Waterborne Infections in Reunion Island, 2010–2017

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Abstract. Gram-negative bacilli *Vibrio* spp., *Aeromonas* spp., and *Shewanella* spp. are a major cause of severe waterborne infection. The aim of this study was to assess the clinical and microbiological characteristics and prognosis of patients hospitalized in Reunion Island for a waterborne infection. This retrospective study was conducted in the two university hospitals of Reunion Island between January 2010 and March 2017. Patients diagnosed with a *Vibrio*, *Aeromonas*, or *Shewanella* infection were evaluated. Over the study period, 112 aquatic strains were isolated at Reunion Island: *Aeromonas* spp. were found in 91 patients (81.3%), *Shewanella* spp. in 13 patients (11.6%), and *Vibrio* spp. in eight patients (7.2%). The in-hospital mortality rate was 11.6%. The main sites of infection were skin and soft tissue (44.6%) and the abdomen (19.6%). Infections were polymicrobial in 70 cases (62.5%). The most commonly prescribed empiric antibiotic regimen was amoxicillin–clavulanate (34.8%). Eighty-four percent of the aquatic strains were resistant to amoxicillin–clavulanate and more than > 95% were susceptible to third or fourth generation cephalosporins and fluoroquinolones. After multivariate analysis, the only independent risk factor of in-hospital mortality was the presence of sepsis ($P < 0.0001$). In Reunion Island, the most commonly isolated aquatic microorganisms were *Aeromonas* spp. Sepsis caused by aquatic microorganisms was frequent (> 50%) and associated with higher in-hospital mortality. This study suggests that empiric antibiotic regimens in patients with sepsis or septic shock caused by suspected aquatic microorganisms (tropical climate, skin lesion exposed to seawater. . .) should include broad-spectrum antibiotics (third or fourth generation cephalosporins).

INTRODUCTION

Gram-negative bacilli *Aeromonas* spp., *Vibrio* spp., and *Shewanella* spp. are the most common cause of waterborne infection. Found naturally in aquatic environments,^{1–3} these microorganisms develop mainly in the warm waters of tropical and subtropical regions.^{1–3} Of all aquatic bacteria, *Aeromonas* strains are the most commonly isolated; in some countries, they are detected even in food and running water.^{1,4–6} *Vibrio* and *Shewanella* strains are less common, and their presence in aquatic environments is favored by salt water.^{2,7,8} *Aeromonas* spp., *Vibrio* spp., and *Shewanella* spp. are responsible for all kinds of infections, especially skin and soft tissue infections.⁹ Waterborne infections often occur in immunocompromised patients—for instance, those suffering from diabetes or liver disease, or those undergoing immunosuppressive therapy.^{1–3} Few global epidemiological studies on aquatic bacteria have been published to date,¹⁰ and local epidemiological studies are usually limited to one type of infection^{2,3,7,8} and one type of bacteria.⁹ To fill this void, we present the most common types of waterborne infections in the Indian Ocean region, in particular in Reunion Island, a French overseas territory with a population of 850,000 inhabitants. The aim of this study was to assess the clinical and microbiological characteristics and prognosis of patients with a waterborne infection.

MATERIAL AND METHODS

This observational study was approved by the Institutional Review Board of the Committee of the French Intensive Care Society (CE SRLF17-27) and was declared to the Commission

nationale de l'informatique et des libertés (CNIL MR-003, No. 2000694). The need for informed consent was waived because of the observational and retrospective nature of the study.

Selection of the study sample. This retrospective study was conducted from January 2010 to March 2017 in the two university hospitals of Reunion Island (Félix Guyon and Saint Pierre).

We consecutively screened all hospitalized patients with a positive microbiological sample for *Aeromonas* spp., *Shewanella* spp., or *Vibrio* spp. The exclusion criteria were age < 18 years old and colonization by *Aeromonas* spp., *Shewanella* spp., or *Vibrio* spp. without infection.

