

The antibiotic susceptibility of water-based bacteria *Ralstonia pickettii* and *Ralstonia insidiosa*

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Ralstonia pickettii and *Ralstonia insidiosa* are waterborne bacteria that can survive and grow in various water sources, and that are emerging pathogens in hospital settings. Not much is known about the antibiotic resistance of these bacteria. Previous reports of antimicrobial susceptibility have been largely limited to a few clinical strains with no accounting for genotypic or phenotypic diversity or that these species could vary from the set breakpoints. Etests and disc diffusion tests were carried out to compare the antimicrobial susceptibilities to 12 different antibiotics of 68 different isolates of *R. pickettii* (53) and *R. insidiosa* (15) from varying environments, which have previously been well characterized both phenotypically and genetically. The majority of the *R. pickettii* and *R. insidiosa* isolates showed susceptibility to most of the antibiotics tested in this study. The most effective were found to be the quinolones and sulfamethoxazole/trimethoprim. Antibiotic susceptibility was also found not to vary between environmental niches for *R. pickettii* and *R. insidiosa* isolates.

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INTRODUCTION

Ralstonia pickettii is abundant in the environment having been isolated from a wide array of environmental sources (Ryan *et al.*, 2011c). The bacterium has been recovered from numerous water sources, including municipal drinking water supplies (Lee *et al.*, 2010), bottled water (Falcone-Dias *et al.*, 2012), dental water supplies (Szymańska 2006), hospital water supplies (Kendirli *et al.*, 2004; Ryan *et al.*, 2006), space shuttle water systems (Koenig & Pierson 1997), standard purified water (Penna *et al.*, 2002), laboratory-based high-purity water systems (Adley *et al.*, 2005) and industrial ultra-pure/high purity water (Kulakov *et al.*, 2002; Adley & Saieb 2005; Bohus *et al.*, 2010). *R. pickettii* has also been identified as forming and maintaining biofilm in plastic industrial water piping (Anderson *et al.*, 1990; Adley & Saieb 2005). In addition, the bacterium has been found in a wide variety of clinical environments worldwide and has become recognized as a nosocomial pathogen that is particularly associated with patients who are immunosuppressed or are in some other way debilitated (Ryan *et al.*, 2006). Conditions associated with *R. pickettii* range from range from minor infections to more severe invasive infections such as sepsis or meningitis. Minor respiratory illnesses were found in 34 patients in an outbreak due to contaminated solutions (Labarca *et al.*, 1999). More invasive

infections, such as osteomyelitis (Wertheim & Markovitz 1992), have been reported in a 71-year-old man suffering from chronic renal failure, and in a 75-year-old women in Bulgaria who was also suffering with renal failure and was reported to have a case of *R. pickettii*-related renal failure sepsis (Strateva *et al.*, 2012). Cases of meningitis have also been reported associated with *R. pickettii*, including that of a 54-year-old male who was otherwise healthy (Heagney, 1998). These infections have been recorded in association with contamination of hospital water supplies such as respiratory solutions (water based) and water for injection (Gardner & Shulman, 1984; McNeil *et al.*, 1985; Roberts *et al.*, 1990; Maki *et al.*, 1991; Raveh *et al.*, 1993; Labarca *et al.*, 1999). The majority of tested isolates of *R. pickettii* display multiresistance to common antibiotics (Zellweger *et al.*, 2004). The bacterium has shown itself to be very resilient to treatment in water supplies, with resistance to disinfectants such as chlorhexidine, and under certain circumstances *R. pickettii* has been shown to penetrate 0.2 micron filters (Sundaram *et al.*, 1999, 2002; Adley *et al.*, 2005).

R. pickettii has been shown to survive in low nutrient (oligotrophic) conditions (McAlister *et al.*, 2002). In addition, the bacterium has been shown to possess a wide range of biodegradative abilities that could be used for commercial applications (pollution clear ups) and that could assist in the survival and adaptation of the organism to low nutrient environments (Ryan *et al.*, 2007). Integrating conjugative elements (ICE) (such as Tn4371) have been found in various isolates of this bacterium, signifying a degree of plasticity in their genomes (Ryan *et al.*, 2009; Van Houdt *et al.*, 2012).

