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Studies on antibiotic sensitivity in *Aeromonas* bacteria isolated from Jorapani River flowing through the Siliguri of West Bengal, India

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Abstract

The genus *Aeromonas* consists of glucose fermenting, Gram negative rod that are widespread in freshwater environments and isolated from clinical, environmental and food samples, where they can grow even at low temperatures. Different researchers across the globe have been isolating *Aeromonas* bacteria from various infection sites of cold and warm-blooded animals, including humans. In the present study, twenty-one *Aeromonas* sp. were isolated from two different locations of a polluted river in the northern part of West Bengal and later on, the antibiotic sensitivity of these bacteria was assessed. Out of these twenty-one bacterial isolates, twenty were found to be *Aeromonas hydrophila* and one belonged to *Aeromonas caviae*. Antibiotic sensitivity of all the bacterial isolates were examined and it was seen that all the isolates were resistant to ampicillin and sensitive to gentamycin and novobiocin. 57.1% isolates were resistant to erythromycin, 23.8% isolates were resistant to clindamycin and 19.04% were resistant to nalidixic acid and cephalothin. Our findings reveal a high prevalence of antibiotic resistance in *Aeromonas* isolates collected from a polluted river, indicating the indiscriminate use of antibiotics in the study area.

Keywords: *Aeromonas*, bacteria, antibiotic resistance

Introduction

Aeromoas are oxidase- positive, Gram negative bacteria that are quite common in aquatic environment and believed to be a threat to aquaculture systems (Berger *et al*., 2021)). These bacteria have a wide host range and are increasingly being documented as pathogens of different lower vertebrates like fish, amphibians and reptiles (Guz *et al*., 2021) [20]. The genus *Aeromonas* has been involved in causing human gastroenteritis, septicemia and wound infections (Janda and Abbott, 2010; Faranandez-Bravo and Figueras, 2020) [12, 22] with the ability to infect both immunocompromised and immunocompetent patients (Figueras and Beaz-Hidalgo, 2015) [14].

Antibiotics are being greatly used as chemotherapeutants since World War II and has achieved a great success in medicine against bacterial infection. However, the inappropriate use of antibiotics to treat the infections has become quite rampant globally and resulted in an unavoidable public health concern of modern ages by generating multi drug resistant microorganisms. Wastewater effluents from different sources makes the aquatic environment more susceptible to the spreading of multi drug resistant pathogens (Baquero *et al*., 2008; Baron *et al*., 2017) [2, 4]. The genus *Aeromonas* being a natural resident of these aquatic ecosystems, exhibits wide range of antibiotic resistant determinants with the potential to disseminate these genes through horizontal gene transfer (Bello-Lopez *et al.,* 2019) [5]. Therefore, the *Aeromonas* bacteria could be represented as indicators to ascertain the antimicrobial resistance transmission in aquatic ecosystems (Baron *et al*., 2017; Grilo *et al*., 2020) [4, 19]

The present study has attempted to isolate and identify the *Aeromonas* bacteria from the polluted water of Jorapani River passing through the congested city area of Siliguri, WB, India and at the same time, the levels of antimicrobial resistance of those bacteria have been assessed.

Materials and Methods

Study area

The Jorapani is a small narrow river around five kms in length, flowing through the Siliguri, a sub-himalayan densely populated city in the northern part of West Bengal, India. It originates at Haiderpara of Siliguri near Baikunthapur Forest Division and meets Fuleswari River at Koimari near NJP railway station, Siliguri, the river flows through the highly congested city area and its bank is encroached by the city dwellers in many places. The entire river is under tremendous anthropogenic pressure and extremely polluted by domestic, hospital and industrial wastes.

Isolation of sample bacteria

Water samples were collected in sterile bottle from two different sites of river Jorapani (L1 & L2) during pre-monsoon season, in the month of April. 0.1 ml of sample water taken from sample bottle was incubated at 30°C for 24-48 hrs in a petry plate containing 20 ml of *Aeromonas* isolation medium supplemented with *Aeromonas*–selective supplement (HiMedia Laboratories, Mumbai, India). Healthy colonies grown in the petry plate were selected and transferred to the nutrient broth for overnight at 30°C to obtain presumed *Aeromonas* isolates.

Identification of bacteria by biochemical characterization

The presumptive isolates of *Aeromonas* were confirmed by using Gram stain, test for oxidase and catalase and growth in agar with 0% but not in 6% NaCl by standard routine test (Carnahan and Joseph, 2005) [8]. The isolates were identified to species level using traditional biochemical tests such as esculin hydrolysis, indole production, VP (Vogues Proskauer), lysine decarboxylase, arginine dehydrolase and ornithine decarboxylase; tests for acid production from arabinose, salicin, sucrose and mannitol; and test for gas production from glucose ((Carnahan *et al*, 1991; Abbott *et al*, 1992) [1, 7].

