

Original Research Article

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Emerging Pathogen: *Shewanella* Algae causing Burn Wound Infection - Report of Two Cases from a Tertiary Care Center

Pratibha Shamanna*, Jayashree Ravindran, Jacinta Lalremruati and Muralidaran Sethumadavan

Department of Microbiology, St John's Medical College, Bangalore, Karnataka, India

*Corresponding author

ABSTRACT

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Shewanella algae infections are rare in humans. Repeated isolation of the same organism from two different patients suffering from burn wound infection and successful treatment thereafter confirms the need of utmost microbiological vigilance to identify the unusual pathogen.

Introduction

Shewanella species are found throughout the world in marine environments, and most reported human infections occur in countries in warm climates (Holt *et al.*, 2005). *Shewanella* species have been implicated in skin and soft tissue infections, bacteremia, biliary tract infections, thoracic empyema, endocarditis, dacryocystitis, intracranial abscess, arthritis, peritonitis, ventilator associated pneumonia, and ear infections (Tsai *et al.*, 2008; Tan *et al.*, 2008) *Shewanella* species is a saprophytic gram negative rod, belonging to family Vibrionaceae. It is frequently isolated from nonhuman sources and is rarely considered pathogenic in humans. *Shewanella* species are widely

distributed in nature, with soil and water being their natural habitat. Initially they were considered to be colonisers or saprophytes thriving on previously damaged tissue (Winn *et al.*, 2006).

Shewanella algae and *Shewanella* putrefaciens are the two important species of *Shewanella* that are most frequently implicated in human infections, although more than 80% are caused by *S. algae* (Tsai *et al.*, 2008). *S. algae* was introduced as a new species in 1990 and has been most frequently associated with infections of skin and soft tissues resulting from breaches in dermis, such as ulcers or following trauma (Tsai *et al.*, 2008; Simidu *et al.*, 1990). Although *S. algae* has been reported to be the etiological agent of distinct

human infections it is difficult to give clinical significance to the isolation of this micro-organism as it may be present as a coloniser or a component of mixed bacterial flora (Renu Goyal *et al.*, 2011). Here we present two case series in burns patients who were infected by *S. algae* during admission to burns ward at the same period of time. In the present study *S. algae* was repeatedly isolated from same patients and the patients responded to the targeted treatment supporting that *S. algae* was the true pathogen.

Materials and Methods

Wound swabs taken from raw burnt area sent to microbiology lab, were cultured on 5% Sheep Blood agar, MacConkey agar and liquid Thioglycollate medium. Simultaneously Gram's stain of the smear was made from wound swab to look for the presence of bacteria and tissue cells. The culture plates and thioglycollate broth after inoculation were incubated at 37°C for 18-24 hours.

On Blood agar grey lytic colonies with red tan pigment was noticed after 24 hours of incubation and on MacConkey agar, non-lactose fermenting colonies were seen. Thioglycollate broth showed uniform turbidity. Bacterial colony from BA and MA were tested for oxidase production by strips impregnated with 1% tetra methyl paraphenylenediamine dihydrochloride. The colonies were oxidase positive. Oxidase positive colonies were further identified by grain stain, motility on mannitol motility (MM) medium. Urease on Christensen's agar, Citrate utilization by Cimmon's citrate agar, H₂S production and differentiation between fermenter and non-fermenter on Triple sugar iron agar slant (TSI) and for Indole production. The organism was identified as *Shewanella* species since this is the only non-fermenter which produces H₂S gas in TSI agar and is also oxidase positive (Winn *et al.*, 2006). *Shewanella* species were further

identified as *S. algae* based on Nitrate reduction, metabolism of maltose, mannitol, sucrose, arabinose, ribose, decarboxylation of Amino acids, arginine, lysine and ornithine, hemolysis in 5% sheep blood agar, growth on Salmonella-Shigella agar (SS), growth at 42°C, growth in presence of 6.5% NaCl, gelatin hydrolysis, DNAase production. Antibiotic sensitivity testing was done using Kirby-bauer disc diffusion method following CLSI guidelines with Ampicillin, Amikacin, Gentamicin, Netilmicin, Cephalexin, Cefotaxime, Ceftazidime, Ciprofloxacin, Cefoperazone + Sulbactam, Piperacillin + Tazobactam, Meropenem, Piperacillin, Imipenem, Ertapenem and zone of inhibition was measured and reported as sensitive or resistant (NCCLS, 2003). In both of cases the isolates were identified as *Shewanella* algae based on the ability to produce mucoid colonies with Beta-hemolysis on 5% sheep blood agar, growth at 42°C and in 6% NaCl, inability to produce acid from maltose and ability to reduce nitrite (Rajshree Patel *et al.*, 2012).

