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## CHARACTERIZATION, HEMOLYSIS AND MULTIDRUG RESISTANCE AMONG *AEROMONAS* SPP. ISOLATED FROM BHAVANI RIVER, ERODE, SOUTH INDIA

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### Abstract

A total of 87 strains of *Aeromonas* spp. were identified biochemically. The strains were isolated from 50 samples of water from Bhavani river Erode, Tamil Nadu, India. In the present study among 87 *Aeromonas* spp. the prevalence strain was identified as *A. hydrophila* (60.9%), while the other strains belonged to the species *A. sobria* (20.7%), *A. caviae* (11.5%) and *A. salmonicida* (6.9%). The virulence factors like hemolysin, lipase, and serine protease were present in 96%, 93% and 94% of the strains respectively. Antibiotic susceptibility of *Aeromonas* spp. was determined by disc diffusion method. All *Aeromonas* spp. were examined for resistance against 16 antibiotics. All strains showed 100% of resistance to Ampicillin, Carbenicillin and Cephalothin. The highest resistances encountered were 91.9% to streptomycin, 90.8% to polymyxin-B, 85% to rifampicin while the rest were under 50%. In contrast all the strains were sensitive to cefotaxime. The present work highlights the important incidence of *Aeromonas* spp., with virulence potential and antimicrobial resistance, isolated from river Bhavani.

**Key Words:** *Aeromonas*; Water; Hemolysis; Antibiotic resistance.

### Introduction

*Aeromonas* spp. are widely distributed in nature and were found in soil, drinking water and chlorinated tap water (1, 2). Members of the genus *Aeromonas* have been considered to be opportunistic pathogens they are involved in a number of diarrhoeal and extra intestinal infections including septicemia, wound infections burn associated sepsis, respiratory tract infections and paediatric gastroenteritis (3, 4 and 5). The presence of *A. hydrophila* and *A. sobria*, in drinking water is considered a relevant factor, since it is associated with digestive tract disorders (6). Food of animal origin, seafood and vegetables also act as an important vehicle for *Aeromonas* spp. infections (7, 8). *Aeromonas* spp. infections normally associated with immuno suppressed patients have been reported (9). It also causes infections in fishes (4). Antibiotic resistant *Aeromonas* spp. have been reported by many scientists (10, 11). A number of

virulence factors like hemolysin, protease, lipase and DNase produced by *Aeromonas* spp. play an important role in the development of disease in human and in fish (12, 13).

Although the number of water borne outbreaks caused by *Aeromonas* spp. has been quite limited, the presence of *Aeromonas* spp. in river system should not be ignored. Despite the number of surveys on the incidence of other kind of organisms like *Escherichia coli* and *Salmonella* spp. in water bodies, no report is available in Bhavani river regarding *Aeromonas* spp. All water samples were collected from areas largely used by mankind for drinking, cooking, bathing and other utilities.

The present study therefore carried out to document the prevalence of *Aeromonas* spp. The production of virulence factors and their antimicrobial susceptibility patterns of the strains isolated were also studied.

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## Materials and Methods

### Collection of water samples

A total of 50 samples were examined during this study. Two hundred milliliters of water was collected from Bhavani river by using pre sterilized screw capped glass bottles. All samples were processed within 3 h of collection.

### Examination of water samples

The water samples were enriched in and a loopful of enriched culture was streaked onto Starch Ampicillin agar (14) medium (SAA) (Hi-Media, Mumbai, India). The plates were incubated at 35°C for 24 h. Yellow to honey coloured colonies surrounded by a clear zone of amylolytic activity after flooding the plates with iodine solution were scored as presumptive *Aeromonas* spp. These colonies were picked, twice streaked to purify on trypticase soy agar plates, and transferred to trypticase soy agar slants for further characterization.

### Characterization and identification of *Aeromonas* strains

A total of 87 isolates were examined by biochemically to species level by referring description of work (15). Gram stain, motility, oxidase and catalase production, fermentation of glucose, and resistance to vibriostatic agent O/129 (12). All isolated *Aeromonas* spp. further tested for growth at 37°C; indole production; gas from glucose, Voges-Proskauer; lysine and ornithine decarboxylase; aesculine hydrolysis; H<sub>2</sub>S from cysteine; susceptibility to 30ug of cephaotin; methyl red test; L-arginine hydrolysis; fermentation of L- arabinose, D-mannitol, salicin sucrose, and D-sorbitol (3) casein hydrolysis, gelatinase, and DNase activity (16) and  $\beta$ -hemolysis on sheep blood agar plates (12).

### Hemolytic activity

The *Aeromonas* strains were tested for  $\beta$ -hemolytic activity on blood agar base (Hi-Media, Mumbai, India) supplemented with 5% sheep blood. The culture is streaked on to the plates and incubated at 37°C for 24 h. The presence of clear colorless zone surrounding the colonies considered as  $\beta$ -hemolytic activity (12).

### Proteolytic activity

Casein hydrolysis was tested on Mueller- Hinton agar containing 10 % (w/v) skimmed milk by streaking onto the plates and incubating at 37°C for 24 h. The presence of a transparent zone around the colonies indicated caseinase activity.

