Original Article

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Microbiology Section

Skin and Soft Tissue Infections due to *Shewanella algae* – An Emerging Pathogen

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ABSTRACT

Introduction: *Shewanella* spp. are emerging human pathogens, the predominant species being *Shewanella* ***algae.*** *Shewanella* skin and soft tissue infections are more commonly seen in immunocompromised patients with a pre-existing cutaneous ulcer and most often associated with exposure to marine environments.

Aim: The study was conducted to investigate the epidemiological and clinical characteristics of *Shewanella* skin and soft tissue infections (SSTIs) for a period of five years.

Materials and Methods: All Gram-negative non-fermenting motile isolates which produced pigmented colonies and positive for oxidase and H_2S were further identified with Vitek 2 system.

Results: A total of 16 patients with SSTIs due to *Shewanella* species were identified during the period from 2010 to 2014. Majority of patients were urban, elderly and fisher men. *Shewanella* ***algae*** (n=12, 75%) was the predominant isolate. Skin or mucosal portal of entry was found in all patients and seawater contact was recorded in 56.25% of the patients. 81% of infections were polymicrobial, common concomitant pathogens being gut and marine flora. Peripheral vascular diseases were the predominant risk factors with comorbidities like diabetes, hypertension and hepatobiliary diseases. Third generation cephalosporins, meropenem and gentamicin were the most effective antibiotics while two of the isolates were multidrug resistant. 75% of the infected patients recovered completely and three patients died of complications.

Conclusion: *Shewanella* ***algae*** should be considered as an emerging pathogen of SSTIs mainly in patients with chronic ulcers and at times be multidrug resistant. These infections have a good clinical outcome if prompt medical, surgical and supportive treatment is offered.

Keywords: Comorbidities, Infections, Marine flora, Non-fermenters, Polymicrobial, *Shewanella* species

INTRODUCTION

Shewanella are saprophytic, motile, Gram-negative bacilli, widely distributed in nature, they are associated with aquatic and marine habitats [1]. In 1985, MacDonell and Colwell [1] categorised the genus *Shewanella* and its three species – *S. putrefaciens, S.hanedai* and *S.benthica*. Additionally *Shewanella putrefaciens* (*S. putrefaciens*) is organized into four groups genotypically and strains in group IV are to be recognized as *Shewanella algae* (*S.algae*) by some authors [2]. Eventually majority of the isolates of *S. putrefaciens* were grouped into *S.algae*.

Khashe and Janda [3] reported that *S.algae* is the predominant human clinical isolate (77%) whereas *S.putrefaciens* represents the majority of non-human isolates (89%). Both were initially considered to be colonisers thriving on previously damaged tissue [1], however reports of their clinical significance is emerging. *Shewanella* have been isolated from wounds, urine, faeces, CSF, bile and various other clinical samples [1].

Risk factors and comorbidities associated with *Shewanella* infections have been identified as chronic leg ulcer, peripheral vascular occlusive disease (PVOD), diabetes, chronic liver and kidney diseases. They have been implicated in skin and soft tissue infections (SSTI), ear infection, eye infection, infective arthritis, osteomyelitis, bacteraemia, infective endocarditis and peritonitis [1].

Since data on *Shewanella* SSTIs is scarce in South India, the present study is carried out with an objective to explore the epidemiology, risk factors and clinical features of *Shewanella* SSTIs and to determine the antibiotic resistance potential of these bacteria. This analysis helps in guiding appropriate selection of antibiotic therapy and prevention of these emerging pathogens.

MATERIALS AND METHODS

This prospective study was carried out in surgical department of Amrita Institute of Medical Sciences (AIMS), a tertiary health care centre cum medical college in Kochi, Kerala from 2010 to 2014 on 16 culture positive patients having *Shewanella* SSTIs.

Inclusion criteria

All patients with clinical findings suggestive of SSTIs like ulcers, cellulitis, and abscesses with or without complications were included. Both acute and chronic infections were studied.

Study tools

Relevant information about demographics, clinical features, treatment, and risk factors were recorded using standard proforma. The study was carried out after getting general informed consent and approval by institutional ethical committee.