Abbreviations: CLSI, Clinical and Laboratory Standards Institute; MIC₅₀, the minimal concentration of antibiotic capable of inhibiting 50 % of the isolates tested; MIC₉₀, the minimal concentration of antibiotic capable of inhibiting 90 % of the isolates tested.

Statistical comparisons and full antibiotic susceptibility testing results, including scatterplots, are available with the online version of this paper.

Ralstonia insidiosa is the most closely related bacterium to *R. pickettii* (Ryan *et al.*, 2011a) and has also been isolated from natural water sources such as ponds and rivers, soil, activated sludge (Coenye *et al.* 2003), laboratory-purified water systems (Ryan *et al.*, 2011b), industrial ultra-pure/high purity water (Ryan *et al.*, 2011b) and water distribution systems (Hoefel *et al.*, 2005). It has been reported to be the causative agent of hospital-based infections in two immunocompromised individuals (Van der Beek *et al.*, 2005) and was found to be the cause of sepsis in eight haemodialysis patients in the Czech Republic (Orlíková *et al.*, 2011) due to contaminated haemodialysis solutions. Both bacteria have been found in the lungs of cystic fibrosis sufferers (Coenye *et al.*, 2003).

The treatment of *R. pickettii* infections is often challenging as this bacterium has been reported as being intrinsically resistant to many antimicrobial agents (Zellweger *et al.*, 2004; Ryan *et al.*, 2006), which could be due to the presence of mobile genetic elements (Ryan *et al.*, 2009). The antimicrobial resistance mechanisms in *Ralstonia* species are generally unknown; however, two specific mechanisms of resistance to some β -lactam antibiotics have been identified. Two different types of class D β -lactamases have been identified: *bla*_{OXA-22} and *bla*_{OXA-60} and these genes have contributed to the multidrug-resistant phenotype displayed by *R. pickettii*. *bla*_{OXA-22} has activity against benzylpenicillin, cloxacillin and restricted-spectrum cephalosporins, and *bla*_{OXA-60} has activity against imipenem (Nordmann *et al.*, 2000; Girlich *et al.*, 2004).

R. pickettii and *R. insidiosa* are a growing threat in clinical situations; this is mainly due to their presence in water and their ability to survive in purified/distilled water used to make-up medicinal products in hospitals for patient treatment, their ability to survive in nutrient-limited environments and their ability to survive treatment with common clinical antimicrobial agents such as chlorhexidine (Adley *et al.*, 2005; Ryan *et al.*, 2006, 2011c). It is thought that infection with *Ralstonia* spp. is mainly due to environmental sources such as contaminated water supplies (Ryan *et al.*, 2006), similar to *Brevundimonas diminuta* (Han & Andrade, 2005).

There is limited information on the surveillance and monitoring of the antibiotic resistance of these bacteria, and even less information on any possible differences in antibiotic resistance between clinical and environmental isolates. This study, which used a unique collection of *R. pickettii* and *R. insidiosa* (68 isolates in total) that included clinical and environmental isolates, reports the antimicrobial susceptibility profile of *R. pickettii* and *R. insidiosa* isolates, as well as comparing the Etest and disc diffusion methodologies for determining the antimicrobial susceptibility of these bacteria.

METHODS

***R. pickettii* and *R. insidiosa* strains.** Sixty-eight isolates of a unique culture collection including 53 *R. pickettii* and 15 *R. insidiosa*

were examined; 32 isolates came from industrial purified water, 11 from various laboratory purified water sources, eight from various clinical sources, six were isolated from washing water from an endoscopy unit, two (whole genome sequenced isolates) from a heavy metal-contaminated lake, two were purchased soil strains, five were purchased strains of *R. pickettii* and two were purchased strains of *R. insidiosa*. They came from various sources, including the BCCM/LMG Bacteria Collection (Ghent, Belgium); the Japan Collection of Microorganisms (Hirosawa, Wako-shi, Japan); the National Collection of Type Cultures (London, UK); the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Braunschweig, Germany); the Collection Bactérienne de l'Institut Pasteur (Paris, France), the American type Culture Collection (Manassas, VA, USA); and the Culture Collection, University of Göteborg (Göteborg, Sweden). The full list can be seen in Table 1. The isolation, identification and genotyping of all these isolates is dealt with in a previous paper (Ryan *et al.*, 2011b). Briefly, these isolates were identified using a combination of biochemical and PCR-based methods of identification. Genotyping was carried out using a whole genome approach with RAPD (random amplification of polymorphic DNA) and BOX PCR (Ryan *et al.*, 2011b.). All isolates were stored at -80°C in nutrient broth (Oxoid) with 50 % glycerol. Isolates were grown aerobically on nutrient agar (Oxoid) and incubated overnight at 30°C .