Definitions. Sepsis was defined as infection with an increase in the Sequential Organ Failure Assessment (SOFA) score ≥ 2 points from baseline.¹¹

Septic shock was defined as sepsis requiring vasopressor support to maintain a mean arterial pressure ≥ 65 mm Hg and serum lactate levels ≥ 2 mmol/L in the absence of hypovolemia.¹¹

Data collection. The following demographic and clinical data were collected from consecutive patients infected with aquatic bacteria: demographic characteristics, site of infection, bacterial species, and death.

Rainfall data. Rainfall data consisted in the average rainfall of Reunion Island over the 2010–2017 period (Météo France, La Réunion, Saint-Denis, Bureau of Meteorology).

Microbiological investigations. All microorganism isolates were identified at the species level using biochemical identification galleries (API® 20E; bioMérieux, Marcy l'Etoile, France) or matrix-assisted laser desorption-ionization time-of-flight (MALDI-TOF) mass spectrometry (Bruker Daltonics, Breme, Germany/bioMérieux, La Balme-les-Grottes, France).

Susceptibility testing and empiric antimicrobial therapy. When an infection was suspected, empiric antimicrobial therapy based on local guidelines was initiated. The therapy

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was considered appropriate when all isolated bacteria were susceptible to at least one of the administered drugs after susceptibility testing.

Susceptibility testing was performed using the disk diffusion method based on the criteria of the European Committee on Antimicrobial Susceptibility Testing.¹²

Statistical analysis. Results were expressed as total numbers (percentages) for categorical variables and as median (25th–75th percentiles) for continuous variables. Continuous variables were compared using the Mann–Whitney test or the Kruskal–Wallis test, as appropriate. Categorical variables were compared using the χ^2 test or the Fisher's exact test, as appropriate.

Risk factors found to be predictive of in-hospital mortality in the bivariate analysis with $P < 0.05$ were entered into a multivariate logistic regression analysis using backward selection with a criterion of $P < 0.05$. Collinearity between independent factors was investigated. When identified, the most clinically relevant factor was chosen for use in the multivariate model. A P value < 0.05 was considered significant. Analysis was performed using SPSS statistical software (8.2, Cary, NC).

RESULTS

Study population. Over the study period, 156 patients were hospitalized with a positive microbiological sample for *Aeromonas* spp., *Shewanella* spp., or *Vibrio* spp. Among these patients, 44 were excluded (35 were < 18 years old and nine were colonized). Overall, 112 aquatic strains were isolated at Reunion Island in 112 infected patients: The majority of infections occurred in Reunion Island ($N = 107$, 95.5%). Four

infections (3.6%) developed in Madagascar after swimming in salt water and one (0.9%) did so in the Comoros Archipelago (0.9%).

As shown in Figure 1, the largest number of *Shewanella* infections occurred during the rainy season, between December and June. *Vibrio* or *Aeromonas* infections were relatively well distributed throughout the year, with a peak of *Aeromonas* infections occurring at the end of the rainy season (Figure 1).

Patient characteristics at study inclusion are shown in Table 1. Median age was 59 [46–72] years. The most frequent underlying diseases were diabetes mellitus (32.1%), heavy alcohol use (23.2%), and cancer (22.3%) (Table 1).

Distribution of microorganisms and sites of infection. Most strains were identified phenotypically by extensive biochemical testing. The most commonly isolated strains were *Aeromonas* spp. in 91 cases (81.3%): *Aeromonas hydrophila* (54 strains), *Aeromonas caviae* (29 strains), and *Aeromonas sobria* (four strains). *Shewanella* spp. were isolated in 13 patients (11.6%): *Shewanella putrefaciens* (11 strains) and *Shewanella algae* (two strains). *Vibrio* spp. were isolated in eight patients (7.1%): *Vibrio alginolyticus* (five strains), *Vibrio parahaemolyticus* (one strain), *Vibrio fluvialis* (one strain), and *Vibrio vulnificus* (one strain).

The main sites of infection were skin and soft tissue in 50 cases (44.6%) and the abdomen in 22 cases (19.6%). Bacteremia was present in 39 cases (34.8%) (Table 2).

Infections were polymicrobial in 70 cases (62.5%). They were more frequently polymicrobial in patients with an *Aeromonas* infection (63.7%) or a *Shewanella* infection (76.9%) than in patients with a *Vibrio* infection (25%, $P = 0.049$).

