International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
(A Peer Reviewed, Referred, Indexed and Open Access Journal)
DOI: 10.22192/ijarbs Coden: IJARQG (USA) Volume 9, Issue 6 -2022

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2022.09.06.022

Studies on antibiotic sensitivity and plasmid profiles in *Aeromonas* bacteria isolated from urban sewage system in the Northern part of West Bengal

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Abstract

The genus *Aeromonas* are oxidase-positive, facultatively anaerobic, glucose fermenting, Gram negative bacilli that belong to the family Aeromonadaceae. These are distributed universally in fresh-water environments and isolated from clinical, environmental and food samples, where they can grow even at low temperatures. Different workers across the globe have been detecting *Aeromonas* bacteria from various infection sites of cold and warm-blooded animals, including human. In the present study, twenty one *Aeromonas* bacterial strains were isolated from four different locations of an urban sewage system in the northern part of West Bengal and later on, the antibiotic sensitivity and plasmid profiles of these bacteria were investigated. Out of these twenty one bacterial isolates, nineteen were found to be *Aeromonas hydrophila* and two belonged to *Aeromonas caviae*. Antibiotic sensitivity of all the bacterial isolates were tested and it was found that all the isolates were resistant to ampicillin and sensitive to co-trimoxazole. 71.4% strains were resistant to erythromycin, 23.8% strains were resistant to clindamycin and 14.2% strains were resistant to nalidixic acid and cephalothin. Plasmid profiles of these bacteria were also investigated and it was found that five A*eromonas hydrophila* isolated from a particular location were carrying a low molecular weight plasmid with 15kbp in length. Our findings focus on the prevalence of antibiotic resistance of *Aeromonas* isolates and their acquisition of Plasmid DNA from urban sewage water.

Keywords: *Aeromonas,* bacteria, antibiotic resistant, plasmid

Introduction

Aeromoas are oxidase- positive, Gram negative bacilli that are widespread in aquatic environment and considered to be a threat to aquaculture systems (Barger *et a*l., 2021). These are increasingly being reported as primary or secondary pathogens of different lower vertebrates like fish, amphibians and reptiles (Guz *et al*., 2021). These bacteria have a broad host range and been implicated in the cause of human gastroenteritis and wound infections arising out of a direct contact with contaminated soil and water (Janda and Abbott, 2010; Faranandez-Bravo and Figueras, 2020).

Application of antibiotics as chemotherapeutants since 1930s has achieved a great success in medicine against bacterial infections. However, the usage of antibiotics has become quite rampant globally and resulted in an unavoidable public health problems of modern ages by generating multi drug resistant microorganisms. Wastewater effluents from different sources facilitate the aquatic environment more susceptible to the spreading of multi drug resistant pathogens (Baaquero*et al*., 2008; Baron *et al*., 2017). The genus *Aeromonas* being a natural inhabitants of these aquatic ecosystems, exhibits wide range of antibiotic resistance profiles and have isolated from various sources such as sewage, soil, vegetables and processed food apart from human and other lower vertebrates (Goncalves Pessoa *et al*., 2019;Barger *et al*.,2021). The *Aeromonas* bacterial isolates collected from different sources harbor wide range of antibiotic resistance determinants and share these genes by transmitting mobile genetic elements (MGE) like plasmids, integrons, insertion sequence and transposons ((Piotrowska and Piotrowska, 2015;Stratev and Odeyemi, 2016).

The present study has attempted to isolate and identify the *Aeromonas* bacteria from the sewage water of a particular area and at the same time, their antibiotic susceptibility and plasmid profiles have been assessed.

Materials and Methods

Isolation and biochemical identification of bacteria

Twenty one *Aeromonas* bacteria were isolated from the sewage water of four different locations in the Siliguri Sub division of West Bengal, India.*Aeromonas* isolation medium supplemented with *Aeromonas* –selective supplement (HiMedia Laboratories, Mumbai, India) was used to obtain *Aeromonas* isolates from the four different water samples collected in sterile bottles using the pour plate method.

The presumed *Aeromonas* isolates were confirmed using Gram stain, test for oxidase and catalase and growth in agar with 0% but not in 6% NaCl by standard methods (Carnahan and Joseph 2005). The isolates were identified to species level using traditional biochemical methods that included tests for 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).

Antibiotic susceptibility test by disc diffusion

The antibiotic susceptibility of each isolate measured by the disc diffusion method. Muller-Hinton agar plates (9 cm diameter) were inoculated with 0.1 ml of 18 hrs old culture of test bacterium in nutrient broth. The antimicrobial discs (HiMedia Laboratories) were applied on the bacterial culture plates and incubated at 30°C for overnight. Discs of commonly used antibiotics such as ampicillin (10 µg), erythromycin(15 µg), cephalothin (30µg), clindamycin (2µg), cefotaxime (30µg), cefuroxime (30µg), tetracycline (30µg), nalidixic acid (30µg), streptomycin (10µg), ofloxacin (1µg), co-trimoxazole (25µg)and norfloxacin (10 µg) were used. The zone of inhibition were measure dusing scale provided by the manufacturer and zone diameters were interpreted as sensitive(S), intermediate (I) or resistant(R) based on manufacturer’s instructions.

