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Original Article

Microbiology Section

Skin and Soft Tissue Infections due to *Aeromonas* spp.: An Emerging Pathogen

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ABSTRACT

Introduction: *Aeromonas* species the emerging human pathogens, can cause various diseases like gastrointestinal infections, Skin and Soft-Tissue Infections (SSTIs), respiratory tract infections, urinary tract infection, hepatobiliary tract infection, blood stream infections etc. *Aeromonas* consists of important pathogenic species like *Aeromonas hydrophila* being the most common one followed by *A. sobria, A. veronii, A. caviae and A. salmonicida.* SSTIs due to Aeromonads are most often associated with pre-existing ulcer, traumatic wound and exposure to water.

Aim: To analyse socio-epidemiological factors, clinical features, risk factors and antibiotic resistance potential of *Aeromonas* spp., SSTIs.

Materials and Methods: This prospective study was performed in Microbiology Department of Rajshree Medical Research Institute, Bareilly, Utter Pradesh, India. A total of 39 patients with *Aeromonas* spp., SSTIs were identified during the period from 2020 to 2022. All Gram-negative fermenting motile isolates which are positive for oxidase, H_2S production, indole reaction, lysine decarboxylase were further identified by Vitek 2 compact system (Biomerieux, France). Patient demographics were presented as mean±standard deviation.

Results: Majority of patients hailed from urban areas, were in middle age group and were farmers. *A. hydrophila 24 (62%)* was the predominant isolate. Majority of the infections were superinfection of wound 16 (41%) and chronic non healing ulcer 13 (33.3%). A total of 33.3% of infections were polymicrobial, common concomitant pathogens being, *Pseudomonas aeruginosa* and Methicillin-resistant *Staphylococcus aureus* (MRSA). Trauma and water exposure were main risk factors with co-morbidities like diabetes, hypertension and liver cirrhosis. A 20.5% of patients were immunocompromised. There was one case of Necrotising Fasciitis (NF) which resulted in patient's death. Co-trimoxazole, 3rd and 4th generation cephalosporins. Aztreonam and Tigecycline were the most effective antibiotics while eight of the isolates were Multidrug Resistant (MDR). A 33 patients recovered completely and three patients died of complications.

Conclusion: *Aeromonas hydrophila* must be regarded as an emerging pathogen of SSTIs mainly in patients with pre-existing ulcers and can be MDR. Such infections have a good prognosis if prompt medical, surgical and supportive treatment is given.

Keywords: Co-morbidities, Polymicrobial, Skin and soft-tissue infections

INTRODUCTION

Aeromonas spp., are gram-negative motile and facultative bacilli, widely distributed in aquatic environments, food and soil [1]. All the members of *Aeromonas* spp., genus might be called as aeromonad. Aeromonads belongs to family Aeromonadaceae [2]. They are emerging pathogens which can colonise and infect various hosts [3]. They are becoming renowned as human pathogens. *Aeromonas* spp., consists of important pathogenic spp., like *Aeromonas hydrophila*, *A. sobria, A. veronii, A. caviae* and *A. salmonicida* [4]. In both immunocompromised and immunocompetent persons, aeromonads can cause variety of diseases. They are divided into most common gastrointestinal infections and extra-gastrointestinal infections [5]. Extraintestinal diseases include Skin and Soft-Tissue Infections (SSTIs), respiratory tract infections, urinary tract infection, hepatobiliary tract infection, endocarditis, bacteremia and meningitis [3,6,7].

The SSTIs are frequently encountered infections which consist of infections of skin, subcutaneous tissue, fascia and muscle and even bone. The clinical presentations range from simple cellulitis to rapidly progressive Necrotising Fasciitis (NF) [8]. Among SSTIs due to *Aeromonas* spp., traumatic wound infections are seen most frequently followed by wound exposure to water [9,10]. Most often we encounter polymicrobial infections caused by enteric bacilli, *Staphylococci*, *Pseudomonas aeruginosa* etc.