FIGURE 1. Seasonal isolation of aquatic microorganisms from 2010 to 2017.

TABLE 1
Clinical characteristics of the 112 patients with waterborne infections

Characteristics	Total (N = 112)	<i>Aeromonas</i> spp. (N = 91)	<i>Shewanella</i> spp. (N = 13)	<i>Vibrio</i> spp. (N = 8)	P value
Age (years old)	59 [46–72]	60 [45–73]	61 [55–69]	54 [42–64]	0.568
Male gender	74 (66.1)	58 (63.7)	8 (61.5)	8 (100)	0.108
Water exposure	12 (10.7)	8 (8.8)	3 (23.1)	3 (37.5)	0.39
Underlying condition					
Body mass index > 27 kg/m ²	14 (12.5)	11 (12.1)	2 (15.4)	1 (12.5)	0.945
Heavy alcohol use	26 (23.2)	22 (24.2)	2 (15.4)	2 (25)	0.775
Biliary tract disease	12 (10.7)	12 (13.2)	0	0	0.212
Liver cirrhosis	6 (5.4)	6 (6.6)	0	0	0.481
Chronic respiratory failure	7 (6.3)	5 (5.5)	1 (7.7)	1 (12.5)	0.716
Cancer (< 3 months)	25 (22.3)	25 (27.5)	0	0	0.024
Diabetes mellitus	36 (32.1)	32 (35.2)	3 (23.1)	1 (12.5)	0.23
Immunodepression	12 (10.7)	12 (13.2)	0	0	0.212
Corticosteroid treatment	6 (5.4)	5 (5.5)	0	1 (12.5)	0.462
Organ failure					
Sepsis*	60 (53.6)	50 (54.9)	8 (61.5)	2 (25)	0.22
ICU admission	29 (25.9)	23 (25.3)	4 (30.8)	2 (25)	0.903
Catecholamines	22 (19.6)	17 (18.7)	3 (23.1)	2 (25)	0.862
Renal replacement therapy	9 (8)	6 (6.6)	2 (15.4)	1 (12.5)	0.491
Creatinine level (μmol/L)	97 [70–168]	97 [69–167]	103 [63–264]	93 [78–116]	0.898
Platelet count (G/L)	194 [106–283]	177 [102–274]	222 [166–310]	232 [198–312]	0.244
Leukocyte count (G/L)	11.6 [6.7–16.3]	11.9 [6.8–16.7]	8.6 [6.4–12.3]	7.4 [6.9–16.4]	0.442
Neutrophil count (G/L)	9.1 [4.7–13.6]	9.3 [5–14.1]	8 [4.5–11.1]	4.8 [3.7–13.9]	0.571
Glasgow Coma Scale score	15 [15–15]	15 [15–15]	15 [11–15]	15 [12–15]	0.308
Mechanical ventilation	22 (19.6)	17 (19.7)	3 (23.1)	2 (25)	0.862
Total bilirubin level (mg/dl)	17 [9–41]	20 [10–45]	11 [8–48]	9.5 [9–10]	0.127
Conjugated bilirubin level (μmol/L)	9 [4–28]	11 [5–33]	6 [5–13]	4 [2–5]	0.038
Prothrombin time (%)	74 [55–87]	73 [54–87]	68 [59–79]	84 [83–85]	0.739
C-reactive protein (mg/dl)	127 [63–256]	97 [69–234]	318 [178–329]	74 [41–119]	0.039

ICU = intensive care unit; SOFA = sequential organ failure assessment. Results are expressed as median [25th–75th percentiles] or *n* (%), as appropriate.

* Increase of SOFA score ≥ 2 points from baseline.

A total of 126 co-pathogens were cultured (Table 3). The most commonly isolated co-pathogens were Enterobacteriaceae (50.9%), enterococci (16.1%), and *Staphylococcus aureus* (14.3%). No significant difference was found between the three groups of patients with respect to the cultured co-pathogens, with the exception of Enterobacteriaceae ($P = 0.01$).

