ORIGINAL ARTICLE

Shewanella algae infection in Italy: report of 3 years' evaluation along the coast of the northern Adriatic Sea

A. Torri[1], S. Bertini[1], P. Schiavone[1], F. Congestrì[1], M. Matteucci[1], M. Sparacino[1], G. Testa[1], M. F. Pedna[1] and V. Sambri[1,2]

1) *Unit of Microbiology, The Great Romagna Hub Laboratory, Pievesestina (FC), Italy and* 2) *DIMES, University of Bologna, Bologna, Italy*

Abstract

Shewanella algae are Gram-negative, nonfermentative, motile bacilli, classified in the genus *Shewanella* in 1985. These environmental bacteria are occasionally identified in human infections, with a relatively strong association with exposure to seawater during warm seasons. This report describes a case series of 17 patients with infection correlated to *S. algae* in the coastal area of Romagna, Italy, from 2013 to 2016. The types of infection included otitis, pneumonia, sepsis and soft tissue (wound). Exposure to the marine environment during hot months was confirmed in 12 of 17 patients. An apparent correlation between increased severity of infection and patient age was also observed.

Keywords: Adriatic Sea, otitis, seawater, sepsis, *Shewanella algae*

Original Submission: 8 September 2017; **Revised Submission:** 29 December 2017; **Accepted:** 4 January 2018

Article published online: 12 March 2018

Corresponding author: A. Torri, Unit of Microbiology, Great Romagna Hub Laboratory, Piazza della Liberazione 60, edificio A, 47522, Pievesestina (FC), Italy.

E-mail: arianna.torri@auslromagna.it

The first two authors contributed equally to this article, and both should be considered first author.

Introduction

Shewanella species are Gram-negative, nonfermentative, motile bacilli. The first isolation occurred in 1931 by Derby and Hammer from dairy products; they named this unknown species *Achromobacter putrefaciens* [1]. In 1941, this species was transferred to the genus *Pseudomonas* under the name *P. putrefaciens,* and according to Shewan et al. [2], for the next three decades, they were placed in the *Pseudomonas* IV group. On the basis of phylogenetic studies and small subunit rDNA sequencing, these organisms were reclassified to the *Vibrionaceae* family and described as a new genus, *Shewanella.* The genus *Shewanella* was shown to be a monophyletic taxon within the gamma subgroup of the phylum *Proteobacteria*. From 16S RNA genetic analysis of genera belonging to this group emerged the existence of a new family, *Shewanellaceae,* containing about 50 *Shewanella* spp., most of which are psychrophilic and therefore of little interest to clinical microbiologists [3–5].

Bacteria of the genus *Shewanella* are found throughout the world, mainly in marine environments and other underwater settings; these germs have also been reported in soil, fish, meat, poultry and dairy products [6]. *Shewanella* spp. are an unusual cause of disease in humans; however, reports of *Shewanella* infections have been increasing, likely as the result of better testing. Most human *Shewanella* infections have been reported in warmer areas, including Southeast Asia, Southern Europe, South Africa and the Caribbean [7–10]. Sporadic cases have also been reported from countries with cooler climates, including Australia, Belgium and Denmark [10]. In Denmark, *S. putrefaciens and S. algae* have been reported in seawater, with the frequency of occurrence correlating with sea temperature. These organisms were detected in Denmark only during July to October, when the water temperature is above 13°C [11]. The only *Shewanella* spp. found in human clinical specimens are *S. putrefaciens* and *S. algae,* and more than 80% of the isolates from humans reported in the literature belong to *S. algae* [12–15]. Because these organisms are frequently isolated together with other bacteria, the pathogenic potential of *Shewanella* has been controversial since its very first report. In a murine pathogenicity model, *S. algae* was the most virulent species,

https://doi.org/10.1016/j.nmni.2018.01.002

and it has been speculated that the haemolytic activity of this species could play an important role in its virulence factors [16].