Plasmid isolation

Plasmids were isolated from bacterial cultures grown overnight at 30°C in nutrient broth, with a mini preparation kit (GeNei India) according to manufacturer’s instructions. The plasmid were electrophoresed on 0.8% agarose gel electrophoresis along with a molecular weight marker (supercoiled DNA ladder, SBS Genetech) and visualized after staining with 0.2% ethidium bromide.

Results and Discussion

In the present investigation, twenty one *Aeromonas* bacteria (E1 to E21) were isolated from four different location of sewage water (L1 to L4). Biochemical analysis suggested that among them nineteen were *Aeromonas hydrophila* and two belonged to *Aeromonascaviae***.** (Table. 1)

Table 1. Bacterial isolates collected from four different locations of sewage water

Location	Isolate number	Isolate identity
L1(Near NJP outpost)	E1	*Aeromonas hydrophila*
	E2	*Aer. hydrophila*
	E3	*Aer. hydrophila*
	E4	*Aer. hydrophila*
	E5	*Aer. hydrophila*
L2(Darjeeling more)	E6	*Aer. hydrophila*
	E7	*Aer. hydrophila*
	E8	*Aer. hydrophila*
	E9	*Aer. hydrophila*
	E10	*Aeromonas caviae*
	E11	*Aer. caviae*
L3(Matigara)	E12	*Aer. hydrophila*
	E13	*Aer. hydrophila*
	E14	*Aer. hydrophila*
	E15	*Aer. hydrophila*
	E16	*Aer. hydrophila*
L4 (Near Siliguri Hospital)	E17	*Aer. hydrophila*
	E18	*Aer. hydrophila*
	E19	*Aer. hydrophila*
	E20	*Aer. hydrophila*
	E21	*Aer. hydrophila*

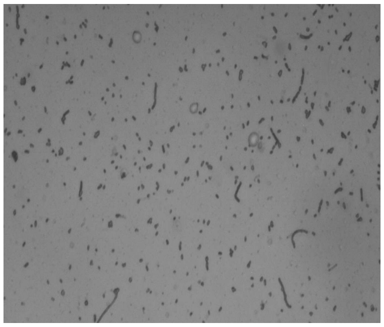

Fig. 1.*Aeromonas caviae*, E10 (X400)

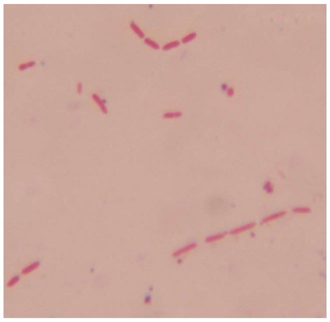

Fig. 2. *Aeromonas hydrophila*, E2 (X1000)

The Antibiotic resistance pattern 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 co-trimoxazole. 71.4% isolates were resistant to erythromycin.23.8% isolates were resistant to clindamycin. Three isolates were resistant to nalidixic acid cephalothin. Two isolates were resistant to cefuroxime, cefotaxime, norfloxacin and tetracycline. Only one isolate was resistant to ofloxacin.(Table 2).

Table 2. Antimicrobial resistance and plasmid profiles in *Aeromonas* isolates collected from sewage water of different locations

Isolates	Antimicrobial resistance	Plasmid sizes (kbp)
E1	Am,E, Na, T	-
E2	Am, E, Na	-
E3	Am,E(I) Nx	-
E4	Am, E, CTX	-
E5	Am, E, CTX	-
E6	Am, CXM	-
E7	Am, CXM	-
E8	Am,E(I)	-
E9	Am,E, Cd	-
E10	Am,E, Cd	-
E11	Am,E, Cd	-
E12	Am, E	15
E13	Am, E	15
E14	Am, E, Ch, Cd	15
E15	Am, E, Ch	15
E16	Am, E, Ch, Cd	15
E17	Am, E(I), Nx	-
E18	Am, E(I), OF	-
E19	Am, E, S	-
E20	Am, E, S, Na	-
E21	Am, E, T	-

Am, Ampicillin; E, Erythromycin; Na, Nalidixic acid; T, Tetracycline, Nx, Norfloxicin; CTX, Cefotaxime; CXM, Cefuroxime; Cd, Clindamycin Ch, Cephalothin; OF, Ofloxacin; S, Streptomycin, I - Intermediate