Empiric antibiotic therapy and susceptibility of microorganisms. In the study cohort, more than 95% of aquatic strains were susceptible to a third or fourth generation cephalosporin, 94.3% to piperacillin/tazobactam, 96.2% to ciprofloxacin, and 96.3% to meropenem—with no significant difference between *Aeromonas* spp., *Shewanella* spp., and *Vibrio* spp. (Table 4). Susceptibility to amoxicillin-clavulanate

was 15.9% and was higher in the *Vibrio* spp. group than in the other two groups ($P < 0.0001$) (Table 4).

The rate of appropriate empiric antimicrobial therapy was 54.8%.

The most commonly prescribed empiric antibiotic regimen was amoxicillin-clavulanate (34.8%). Third generation cephalosporins, piperacillin/tazobactam, and carbapenems were prescribed in 24 patients (21.4%), 21 patients (18.8%), and two patients (1.8%), respectively. A combination therapy with either aminoglycosides, fluoroquinolones, or vancomycin was used in 24 patients (21.4%), seven patients (6.3%), and two patients (1.8%), respectively.

Prognosis. Sixty patients had sepsis (53.6%), 22 had septic shock (19.6%), and 29 were hospitalized in intensive care unit (25.9%), with no significant difference between the three groups of microorganisms.

In-hospital mortality was 11.6%, with no significant difference between the three groups of patients ($P = 0.895$). After univariate analysis, the factors associated with in-hospital mortality were the following: male gender ($P = 0.025$), SOFA score ≥ 2 ($P = 0.0004$), intensive care unit admission ($P < 0.0001$), catecholamines ($P < 0.0001$), renal replacement therapy ($P = 0.001$), higher lactatemia ($P = 0.004$), lower platelet count ($P = 0.003$), lower Glasgow Coma Scale score ($P = 0.004$), mechanical ventilation ($P < 0.0001$), higher bilirubin level ($P = 0.002$), and bacteremia ($P = 0.03$).

After multivariate analysis, SOFA score ≥ 2 (OR: 7.5; 95% confidence interval [CI]: 1.8–37.1; $P < 0.0001$) was the only independent risk factor of in-hospital mortality. The Hosmer–Lemeshow goodness-of-fit test P value was 0.76. The Nagelkerke and Cox/Snell R squares were 0.332 and 0.17, respectively.

TABLE 2
Sites of infection

Sites of infection	Total (N = 112)	<i>Aeromonas</i> spp. (N = 91)	<i>Shewanella</i> spp. (N = 13)	<i>Vibrio</i> spp. (N = 8)	P value
Pulmonary	9 (8)	4 (4.4)	3 (23.1)	2 (25)	0.013
Catheter	4 (3.6)	4 (4.4)	0	0	0.62
Skin and soft tissue	50 (44.6)	40 (44)	6 (46.2)	4 (50)	0.941
Urinary tract	8 (7)	7 (7.7)	0	1 (12.5)	0.5
Abdominal or biliary tract	22 (20)	20 (22)	2 (15.4)	0	0.298
Bacteremia	39 (32)	32 (35.2)	4 (30.8)	3 (37.5)	0.94
Bone	7 (5)	5 (5.5)	1 (7.7)	1 (12.5)	0.716
Meningitis	1 (0.9)	0 (0)	1 (7.7)	0	
Feces	1 (0.9)	1 (1.1)	0	0	0.89

Results are expressed as number (%).

TABLE 3
Presence of co-pathogens

Microorganisms	Total (N = 112)	<i>Aeromonas</i> spp. (N = 91)	<i>Shewanella</i> spp. (N = 13)	<i>Vibrio</i> spp. (N = 8)	P value
<i>Staphylococcus aureus</i>	16 (14.3)	12 (13.1)	3 (23.1)	1 (12.5)	0.628
Streptococci	6 (5.4)	5 (5.5)	1 (7.7)	0	0.742
Non-fermenting Gram-negative bacilli	12 (10.7)	9 (9.9)	3 (23.1)	0	0.212
Enterobacteriaceae	57 (50.9)	49 (53.8)	8 (61.5)	0	0.01
Anaerobes	8 (7.1)	6 (6.6)	2 (15.4)	0	0.37
Enterococci	18 (16.1)	13 (14.3)	4 (30.8)	1 (12.5)	0.305
Fungi	4 (3.6)	4 (4.4)	0	0	0.620
Other	5 (4.5)	3 (3.3)	2 (15.4)	0	0.117

Results are expressed as n (%).