Case-1

22yr old male patient, cook by occupation with history of accidental thermal burns sustained 3 months back at his residence was admitted to Victoria hospital and later referred to St John's Hospital for further treatment and follow up, came with raw area involving face, neck, chest.

On local examination: Post burns raw area with slough, granulation tissue along with discharge was noticed involving mainly anterior chest, upper abdomen, right posterior chest, scalp area and bilateral medial aspect of forearm. There was restriction in lateral shoulder abduction and neck movements. Also grade III contracture of ear was observed.

Investigations revealed Hb-10.6%, PCV-33.20, S. Creatinine 0.1, B. Urea 181,

Na/K/CL-35/4.6/100, and HIV and HBsAg status – Non reactive.

Wound swab taken from discharge site for culture and sensitivity in Gram's stain-showed numerous pus cells, numerous gram positive cocci in singles, pairs, clusters, numerous gram negative bacilli. Wound swab culture showed growth of *Shewanella* algae and *Staphylococcus aureus*.

Shewanella algae were sensitive to Piperacillin + Tazobactam, Piperacillin, Cefoperazone + Salbactam, Gentamycin, *Meropenem*, intermediate to Amikacin. *Staphylococcus aureus* was sensitive to Netilmicin, Amikacin, Tetracycline, Chloramphenicol, Erythromycin, Vancomycin, Teicoplanin. Repeat sample sent also grew *Shewanella* algae with same sensitivity pattern. A provisional diagnosis 22% burnswith *S. algae* infection was made. The patient underwent Split skin grafting (SSG) in anterior chest and bilateral arm and SSG on the back. Post operatively graft was healthy and patient advised for discharge and regular follow up.

Case-2

An 8yr old female child came with alleged history of flame burns, child clothes caught fire while playing near campfire.

On local examination: 2° deep to 3° burns over the right thigh anterior aspect, left anterior thigh, lateral aspect and left lower anterior trunk.

Investigations revealed: HB-9.7%, S.Creat-0.6%, Na⁺ 128, K-3.6, CL-96, HIV, HBsAg-Non -reactive. Wound swab taken from discharge site on Gram's stain-showed scanty pus cells, scanty gram negative bacilli. Wound swab culture grew *Shewanella* algae and *Klebsiella* species. *Shewanella* algae was sensitive to Piperacillin + Tazobactam, Piperacillin, Cefoperazone + Salbactam,

Meropenem and *Klebsiella* species was resistant to all gram negative drugs. A provisional diagnosis of 15% thermal burns with infection was made. Patient was taken for 6% epifacial excision of left thigh 9% total excision over abdomen with homograft and right thigh with autograft done. After a week homograft over left thigh was replaced with autograft. Post operatively graft was taken up well and child was advised discharge with regular follow up.

Results and Discussion

Although *Pseudomonas aeruginosa* is the most common oxidase positive non fermenter isolated from clinical specimens, *Shewanella* species has recently attracted attention of clinical microbiologists. Recognition of this rare organism has been hampered as the isolation of *Shewanella* species from clinical samples has long been considered merely colonization rather than active infective agent (Mukhopadhyay *et al.*, 2007). The organism generally resides in marine and aquatic environment so the route of transmission in these cases is not clear. There have been only four reports of isolation of *Shewanella* from India and these were from patients with infective endocarditis, peritonitis, chronic obstructive pulmonary disease respectively (Mukhopadhyay *et al.*, 2007; Dhawan *et al.*, 1998). *Shewanella* algae and *Shewanella putrefaciens* are the two species of *Shewanella* that are most frequently implicated in human infections. Among *Shewanella* species, *S. algae* causes 80% human infections (Khashe and Micheal, 1998).