Drug resistance in genus *Aeromonas* isolated from environmental samples have been reported earlier (Galler *et al*., 2018; Conte *et al.,* 2021).Being residents of aquatic ecosystems, these microorganisms can be used as ecological indicators of water pollution as they possess antibiotic resistance genes obtained from wastewater effluents(Baron *et al*.,2017;Grilo*et al.,* 2020). In the present investigation, a wide spectra of antibiotic resistance have been detected in *Aeromonas* bacterial isolates collected from different locations of sewage system. Out of thirteen antibiotics tested, twelve were found to be resistant to *Aeromonas* bacteria isolated from different locations. Only antibiotic co-trimoxazole was found to be sensitive to all the twenty one bacterial isolates. Earlier, antibiotic resistance profiles of *Aeromonas* bacteria, isolated from different river water body with varying altitudes were evaluated in the Darjeeling districts of West Bengal (Bhowmick and Bhattacharya, 2017). The present study confirmed the antibiotic resistance pattern of the earlier findings because ten antibiotics tested in both the studies showed similar result. The resemblance in both the findings indicated the indiscriminate use of such antibiotics in this region.

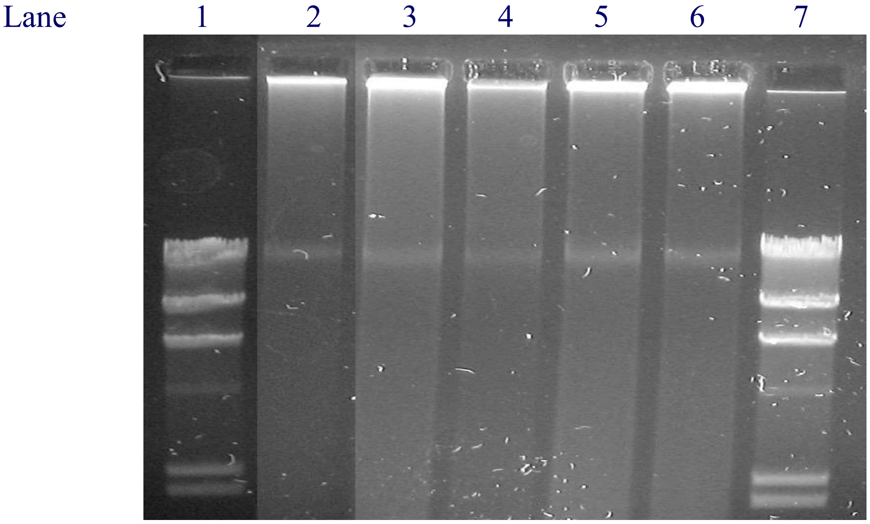

Fig. 3 Plasmid profiles in the *Aeromonas* bacteria isolated from Location 3 in the sewage water; Lane 1 and 7: supercoiled DNA ladder, 2: E12, 3: E13, 4: E14, 5: E15, 6: E16

In the present study, five *Aeromonas hydrophila* isolates collected from location L3, harbor a low molecular weight plasmid of 15kbp (Fig.1)All the five isolates are resistant to ampicillin and erythromycin. Three among them are resistant to cephalothin and two to clindamycin. Das et al(Das *et al*, 2009) detected 23 and 64 kbp plasmids from the *Aeromonas* bacteria isolated from the ulcers of the Epizootic Ulcerative Syndrome (EUS) affected fish and successfully transferred these plasmids to the susceptible species in vitro. Marti and Balcazar (2012) completely sequenced a26.6 kbp plasmid (pP2G1) of *Aeromonas* sp collected from environmental isolates, which conferred resistance to β-lactems, aminoglycosides and quinolones, chloramphenicol, macrolides, rifampin and sulphonamides. *Aeromonas hydrophila* isolates harboring 15kbp plasmid with varying antibiotic susceptibility profiles indicates the possibility of conferring antibiotic resistant determinants or other virulence factors

Conclusion

Aeromonas are ubiquitous microorganisms and more prone to acquire multi-drug resistance, representing a threat to human health in future. Our findings contribute to highlighting the prevalence of antibiotic susceptibility of *Aeromonas* bacterial isolates in a particular region, depicting the widespread use of such antimicrobial agents in that area. Our study also assessed the plasmid profiles of *Aeromonas* bacteria collected from different locations of sewage water. The further characterization of these plasmids may enlighten their role as a vehicle of antibiotic resistant determinants or virulence factors to other susceptible species by horizontal gene transfer.

Acknowledgment

The author is thankful to the Fish Biology Laboratory, Department of Zoology, University of North Bengal, WB, India and Department of Zoology, Burdwan Raj College, WB, India for providing research facilities and resources.

Conflict of internet

The author declares no conflict of interest

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DOI:10.22192/ijarbs.2022.09.06.022

How to cite this article:
Arindam Das. (2022). Studies on antibiotic sensitivity and plasmid profiles in *Aeromonas* bacteria isolated from urban sewage system in the Northern part of West Bengal. Int. J. Adv. Res. Biol. Sci. 9(6): 190-196.
DOI: http://dx.doi.org/10.22192/ijarbs.2022.09.06.022