### Gelatinase activity

Gelatinase activity was tested by using gelatin agar plate, the cultures were streaked onto the plates and incubated at 22°C for 24 h. Then the plates were immersed with mercuric chloride solution. The presence of transparent zone around the colonies indicated gelatinase activity.

### Lipolytic activity

Lipase activity was determined by streaking the culture on to the plate's containing 0.5% tributyrin emulsified with 0.2% Triton X- 100 and incubated at 37°C for 24 h. (17). The presence of transparent zone around the colonies indicated lipase activity.

### Nuclease activity

DNases activity were determined on DNase agar plates with 0.005% methyl green. The culture is streaked on to the plates and incubated at 37°C for 24 h. A pink halo around the colonies indicated nuclease activity.

### Antimicrobial susceptibility test

The antimicrobial resistance of all strains to various antibiotic was determined by the disc diffusion method (18). The antibiotics and concentration ranges of the discs (Hi-media) tested were as follows: amikacin (30 $\mu$ g), ampicillin (10 $\mu$ g), Carbenicillin (100 $\mu$ g), cephalothin (30 $\mu$ g), cefotaxime (30 $\mu$ g), ciprofloxacin (5 $\mu$ g), chloramphenicol (30 $\mu$ g), erythromycin (15 $\mu$ g), streptomycin (10 $\mu$ g), gentamycin (10 $\mu$ g), kanamycin (30 $\mu$ g), neomycin (30 $\mu$ g), polymyxin-B (300U), rifampicin (5 $\mu$ g), tetracycline (30 $\mu$ g), piperacillin (100 $\mu$ g). After enrichment in tryptic soy broth (Hi-media) for 6-8 h at 37°C, the cultures were streaked on Mueller- Hinton agar (Hi-media) plates using a sterile cotton swab. The antibiotic discs were placed on the agar surfaces sufficiently separated from each other so as to prevent over-lapping of the inhibition zones. After 30 minutes, the plates were inverted and incubated at 37°C for 24h. Results were recorded by measuring the inhibition zones, comparing with interpretive chart of the Kirby-Bauer sensitivity test method.

## Results

A total of 50 numbers of water samples were collected from river Bhavani during the period of April 2008 –May 2009. Only 86% of water samples showed the presence of *Aeromonas* spp. (Table 1). A total of 87 *Aeromonas* spp. were isolated from water samples. Yellow to honey coloured colonies surrounded by clear halo zone of amylolytic activity were selected for

further analysis. The most commonly found *Aeromonas* spp. were *Aeromonas hydrophila* (60.9%), *Aeromonas sobria* (20.7%), *Aeromonas caviae* (11.5%) and *Aeromonas salmonicida* (6.9%) were also recorded (Table 2).

Table 1: Percent incidence of *Aeromonas* spp in water from Bhavani river

Source	No. of water samples	Positive for <i>Aeromonas</i> spp.	%
Water	50	43	86

Table 2: Percentage incidence of *Aeromonas* strains isolated from Bhavani river water samples

<i>Aeromonas</i> strains	Number of isolates	Percentage of Occurrence (%)
<i>Aeromonas hydrophila</i>	53	60.90
<i>Aeromonas sobria</i>	18	20.70
<i>Aeromonas caviae</i>	10	11.50
<i>Aeromonas salmonicida</i>	6	06.90

All *Aeromonas* spp.(100%) showed DNase activity. The virulence factors like hemolysin, lipase, and serine protease were present in 96%, 93% and 94% of the strains respectively. However, protease activity was different when evaluated with the caseinase test (66%) than with gelatinase (94%) (Table 3).

Table 3: Incidence of virulence factors (%) in *Aeromonas* spp. isolated from Bhavani river

<i>Aeromonas</i> strains	β-hemolysis test (37°C)	Lipase test	Serine Protease test	Caseinase test	Gelatinase test	DNase test
<i>A. hydrophila</i> , n=53	100	100	100	51	92	100
<i>A. sobria</i> , n=18	94	88	88	100	100	100
<i>A. caviae</i> , n=10	80	70	70	100	100	100
<i>A. salmonicida</i> , n=6	100	83	100	50	83	100
All the strains	96	93	94	66	94	100

The resistance patterns obtained with the 87 *Aeromonas* spp. against 16 antibiotics are shown in table 3. A 100% of resistance was observed in *Aeromonas* spp. against Ampicillin, Carbenicillin and Cephalothin. In addition the highest resistances showed by *Aeromonas* spp. encountered were 91.9% to streptomycin, 90.8% to polymyxin-B, and 85% to rifampicin (Table 4).

Table 4: Percentage antimicrobial resistance of *Aeromonas* spp. isolated from Bhavani River.