As limited data on *Aeromonas* spp., SSTIs is available in India especially northern part [11-13]. This study was conducted with an aim to explore epidemiology, risk factors and clinical features and to evaluate antibiotic resistance potential of these *Aeromonas* bacteria. This investigation helps in guiding appropriate selection of antibiotic therapy and prevention of these emerging human pathogens.

MATERIALS AND METHODS

This prospective study was performed in Microbiology Department of Rajshree Medical Research Institute (RMRI), a tertiary health care center. It is a 1080 bedded hospital located in Bareilly, Utter Pradesh, India. The study was carried out for a period of two years from August 2020 to July 2022. We took general informed consent from the patients and the study was performed after getting approval by Institutional Ethical Committee (Reference number- RMRI/IEC/54/2020).

Inclusion criteria:

- Patients with clinical features indicative of SSTIs such as cellulitis, gangrene, abscess.
- Patients with or without complications and both acute and chronic infections.

Exclusion criteria:

- Patients presenting with gastrointestinal infection.
- Patients presenting with extraintestinal infections other than SSTIs.

As the present study duration based study, hence all the consecutive patients having SSTIs were enrolled during the study period. All relevant data regarding demographic and clinical characteristics, risk factors were collected from hospital information system.

Study Procedure

All samples were processed by standard clinical laboratory condition [14]. Samples were subjected to Gram's stain which showed Gram-negative bacilli and hanging drop preparation from the colonies showed motility. They were oxidase and catalase positive. On nutrient agar, buff-colored, convex colonies 3-5 mm in diameter were seen after overnight incubation at 37°C. On sheep blood agar, beta-haemolysis was produced. Growth on MacConkey agar showed pink colonies due to lactose fermentation. All the *Aeromonas* spp., isolated by conventional methods were confirmed using VITEK 2® compact system (Biomerieux, France), only if probabilities of identifications were ≥96%.

The Minimum Inhibitory Concentration (MIC) values were determined for following antibiotics: amikacin, ceftazidime, ciprofloxacin, ceftriaxone, colistin, gentamycin, imipenem, levofloxacin, meropenem, piperacillin, ampicillin, cefoperazone/sulbactam, trimethoprim/sulfamethoxazole, tetracycline, tigecycline, ticarcillin, tobramycin, piperacillin/tazobactam, aztreonam, doripenem and cefepime by broth microdilution method using VITEK 2® compact system. The results were analysed as per Clinical and Laboratory Standards Institute (CLSI) guidelines [15,16].

For colistin, E-strips were also used to determine MICs. Interpretative criteria for colistin were taken from Fosse T et al., (MIC of ≤2 µg/mL was considered susceptible) [17].

E test was done for the antibiotics ampicillin sulbactum, cefoperazone sulbactum, tigecycline, ticarcillin and tobramycin to determine MICs. Interpretative criteria for these antibiotics were derived from those described for the Enterobacteriaceae by the Food and Drug Administration and by the CLSI M100 [18,19]. Disc diffusion test was also performed for all the antibiotics and results were analysed as per CLSI guidelines [20].

STATISTICAL ANALYSIS

Patient demographics were presented as mean±standard deviation. Clinical characteristics, co-morbid conditions were presented in frequency and percentages.

RESULTS

The epidemiological, microbiological and clinical characteristics of infected 39 patients were outlined in [Table/Fig-1,2].

Character	Number of cases (N=39)	Percentage (%)
Age (years)		
10-20	2	5.12
21-40	18	46.15
41-60	16	41
61-80	3	7.6
Gender		
Male	26	66.6
Female	13	33.3
Occupation		
Farmer	13	33.3
Labourer	11	28.2
Fisherman	9	23
Housewife	4	10.2
Students	2	5.1
Location		
General surgery	15	38.4
Endocrinology	15	38.4
Plastic surgery	7	17.9
Oncology	1	2.5
Orthopeadics	1	2.5

[Table/Fig-1]: Epidemiological characteristics of 39 patients with *Aeromonas* spp., SSTIs.