The major risk factor linked to *Shewanella* infection is an intimate association between humans and the marine environment or its contents; this correlation has been described in the literature [11,17–22]. *S. algae* has also been isolated from the heart of a dolphin with meningoencephalitis along the cost of the Adriatic Sea; indeed, this is additional evidence of the potential pathogenic role of *S. algae* for mammals in the marine environment [23]. The clinical picture of human infection caused by *S. algae* is generally quite similar to those caused by various species of the *Vibrionaceae* family: skin and soft tissue infections associated with ulcers or trauma; ear infections present as acute infections or acute exacerbations in chronic otitis media; and bacteraemia, the course of which in *S. algae* blood infection is usually benign [6]. To our knowledge, to date, only a single case of isolation of *S. putrefaciens* has been described from Italy. This event was related to a patient with soft tissue infection and bacteraemia who, a few days before the infection, had just returned from a holiday on the Adriatic shore [9].

This study was conducted to investigate the epidemiologic and clinical characteristics of *S. algae* isolates obtained from clinical specimens submitted for routine microbiologic diagnosis over a period of 3 years (2013–2016) along the Romagna Adriatic shore in northern Italy. To our knowledge, this is the first report of human infection clearly caused by *S. algae* in Italy.

Materials and methods

The *S. algae* isolates were identified from samples routinely submitted to the microbiology unit of the Great Romagna Hub Laboratory, Pievesestina-Cesena, northern Italy. Details about the standard diagnostic methods used to manage the samples positive for *S. algae* isolates are provided online in Supplementary Appendix S1. The identification of *S. algae* from the growing brown mucous colonies was obtained (99% confidence) using a VITEK MS instrument (bioMérieux, Marcy l'Etoile, France). The antimicrobial susceptibility of *S. algae* isolates was determined with a VITEK 2 system (bioMérieux). The Etest was used as a confirmatory test for susceptibility to carbapenems and was interpreted following European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines for other non-*Enterobacteriaceae* glucose nonfermenting Gram-negative bacilli.

Results

A total of 18 *S. algae* strains were isolated from 17 immunocompetent patients seeking care at the healthcare facilities of the Great Romagna area in northern Italy from 1 July 2013 to 30 November 2016. The Great Romagna area is located along the coast of the western Adriatic Sea. The microbiology unit of the Great Romagna Hub Laboratory serves the entire network of these public facilities. All the isolates were found during April to November, with a peak in July (Fig. 1). The *S. algae* strains were identified in the following samples: blood cultures (n = 5), skin and soft tissue swabs (n = 5), ear swabs (n = 5), bronchial aspiration (n = 1) and sputum (n = 2). In one patient, *S. algae* was isolated from two different types of specimens: blood culture and bronchial aspiration.

Most patients with *S. algae* infection were older than 60 years (n = 12), with a mean age of 68 years. One patient was 43 years old, and the remaining four were children (mean age, 7 years and 6 months). Nine patients were female and eight were male.

Recent (<4 weeks) exposure to seawater along the local coast of the Adriatic Sea was reported for 13 patients. No precise information is available in the scientific literature regarding the incubation time of *S. algae* infections, and consequently we can only hypothesize that for these 13 subjects, the infection was a direct consequence of contact with polluted seawater. All of the paediatric patients had otitis externa. The adult patients had different clinical pictures: five had skin and soft tissue infections, five had bacteraemia, two had acute pneumonia and one had external otitis. It is noteworthy that *S. algae* was associated with other pathogens in nine of the 17 patients. All patients had a positive final outcome, with the exception of a 92-year-old woman with sepsis, who died 8 days after *S. algae* was cultured from a blood sample. Table 1 summarizes the clinical picture of each patient.

All the *S. algae* isolates were susceptible to third-generation cephalosporins and gentamycin. In addition, amikacin, carbapenems and piperacillin/tazobactam showed very good activity

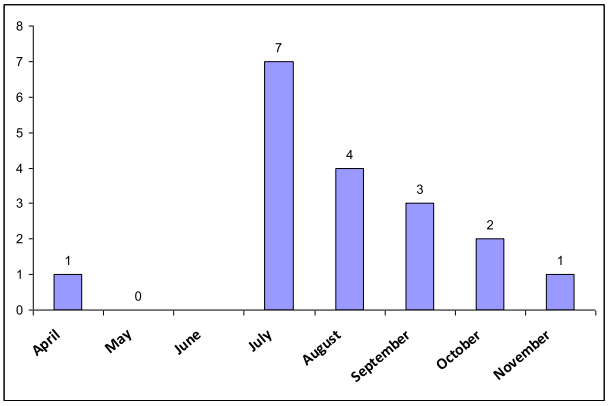

FIG. 1. Seasonal trends of *Shewanella algae* isolates.

against most of the isolated strains. Amoxicillin/clavulanic acid was efficacious against <50% of the isolates (5/13). Phosphomycin showed no activity against the seven strains evaluated. The *in vitro* activity of tigecycline and colistin was only evaluated against five and six isolates, respectively. The antimicrobial susceptibility data of the 17 *S. algae* isolates are detailed in Table 2.

