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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 spp.,* 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.veroni, A.caviae and A.salmonicida*. SSTIs due to Aeromonads are most often associated with pre-existing ulcer, traumatic wound and exposure to water.

Aim: The present study was carried out to analyse socio-epidemiological factors, clinical features, risk factors and antibiotic resistance potential of *Aeromonas spp.*, SSTIs.

Materials and Methods: In this prospective study, 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). The study period was 2 years.

Results: A total of 39 patients with *Aeromonas spp.,* SSTIs were identified during the period from 2020 to 2022. Majority of patients hailed from urban areas, were in middle age group and were farmers. *A.hydrophila* (n=24, 62%) was the predominant isolate. Majority of the infections were superinfection of wound (n=16, 41%) and chronic non healing ulcer (n=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). Thirty-three 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.veroni, 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]. Extra-intestinal diseases include SSTIs, respiratory tract infections, urinary tract infection, hepatobiliary tract infection, endocarditis, bacteremia, meningitis [3,6,7].

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 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 Sciences (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:

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

Exclusion criteria:

1. Patients presenting with gastrointestinal infection.
2. Patients presenting with extraintestinal infections other than SSTIs.

Sample size with justification: Duration based study; hence all the consecutive patients having SSTIs were enrolled during the study period.

Study tools: All relevant data regarding demographic and clinical characteristics, risk factors were collected from hospital information system.

Laboratory procedures: 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 [18] and by the CLSI M100 [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.

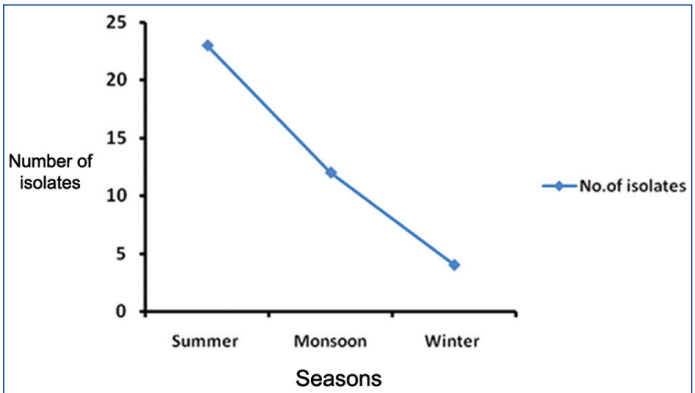
Character	Number of cases (N=39)	Percentage (%)
Type of SSTI		
Wound infection	16	41
Superinfection of CNHU*	13	33.3
Cellulitis	7	17.9
Gangrene	2	5.1
Necrotising fascitis	1	2.5
Type of infection		
Monomicrobial	26	66.6
Polymicrobial *Pseudomonas spp.* (6) MRSA (4) *Proteus spp.* (1) *Acinetobacter spp.* (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

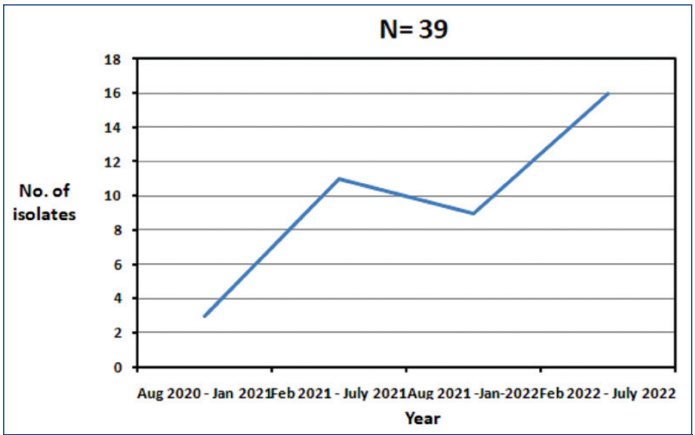
Epidemiological findings: The mean (SD) age of the patients was 41.97 (±12.94) years (range: 18-72 years). Among 39 patients who were infected with *Aeromonas spp.,* 66.6% (26/39) were male patients. Occupational analysis displayed, high frequency among farmers (n=13, 33.3%) followed by labourers (n=11, 28.2%). We found *Aeromonas spp.,* SSTIs occurring more commonly in summer and monsoon [Table/Fig-3]. [Table/Fig-4] revealed significant increase in *Aeromonas spp., SSTIs* over 2-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* (n=24, 62%) as a most common isolate followed by *A.caviae* (n=7, 18%) and *A.sobria* (n=6, 15%) [Table/Fig-6]. *Pseudomonas aeruginosa* and MRSA were predominant isolates grown along with *Aeromonas spp.,* in polymicrobial infection.