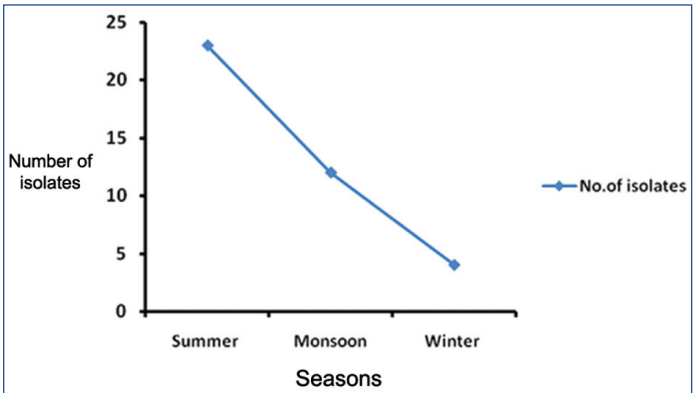
Character	Number of cases (N=39)	Percentage (%)
Type of SSTI		
Wound infection	16	41
Superinfection of CNHU*	13	33.3
Cellulitis	7	17.9
Gangrene	2	5.1
Necrotising fascitis	1	2.5
Type of infection		
Monomicrobial	26	66.6
Polymicrobial *Pseudomonas* spp. (6) MRSA (4) *Proteus* spp. (1) *Acinetobacter* spp. (2)	13	33.3
Risk factor/Cause of infection		
Trauma	19	48.7
Water exposure	12	30.7
Immunocompromised status	8	20.5
Co-morbid conditions		
Diabetes	13	33.3
Hypertension	7	17.9
Liver cirrhosis	3	7.6
Solid tumour	1	2.5
Receiving immunosuppressants	1	2.5
No co-morbidity	14	35.8
Management		
Wound debridement+antibiotic therapy	21	53.8
Only antibiotic therapy	9	23
Reconstructive surgery	7	17.9
Amputation	2	5.1
Clinical outcome		
Cured	36	92.3
Mortality	3	7.6

[Table/Fig-2]: Clinical characteristics of 39 patients with *Aeromonas* spp., SSTIs.
CNHU*: Chronic non healing ulcer

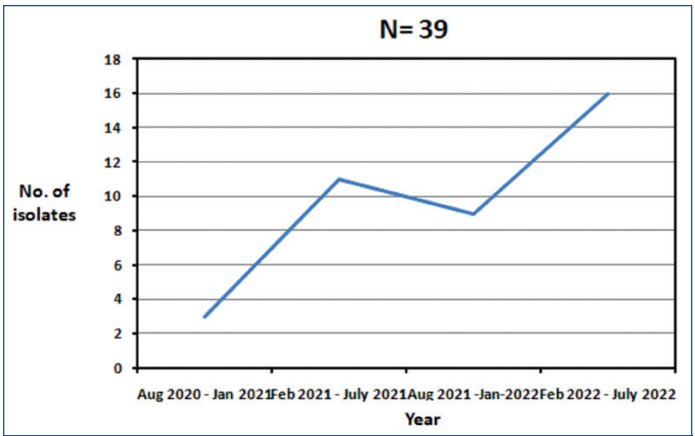
Epidemiological findings: The mean (SD) age of the patients was 41.97 (±12.94) years (range: 18-72 years). Among 39 patients who were infected with *Aeromonas* spp., 26 (66.6%) were male patients. Occupational analysis displayed, high frequency among farmers 13 (33.3%) followed by labourers 11 (28.2%). We found *Aeromonas* spp., SSTIs occurring more commonly in summer and monsoon [Table/Fig-3]. The [Table/Fig-4] revealed significant increase in *Aeromonas* spp., SSTIs over two-year period.

Microbiological findings: Great number of isolates were from tissue (54%) followed by pus (41%) samples. Distribution of isolates according to sample source is shown in [Table/Fig-5]. We found *A. hydrophila* 24 (62%) as a most common isolate followed by *A. caviae* 7 (18%) and *A. sobria* 6 (15%) [Table/Fig-6]. *Pseudomonas aeruginosa* and MRSA were predominant isolates grown along with *Aeromonas* spp., in polymicrobial infection.