Antimicrobial susceptibility disc diffusion tests. The antibiotic discs were purchased from Oxoid. They are listed in Table 2. All tests were carried out on Müller–Hinton (MH) agar (Oxoid) according to the Clinical and Laboratory Standards Institute (CLSI) standards (CLSI, 2013). As there are no CLSI susceptibility breakpoints available for *R. pickettii* or *R. insidiosa*, the antibiotic susceptibility results were interpreted using the CLSI criteria for *Pseudomonas* sp., *Burkholderia cepacia* and *Acinetobacter* spp. Zone diameters for the disc susceptibility tests were measured with vernier callipers. All testing was carried out in triplicate and all discs were within their use by date. *Pseudomonas aeruginosa* ATCC 27853 was included with each testing session. All results were found to be within recommended limits, demonstrating the validity of the testing procedures used.

MIC determination using Etests. The MICs of the 68 isolates of *R. pickettii* and *R. insidiosa* to the 12 antibiotics listed in Table 3 were determined using Etests (Biodisk) on MH agar. The MH agar plates were inoculated using a sterile cotton swab with suspensions of the cultures (made to 0.5 McFarlane standard) diluted with sterile tryptone soya broth (Oxoid) and resulted in confluent growth. The MICs were read after incubation overnight at 35°C . The MICs for the bactericidal drugs (aztreonam, ceftazidime, ciprofloxacin, cefotaxime, gentamicin, meropenem, ofloxacin and piperacillin) were defined as the point of intersection between the ellipse edge and the Etest strip where there was complete inhibition of all growth. The MIC for the bacteriostatic drugs (tetracycline, minocycline and sulfamethoxazole/trimethoprim) was read at 80 % inhibition. There are no CLSI susceptibility breakpoints for *R. pickettii* or *R. insidiosa*, so the antibiotic susceptibility testing results were interpreted using the CLSI criteria for *Pseudomonas* sp., *Burkholderia cepacia* and *Acinetobacter* spp. (CLSI, 2013). *Pseudomonas aeruginosa* ATCC 27853 was used as a control.

Statistical analysis. Simple linear regression analysis was applied to define linear functions correlating the zone of inhibition (mm) with MICs obtained by disc diffusion and Etest (mg l^{-1}). The strength of the linear association between pairs of variables was determined by coefficients of determination (*R*-square): *R*-square $\geq 50\%$, strong correlation; *R*-square 25–49%, moderate correlation; and *R*-square $< 25\%$, weak correlation. The disc diffusion method was accepted as the reference method. Categorical agreement was defined if the tests results were within the same susceptibility category, and errors of disc