DISCUSSION

To date, only one study, conducted in Australia, has examined the epidemiology of waterborne infections.¹⁰ In our study of waterborne infections in Reunion Island, the main findings were as follows: 1) the aquatic bacteria most likely to cause infection are *Aeromonas* spp., 2) waterborne infections are often severe (> 50%), and 3) amoxicillin-clavulanate is the most commonly prescribed probabilistic antibiotic treatment (35%), but it is often ineffective on aquatic bacteria (84%).

The most frequently isolated aquatic bacteria in our study were *Aeromonas* spp., a finding that was also reported by Mc Auliffe et al.¹⁰ Comorbidities such as diabetes, alcoholism, neoplasia, and immunosuppression were common in our patients, as has been observed elsewhere.^{2,3,9,10}

In our series, 38% of patients with a *Vibrio* infection had a cutaneous entry point after contact with seawater. This is a common finding in studies that specifically evaluate non-cholera *Vibrio* infections.¹³ Skin and soft tissue infections were the most common in our series, with an incidence comparable

to that found in the study by Lamy et al.,¹⁴ which focused specifically on *Aeromonas* infections (44%). However, the incidence of skin and soft tissue infection was significantly lower than that reported by Mc Auliffe et al. (80%).¹¹

Whereas the very low incidence of waterborne gastroenteritis in our study (< 1%) is similar to that found by Mc Auliffe et al.¹⁰ (5.4%), it is much lower than that reported by Lamy et al.¹⁴ (19%). This low incidence may be because of the fact that unlike the situation for children, the disease is not usually severe in adults, and hence rarely leads to hospitalization.¹⁵

In sensitivity analysis, only a small proportion of the isolated strains were sensitive to amoxicillin-clavulanate (16%), which makes sense given that the majority of these strains were *Aeromonas* spp.^{14,16} However, this poses a therapeutic problem because the most of the waterborne infections are skin and soft tissue infections, these being often treated probabilistically with amoxicillin-clavulanate. In addition, waterborne infections are most often polymicrobial, and there are no specific signs associated with aquatic bacteria.^{2,10} In cases of severe waterborne skin and soft tissue infection, antibiotic therapy should include broad-spectrum antibiotics such as third or fourth generation cephalosporins, which are effective on these bacteria in the vast majority of cases. In fact, antibiotic therapy may require a combination of molecules because infections are often polymicrobial, but also because *Aeromonas* strains can produce AmpC β -lactamase, which has been shown to occur frequently.¹⁷

Shewanella strains are most often susceptible to broad-spectrum antibiotics, such as third or fourth generation cephalosporins and carbapenems. It should be noted, however, that these strains can produce oxacillinase, which makes them resistant to carbapenems¹⁸ and may, therefore, lead to therapeutic failure.¹⁹ The study by Wong et al. suggests that fluoroquinolones can lead to a decrease in mortality from *Vibrio* infections.²⁰ In the case of patients with marine injury with sepsis or septic shock, the use of fluoroquinolone should be discussed. Cefepime may be an interesting

TABLE 4
Antibiotic susceptibility of clinical *Aeromonas* spp., *Shewanella* spp., and *Vibrio* spp. isolates