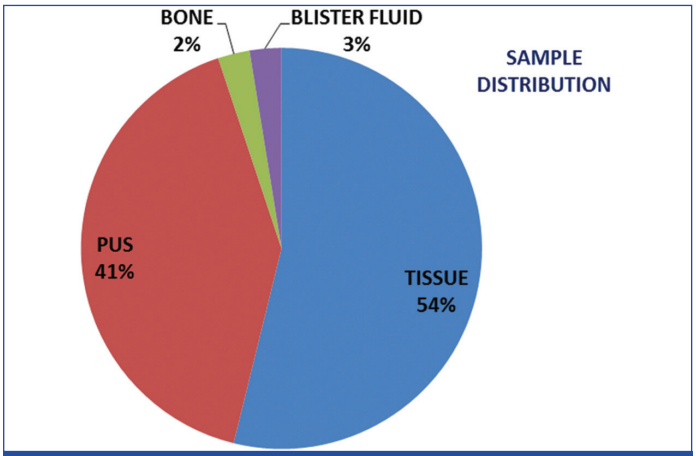
The antibiotic resistance patterns of *Aeromonas* spp., isolates from clinical samples against different antibiotics are shown in [Table/Fig-7a,b]. It showed maximum resistance to ampicillin (92%), ticarcillin (85%) followed by doripenem (48%) and piperacillin-tazobactum (38%). Major effective antibiotics showing more than 95% sensitivity were co-trimoxazole, 3rd and 4th generation cephalosporins, aztreonam and tigecycline. Sensitivity rate ranging between 85-95% seen for fluoroquinolones, colistin, aminoglycosides and cefoperazone-sulbactum. We got eight Multidrug Resistant (MDR) isolates which were susceptible to only co-trimoxazole and colistin.



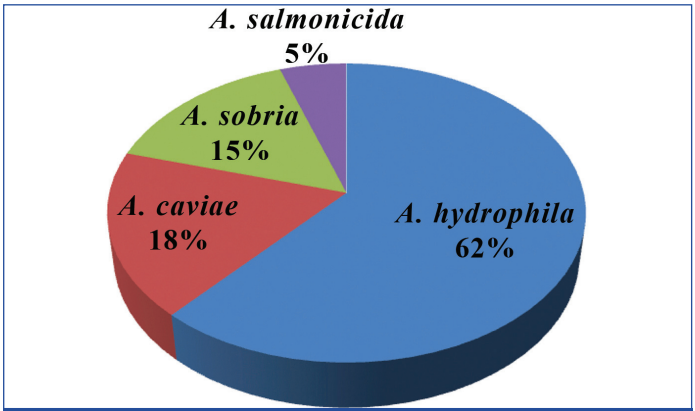
[Table/Fig-3]: Seasonal variations of *Aeromonas* spp., SSTIs in present study.



[Table/Fig-4]: Trends in prevalence of *Aeromonas* spp. SSTIs during two year period.



[Table/Fig-5]: Sample wise distribution of the clinical isolates.

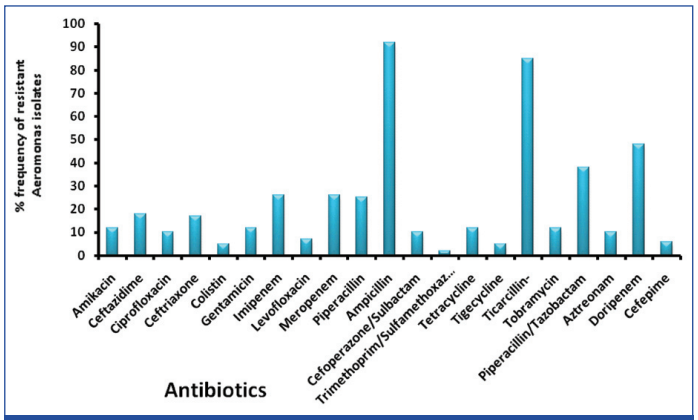


[Table/Fig-6]: Species distribution of the clinical isolates.