Table 1. *Ralstonia* strains used in this work

Strain	Source
<i>R. pickettii</i> JCM 5969, NCTC 11149, DSM 6297, CIP 73.23, CCUG 3318	Culture Collections
<i>R. pickettii</i> CCM 2846, CCUG 18841	Culture Collections
<i>R. insidiosa</i> ATCC 4199	Culture Collection
<i>R. insidiosa</i> LMG 21421	Culture Collection
<i>R. pickettii</i> ULC193, ULC194, ULC277, ULC297, ULC298, ULC224, ULC421	Isolated from the lung sputum of cystic fibrosis patients at the Microbiology Laboratory of Limerick Regional Hospital, Limerick Ireland (2002–2003)
<i>R. pickettii</i> ULCSF1	Isolated from cerebrospinal fluid at the University of Pittsburgh Medical Center, Pittsburgh, USA (2009)
<i>R. pickettii</i> ULI785, ULI788, ULI790, ULI791, ULI796, ULI800, ULI801, ULI804, ULI806, ULI807, ULI818, ULI159, ULI162, ULI165, ULI167, ULI169, ULI171, ULI174, ULI181, ULI187, ULI188, ULI193	Isolated from various industrial purified water systems (Ireland)
<i>R. insidiosa</i> ULI821, ULI797, ULI785, ULI181, ULI794, ULI185, ULI166, ULI819, ULI784, ULI163, ULI795	Isolated from various industrial purified water systems (Ireland)
<i>R. pickettii</i> ULM001, ULM002, ULM003, ULM004, ULM005, ULM006	Isolated from various Millipore purified water systems (France)
<i>R. pickettii</i> ULM007, ULM008, ULM009, ULM010, ULM011	Isolated from various Millipore laboratory purified water systems (Ireland)
<i>R. insidiosa</i> ULM008, ULM009	Isolated from various Millipore laboratory purified water systems (Ireland)
<i>R. pickettii</i> 12J, 12D	Heavy metal polluted lake, USA
<i>R. pickettii</i> 1177203, 1179198, 1179199, 1179204, 1179250, 1179251	Endoscopy unit washing water, UK

diffusion and Etest methods were determined as follows: very major error, resistant by disc diffusion method, susceptible by Etest method; major error, susceptible by disc diffusion method, resistant by Etest method; and minor error, intermediate result obtained by one method but not the other (Tatman-Otkun *et al.*, 2005). Percentage errors were calculated based on the total number of isolates that were tested. A good agreement was defined as complete category agreement over 90 %, and the total of very major and major errors below 5 %. The Pearson correlation was calculated using SPSS v20.

RESULTS

Antimicrobial susceptibility disc diffusion tests

The antibiotyping results of *R. pickettii* and *R. insidiosa* from this study (Table 2, and Table S1, Table S2 and Figs

S1–S24 available in JMM Online) generally agree with the results of a previous study of antibiotic susceptibility carried out on *R. pickettii* and on the antibiotic susceptibility of two isolates of *R. insidiosa*, which infected two immunocompromised individuals in Belgium (Van der Beek *et al.*, 2005). Almost all *R. pickettii* isolates were susceptible to ceftazidime (five isolates resistant), ciprofloxacin, cefotaxime, ofloxacin, meropenem (seven isolates resistant), minocycline (one isolate resistant), sulfamethoxazole/trimethoprim and tetracycline. All isolates were resistant to aztreonam, with 48 isolates resistant to gentamicin and 23 isolates resistant to ticarcillin/clavulanic acid mix. Susceptibility profiles for *R. insidiosa* were similar except in relation to tetracycline, where 13 isolates were shown to be resistant. Susceptibility profiles for species of

Table 2. Antibiotic resistance profile of 53 *R. pickettii* and 15 *R. insidiosa* isolates using disc diffusion testing

CLSI breakpoints for resistance for *P. aeruginosa* (table 2B-1) were used for the antibiotics, except for ceftazidime, meropenem, minocycline and trimethoprim/sulfamethoxazole where breakpoints for resistance for *Burkholderia cepacia* (table 2B-3) were used, and for cefotaxime and tetracycline where breakpoints for resistance for *Acinetobacter* spp. (table 2B-2) were used (CLSI, 2013). Atm, Aztreonam (30 µg ml⁻¹); Caz, ceftazidime (30 µg ml⁻¹); Cip, ciprofloxacin (5 µg ml⁻¹); Cn, gentamicin (10 µg ml⁻¹); Ctx, cefotaxime (30 µg ml⁻¹); Men, meropenem (10 µg ml⁻¹); Mh, minocycline (30 µg ml⁻¹); Of, ofloxacin (5 µg ml⁻¹); Prl, piperacillin (100 µg ml⁻¹); SxT, sulfamethoxazole/trimethoprim (23.75/1.25 µg ml⁻¹); Te, tetracycline (30 µg ml⁻¹); Tic, ticarcillin/clavulanic acid (75/10 µg ml⁻¹).