TABLE 2. Antibiotic susceptibility of 17 strains of *Shewanella algae*

Antibiotic	MIC (mg/L), mean (range)	No. of clinically sensitive isolates of *S. algae* strains/no. tested
Amikacin	≤2 (≤2 to 4)	15/17
Amoxicillin/clavulanic acid	16 (≤2 to ≥32)	5/13
Cefepime	≤1 (≤1)	17/17
Cefotaxime	≤1 (≤1)	16/16
Ceftazidime	≤1 (≤1)	16/16
Ciprofloxacin	≤0.25 (≤0.25 to 2.0)	15/16
Phosphomycin	≥256 (≥256)	0/7
Gentamycin	≤1 (≤1)	17/17
Imipenem	1 (≤0.25 to ≥32)	14/17
Meropenem	1 (≤0.25 to 4.0)	16/17
Piperacillin/tazobactam	≤4 (≤4 to ≥128)	15/16
Tigecycline	≤0.5 (≤0.5)	5/5

MIC, minimum inhibitory concentration.

Discussion

Shewanella spp. infections have been reported worldwide. To our knowledge, this is the first report of infections caused by *S. algae* in Italy, where a single sporadic case of bacteraemia associated with soft tissue infection by *S. putrefaciens* was described 14 years ago [9]. The most frequent clinical features of *S. algae*–related human disease include soft tissue infections, otitis externa, invasive bacteraemia and sepsis with hepatic and biliary involvement. Because *Shewanella* spp. are mostly environmental microbes whose pathogenic mechanisms remain to be completely elucidated, most cases of human infection develop in people with underlying diseases, and infection is often related to massive exposure to seawater [6].

Bacteria that today is included in the genus *Shewanella* were first described in 1931 [1] as *Achromobacter putrefaciens*. Later they were classified as *P. putrefaciens,* and finally the novel genus *Shewanella* was proposed in 1985 [6]. The only *Shewanella* spp. relevant for human diseases so far are *S. putrefaciens* and *S. algae,* with a higher frequency of reported cases due to the latter species. This potentially higher pathogenic capability for humans has been correlated with the production of a haemolysin [24]. Most automated identification systems based on biochemical reactions are unable to distinguish between *S. putrefaciens* and *S. algae* because the latter species is not included in their database [7]. However, today, identification is based on matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, as these systems include the *S. algae* spectrum in their database [25]. This new ability to correctly and precisely identify *S. algae* has likely contributed to the increased frequency of descriptions of infections caused by *S. algae* in the last decade [26]. Our study describes a series of 17 cases of infection due to *S. algae* detected from 2013 to 2016 along the shores of northern Adriatic Sea in Romagna, Italy.

As reported previously in other geographical areas, human cases of *S. algae* infection are overwhelmingly linked with exposure to the marine environment. The mode of contact reported in the literature includes recreational or occupational exposure, consumption of seafood, introduction of *Shewanella* species into puncture wounds caused by marine life (sea urchins, fish) or direct exposure to seawater. In our study, more than 70% (12/17) of the patients reported massive contact with seawater within 4 weeks of the isolation of *S. algae*. This fact also correlates with the time period during which *S. algae* has

TABLE 1. Clinical characteristics of 17 patients with *Shewanella algae* infection