Antibiotic susceptibility testing

The antibiotic susceptibilities of each isolate were tested by the disc diffusion method. Muller-Hinton agar plates (9 cm diameter) were inoculated with 0.1 ml of 24 hrs old culture of test bacterium in nutrient broth. The discs of antibiotics (HiMedia Laboratories) were put on the bacterial culture plates and kept on 30°C for overnight. Discs of ampicillin (10 µg), cefuroxime (30 µg), ceftazidime (30 µg), cephalothin (30 µg), clindamycin (2 µg), erythromycin (15 µg), gentamycin (10 µg), kanamycin (30 µg), nalidixic acid (30 µg), novobiocin (30µg) oxacillin (1µg), streptomycin (10 µg) and tetracycline (30 µg) were selected for the test. The zone of inhibition was assessed (Saha and Pal, 2002) [25] and zone diameters were interpreted as sensitive (S), intermediate (I) or resistant(R) based on manufacturer's instructions.

Results

In the present investigation, twenty-one *Aeromonas* bacteria (A1 to A21) were isolated from two different sites (L1 & L2) of Jorapani River within the city.

Biochemical analysis suggested that among them twenty were *Aeromonas hydrophila* and one belonged to *Aeromonas caviae***.** (Table. 1). The only *Aeromonas caviae* was collected from L2 site of the river.

Table 1: Bacterial isolates collected from two different locations (L1 &L2) of Jorapani River

Location	Isolate number	Isolate identity
L1 (Haiderpara)	A1	*Aeromonas hydrophila*
	A2	*Aer. hydrophila*
	A3	*Aer. hydrophila*
	A4	*Aer. hydrophila*
	A5	*Aer. hydrophila*
	A6	*Aer. hydrophila*
	A7	*Aer. hydrophila*
	A8	*Aer. hydrophila*
	A9	*Aer. hydrophila*
	A10	*Aer. hydrophila*
L2 (NJP police out post)	A11	*Aer. hydrophila*
	A12	*Aer. caviae*
	A13	*Aer. hydrophila*
	A14	*Aer. hydrophila*
	A15	*Aer. hydrophila*
	A16	*Aer. hydrophila*
	A17	*Aer. hydrophila*
	A18	*Aer. hydrophila*
	A19	*Aer. hydrophila*
	A20	*Aer. hydrophila*
	A21	*Aer. hydrophila*

The antibiotic resistance patterns of the *Aeromonas* isolates for thirteen different antibiotics were tested. All the bacterial isolates were found to be resistant to antibiotic ampicillin and sensitive to gentamycin and novobiocin. 57.1% isolates were resistant to erythromycin and 23.8% isolates were resistant to clindamycin. Nalidixic acid and cephalothin resistance were found among 19.04% of the isolates. Three isolates were resistant to tetracycline and two were resistant to cefuroxime, ceftadzidime and streptomycin. Only one isolate was resistant to oxacillin and kanamycin (Table 2).

Table 2: Antimicrobial resistance in *Aeromonas* isolates collected from two different locations of Jorapani River

Isolate	Antimicrobial resistance
A1	Am, E, Na,
A2	Am, E, K, Na
A3	Am, E(I)
A4	Am, E(I), T
A5	Am, E(I), T
A6	Am, CXM
A7	Am, CXM
A8	Am, E(I), T
A9	Am, E, Na
A10	Am, E, Na, Ox
A11	Am, E, Cd,
A12	Am, E, S
A13	Am, E, S
A14	Am, E, Ch, Cd
A15	Am, E, Ch
A16	Am, E, Ch, Cd
A17	Am, E(I), Na, Cd
A18	Am, E (I), Cd
A19	Am, E, CAZ
A20	Am, E, S, Na
A21	Am, E(I), Ch, CAZ

Am, Ampicillin; E, Erythromycin; Na, Nalidixic acid; T, Tetracycline; N, Novobiocin; CAZ, Ceftazidime; CXM, Cefuroxime; Cd, Clindamycin Ch, Cephalothin; Ox, Oxacilin; K, Kanamycin; G, Gentamycin; S, Streptomycin; I - Intermediate