Laboratory procedures

All samples were processed by standard clinical laboratory conditions [1]. Samples were subjected to Gram's stain and cultured on blood agar and MacConkey agar. Then orange pigmented non-lactose fermenting colonies were tested for motility, oxidase and H_2S production. Motile, oxidase and H_2S positive Gram-negative non-fermenters were presumptively identified as *Shewanella* spp. The genus *Shewanella* was further differentiated into *S. algae* and *S. putrefaciens* based on growth characteristics. *S.algae* is positive for growth in the presence of 6.5% NaCl, growth at 42°C and haemolysis on BA [3,4]. All the isolates were confirmed using Vitek 2 compact system (Biomerieux, France), where the isolates' identification was confirmed only when probabilities of identifications were ≥ 92%.

The minimum inhibitory concentration (MIC) values were determined for commonly used antibiotics like ampicillin/sulbactam, piperacillin, piperacillin/tazobactam, ceftazidime, ceftriaxone, cefepime, cipro-

floxacin, levofloxacin, carbapenems, gentamicin, and trimethoprim-sulfamethoxazole by Broth microdilution method using Vitek 2 compact system. Later the results were confirmed by disk diffusion method on Mueller-Hinton agar as per Clinical and Laboratory Standards Institute (CLSI) guidelines. There are no recognized CLSI MIC interpretive standards specified for this bacterial genus and the MIC breakpoints of ampicillin/sulbactam for non-fermentative Gram-negative bacilli are also lacking in the CLSI database. Hence MIC values for above antibiotics were interpreted using CLSI approved standard M100-S23 [5] categories for other Non-Enterobacteriaceae in accordance with previous studies [6,7] and as per the standards recommended by US Food and Drug Administration for ampicillin/sulbactam [8].

RESULTS

The epidemiological and clinical characteristics of infected patients were summarized in [Table/Fig-1].

Epidemiological findings

The mean age of the study population was 54.5 years with slight preponderance for males. We found 56.25% (n=9) of the infected patients hailed from urban areas. Occupational analysis revealed high incidence among fishermen (n=6, 37.5%).

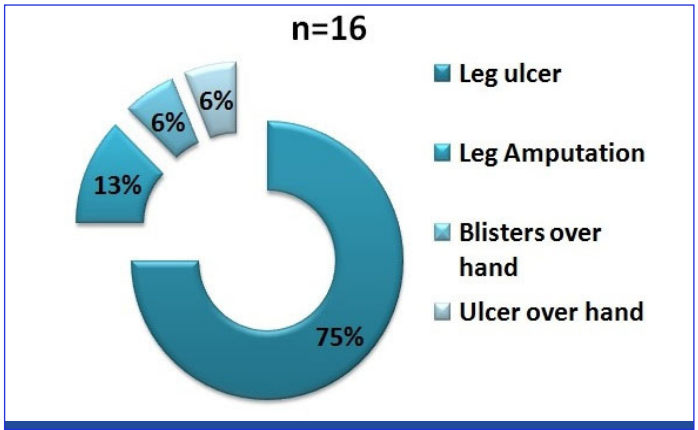
The probable modes of infection found in this study were mucocutaneous abrasions or penetrating trauma with and without history of sea water exposure [Table/Fig-2]. [Table/Fig-3] revealed significant increase in *Shewanella* SSTIs over 5-year period and [Table/Fig-4] showed soft tissue infections occurring more frequently in summer and monsoon.

Microbiological findings

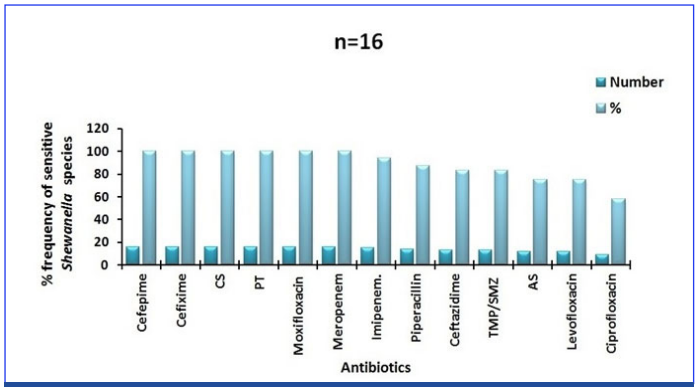
Shewanella species were isolated from 18 clinical specimens from 16 patients. In two patients, it was isolated twice from two different samples. None of these patients had grown *Shewanella* from blood. All sixteen isolates were considered as pathogens. *S.algae* (n=12,