Patient	Age/sex	Additional microorganisms isolated from same sample	Clinical picture	Exposure to seawater within 4 weeks	Final outcome
1	6/F	None	Otitis externa	Yes	Positive
2	7/M	*Vibrio alginolyticus*	Otitis externa	Yes	Positive
3	8/F	*V. alginolyticus*	Otitis externa	Yes	Positive
4	9/F	*Klebsiella oxytoca*	Otitis externa	Yes	Positive
5	71/M	None	Otitis externa	Yes	Positive
6	83/M	None	Acute pneumonia	No	Positive
7	75/M	None	Acute pneumonia	Yes	Positive
8	80/M	None	Sepsis	No	Positive
9	92/F	None	Sepsis	No	Died from acute sepsis
10	82/F	None	Sepsis	Yes	Positive
11[a]	68/F	None; *Pseudomonas aeruginosa*	Sepsis; acute pneumonia	Yes	Positive
12	78/M	None	Sepsis	No	Positive
13	70/F	*Staphylococcus aureus, Acinetobactecter baumannii, Escherichia coli*	Skin and soft tissue infection	Yes	Positive
14	73/F	*Myroides* spp.	Skin and soft tissue infection	Yes	Positive
15	65/F	*Staphylococcus aureus*	Skin and soft tissue infection	No	Positive
16	66/M	*Morganella morganii*	Skin and soft tissue infection	Yes	Positive
17	43/M	None	Skin and soft tissue infection	Yes	Positive

[a]Only patient from whom *S. algae* was isolated from two different samples (blood and bronchial aspiration).

been identified (April to November, with a peak from July to September), which corresponds to the months with the highest frequency of sea-bathing activities along the coast of the northern Adriatic Sea (Fig. 1). Because not all human cases of infection by *S. algae* are linked to exposure to seawater, and because other studies have not reported such a strong association, other possible ways to acquire this infection have been hypothesized [6]. Such hypothetical modes of transmission could also apply to the five patients in this series who did not report contact with the marine environment. The presence of *S. algae* in the marine environment correlates with water temperature [10]. In the northern Adriatic Sea, the average temperature is above 13°C from April until November, with a peak (constantly above 20°C) from June to September detected since 2011. This climatic feature is compatible with a possible increase of *S. algae* concentration into seawater (http://www.isprambiente.gov.it).

The isolation of *S. algae* was frequently associated with other pathogenic or potentially pathogenic microbes (8/17 cases); this was more relevant in cases of less invasive infection (3/5 otitis externa, 4/5 wound infection) than in cases of bacteraemia or acute pneumonia (1/6). This fact clearly suggests that in superficial infections, the pathogenic role of *S. algae* is less defined because the pathogen is part of a mixed infecting flora and is a member of the microbiota found in the deeper part of skin and soft tissue and wound infections. However, *S. algae* likely plays a relevant pathogenic role on its own in invasive infections. As previously described in a Danish study [12], *S. algae* causes otitis, particularly in younger children, whereas invasive infections apparently develop only in the older population. Most of the cases of *Shewanella*-related bacteraemia are community acquired [27,28], and in the absence of underlying complications, these infection have an overall benign clinical course [28–30], as was indeed found in the population we studied. In our cases series, only one patient died from sepsis due to *S. algae*. This unfavourable outcome was correlated with the extreme age (92 years) of the patient.

S. algae was also identified in specimens from infected wounds; this is a common site for isolation of *Shewanella* spp., which are frequently found as members of the polymicrobial flora in skin and soft tissue infections [10,21,22,30]. This characteristic is likely linked to the exposure of broken skin to environmental bacteria; in this limited cases series, four of five patients with wound infections reported recent exposure to the marine environment.

Acute pneumonia is infrequently caused by *S. algae*. Patients can come into contact via the respiratory route in near-drowning events in saltwater, via head submersion resulting from recreational activities or via environmental exposure due to trauma [31]. In this study, only two strains were found as the unique pathogen in sputum samples, thus confirming the relatively infrequent role of *S. algae* in acute respiratory infections. The *in vitro* antimicrobial susceptibility of *S. algae* was reviewed in 2013 by Vignier et al. [10], who found that most isolates were sensitive to third-generation cephalosporin, piperacillin/tazobactam, ciprofloxacin and gentamycin. The isolates that we evaluated showed almost identical patterns of antimicrobial sensitivity. Erythromycin, chloramphenicol and tetracycline, although reported in the literature as effective against *S. algae* [10,28], were not included in our "Antimicrobial Susceptibility Testing (AST)" evaluation because they do not belong the first line of drugs used in clinical practice for the standard of cure for the illnesses caused by *S. algae*. As reported in the literature, *S. algae* showed a propensity towards resistance to carbapenems [30]. The mechanism of resistance may be related to a carbapenem-hydrolyzing Amber class D β-lactamase [32]. This feature was confirmed among the isolates described in this study that were susceptible to imipenem (14/17 susceptible isolates), with meropenem being more effective (16/17 susceptible isolates).