	Atm	Caz	Cip	Cn	Ctx	Men	Mh	Of	Prl	SxT	Te	Tic
<i>R. pickettii</i> (n=53)	53 (100 %)	5 (9 %)	–	48 (91 %)	–	7 (13 %)	1 (2 %)	–	–	–	–	30 (57 %)
<i>R. insidiosa</i> (n=15)	15 (100 %)	–	–	14 (93 %)	–	2 (13 %)	–	–	–	–	13 (86 %)	12 (80 %)

Table 3. MIC₅₀, MIC₉₀ and ranges for 53 *R. pickettii* and 15 *R. insidiosa* isolates (all values in µg ml⁻¹)

Antibiotic	Breakpoint for resistance*	<i>Ralstonia pickettii</i> (n=53)				<i>Ralstonia insidiosa</i> (n=15)			
		MIC ₅₀	MIC ₉₀	Range	Percentage resistant	MIC ₅₀	MIC ₉₀	Range	Percentage resistant
Aztreonam	≥ 32	>256	>256	24– >256	99.9	>256	>256	>256	100
Cefotaxime	≥ 64	0.5	1	0.03–2	0	1	1	0.25–1	0
Ceftazidime	≥ 32	4	12	0.75–21	9.4	4	6	3–8	0
Ciprofloxacin	≥ 4	0.06	0.12	0.002–0.12	0	0.12	0.12	0.06–0.12	0
Gentamicin	≥ 16	>256	>256	0.06– >256	99.9	>256	>256	>256	100
Meropenem	≥ 16	2	8	0.125– >256	13.2	2	4	0.032– >256	13.3
Minocycline	≥ 16	0.38	1.0	0.16–1.0	0	1	1	0.19–1	0
Ofloxacin	≥ 8	0.25	0.5	0.006–6	0	0.38	0.5	0.25–0.5	0
Piperacillin	≥ 128	3	16	0.092–18	2	8	12	0.064–16	0
Tetracycline	≥ 16	1.5	3	0.023–3	0	3	3	0.38–6	13.3
Ticarcillin/clavulanic acid	≥ 128/2	>256	>256	0.016– >256	55	>256	>256	>256	93.3
Trimethoprim/sulfamethoxazole	≥ 4/76	0.012	0.023	0.002–0.38	0	0.012	0.023	0.008–0.032	0

*CLSI breakpoints for resistance for *P. aeruginosa* (table 2B-1) was used for the antibiotics, except for ceftazidime, meropenem, minocycline and trimethoprim/sulfamethoxazole where breakpoints for resistance for *Burkholderia cepacia* (table 2B-3) were used, and cefotaxime and tetracycline where breakpoints for resistance for *Acinetobacter* spp. (table 2B-2) were used (CLSI, 2013).

the genus *Ralstonia* reported in the literature demonstrate susceptibility to cefotaxime, ciprofloxacin, ofloxacin and trimethoprim/sulfamethoxazole, and resistance to gentamicin and aztreonam. The major variation was with relation to tetracycline, where *R. insidiosa* was reported to be susceptible in all cases, whereas our results show resistance. Little variation was found due to the environmental niche.

MIC determination using Etests

The Etest MIC results are presented in the Table 3 and used the CLSI breakpoints for *P. aeruginosa*, *Acinetobacter* and *Burkholderia cepacia* for interpretation (CLSI, 2013). Nearly all the isolates of both bacteria were highly resistant to the aminoglycoside gentamicin (>256 µg ml⁻¹, 51 isolates of *R. pickettii* and 15 isolates of *R. insidiosa*) and the β-lactam antibiotic aztreonam (>256 µg ml⁻¹, 52 isolates of *R. pickettii* and 15 isolates of *R. insidiosa*) and variably resistant to the ticarcillin/clavulanic acid mix (>256 µg ml⁻¹, 31 isolates of *R. pickettii* and 14 isolates of *R. insidiosa*). For the carbapenem antibiotic, meropenem, six isolates of *R. pickettii* and one isolate of *R. insidiosa* were found to be resistant and the rest susceptible. All isolates were susceptible to the quinolones (ciprofloxacin and ofloxacin), the tetracyclines (tetracycline and minocycline), the cephalosporins (cefotaxime and ceftazidime), the folate pathway inhibitor (trimethoprim/sulfamethoxazole) and the extended spectrum β-lactam antibiotic of the ureidopenicillin class (piperacillin).