Clinical findings: Majority of the patients had surgical and endocrinology admission 15 (38.4%). As shown in [Table/Fig-1], majority of the infections were superinfection of wound 16 (41%) and chronic non healing ulcer 13 (33.3%). We encountered one case of NF which was co-infected with *A. hydrophil* and *Pseudomonas aeruginosa*. We found trauma 19 (48.7%) as a major risk factor followed by water exposure 12 (30.7%). The present study also showed that 64% of infected patients had considerable pre-existing co-morbidities, diabetes and hypertension being the most common. Outcome analysis showed that 36 patients were cured and remaining three cases died of infection. Wound debridement and antibiotic therapy resulted in complete recovery in 53.8% patients and 5.1% patients required amputation.

S. No.	Antimicrobial agent	Sensitive n (%)	Resistance n (%)
1	Amikacin	34 (87)	5 (12)
2	Ceftazidime	32 (82)	7 (18)
3	Ciprofloxacin	35 (89)	4 (10)
4	Ceftriaxone	32 (82)	7 (17)
5	Colistin	37 (94)	2 (5)
6	Gentamicin	34 (87)	5 (12)
7	Imipenem	29 (74)	10 (25)
8	Levofloxacin	36 (92)	3 (7)
9	Meropenem	29 (74)	10 (25)
10	Piperacillin	29 (74)	10 (25)
11	Cefoperazone/Sulbactum	35 (89)	4 (10)
12	Trimethoprim/Sulfamethoxazole	38 (97)	1 (2)
13	Tetracyclin	34 (87)	5 (12)
14	Tigecyclin	37 (94)	2 (5)
15	Ticarcillin	6 (15)	33 (85)
16	Tobramycin	34 (87)	5 (12)
17	Piperacillin/Tazobactum	24 (61)	15 (38)
18	Aztreonam	35 (90)	4 (10)
19	Doripenem	21 (54)	18 (46)
20	Cefepime	36 (92)	3 (8)
21	Ampicillin	4 (10)	35 (90)

[Table/Fig-7a]: Percentage of antibiotic resistance pattern of *Aeromonas* isolates by disc diffusion test (Kirby-Bauer method).



[Table/Fig-7b]: Percentage of antibiotic resistance pattern of *Aeromonas* spp., isolates from SSTIs.

DISCUSSION

The genus *Aeromonas* spp., is now added to Aeromonadaceae family which contains Gram-negative bacilli [21]. They are ubiquitous in nature especially in marine environments like fresh and brackish water, food and soil [1,22,23]. *A. hydrophila, A. caviae, A. veronii* and *A. sobria* are responsible for more than 85% of human infections [24].

Most of the *Aeromonas* spp., are regarded as emerging pathogens; in particular *A. hydrophila* because they cause different diseases, mainly gastroenteritis, wound infections, cellulitis and septicemia. They infect both immunocompromised and immunocompetent persons. SSTI was the most frequent extraintestinal manifestation caused by *Aeromonas* spp., [22,25,26].

We found that immune status was not a risk factor for *Aeromonas* spp., infections similar to previous study [2]. *Aeromonas* spp., had different virulence factors which allow them to adhere, colonise, invade and destroy the host cells and therefore evade the host immune response [3,27].

The present study recorded more infections in middle aged patients and in men which is related to their outdoor activities similar to previous study [6].

Even though, previous literature showed that most of the *Aeromonas* spp., SSTIs are due to water exposure, only 30.7% of the patients in present study had such history. Present investigations indicate that *Aeromonas* spp., can also cause traumatic wound infections. A total of 48.7% of SSTIs are due to trauma in this study similar to previous studies [10]. This might be due to contact with the soil in which *Aeromonas* spp., is naturally present and can act as a source of infection.

We observed a significant increasing trend in prevalence rate of *Aeromonas* spp., *SSTIs* from 8% in 2020 to 41% in 2022 and are related to changes in socio-epidemiological factors, increased co-morbidities and emerging drug resistant strains. We found high infection rates during summer and monsoon seasons due to increased exposure to water.