This study is novel in that it provides evidence for the first time of the pathogenic role played by *S. algae* in Italy; in particular, it confirms that isolation of this microbe mainly occurs in patients exposed to the marine environment when the seawater temperature is elevated. Although the number of patients is small and we could therefore not perform statistical analysis, we found an association between age and the severity of the *S. algae* infection. Otitis was the predominant clinical feature in children, whereas invasive infections and infections of skin and soft tissue were prevalent in older subjects. Moreover, the isolation of *S. algae* in clinical samples only during the summer period correlates with data reported in the literature, which demonstrate a connection between an increase in water temperature and the frequency of identification of this organism in human infections. *S. algae* should be considered an emerging opportunistic pathogen linked to environmental conditions in the coastal area of Romagna.

Conflict of interest

None declared.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.nmni.2018.01.002.

References

[1] Derby HA, Hammer HA. Bacteriology of butter. IV. Bacteriological studies on surface taint butter. Iowa Agric Exp Stn Res Bull 1931;145: 389–416.

[2] Shewan JM, Hobbs G, Hodgkiss W. A determinative scheme for the identification of certain genera of gram-negative bacteria, with special reference to *Pseudomonadaceae*. J Appl Bacteriol 1960;23:379–90.

[3] MacDonell MT, Colwell RR. Phylogeny of the *Vibrionaceae*, and recommendation for two new genera, *Listonella* and *Shewanella*. Syst Appl Microbiol 1985;6:171–82.

[4] Gauthier G, Gauthier M, Christen R. Phylogenetic analysis of the genera *Alteromonas, Shewanella*, and *Moritella* using genes coding for small-subunit rRNA sequences and division of the genus *Alteromonas* into two genera, *Alteromonas* (emended) and *Pseudoalteromonas* gen. nov., and proposal of twelve new species combinations. Int J Syst Bacteriol 1995;45:755–61.

[5] Ivanova EP, Flavier S, Christen R. Phylogenetic relationships among marine *Alteromonas*-like proteobacteria: emended description of the family *Alteromonadaceae* and proposal of *Pseudoalteromonadaceae* fam. nov., *Colwelliaceae* fam. nov., *Shewanellaceae* fam. nov., *Moritellaceae* fam. nov., *Ferrimonadaceae* fam. nov., *Idiomarinaceae* fam. nov. and *Psychromonadaceae* fam. nov. Int J Syst Evol Microbiol 2004;54:1773–88.

[6] Janda JM, Abbott SL. The genus *Shewanella*: from the briny depths below to human pathogen. Crit Rev Microbiol 2014;40:293–312.

[7] Holt HM, Gahrn-Hansen B, Bruun B. *Shewanella algae* and *Shewanella putrefaciens*: clinical and microbiological characteristics. Clin Microbiol Infect 2005;11:347–52.

[8] Finkelstein R, Oren I. Soft tissue infections caused by marine bacterial pathogens: epidemiology, diagnosis, and management. Curr Infect Dis Rep 2011;13:470–7.

[9] Pagani L, Lang A, Vedovelli C, Molino O, Rimenti G, Pristeran R, et al. Soft tissue infection and bacteremia caused by *Shewanella putrefaciens*. J Clin Microbiol 2003;41:2240–1.

[10] Vignier N, Barreau M, Olive C, Baubion E, Théodose R, Hochedez P, et al. Human infection with *Shewanella putrefaciens* and *S. algae*: report of 16 cases in Martinique and review of the literature. Am J Trop Med Hyg 2013;89:151–6.

[11] Gram L, Bundvad A, Melchiorsen J, Johansen C, Fonnesbech VB. Occurrence of *Shewanella algae* in Danish coastal water and effects of water temperature and culture conditions on its survival. Appl Environ Microbiol 1999;65:3896–900.

[12] Holt HM, Søgaard P, Gahrn-Hansen B. Ear infections with *Shewanella alga*: a bacteriologic, clinical and epidemiologic study of 67 cases. Clin Microbiol Infect 1997;3:329–34.