Discussion

Wastewater obtained from industrial areas, healthcare clinics, livestock practices and aquaculture farms promote the spread of multidrug- resistant organisms in the aquatic environments (Baron *et al.,* 2017) [4]. *Aeromonas* bacteria, considered as an emergent pathogen is abundantly found in aquatic environment and have been recovered from wastewater (Figueira *et al*., 2011)) [13], drinking water (Carvalho *et al*., 2012) [9], rivers (Gomes *et al*., 2021) [16], lakes and estuaries (Henriues *et al*., 2006) [21] Being a natural inhabitants of these ecosystems, they acquire broad spectrum of antibiotic resistant determinants and transfer these genes by sharing mobile genetic elements such as plasmids, integrons, and transposons (Piotrowska and Popowska, 2015) [24]. In the present work, a wide range of antibiotic resistance have been detected in *Aeromonas* bacteria isolated from two different locations of highly polluted river Jorapani traversing through the populated city area. Out of thirteen antibiotics tested, eleven were found to be resistant to *Aeromonas* bacteria collected from both the locations (L1 & L2). Antibiotic resistance profiles of *Aeromonas* bacteria from the ulcers of Epizootic Ulcerative Syndrome (EUS) affected fish obtained from Fish farm were studied in this region (Das *et al*., 2009) [11] which showed resistance to antibiotics ampicillin & erythromycin (100%), kanamycin (33%), tetracycline (33%), novobiocin (93%), rifampicin (80%) and sulphadiazine (93%). Pal and Bhattacharjee (2011) [23] isolated *Aeromonas* sp. from different sources of water body of Darjeeling and Jalpaiguri districts of West Bengal and their investigation revealed the antibiotic resistance to ampcillin (70%), chloramphenicol (6%), tetracycline (34%), kanamycin (13%), gentamycin (3%) and co-trimoxazole (42%). In the present investigation, all the *Aeromonas* isolates from both the locations were resistant to β-lactem antibiotic, ampicillin (Figure 1). High resistance to β-lactem antibiotics in

Aeromonas has been confirmed by previous two studies along with other investigations (Gomes *et al*.,), posing a serious threat to public health throughout the world. Presence of unstable β-lactem ring in the structure of β-lactem antibiotics is degraded by β lactamases produced by *Aeromonas* bacteria (Goni-Urriza *et al*., 2000) [18]. All the isolates were sensitive to antibiotics gentamycin and novobiocin and only isolate was resistant to kanamycin.

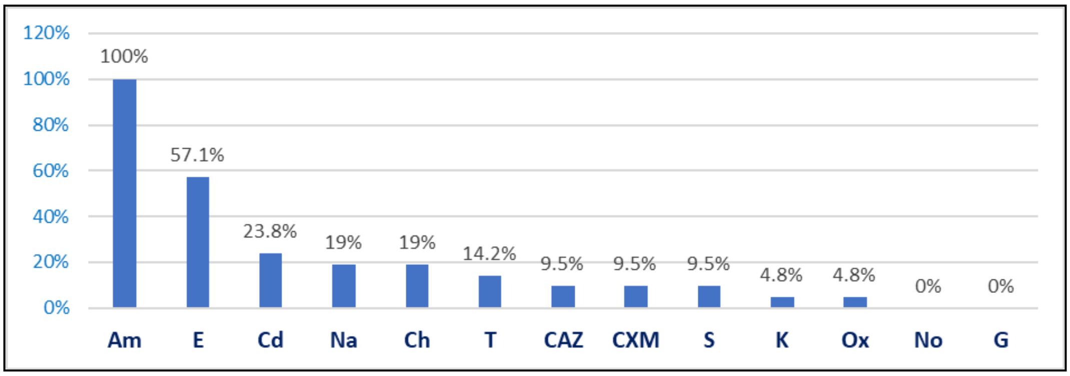

Fig 1: Percentage of resistance to different antibiotics by twenty-one *Aeromonas* isolates collected from location L1 & L2

Erythromycin resistant was found in 57.1% of the isolates (Figure 1). This finding deviated from the results of the former two studies in which resistance to four antibiotics mentioned above was comparatively high. In the present work, nalidixic acid resistance was observed by 19.04% bacterial isolates but in the earlier two studies all the isolates were sensitive to nalidixic acid. First generation Cephalosporin, cephlalothin resistance was detected in 19.04% of the isolates whereas, a second-generation cephalosporin, cefuroxime and third generation, ceftazidime was measured in 9.52% of the isolates (Figure 1). This finding indicated the chance of severe use of this group of antibiotics in this region. Antibiotic resistance profile of *Aeromonas* sp. isolated from different river water with varying altitude were evaluated in Darjeeling district of West Bengal (Bhowmick and Bhattacharaya, 2017) [6], suggesting high resistance to ampicillin, ceftazidime, cefurozime, clindamycin, cephalothin and nalidixic acid. The antibiotic resistance profile observed in the present work is consistent with the previous investigation, indicating the overuse of these antibiotics along with the reduced use of kanamycin, gentamycin and novobiocin in this area.

Conclusion

Aeromonas are ubiquitous microorganisms with a wide host spectrum and becoming a serious concern to the present-day medical science. In the present work, multi-drug resistant *Aeromnas* bacteria were recovered from the polluted river water passing through the congested city area. High anthropogenic activities in the catchment area of the river make it vulnerable to acquire multi-drug resistance isolates as these bacteria are widespread in all types of water bodies and are commonly associated with many aquatic vertebrates including humans. Resistance to some higher generation antibiotics was observed in the present work which means their use increased with time. It is a high time to use antibiotics judiciously or find out an alternative way to fight infection otherwise we have to face a terrible medical crisis in the days to come.

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