Patient Sl.No with age, sex, residence & occupation.	Site of micro-bial isolati-on	Micro-organisms isolated	Type of SSTI & Organ effected	Risk factor / Cause of infection	Comorbid conditions	Treatment offered	Clinical outcome.
Case 1, 67Y, M, R, Farmer	Pus	*S.algae, P. aeruginosa, MRSA*	Superinfection of CNHU, L. Leg	PVOD	HTN	Linezolid plus Gentamycin@ Wound dressing*	Cured
Case 2, 62, M, R, Fisherman	Bone Tissue	*S.algae, P. vulgaris, E.coli*	L. 3rd & 4th toe gangrene & cellulitis	PN & PVOD	DM, HTN, DLP	Amputation	Died (cardiac arrest)
Case 3, 63Y, M, R, Fisherman	Deep Tissue	*S.algae, MRSA, P.aeruginosa*	Superinfection of ulcer of Rt. toe amputated stump	PN PVOD	DM, HTN, DLP, CRF	Cefepime, 2g IV q12h x10 days Wound dressing*	Cured
Case 4, 50Y, M, R, Fisherman	Deep Tissue	*S.algae, P.aeruginosa, E.coli*	Infection of Pyoderma gangrenosum, Rt Leg	PVOD	DM,HTN, DLP	Meropenem 1gm IV q 8h x 14 days	Cured
Case 5,62Y, F U, Housewife	Deep Tissue	*S.algae,*	Blisters, cellulitis dorsum of Rt. hand	Deep burns	DM	Piperacillin/tazobactam 4.5 g IV q8h x 10 days ; Regular dressing	Cured
Case 6, 64Y, M, U, Labourer	Pus	*S.algae, E.coli*	Cellulitis, L. Leg	PN	DM, CLD, Neutropenia	Meropenem 500 mg q8h & clindamycin 600 mg q8h IV x 10 days	Died due to sepsis
Case 7, 52Y, M,U, Bakery owner	Pus	*S.putrefaciens A.caviae*	Necrotizing fasciitis, L.Foot	PN	DM,CLD, HTN, DLP	Surgical debridement, Skin grafting. Moxifloxacin 400mg & Clindamycin 300mg q24h PO x 10 days each	Cured
Case 8, 57Y, F,U, Housewife	Bone & Tissue	*S.algae, P.vulgaris P.mirabilis*	Superinfection of L.great toe ulcer	PVOD	HTN	Moxifloxacin 400mg q24h PO x 14 days plus Clindamycin 600mg q8h PO x 14 days	Amputation
Case 9, 40Y, M, U, Conductor	Pus	*Shewanella sp P. vulgaris, MRSA*	Superinfection of Ulcer, Rt.Leg	PVOD	HTN	Linezolid 600 mg q12h PO x 4 days plus Meropenem 1gm IV q8h x 14 days	Cured
Case 10, 30Y, M, U, Student	Pus	*Shewanella sp K. pneumonia, E.coli*	Wound infection, L. Leg	PT (RTA)	NC	Levofloxacin 750mg q24hr PO x 14 days plus Linezolid 300mg q12h PO x 6 days	Cured
Case 11, 57Y, M, R, Fisherman	Tissue	*S.putrefaciens A. hydrophila, P.aeruginosa*	Wound infection, Rt. Hand	PT (RTA), PVOD	DM HTN	Levofloxacin 750mg PO q24hr x 14 days	Cured
Case 12, 62Y, F, U, housewife	Pus	*S.algae*	Wound infection, Rt. Leg	PVOD	HTN, DM	Cefepime 2g IV q12h x10 days Regular wound dressing*	Cured
Case 13, 67Y,M, R, Fisherman	Tissue	*S.algae E.coli, P.mirabilis*	Superinfection of NHU, chin of the Rt. tibia	PVOD,	HTN	Ampicillin- Cloxacillin 500 mg q8hr PO x 5days	Died due to Sepsis
Case 14, 28Y,M, U, Student	Tissue	*S.algae*	Superinfection of ulcer, dorsum of Rt. foot	PT (RTA)	NC	Ampicillin- Cloxacillin 500 mg PO q8h x5days	cured
Case 15, 52Y, M, U, Fisherman	Pus	*Case 15, 52Y, M, U, Fisherman*	Superinfection of Ulcer, Rt. leg	PN	NC	Levofloxacin 750mg q24h PO x 14 days	Cured
Case 16, 57Y, M,R, Farmer	Tissue	*S.algae Streptococcus sp, K. pneumonia*	Cellulitis, Rt. Leg	Shoe bite injury	DM	Amoxicillin-Clavulanate 625 mg, q8hr PO x10 days	cured

