

## Cellulitis due to *Shewanella algae*: Crucial diagnostic clues from basic microbiological tests

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Received 22 February 2019; received in revised form 6 August 2019; accepted 8 August 2019

**Abstract.** *Shewanella* spp. are infrequently implicated in human infections but they are emerging pathogens with particular significance in regions with warm climates, such as Southeast Asia. This is a case of a middle-aged diabetic and hypertensive man who presented with worsening congestive heart failure symptoms associated with fever and a painful right leg. His right leg had numerous scabs and was tender, warm and erythematous. He was provisionally diagnosed with decompensated heart failure precipitated by cellulitis and uncontrolled hypertension. His blood grew non-fermentative, oxidase-positive and motile gram-negative bacilli which produced hydrogen sulfide on triple sugar iron agar. When cultured on blood agar, mucoid and weakly  $\beta$ -haemolytic colonies were observed after 48 hours. API 20 NE named the isolate as *Shewanella putrefaciens* but 16S rRNA sequence analysis identified the organism as *Shewanella algae*. The patient was treated with a 10-day course of ceftazidime, which resulted in the resolution of the cellulitis.

### INTRODUCTION

*Shewanella* spp. have a worldwide distribution and they reside naturally in all forms of water (particularly seawater) and soil (Vignier *et al.*, 2013). In recent years, more and more cases of *Shewanella* infections have been reported, essentially making *Shewanella* spp. emerging pathogens (Torri *et al.*, 2018). Although *Shewanella* is a relatively “modern” genus, having been first proposed in the 1980s, some of the bacteria currently assigned to this genus have actually been recognized for more than eight decades (Janda & Abbott, 2014). Over the years, the genus has undergone numerous name changes, from *Achromobacter* to *Pseudomonas* to *Alteromonas*, before finally being designated as *Shewanella* (Janda & Abbott, 2014). Even though more than 50 species of *Shewanella* have been described,

the only species that have been isolated from clinical specimens are *S. putrefaciens* and *S. algae* (Vignier *et al.*, 2013; Janda & Abbott, 2014). Most *Shewanella* infections in humans have been reported from countries with warm climates or from temperate countries during particularly warm summers (Finkelstein & Oren, 2011). Thus, it is imperative for microbiologists and physicians practicing in tropical countries such as Malaysia to be aware of this genus.

### CASE REPORT

A 52-year-old Chinese gentleman with long-standing hypertension and diabetes mellitus presented to Hospital Canselor Tuanku Muhriz with a 7-day history of bilateral lower limb swelling which was painful and more pronounced on the right. Fever with chills and

rigors were also noted a day prior to his presentation to the hospital. He also complained of worsening orthopnoea and paroxysmal nocturnal dyspnoea during the past week. On examination, the patient was alert and conscious but tachypnoeic (respiratory rate: 23 breaths/min), hypertensive (blood pressure: 198/116 mm Hg), tachycardic (pulse rate: 118 beats/min) and febrile (temperature: 38.5°C). Pitting oedema was noted up till the upper shins and more severe on the right leg. The right leg had numerous scabs indicating healed/healing skin lesions and was also noticeably tender, warm and erythematous (Figure 1). Auscultation of the lungs revealed bibasal crepitations and poor inspiratory efforts. Other systemic examinations were unremarkable.

A full blood count investigation revealed a high white cell count ( $15.4 \times 10^9/L$ ) with a predominance of neutrophils. A chest X-ray showed cardiomegaly with bilateral lower zone haziness. There was no evidence of deep vein thrombosis from Doppler ultrasound scans of both lower limbs. A blood

specimen was sent to the microbiology laboratory for bacteriological culture. Based on the clinical manifestations and initial laboratory investigation results, the patient was provisionally diagnosed with decompensated heart failure precipitated by cellulitis and uncontrolled hypertension. He was promptly started on intravenous ampicillin-sulbactam 1.2 g q8h, oral metformin 1 g bd, oral amlodipine 10 mg od, oral perindopril 8 mg od and subcutaneous isophane insulin 16 units at night.

On the second day of incubation, the aerobic BACTEC vial grew gram-negative bacilli which formed mucoid and weakly  $\beta$ -haemolytic colonies on sheep blood agar, which became more apparent after 48 hours of incubation at 35°C (Figure 2). The bacteria were also able to grow on sheep blood agar incubated at 42°C. Other notable characteristics of the bacteria were oxidase-positivity, umbrella-like motility in semi-solid agar, mucoid non-lactose fermenting colonies on MacConkey agar and an alkaline/alkaline reaction with hydrogen sulfide ( $H_2S$ ) production on triple sugar iron (TSI) agar



Figure 1. Bilateral lower limb swelling (more pronounced on the right leg), with redness and scabs on the right leg.