DISCUSSION

R. pickettii and *R. insidiosa* are a growing problem in hospital (and industrial) settings due their ability to survive and thrive in water. In our results (Table 4, Table S1, Table S2, Figs S1–S24) cefotaxime ($P<0.001$), ciprofloxacin ($P<0.001$), ofloxacin ($P=0.421$), tetracycline ($P=0.934$) and trimethoprim/sulfamethoxazole ($P<0.001$) showed 100 % correlation of the disc diffusion and MIC results for *R. pickettii*. Aztreonam ($P<0.001$), ciprofloxacin ($P<0.05$), meropenem ($P<0.001$), minocycline ($P=0.818$), ofloxacin ($P=0.069$) and trimethoprim/sulfamethoxazole ($P<0.05$) showed 100 % correlation of the disc diffusion and MIC results for *R. insidiosa*. Poor correlation rates were found with ceftazidime, gentamicin, meropenem, piperacillin and ticarcillin/clavulanic acid for *R. pickettii*, and cefotaxime, gentamicin and tetracycline for *R. insidiosa*. Similar variation between MIC and disc diffusion results was found in a comparable study that was carried out on *Stenotrophomonas maltophilia* (Nicodemo *et al.*, 2004, Tatman-Otkun *et al.*, 2005).

No major differences were observed between the MICs of different *R. pickettii* isolates from differing environmental niches except for the ticarcillin/clavulanic acid mix where the isolates from the industrial purified water supplies and the purchased isolates were all resistant, whereas the

Table 4. Categorical agreement and error rates for strains of *R. pickettii* and *R. insidiosa* tested

Antibiotic	<i>R. pickettii</i> (n=53)				<i>R. insidiosa</i> (n=15)			
	Very major (%)	Major (%)	Minor (%)	Correlation (%)	Very major (%)	Major (%)	Minor (%)	Correlation (%)
Aztreonam	0	0	1.88	98.12	0	0	0	100
Cefotaxime	0	0	0	100	6.67	0	0	93.33
Ceftazidime	9.43	0	39.62	50.95	0	0	6.67	93.33
Ciprofloxacin	0	0	0	100	0	0	0	100
Gentamicin	0	5.66	0	94.34	0	6.67	0	93.33
Meropenem	11.32	9.43	20.75	58.5	0	0	0	100
Minocycline	0	0	7.55	92.45	0	0	0	100
Ofloxacin	0	0	0	100	0	0	0	100
Piperacillin	0	0	30.19	69.81	0	0	60	40
Tetracycline	0	0	0	100	86.67	0	0	13.33
Ticarcillin/clavulanic acid	16.98	0	47.17	35.85	0	0	20	80
Trimethoprim/sulfamethoxazole	0	0	0	100	0	0	0	100

clinical isolates, and hospital and laboratory purified water isolates were susceptible. This is interesting as it suggests the possibility of a mechanism of resistance in environmental isolates that is not present in clinical isolates.

The MICs of some antibiotics found in the present study were broadly similar to those reported previously for *R. pickettii*. For example Sader & Jones (2005), Gales *et al.* (2005) and Fung-Tomc *et al.* (1997) reported the MIC₅₀ (the minimal concentration of antibiotic capable of inhibiting 50 % of the isolates tested) and the MIC₉₀ (the minimal concentration of antibiotic capable of inhibiting 90 % of the isolates tested) of ceftazidime as >16 µg ml⁻¹, whereas our isolates had an MIC₅₀ of 4 µg ml⁻¹ and an MIC₉₀ of 12 µg ml⁻¹. This was also true for meropenem, tetracycline and ticarcillin/clavulanic acid, where similar levels of resistance were found to those in our study (Fung-Tomc *et al.*, 1997; Sader & Jones 2005; Gales *et al.*, 2005). However, for trimethoprim/sulfamethoxazole (an MIC₅₀ of 0.012 µg ml⁻¹ and an MIC₉₀ of 0.023 µg ml⁻¹ in our study and an MIC₅₀ of ≤0.5 and an MIC₉₀ of 1/>2 µg ml⁻¹ in published studies) and ciprofloxacin (an MIC₅₀ of 0.06 µg ml⁻¹ and an MIC₉₀ of 0.12 µg ml⁻¹ in our study and an MIC₅₀ of 0.25/0.5/2 µg ml⁻¹ and an MIC₉₀ of 2/4 µg ml⁻¹ in published studies) higher levels of resistance were found than in our study. Also, in our study much higher levels of resistance were found to aztreonam (an MIC₅₀ and an MIC₉₀ of >256 µg ml⁻¹ and an MIC₅₀ of >8 and an MIC₉₀ of 16/64 µg ml⁻¹ in the previously published studies) and gentamicin (MIC₅₀ and an MIC₉₀ of >256 µg ml⁻¹ and an MIC₅₀ and an MIC₉₀ of >8 µg ml⁻¹ in the previously published studies) than in the published studies. These studies were, however, carried out with a limited number of clinical isolates (38, 10 and 14, respectively) compared with our study's 53 environmental and clinical isolates.