[Table/Fig-1]: Epidemiological and clinical characteristics of 16 patients with *Shewanella* SSTIs
@ =Linezolid 600 mg po, q12h X 5 days, followed by 300 mg for 5 more days
plus Gentamycin 2 mg/kg loading dose, followed by 1 mg/kg IV q8h x14 days
*=Wound dressing with topical nadifloxacin, septigrass and gentamicin after cleaning with normal saline.
Y =year; F/M = male/female; R/U= Rural/Urban; Rt=Right; L=left
DM=Diabetes mellitus; HTN=Hypertension; CLD=Chronic liver disease; CRF=Chronic renal failure, DLP= Dyslipidemia; NC= No comorbidities PVOD=Peripheral vascular occlusive disease; PN=Peripheral neuropathy; PT=Penetrating trauma; RTA=Road traffic accident; CNHU= Chronic Non-healing Ulcer



[Table/Fig-2]: Graph showing portals of entry for SSTI due to *Shewanella*



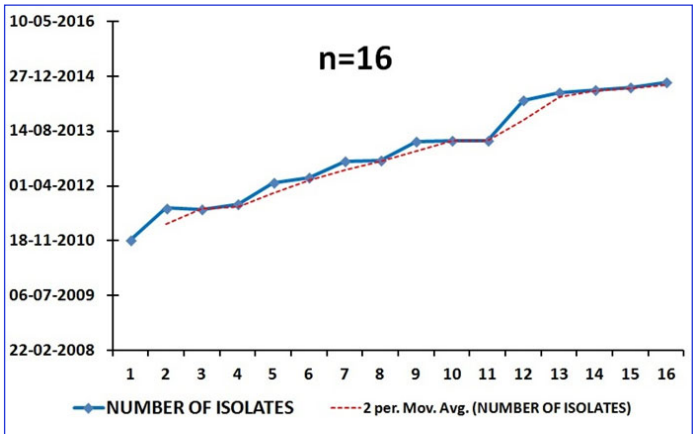
[Table/Fig-5]: Antibiotic resistance pattern of *Shewanella* isolates from SSTIs
CS = Cefoperazone-Sulbactam; PT = Piperacillin-Tazobactam;
TMP/SMZ = Trimethoprim- Sulfamethoxazole; AS = Ampicillin-Sulbactam

75%) was the predominant isolate, mixed growth was seen in 13 cases and pure growth of *S. algae* in three cases. The concomitant pathogens grown were summarized in [Table/Fig-1], which reveals that *P. aeruginosa* and *Proteus species* were the predominant coisolates.

