei is a biothreat agent and causes melioidosis, a globally emerging infectious disease with high case fatality rates, and *B. pseudomallei* infections are difficult to treat.[1,2] Although curiously rare in *B. pseudomallei*, drug resistance is observed and primarily due to efflux, enzymatic modification or target mutation.[3] Efflux is the dominant resistance mechanism.[3,4] Melioidosis treatment requires an acute-phase therapy limited mostly to β-lactam antibiotics such as ceftazidime and carbapenems, followed by a prolonged eradication-phase therapy, which nowadays is mostly limited to trimethoprim/sulfamethoxazole.[2] While extensively employed for treatment of various Gram-negative infections, several studies have shown that fluoroquinolones are not useful for melioidosis therapy.[5] A previous study published in this Journal showed that the *in vitro* activity of ciprofloxacin against *B. pseudomallei* is very weak and the activity of newer fluoroquinolones such as gatifloxacin and moxifloxacin is rather limited.[6] The reasons for therapeutic inefficacy of fluoroquinolones with *B. pseudomallei* are unknown. Acquired fluoroquinolone resistance in *B. pseudomallei* predominantly involves expression of the BpeEF-OprC efflux pump, although target mutations affecting the quinolone resistance-determining region of GyrA have been reported.[4,7] Finafloxacin is a fluoroquinolone that was approved by the US FDA in 2014 for the treatment of *Pseudomonas aeruginosa*-mediated acute otitis externa.[8] A unique property of finafloxacin is that it exhibits increased activity at acidic pH and, when compared with other fluoroquinolones, superior antibacterial activity in acidic conditions (e.g. pH 5.8).[8] These

Table 1. *In vitro* susceptibility of isogenic *B. pseudomallei* strains expressing or lacking defined efflux pumps

			MIC (mg/L)[a]					
			ciprofloxacin		moxifloxacin		finafloxacin	
Strain[b]	Genotype	Efflux pump(s) expressed	pH 5.8	pH 7.2	pH 5.8	pH 7.2	pH 5.8	pH 7.2
1026b	*amrA*$^+$*B*$^+$-*oprA*$^+$ *bpeA*$^+$*B*$^+$-*oprB*$^+$ *bpeE*$^+$*F*$^+$-*oprC*$^+$	AmrAB-OprA→[c] BpeAB-OprB→	64	2	16	2	0.125	0.25
Bp340	Δ(*amrRAB-oprA*)	BpeAB-OprB→	32	2	16	1	0.25	2[d]
Bp227	Δ(*bpeAB-oprB*)	AmrAB-OprA→	16	0.5	8	0.5	0.0625	0.0625
Bp207	Δ(*amrRAB-oprA*) Δ(*bpeAB-oprB*)	none known[e]	4	0.25	2	0.125	0.03125	0.03125
Bp58	Δ*bpeR* Δ(*amrRAB-oprA*)	BpeAB-OprB↑	128	4	64	4	0.5	4
Bp282	Δ(*amrRAB-oprA*) Δ(*bpeAB-oprB*) *bpeT*	BpeEF-OprC↑	128	32	128	16	2	2
Bp320	Δ(*amrRAB-oprA*) Δ(*bpeAB-oprB*) Δ(*bpeEF-oprC*) *bpeT*	none known	0.125	0.0625	0.25	0.125	≤0.0078125	≤0.0078125
E. coli ATCC 25922		N/A	0.25	0.03125	0.5	0.125	≤0.0078125	0.015625

N/A, not available.
[a]MIC testing for each strain was performed in biological triplicate on three separate days. The reported values represent the median of the three values read for each isolate.
[b]All strains used in this study are derived from 1026b and with the exception of Bp282 and Bp320 their construction has been described.[11] The latter two strains were derived as described below. Bp282 is a ciprofloxacin-resistant derivative of Bp207 obtained using passive selection. It contains a *bpeT* point mutation causing a S280P BpeT amino acid substitution. The passive selection experiment was conducted prior to 4 December 2012, and its performance and mutant possession did not require US Federal Select Agent Program approval. Bp320 is a Δ(*bpeEF-oprC*) derivative of Δ(*amrAB-oprA*) Δ(*bpeAB-oprB*) strain Bp282 and was constructed using previously described methods.[11] This strain does not express any known efflux pumps.
[c]Horizontal arrows indicate endogenous levels of efflux pump expression and vertical arrows indicate constitutive expression due to regulatory mutations, *bpeR* in Bp58 and *bpeT* in Bp282.
[d]For unknown reasons the MIC of finafloxacin at pH 7.2 is higher for Bp340 than for the parental strain 1026b, whereas the MICs of the comparators ciprofloxacin and moxifloxacin remain unchanged at this pH.
[e]Bp207 is 1026b Δ(*amrAB-oprA*) Δ(*bpeAB-oprB*) and does not express any known efflux pump. Higher MICs for this strain when compared with Bp320 may be due to slight up-regulation of BpeEF-OprC in this mutant, but this has not yet been established.