Resistance to the ticarcillin/clavulanic acid mix in some strains could possibly be due to the presence of the *bla*_{OXA-22} and *bla*_{OXA-60} genes, which have been identified in both

R. pickettii 12J (Rpic_3817 and Rpic_3962) and 12D (Rpic12D_3930 and Rpic12D_4075) genomes. Mutational studies have shown that these genes confer resistance to ticarcillin and to a ticarcillin/clavulanic acid mix. These *bla*_{OXA-22} and *bla*_{OXA-60} gene products have also been shown to raise MIC values for carbapenems, such as meropenem and imipenem, and for cephalosporins, such as cefepime and cefotaxime (Nordmann *et al.*, 2000; Girlich *et al.*, 2004).

Resistance to aztreonam in Gram-negative bacteria (like *R. pickettii* and *R. insidiosa*) is usually due to extended-spectrum β-lactamases (Franceschini *et al.*, 1998). Several genes for these extended-spectrum β-lactamases can be found in the genomes of *R. pickettii* 12J (21 proteins) and 12D (20 proteins).

Resistance to the aminoglycosides (like gentamicin) could possibly be due to a multidrug efflux pump found in both the genomes of *R. pickettii* 12J (Rpic_3744-Rpic_3747) and 12D (Rpic12D_3421-Rpic12D_3424) that is similar to that of the BpeAB–OprB (BpeR 45 % similarity, BpeA 53 % similarity, BpeB 58 % similarity and OprB 52 % similarity) and AmrAB–OprA (AmrR 36 % similarity, AmrA 48 % similarity, AmrB 55 % similarity and OprA 47 % similarity) pumps of *Burkholderia pseudomallei* (Chan *et al.*, 2004; Mima & Schweizer, 2010). In some strains of *Burkholderia pseudomallei* the BpeAB–OprB system was found to mediate aminoglycoside resistance, while in others the AmrAB–OprA was found to carry out the same task.

These results indicate that sulfamethoxazole/trimethoprim and the fluoroquinolone ciprofloxacin are the best antibiotics with which to treat infections with *R. pickettii* and *R. insidiosa*. This is shown by the agreement of the statistical analysis with the experimental data shown. This agreed with the literature data, which showed that ciprofloxacin worked to treat *R. pickettii* infections after the failure of other treatments (Kendirli *et al.*, 2004; Woo *et al.*, 2002). However, issues such as pharmacokinetics and

pharmacodynamics and clinical experience should be taken into consideration when choosing the best antibiotic to treat infections.

This is believed to be the first in-depth study on the antibiotic resistance of a significant number of *R. pickettii* isolates, which is vital in determining the appropriate treatment in cases of infection. Our results suggest that infection with *R. pickettii* and/or *R. insidiosa* could be treated orally with quinolones or trimethoprim/sulfamethoxazole, which would reduce the invasiveness of treatment (Table 3). In-depth clinical studies are essential to confirm the *in vivo* effectiveness of these antibiotics for the treatment of *R. pickettii*/*R. insidiosa* infections, and to assess the correlation between the susceptibility testing results and the clinical outcomes of treatment. These results indicate that there is little difference in antibiotic resistance between clinical and environmental isolates of the two bacteria; this is of interest as most clinical infections with *R. pickettii* are thought to come from environmental sources such as purified water supplies.

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