	Total (N = 112)	<i>Aeromonas</i> spp. (N = 91)	<i>Shewanella</i> spp. (N = 13)	<i>Vibrio</i> spp. (N = 8)	P value
Amikacin	111/111 (100)	90/90 (100)	13/13 (100)	8/8 (100)	0.89
Ampicillin	3/46 (6.5)	0/38 (0)	0/1 (0)	3/7 (42.9)	< 0.0001
Amoxicillin-clavulanate	7/44 (15.9)	2/37 (5.4)	0/1 (0)	5/6 (83.3)	< 0.0001
Aztreonam	63/88 (71.6)	52/72 (72.2)	7/12 (58.3)	4/4 (100)	0.267
Cefotaxim	46/47 (97.9)	38/39 (97.4)	1/1 (100)	7/7 (100)	0.901
Ceftazidime	106/111 (95.5)	85/90 (97.4)	13/13 (100)	8/8 (100)	0.543
Ceftriaxone	25/26 (96.2)	20/21 (95.2)	0/0	5/5 (100)	0.248
Cotrimoxazole	90/100 (90)	77/86 (89.5)	8/9 (88.9)	5/5 (100)	0.745
Ciprofloxacin	102/106 (96.2)	83/86 (96.5)	12/13 (92.3)	7/7 (100)	0.656
Cefepime	89/90 (98.9)	72/73 (98.6)	10/10 (100)	7/7 (100)	0.889
Colistin	28/33 (84.8)	21/25 (84)	2/2 (100)	5/6 (83.3)	0.826
Fosfomycin	41/53 (77.4)	37/40 (92.5)	0/8 (0)	4/5 (80)	< 0.0001
Gentamicin	108/110 (98.2)	88/89 (98.9)	12/13 (92.3)	8/8 (100)	0.234
Imipenem	98/107 (91.6)	79/87 (90.8)	11/12 (91.7)	8/8 (100)	0.669
Meropenem	52/54 (96.3)	42/43 (97.7)	8/9 (88.9)	2/2 (100)	0.429
Ofloxacin	41/43 (95.3)	36/37 (97.3)	1/1 (100)	4/5 (80)	0.221
Piperacillin	60/84 (71.4)	43/63 (68.3)	11/13 (84.6)	6/8 (75)	0.48
Piperacillin-tazobactam	99/105 (94.3)	81/87 (93.1)	13/13 (100)	5/5 (100)	0.518
Ticarcillin	15/111 (13.5)	2/91 (2.2)	11/13 (84.6)	2/7 (28.6)	< 0.0001
Ticarcillin-clavulanate	56/100 (56)	41/82 (50)	11/13 (84.6)	4/5 (80)	0.035
Tobramycin	97/100 (97)	82/84 (97.6)	12/12 (100)	3/4 (75)	0.028

Results are expressed as no. positive/no. tested (%).

molecule for the treatment of *Aeromonas* infections. Indeed, cefepime is little affected by cephalosporinase, and minimal inhibitory concentrations of the molecule required for the treatment of *Aeromonas* and *Shewanella* infections are very low.^{20–22}

McAuliffe et al. did not evaluate the prognosis of patients in their study. By contrast, we found that waterborne infections are often severe: sepsis was present in more than 50% of the examined cases, often leading to hospitalization in intensive care (25.9%), with an in-hospital mortality rate of 11.6%. The different causative organisms did not seem to be associated with significantly different virulence. Nevertheless, Wu et al.²³ have found that mortality is higher with *Aeromonas dhakensis* strains than with other *Aeromonas* strains. Similarly, mortality has been found to be higher with *Vibrio vulnificus* strains than with other *Vibrio* strains,²⁴ and *S. algae* strains have been shown to be more virulent than *S. putrefaciens* strains.²⁵

This study has many limitations. Its retrospective nature is clearly a weakness. Moreover, the number of infections is relatively low, which limits the power of statistical analysis. The typing of the strains may be criticized because in most of the cases it was performed phenotypically, and hence without genetic analysis. Indeed, it has been shown that many strains identified as *S. putrefaciens* by MALDI-TOF are identified as *S. algae* after analysis of 16S RNA.²⁶

This study was performed on a tropical island in the Indian Ocean and cannot provide any definitive conclusions for other much larger island continents, such as Australia, and other tropical regions.

CONCLUSION

In Reunion Island, *Aeromonas* spp. were the most commonly isolated aquatic microorganisms. Infections were often severe and associated with sepsis in > 50% of cases. Amoxicillin–clavulanate was the most frequently prescribed antibiotic, but was often ineffective on aquatic microorganisms. This study suggests that empiric antibiotic regimens in patients with sepsis or septic shock caused by suspected aquatic microorganisms (tropical climate and skin lesion exposed to seawater) should include broad-spectrum antibiotics such as third or fourth generation cephalosporins.

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