attributes support the notion that the drug is suitable for the treatment of infections in acidic environments. *B. pseudomallei* is an intracellular pathogen that can replicate within host organelles where the local pH is acidic (e.g. in macrophages). A previous study indicated that finafloxacin possesses potent bactericidal activity against *B. pseudomallei*, and bacterial burdens 24 h post-challenge were lower in finafloxacin-treated animals than in those treated with ciprofloxacin or trimethoprim/sulfamethoxazole.[9]

Because efflux is an inherent liability of fluoroquinolones in Gram-negative bacteria, in this study we compared the *in vitro* efficacy of finafloxacin using a defined, isogenic panel of efflux-proficient or efflux-compromised *B. pseudomallei* mutants based on prototype strain 1026b. *B. pseudomallei* encodes 10 efflux pumps of the resistance nodulation cell-division (RND) family, but only three of these (AmrAB-OprA, BpeAB-OprB and BpeEF-OprC) have been characterized in some detail.[4] AmrAB-OprA is expressed at significant levels in wild-type strains (e.g. 1026b; Table 1), where it is responsible for the intrinsic aminoglycoside and macrolide resistance observed in the vast majority of clinical and environmental isolates. BpeAB-OprB is expressed at very low levels in wild-type strains and de-repressed in *bpeR* regulatory mutants (e.g. Bp58). BpeAF-OprC is not expressed in wild-type strains, but up-regulated in *bpeT* regulatory mutants (e.g. Bp282). Whereas AmrAB-OprA and BpeEF-OprC are expressed in MDR clinical isolates, the clinical significance of BpeAB-OprB remains yet to be established.[4] Although the BpeEF-OprC pump is known to be the major fluoroquinolone resistance mechanism in *B. pseudomallei*, we included BpeAB-OprB mutants in our studies because when expressed this pump confers low-level fluoroquinolone resistance. MIC values of ciprofloxacin and the comparators moxifloxacin and finafloxacin were determined using Merlin Micronaut-S microtitre plates and broth microdilution. *B. pseudomallei* efflux-proficient and efflux-deficient strains, including prototype 1026b, were grown to mid-log phase in cation-adjusted Mueller–Hinton II broth (MHB; Becton Dickinson and Company, Sparks, MD, USA) and inoculated into MHB adjusted to pH 5.8 or 7.2. Growth was visually inspected after 20 h of incubation at 37 °C.

Finafloxacin MIC values were generally lower than those of ciprofloxacin and moxifloxacin at pH 7.2, and substantially lower at pH 5.8. All three antibiotics appear to be extruded to some extent by the BpeAB-OprB and BpeEF-OprC efflux pumps, but not AmrAB-OprA. The data confirm that BpeEF-OprC is a major fluoroquinolone resistance mechanism in *B. pseudomallei*, which bestows high-level ciprofloxacin and moxifloxacin resistance. At pH 7.2 the MICs of both fluoroquinolones are 2 mg/L for 1026b versus 32 mg/L (ciprofloxacin) and 16 mg/L (moxifloxacin) for the BpeEF-OprC-expressing strain Bp282. While the finafloxacin MIC value is higher for the BpeEF-OprC-expressing strain when compared with the 1026b prototype control (2 versus 0.25 mg/L at pH 7.2 and 2 versus 0.125 mg/L at pH 5.8, respectively), the susceptibility of *B. pseudomallei* to finafloxacin is less affected by efflux than ciprofloxacin and moxifloxacin, especially at acidic pH.

In summary, our data show that finafloxacin maintains *in vitro* efficacy against *B. pseudomallei* in the face of efflux-mediated fluoroquinolone resistance, especially under acidic conditions. This corroborates previously reported findings in this Journal for *Escherichia coli*, which demonstrated that finafloxacin shows higher activity, notably at pH 5.8, against mutants expressing known fluoroquinolone resistance determinants, including efflux.[10] In contrast to ciprofloxacin and moxifloxacin, finafloxacin may therefore retain its therapeutic potential against *B. pseudomallei* even in BpeEF-OprC-expressing, fluoroquinolone-resistant strains. Additional studies are warranted to examine the potential utility of finafloxacin in augmenting the limited arsenal of antibiotics available for melioidosis prophylaxis and therapy.

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Effect of faecal microbiota transplantation on mouse gut colonization with carbapenemase-producing *Escherichia coli*

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