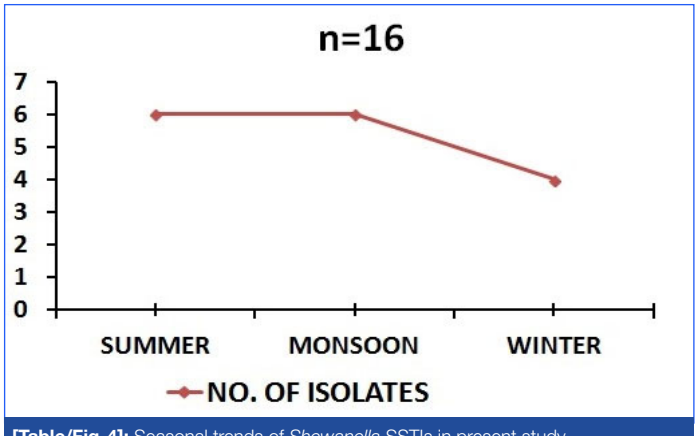
Antibiogram pattern showed that 3rd generation cephalosporins, moxifloxacin and carbapenems were the major effective drugs. It displayed maximum resistance to ciprofloxacin (n=7, 42%) with MIC values=4 µg/mL (S=1µg/mL). We found one imipenem resistant isolate and 2 multidrug resistant isolates of *S.algae* which were sensitive to only meropenem and cefepime [Table/Fig-5].

Clinical findings

All the 16 patients presented with SSTIs involving lower (14) and upper (2) extremities. Twelve cases were chronic in nature. As shown in [Table/Fig-1], majority of the infections were super infection of cutaneous ulcer and cellulitis. It also shows that 81.25% of infected patients had significant pre-existing comorbidities, diabetes and hypertension being the most common. The predominant risk factors observed were peripheral vascular occlusive disease (PVOD) and peripheral neuropathy. Regarding antibiotic treatment, majority of the patients received broad-spectrum ß-lactam antibiotics and combination of newer fluoroquinolones and aminoglycosides. [Table/Fig-1] summarizes the clinical outcome of affected patients where one patient underwent toe amputation and three patients died of complications.



[Table/Fig-3]: Trends in prevalence of *Shewanella* SSTIs during 5 years period



[Table/Fig-4]: Seasonal trends of *Shewanella* SSTIs in present study

DISCUSSION

Until the early nineties, the genus *Shewanella* was clinically associated with human infections with the species designation of *S.putrefaciens* [9]. However, Nozue et al., [10] differentiated the genus into *S.putrefaciens* and *S.algae* based on mol% G+C content and biochemical properties. Since the description of first human infection by *S.algae* [11], more than 90% strains of *S.algae* were reported as human pathogens. Nath et al., [12] from India reported two cases of gastroenteritis where *S.algae* was isolated from the rectal swab. Similarly Gautam et al., [13] recorded first case report from India where the same species was isolated from bed sores. *S.algae* is found to be more pathogenic due its ability for adhesion and bacterial haemolysin production [3].

The present study recorded more infections in elderly patients, probably due to their weakened immune system. Majority of the patients were men similar to previous studies [14] and is attributed to their outdoor activities. The significant difference in infection rates of urban and rural patients is due to relatively less farming and field activities in Kochi. Occupational analyses showed high prevalence rates among fishermen and labourers due to their high risk of exposure to marine water. We observed a significant increasing trend in prevalence rate of *Shewanella* SSTIs from 6.25% in 2010 to 31.25% in 2014 and is related to changes in socio-epidemiological factors, increased comorbidities and emerging drug resistant strains. We found high infection rates during summer and monsoon seasons due to increased exposure to marine water. In Kochi, summer is an ideal time for swimming and taking holy drips in river banks as the river water is at its lowest. Monsoon season seems to be a good time for fishermen as it fetches good catch for them.

Breakdowns of skin integrity open the door for bacteria to cause SSTIs. A previously existing leg ulcer acted as common entry portal in most of the studies [14,15]. The present study recorded chronic leg ulcers in 75% patients similar to the findings (82%) of Vignier et al., [15]. *Shewanella* species has a predilection for causing infections in tissues with poor circulation. We found PVOD and peripheral neuropathy as major risk factors.

Since *Shewanella* species are associated with marine environments, infection in man is supposed to be associated with exposure to this ecosystem. But we found only 50% to 60% of published case

reports [9,11,14] revealing such a potential mode of infection. The finding of sea water exposure (56.25%) in present study is similar to the finding (44%) of Vignier et al., [15]. Travel is considered as a risk factor by Wagner et al., [16] who reported infection of leg ulcer by *S.algae* in a traveller with history of frequent bathing in the Mediterranean Sea during her holiday trip. The present study reports a similar case where a conductor had infection of venous leg ulcer by *S.algae*. Exposure to Kerala backwaters of Arabian Sea during his journey would have been the contributing factor.