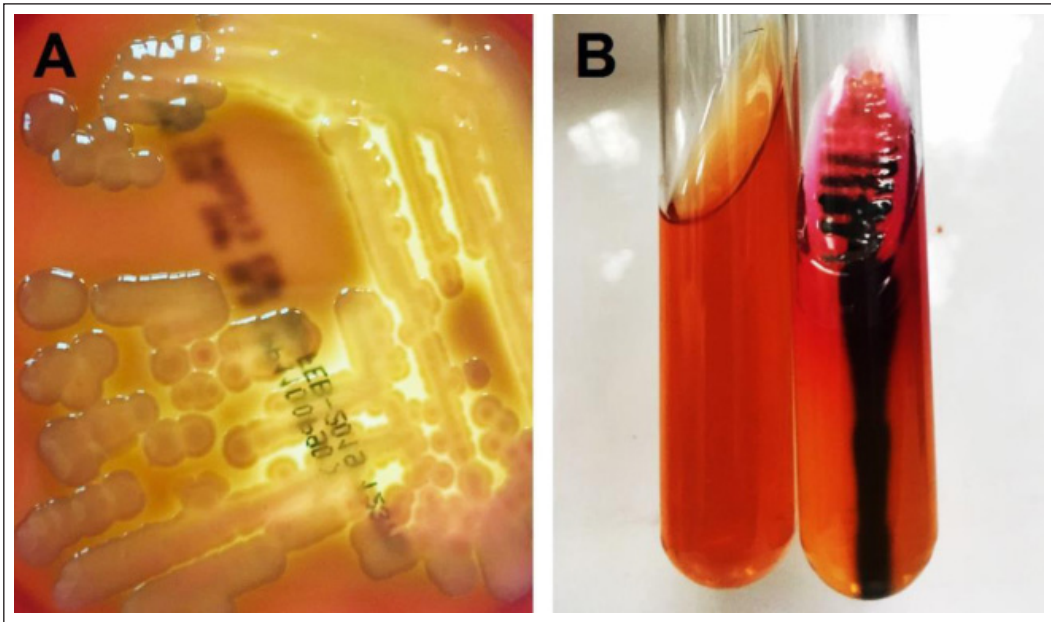


Figure 2. A: The bacteria formed mucoid and weakly  $\beta$ -haemolytic colonies on sheep blood agar after 48 hours of incubation. B: The triple sugar iron agar slant on the right has been inoculated with the bacteria and an alkaline/alkaline reaction with hydrogen sulfide production was observed after overnight incubation; an uninoculated agar slant is shown on the left for comparison.

(Figure 2). Biochemical identification using API 20 NE (bioMérieux, France) was attempted and the organism was identified as *Shewanella putrefaciens* group (numerical profile: 040200441, %ID: 99.3). However, when 16S rRNA sequencing was performed, the bacterial isolate showed 96% homology to *Shewanella algae* strain CCU101 (GenBank accession no. CP018456.1).

Antibiotic susceptibility testing was performed using Etest (AB Biodisk, Sweden) strips and the minimal inhibitory concentration (MIC) values for ceftazidime, cefepime and gentamicin were 0.5 mg/mL, 0.032 mg/mL and 0.75 mg/mL, respectively. When interpreted according to the Clinical & Laboratory Standards Institute (CLSI) breakpoints for “other non-Enterobacteriaceae”, the organism was susceptible to all three antibiotics tested (CLSI, 2017). Based on the blood culture and antibiotic susceptibility results, the managing team changed the antibiotic to intravenous ceftazidime 1 g q8h for 10 days, which resulted in the resolution of the cellulitis.

## DISCUSSION

Most of the earlier reports in the published literature on *Shewanella* infections name *S. putrefaciens* as the causative agent of human infections but this could be due to misidentification because it is likely that more than 80% of these infections were actually caused by *S. algae* (Holt *et al.*, 2005). The most common clinical manifestation described in the published literature is infection of skin and soft tissue, with breaches in the skin (due to trauma or ulcers) and exposure to seawater being notable risk factors (Finkelstein & Oren, 2011; Torri *et al.*, 2018). Our patient presented with cellulitis (a type of skin and soft tissue infection) and had scabs on his right lower limb (indicating skin breaches) which could have been the portal of entry for *S. algae*. Although he had no recent history of exposure to a marine environment, such a history need not necessarily be present (Janda & Abbott, 2014). Knowing the correct species is not merely of academic interest, because of the higher pathogenic potential believed to be

associated with *S. algae* (Khashe & Janda, 1998; Holt *et al.*, 2005). Thus, clinicians should treat *S. algae* infections more aggressively.