Important differential characteristics between the two species include the ability of *S. algae* to produce mucoid colonies with β -hemolysis on 5% sheep blood agar, to grow at 42°C and in 6% NaCl and to reduce nitrate, and an inability to produce acid from maltose all of which are in contrast to the characteristics of *Shewanella putrefaciens* (Fig. 1–4).

Fig.1 Blood agar showing lytic colonies with red tan pigment



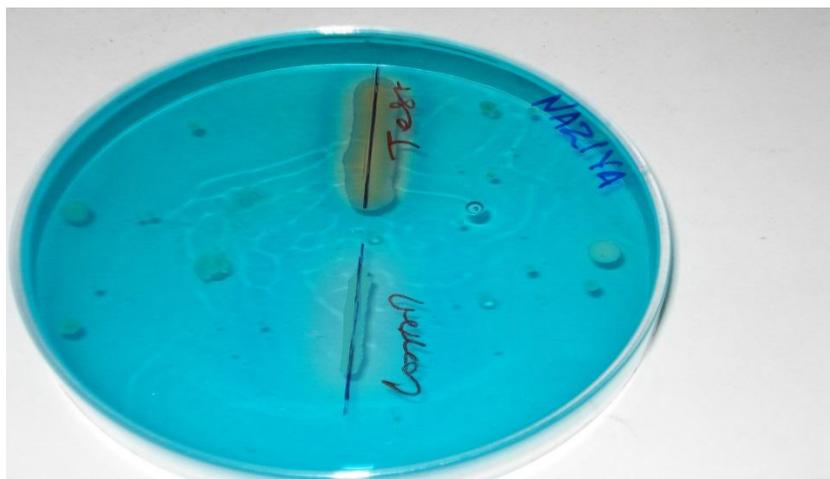
Fig.2 MacConkey's agar showing non-lactose fermenting colonies



Fig.3 Biochemical properties of *Shewanella* algae. a) Showing OF (oxidative fermentative media with 1% glucose), MM (mannitol motility media), TSI (triple sugar iron media), Indole, Citrate, Urease. b) Ornithine decarboxylase test with control



Fig.4 DNase test with control



Automated identification systems fail to differentiate between *Shewanella* algae and *Shewanella putrefaciens*, as *S. algae* is not included in the databases of these systems. Presumably for this reason most *Shewanella* infections reported during recent years have been attributed to *Shewanella putrefaciens*.

However in studies where extensive phenotypic characterisation is performed most human infections are caused by *Shewanella* algae. As the two species have different pathogenic potential for humans, correct identification is important, and this is possible only in routine clinical microbiology laboratories (Holt *et al.*, 2003).

In present study also *Shewanella* algae was isolated in both the burn wound cases, as most common infection due to *Shewanella* species are infections of skin and soft tissue and are usually associated with breaches in the skin such as ulcers or following trauma like in other studies.

There are no standard guidelines for treatment of *Shewanella* infection. In both of our cases the organism was sensitive to Piperacillin, Piperacillin + Tazobactam, Meropenem and Gentamicin, intermediate sensitive to Amikacin and resistant to other gram negative antibiotics. Previously published studies reported that

Shewanella algae are susceptible to aminoglycosides, carbapenems, erythromycin, and quinolones with variable susceptibility to penicillin and cephalosporin. However rapid development of resistant to Imipenem and Piperacillin + Tazobactam has been reported (Ostwal *et al.*, 2015).

Though burn wound infections due to *Shewanella* algae have been reported from several countries, such reports from India are rare. The main reason for this is that in most of the hospitals, all gram negative and oxidase positive organisms are reported as *Pseudomonas* species and no further identification is done.

To conclude the isolation of *Shewanella* algae from above two cases strengthens the assumption that the organism should be regarded as a potential emerging pathogen. Utmost microbiological vigilance to diagnose the unusual pathogen will facilitate the administration of appropriate treatment at the earliest thereby getting satisfactory clinical response and prevention of further complications.

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