The pattern of pathogens grown probably reflects how the infection occurs. This is exemplified in two of our patients, from whom *Aeromonas* species were coisolated, because of their similar ecology. Similarly in a case of burns patient, source may be contaminated water, which might be used for pain relief. Similar case was reported in previous literature [17]. Trauma wound infection seen in three of our patients could have occurred due to contact with the soil, in a road accident. Because *Shewanella* is naturally present in the soil, the source of infection in these cases could be soil. An identical case of post-trauma ulcer was reported by Sharma et al., [17]. And few studies could not show exact source of infection similar to our findings in one patient. Gautam et al., [13] reported a case of primary *S.algae* SSTI, with no history of sea water exposure, after a long stay in hospital. We encountered similar incident in two of our patients. This finding highlights the fact that other causes that lead to emergence of *S.algae* should always be looked for [13].

In present literature, *S.algae* was a predominant isolate (75%) similar to reports (74%) of earlier investigations [3,18]. An intriguing finding in our study is *S.algae* was the only species isolated since 2013, thus contributing to 50% of the *Shewanella* SSTIs. The above findings underscore the importance of emerging highly pathogenic strains of *S.algae* potential for multi-drug resistance [3].

We recognized comorbidities in 81.25% of infected patients similar to earlier reports (80%) [14,15]. Patients with chronic liver disease (CLD) usually have chronic leg edema, which could predispose to minor trauma providing an entry portal for bacteria. The present literature reported SSTIs in 18.5% of patients with CLD which corroborates the results of Tsai et al., [14] who reported 11% of SSTIs in this group. The clinical significance of an isolate is related to specimen type from which it was isolated [17]. In our study, all the 16 isolates were grown from the samples which had least chances of contamination or colonization.

The clinical significance of co-isolates cannot be ruled out as polymicrobial infections lead to increased microbial resistance, limiting the treatment options and significantly increase morbidity, mortality and hospital costs. A study conducted by Vignier et al., [15] reported that 50% of the *Shewanella* infections were polymicrobial in comparison to 81% in our study, which is related to socio-epidemiological differences, differences in risk factors and associated comorbidities. However in both the studies, we found *Aeromonas* species and coliform bacilli to be predominant concomitant pathogens belonging to marine and gut flora respectively.

Available data suggest that *Shewanella* species are susceptible to aminoglycosides, quinolones, extended-spectrum cephalosporins, ß-lactamase inhibitor combinations and carbapenems [4,7]. However recent epidemiological data showed an increasing frequency of drug resistance especially to piperacillin-tazobactam and imipenem and was attributed to the presence of derepressed ambler class D beta-lactamases in them [6,14,19,20].

Frincy et al. [21] from India reported an isolate of *S.putrefaciens*, which was resistant to imipenem and meropenem, from ascitic fluid of a patient with CLD. We found one isolate of imipenem resistant *S.algae* in present study.

The sensitivity findings of our study are in complete agreement with those reported by other investigators [7,15,16]. There are reports of multidrug resistance in *Shewanella* [10,12,22]. In present study, we found two isolates of *S.algae which were multi-drug resistant*. *Shewanella* are known to carry plasmid-borne quinolone resistance (qnr) progenitor genes [7]. It underscores the need to have close watch at possible emergence of quinolone resistance in *Shewanella*. Considering above data the microbiologists should devise a new 'standard surgical regime' for treatment and empirical therapy. The treatment outcome observed in our study is similar to other studies [14,23].

CONCLUSION

This study provides us an insight to current state of *Shewanella* SSTIs, highlighting *S.algae* as an emerging pathogen. It underscores the significance of distinguishing *S.algae* from *S.putrefaciens* due to their differences in pathogenicity and treatment modalities. Finding of quinolone resistance in present study underlines the need for continued vigilance and stringent control of emergence of quinolone resistant strains of *Shewanella*. The microbiologists should be aware of the fact that *Shewanella* can at times be multidrug resistant while suggesting empiric antibiotic therapy. These infections have a good clinical outcome if detected promptly and offered apt medical, surgical and conservative treatment.

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