Antibiotics with concentration	<i>A. hydrophila</i> n=53	<i>A. sobria</i> n=18	<i>A. caviae</i> n=10	<i>A. salmonicida</i> n=6	Resistant strains
Amikacin (30µg)	0	38.8	40	33	14.9
Ampicillin (10µg)	100	100	100	100	100
Carbenicillin (100µg)	100	100	100	100	100
Cephalothin (30µg)	100	100	100	100	100
Cefotaxime (30µg)	0	0	0	0	0
Ciprofloxacin (5µg)	49	50	50	33	48.2
Chloramphenicol (30µg)	0	11	20	16.6	5.74
Erythromycin (15µg)	52.8	44.4	40	50	49.4
Streptomycin (10µg)	100	77.7	80	83.3	9.9
Gentamycin (10µg)	37.7	55.5	60	66.6	45.9
Kanamycin (30µg)	0	38.8	20	33	12.6
Neomycin (30µg)	33.9	44.4	40	50	37.9
Polymyxin-B (300 U)	100	77.7	80	66.6	90.80
Rifampicin (5µg)	100	55.5	70	66.6	85.05
Tetracycline (30µg)	33.9	50	50	50	19.54
Piperacillin (100µg)	0	27.7	10	16.6	18.3

Least resistance was observed against Chloramphenicol (5.7%), Kanamycin (12.6%), Tetracycline (19.54%), Neomycin (37.9%), Gentamycin (45.9%), Erythromycin (49.4%). *Aeromonas* strains showed 14.9%, 48% resistance to amikacin, ciprofloxacin respectively. In contrast all the strains were sensitive to cefotaxime. Only 18.3 % of the strains showed resistance to Piperacillin.

## Discussion

A total of 87 *Aeromonas* spp. belonging to four different species isolated from samples of Bhavani river water. The prevalence of *Aeromonas* spp. revealed remarkable diversity in this water system, which includes potential pathogens like *A. hydrophila*. In the present study this particular species accounts for 60.9%. The prevalence of *A. hydrophila* from drinking water samples already have been reported (2,19). *Aeromonas sobria* (20.7%), *Aeromonas caviae* (11.5%) and *Aeromonas salmonicida* (6.9%). have reported in our study (Table 2). Occurrence of *A. sobria* in water has been already reported (20). *A. salmonicida* has been considered as a fish pathogen (21,22) but also reported in water (23,1). The interesting observation of *Aeromonas* spp. in river bhavani which suggests the possible release of waste waters into Bhavani river that might enhanced the population of *Aeromonas* spp.

The virulence factors by *Aeromonas* spp. has been identified by production of exo enzymes, although the importance and exact mechanism of each factor associated to the virulence has not been well established (24). Our study showed that *Aeromonas* spp. produced hemolysin, Lipase, Serine protease, gelatinase and caseinase (Table 3). *Aeromonas hydrophila* and *Aeromonas salmonicida* had a high occurrence of hemolytic activity. The presence of hemolytic activity in *Aeromonas* spp. has been reported. 66% of the *Aeromonas* spp. showed hemolytic activity was noted by many scientists (25,1,26, 27). Hemolytic *A. hydrophila* from water sample was also reported (28). 97% correlation between hemolysis and enterotoxin production was noticed in many reports (29).

The lipases are important for bacterial nutrition (24) but they also involved in virulence character. In the present study *A. caviae* alone showed 70% of the activity. The proteolytic activity is important to induce pathology in living system. In our study, proteolytic activity of the isolates was evaluated by determining caseinase and gelatinase production. This study revealed

(94%) activity showed for serine protease and gelatinase. But only 66% of the strains produced caseinase. 61% of the *Aeromonas* isolates produced protease in this study (30). The DNase activity was detected in 100% of the strains. This may indicate their virulence ability.

The antibiotic resistance testing revealed that all *Aeromonas* spp. were resistant to  $\beta$ -lactam antibiotics (1) (Table 4). However, the resistance to other antibiotics was variable. Only 18.3% of the strains showed resistance to piperacillin. Penicillin resistance *Aeromonas* spp. from food samples have been reported (31). None of the strains were shown resistant to cefoxime antibiotic. Our results are consistent with data reported by (32, 33, 34) found that strains of *A. salmonicida* isolated from environment samples were 100% susceptible to ampicillin, cephalothin, polymyxin, rifampicin. But in our study it showed a different degree of resistance to these antibiotics, ranging from 16% to 100%. (28) reported all *Aeromonas* spp. were found to be susceptible to amikacin and ciprofloxacin in his studies. In the present study 14.9%, 48% strains showed resistance to amikacin, ciprofloxacin respectively. Resistance to streptomycin was high (91.9%). Our results correlated with his findings (35). Least resistance was observed against Chloramphenicol (5.7%), Kanamycin (12.6%), Tetracycline (19.54%), Neomycin (37.9%), Gentamycin (45.9%), Erythromycin (49.4%). Our results are not correlated with the findings of this study (10).

The present study highlights the presence of *Aeromonas* spp in water system intended for human consumption in the city. High prevalence of multiple antibiotic resistant, hemolysis and protease producing *Aeromonas* spp. was noticed. The productions of hemolysis and resistant to various antibiotics are normally associated to enteric infections caused by species of *Aeromonas*.

## Conclusion

The result obtained in the present study indicates that different *Aeromonas* spp. is able to multiply in water distribution systems. The increased occurrence of these strains should not be ignored. These microorganisms could reach the food product, becoming a potential human pathogen. It should be kept in mind that these microorganisms in water system might be a potential risk for public health.

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