In current study, *A. hydrophila* was a major isolate (62%) similar to previous investigation [6]. It was found interesting that, since January 2022 *A. hydrophila* was the only species isolated and added to more than 50% of the *Aeromonas* spp., SSTIs. These findings highlight the significance of emerging extremely pathogenic strains of *A. hydrophila* potential for MDR.

Unlike other studies most of the SSTIs in this study were monomicrobial (66.6%) [5,28]. *Pseudomonas aeruginosa* was the predominant co-pathogen followed by MRSA. *Aeromonas* spp., elaborates lytic enzymes like caseinase and elastase which may invade tissue and cause NF [29].

We encountered a single case of NF where MDR *Pseudomonas aeruginosa* was a co-pathogen isolated from tissue debris as well as blood. The person died of septicemia. Though, *Aeromonas* spp., causes NF very rarely, it has poor prognosis because of its invasive property, high virulence and MDR as occurred in present study. It underlines the importance of prompt diagnosis and early surgical intervention [30].

In present study, 92% of isolates showed resistance to ampicillin similar to previous studies due to the production of beta-lactamase enzyme [16,31]. The most active antibiotics in current study with sensitivity rates more than 95% were co-trimoxazole, 3rd and 4th generation cephalosporins, aztreonam and tigecycline similar to previous studies [28,32-34].

I present study, 21% of clinical isolates were MDR, mainly seen in *A. hydrophila.* Ugarte-Torres A et al., quoted that one of the major virulence factors of *A. hydrophila* is development of MDR [30]. It's mechanism is attributed to production of inducible chromosomal β-lactamase and an extended-spectrum beta-lactamase and a metallo-β-lactamase active against carbapenems [35-38].

Sensitivity rate ranging between 85-95% seen for fluoroquinolones as seen in previous literature [25]. Present findings suggest that antibiotic sensitivity testing should be done for all clinically significant strains as resistance to various antibiotics are strain dependent.

In this study, the outcomes were favourable. Of the 39 patients with *Aeromonas* spp., SSTIs, only three patients died one with a complication of NF and other two due to co-morbid diseases. Two patients required amputation and both of them had diabetes mellitus as a risk factor. In the present study, 53.8% of the patients received wound debridement plus antibiotic therapy and it is likelily that the favourable result among the majority was atleast in part due to surgical treatment. The above results are in line with the findings of Chao CM et al., [6]. Previous studies on *Aeromonas* spp., SSTIs in different states of India are shown in [Table/Fig-8] [11-13,39-43].

Author's and year of the study	Place of study	Number of cases presented with *Aeromonas* SSTIs	Clinical presentation
Vithiya G and Raja S, [39] 2022	Madurai	9	Cellulitis, gangrene
Veeren G et al., [40] 2022	Chennai	15	Cellulitis, Necrotising fasciitis
Kumar S et al., [41] 2012	Kolkata	1	Necrotising fasciitis (NF)
Jangla SM and Mishra SC, [42] 2020	Mumbai	1	Myonecrosis
Saurabh A et al., [11] 2017	Dehradun	1	Cellulitis
Sood S and Nerurkar V, [12] 2014	Rajasthan	1	Necrotising fasciitis (NF)
Behera B et al., [13] 2011	New Delhi	1	Post-traumatic abscess
Mukhopadhyay C et al., [43] 2008	Karnataka	7	Abscess over the lower leg, cellulitis,
Present study	Uttar Pradesh	39	Wound infection, cellulitis, gangrene, Necrotising fascitis

[Table/Fig-8]: Review of literature on *Aeromonas* spp., SSTIs in different states of India [11-13,39-43].

Limitation(s)

The isolates were not subjected to molecular methods for confirmation.

CONCLUSION(S)

The present work gives us an intuition to current state of *Aeromonas* spp., SSTIs, highlighting *A. hydrophila* as an emerging human pathogen. It underscores the significance of distinguishing various species of *Aeromonas* spp., due to their differences in pathogenicity and treatment modalities. And also, we should be aware of the fact that *Aeromonas* spp., can at times be MDR while giving empiric antibiotic therapy. These infections have a good prognosis if prompt medical, surgical and supportive treatment is given.

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