[13] Nozue H, Hayashi T, Hashimoto Y, Ezaki T, Hamasaki K, Ohwada K, et al. Isolation and characterization of *Shewanella alga* from human clinical specimens and emendation of the description of *S. alga* Simidu et al., 1990, 335. Int J Syst Bacteriol 1992;42:628–34.

[14] Khashe S, Janda JM. Biochemical and pathogenic properties of *Shewanella alga* and *Shewanella putrefaciens*. J Clin Microbiol 1998;36:783–7.

[15] Vogel BF, Jorgensen K, Christensen H, Olsen JE, Gram L. Differentiation of *Shewanella putrefaciens* and *Shewanella alga* on the basis of whole-cell protein profiles, ribotyping, phenotypic characterization, and 16S rRNA gene sequence analysis. Appl Environ Microbiol 1997;63:2189–99.

[16] Sharma KK, Kalawat U. Emerging infections: *Shewanella*—a series of five cases. J Lab Physicians 2010;2:61–5.

[17] Heller HM, Tortora G, Burger H. *Pseudomonas putrefaciens* bacteremia associated with shellfish contact. Am J Med 1990;88:85–6.

[18] Rosenthal SL, Zuger JH, Apollo E. Respiratory colonization with *Pseudomonas putrefaciens* after near-drowning in salt water. Am J Clin Pathol 1975;64:382–4.

[19] Leong J, Mirkazemi M, Kimble F. *Shewanella putrefaciens* hand infection. Aust N Z J Surg 2000;70:816–7.

[20] Bulut C, Ertem GT, Gökcek C, Tulek N, Bayar MA, Karakoc E. A rare cause of wound infection: *Shewanella putrefaciens*. Scand J Infect Dis 2004;36:692–4.

[21] Chen YS, Liu YC, Yen MY, Wang JH, Wann SR, Cheng DL. Skin and soft-tissue manifestations of *Shewanella putrefaciens* infection. Clin Infect Dis 1997;25:225–9.

[22] Dominguez H, Vogel BF, Gram L, Hoffmann S, Schaebel S. *Shewanella alga* bacteremia in two patients with lower leg ulcers. Clin Infect Dis 1996;22:1036–9.

[23] Di Renzo L, Di Francesco G, Profico C, Di Francesco CE, Ferri N, Averaimo D, et al. *Vibrio parahaemolyticus*– and *V. alginolyticus*–associated meningo-encephalitis in a bottlenose dolphin (*Tursiops truncatus*) from the Adriatic coast of Italy. Res Vet Sci 2017;115:363–5.

[24] Goyal R, Kaur N, Thakur R. Human soft tissue infection by the emerging pathogen *Shewanella algae*. J Infect Dev Ctries 2011;5:310–2.

[25] Tang TH, Cheng NH, Ho RT, Chan HS, Lam KW, Xavier J, et al. *Shewanella*-related bacteremia and Fournier's gangrene: a case report. Open Forum Infect Dis 2016;3:ofw148.

[26] Hau HH, Gralnick JA. Ecology and biotechnology of the genus *Shewanella*. Annu Rev Microbiol 2007;61:237–58.

[27] Brink AJ, Van Straten A, Van Rensburg AJ. *Shewanella (Pseudomonas) putrefaciens* bacteriemia. Clin Infec Dis 1995;20:1327–32.

[28] To KK, Wong SS, Cheng VC, Tang BS, Li IW, Chan JF, et al. Epidemiology and clinical features of *Shewanella* infection over an eight-year period. Scand J Infect Dis 2010;42:757–62.

[29] Otsuka T, Noda T, Noguchi A, Nakamura H, Ibaraki K, Yamaoka K. *Shewanella* infection in decompensated liver disease: a septic case. J Clin Gastroenterol 2007;42:87–90.

[30] Tsai MS, You HL, Tang YF, Liu JW. *Shewanella* soft tissue infection: case report and literature review. Int J Infect Dis 2008;12:e119–24.

[31] Jorens PG, Goovaerts K, Ieven M. *Shewanella putrefaciens* isolated in a case of ventilator-associated pneumonia. Respiration 2004;71: 199–201.

[32] Kim DM, Kang CL, Lee CS, Kim HB, Kim EC, Kim NJ, et al. Treatment failure due to emergence of resistence to carbapenem during therapy for *Shewanella algae* bacteremia. J Clin Microbiol 2006;44:1172–4.