Fortunately, clues which can potentially identify the genus as well as differentiate the two clinically important species can be unearthed easily with basic microbiological tests. Although gram-negative bacteria which produce H<sub>2</sub>S are not uncommon, these bacteria are typically fermentative, oxidase-negative and belong to the Enterobacteriaceae family (e.g. *Salmonella* spp. and *Proteus* spp.). *Shewanella* spp. are the only non-fermentative and oxidase-positive gram-negative bacilli known to produce H<sub>2</sub>S (Sharma & Kalawat, 2010). Next, examining colony characteristics on blood agar will provide additional clues to aid speciation, with mucoid and β-haemolytic colonies (especially following 48 hours of incubation) being characteristic for *S. algae* (Holt *et al.*, 2005). In fact, this haemolytic ability of *S. algae* has been postulated to be one of the reasons *S. algae* is considered more virulent than *S. putrefaciens* (Khashe & Janda, 1998).

Relying on commercial biochemical bacterial identification systems to positively identify *S. algae* may result in the isolate being named as *S. putrefaciens* instead. This is because the databases for common commercial identification kits/systems (e.g. API 20 E, API 20 NE and Vitek) contain *S. putrefaciens* but not *S. algae* (Holt *et al.*, 2005). Accurate bacteriological identification can therefore be achieved through molecular typing such as 16S rRNA sequencing, which we performed on our isolate (Vignier *et al.*, 2013). However, microbiologists with limited facilities or resources need not resort to molecular testing if they are able to correlate the basic laboratory test findings mentioned earlier with any *S. putrefaciens* result generated by a commercial biochemical identification kit or system. The ability to grow at 42°C (which we also demonstrated) and in NaCl 6% w/v are additional laboratory findings suggestive of *S. algae* (Holt *et al.*, 2005).

More than 90% of *Shewanella* isolates are susceptible to carbapenems, erythromycin,

aminoglycosides and ciprofloxacin but resistant to penicillin (Vignier *et al.*, 2013). Cephalosporin susceptibility is variable, with more isolates being susceptible to 3<sup>rd</sup> and 4<sup>th</sup> generation agents than to the earlier generation agents (Holt *et al.*, 2005). Our own *S. algae* isolate was susceptible to ceftazidime and cefepime, which are 3<sup>rd</sup> and 4<sup>th</sup> generation cephalosporins, respectively. Although our patient was initially treated with ampicillin-sulbactam, we did not perform *in-vitro* susceptibility testing for this antibiotic due to the lack of breakpoints (CLSI, 2017). However, it is likely that the susceptibility to ampicillin-sulbactam may be poor, judging from reports that < 50% of *S. algae* isolates have been found to be susceptible to co-amoxycylav, a closely related β-lactam/β-lactamase inhibitor combination (Torri *et al.*, 2018). We also did not perform susceptibility testing for erythromycin, ciprofloxacin and carbapenems because these drugs either had no published breakpoints or were classified as “Group B” agents (i.e. agents which should be tested and reported selectively) for isolates categorized as “other non-Enterobacteriaceae” (CLSI, 2017).

## CONCLUSION

A high index of suspicion is essential to detect *S. algae*. Non-fermentative and oxidase-positive gram-negative bacilli should not be routinely reported as “*Pseudomonas* species” especially when isolated from patients with soft tissue infections. This practice may result in such patients being preferentially treated with an anti-pseudomonal antibiotic such as ceftazidime, when the susceptibility of *S. algae* towards cephalosporins is uncertain without formal antibiotic susceptibility testing. By correlating basic laboratory test findings (e.g. motility, oxidase reaction, TSI reaction and colony characteristics) with any *S. putrefaciens* result generated by a commercial biochemical identification kit/system, district or small microbiology laboratories should be able to confidently identify *S. algae*.

*Acknowledgements.* We are grateful to the Dean of the Faculty of Medicine, Universiti Kebangsaan Malaysia for her permission to publish this article. We would also like to thank Dr. Le Jie Lee from Prima Nexus Sdn. Bhd. for his technical assistance.

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