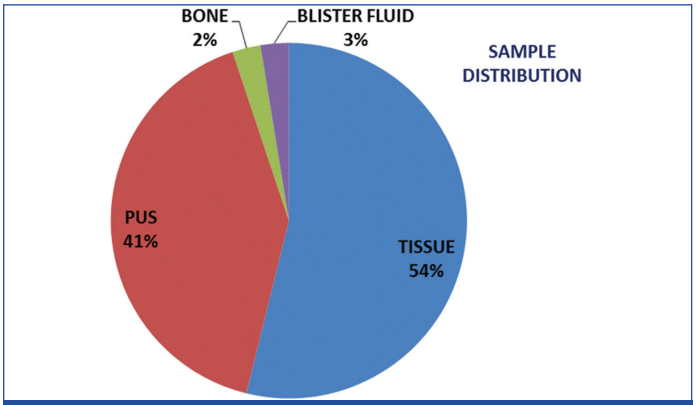
The antibiotic resistance patterns of *Aeromonas spp.,* isolates from clinical samples against different antibiotics are shown in [Table/Fig-7]. It showed maximum resistance to Ampicillin (92%), Ticarcillin (85%) followed by Doripenem (48%) and Piperacillin-Tazobactum (38%). Major effective antibiotics showing more than 95% sensitivity were Co-trimoxazole, 3rd and 4th generation cephalosporins, Aztreonam and Tigecycline. Sensitivity rate ranging between 85% to 95% seen for fluoroquinolones, colistin, aminoglycosides and cefoperazone-sulbactum. We got eight MDR isolates which were susceptible to only co-trimoxazole and colistin.



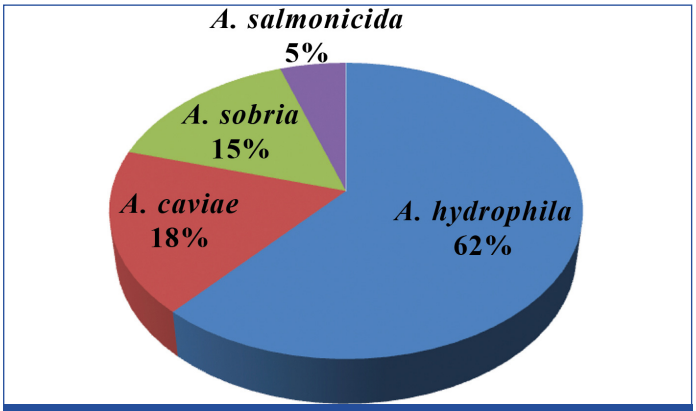
[Table/Fig-3]: Seasonal variations of *Aeromonas* spp., SSTIs in present study.



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



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

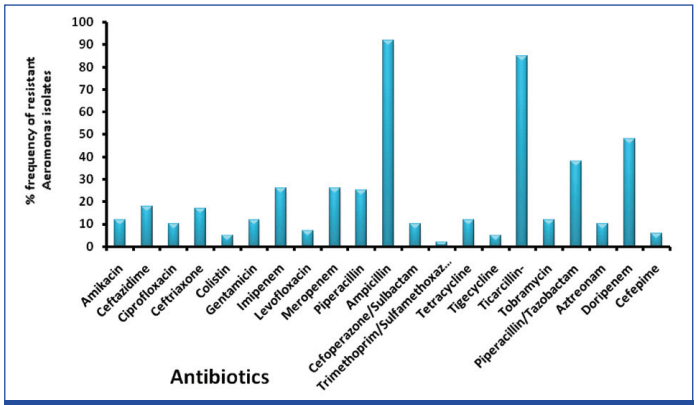


[Table/Fig-6]: Species distribution of the clinical isolates.

Clinical findings: Majority of the patients had surgical and endocrinology admission (n=15, 38.4%). As shown in [Table/Fig-1], majority of the infections were superinfection of wound (n=16, 41%) and chronic non healing ulcer (n=13, 33.3%). We encountered one case of NF which was co-infected with *A.hydrophila* and *Pseudomonas aeruginosa*. We found trauma (n=19, 48.7%) as a

Sl 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-7]: Percentage of antibiotic resistance pattern of *Aeromonas spp.*, isolates from SSTIs.

major risk factor followed by water exposure (n=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 3 cases died of infection. Wound debridement and antibiotic therapy resulted in complete recovery in 53.8% patients and 5.1% patients required amputation.

DISCUSSION

The genus *Aeromonas spp.,* is now added to Aeromonadaceae family [21], which contains Gram-negative bacilli. They are ubiquitous in nature especially in marine environments [22] like fresh and brackish water, food and soil [1,23]. *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 extra-intestinal 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 our study had such history. Our 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 multi-drug resistance.

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 multidrug resistance as occurred in our study. It underlines the importance of prompt diagnosis and early surgical intervention [30].

Ninety two percent of our 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 [28,32,33,34].

Twenty one percent of our 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 multidrug resistance [30]. It's mechanism is attributed to production of inducible chromosomal β-lactamase [35,36], and an extended-spectrum beta-lactamase and a metallo-β-lactamase active against carbapenems [37,38].

Sensitivity rate ranging between 85% to 95% seen for fluoroquinolones as seen in previous literature [25]. Our 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].

Year of study	Place of study	Number of cases presented with Aeromonas SSTIs	Clinical presentation
Vithiya G et al., [39] 2022	Madurai	9	Cellulitis, gangrene
Veeren G et al., [40] 2022	Chennai	15	Cellulitis, Necrotising fasciitis
Kumar S et al., [41] 2021	Kolkata	1	Necrotising fasciitis (NF)
Jangla SM et al., [42] 2020	Mumbai	1	Myonecrosis
Saurabh A et al., [11] 2017	Dehradun	1	Cellulitis
Sood S et al., [12] 2014	Rajasthan	1	Necrotising fasciitis (NF)
Behera B et al., [13] 2011	New Delhi	1	Posttraumatic 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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