

A Comparative Study of Antibiotic Resistance in Bacterial Isolates from Industrially Polluted and Unpolluted Water

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Abstract: The antibiotic resistance shown by bacterial isolates from aquatic sources was studied. Resistance towards ampicillin was higher compared to the other antibiotics viz. amikacin, chloramphenicol, gentamicin and tetracycline. Compared to the isolates from unpolluted water sources the isolates from industrially polluted water showed higher incidence of resistance towards antibiotics such as ampicillin, chloramphenicol, gentamicin and tetracycline. Antibiotic resistance pattern of the isolates was compared. Incidence of multiple drug resistance to three or more drugs was more in isolates from polluted water. The results suggest that industrially polluted water bodies can act as reservoirs of multidrug resistant bacteria from where they can spread to the environment.

Key words: Antibiotic resistance, industrially polluted, unpolluted, multiple drug resistance.

Introduction

The major industrial water pollutants include a wide range of chemicals, metals and salts. Contamination of water with the pollutants can favour the selective proliferation of pollutant resistant microorganisms in the aquatic environment. Hence it can be supposed that the presence in excess of heavy metals or other pollutants in water can support the proliferation of those bacteria which are resistant to them. It is likely that the plasmids responsible for resistance towards these pollutants such as heavy metals can code antibiotic resistance also as in the case of *Staphylococcus aureus* (Richmond, 1972). Antibiotic resistance is a serious concern of clinicians worldwide. Use and misuse of antibiotics for many decades have resulted in the spread of multidrug resistant bacteria in the natural ecosystems. So screening for antibiotic resistant bacteria in the natural ecosystems is relevant, and worldwide studies have been carried out to

identify the environmental reservoirs of bacterial antibiotic resistance (Goni-Urriza et al., 2000). For the above mentioned reason one of the possible natural reservoir of antibiotic resistant bacteria can be industrially polluted water. It has been reported that multi-drug resistant coagulase negative *Staphylococcus* might constitute a reservoir for disseminating antibiotic resistance into the community (Kessie et al., 1998). Based on this assumption a study was carried out to compare incidence of drug resistance in bacterial isolates collected from industrially polluted and unpolluted water samples.

Materials and Methods

Water Samples

Samples of industrially polluted and unpolluted water were collected from different places in Kottayam, a district in Kerala, India. The industrially polluted samples were collected from two different rivers, one

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continuously receiving waste water from a newsprint factory and the other from the nearby metal industries. Samples were collected from the vicinity of the wastewater outlets from the factories.

Isolation of Bacteria

Bacteria were isolated from water by pour-plating in nutrient agar. The plates were incubated at room temperature ($30 \pm 4^\circ\text{C}$) for 48 hours. Colonies obtained were random selected, picked and purified by streaking on nutrient agar plates. The isolates were maintained on nutrient agar slants under refrigerated condition.

Identification of Bacteria

The bacterial isolates were tentatively identified by studying cultural, morphological and physiological and biochemical properties. Guidelines in the Bergey's Manual of Systematic Bacteriology (Holt, 1984) were followed.

Antibiotic Susceptibility Tests

Antibiotic sensitivity of the isolates was tested by Kirby-Bauer disc diffusion method (Buer et al., 1966). The Mueller-Hinton agar plates were lawn inoculated with overnight incubated bacterial broth cultures. The commercially available antibiotic discs (Himedia) were then placed on the plates. The antibiotics discs used and

their disc content were: ampicillin, 30 μg ; ampicillin, 10 μg ; chloramphenicol, 30 μg ; gentamicin, 10 μg ; gentamycin, 10 μg and tetracycline, 30 μg . The plates were incubated for 48 hours at room temperature ($30 \pm 4^\circ\text{C}$) and examined for the zone of clearance around each disc. The diameter of zone of clearance was noted and the results were interpreted using standard interpretative chart provided by Himedia.

Results and Discussion

Of the fifty bacterial isolates from aquatic sources, 25 were from the polluted and the other 25 were from the unpolluted water sources. Resistance towards different antibiotics in bacterial isolates from aquatic sources is given in Table 1. Resistance towards ampicillin was higher compared to the other antibiotics viz. amikacin, chloramphenicol, gentamicin and tetracycline. Table 2 shows comparative analysis of incidence of antibiotic resistance in isolates from polluted and unpolluted water samples. Compared to the isolates from unpolluted water sources the isolates from industrially polluted water showed higher incidence of resistance towards antibiotics such as ampicillin, chloramphenicol, gentamicin and tetracycline. Antibiotic resistance patterns shown by the isolates from polluted and unpolluted water samples are given in Table 3.

Table 1: Antibiotic resistance shown by different bacteria isolated from aquatic environments

Name of bacteria	No. of isolates	Number of isolates resistant to different antibiotics*				
		A	Ak.	G	C	T
<i>Bacillus</i> spp.	13	10	2	1	0	3
<i>Aeromonas</i> spp.	9	6	3	0	3	2
<i>Staphylococcus</i> spp.	8	7	3	2	2	2
<i>Micrococcus</i> spp.	4	4	1	2	3	0
<i>Enterococcus</i> spp.	3	1	2	0	1	1
<i>Citrobacte</i> spp.	2	1	0	0	0	1
Unidentified	11	3	0	0	0	1

* A: Ampicillin, Ak: Amikacin, C: Chloramphenicol, G: Gentamicin, T: Tetracycline.

Table 2: Incidence of resistance towards different antibiotics in bacterial isolates from polluted and unpolluted water samples

Sl.No.	Antibiotics	Total number of isolates resistant to the drug	Source of isolates	
			Unpolluted water	Polluted water
1	Ampicillin	39	17	22
2	Amikacin	14	11	3
3	Chloramphenicol	9	2	7
4	Gentamicin	11	0	11
5	Tetracycline	28	13	15

Table 3: Antibiotic resistance pattern of isolates from polluted and unpolluted water samples

Sl.No.	Antibiotic resistance pattern	No. of isolates showing the pattern	Source	
			Unpolluted water	Polluted water
1	Resistant to none of the drugs	6	3	3
2	Resistant to one drug	9	7	
3	Resistant to two drugs	12	7	5
4	Resistant to three drugs	12	4	8
5	Resistant to four drugs	7	1	6
6	Resistant to five drugs	4	1	3

Resistance to three or more drugs was more among isolates from polluted water samples compared to those from unpolluted water samples. These results show the higher incidence of multiple drug resistance in bacterial isolates from polluted water sources compared with the isolates from unpolluted water sources. Hence it can be assumed that there may be some factors favouring the proliferation of multidrug resistant bacteria in the polluted water systems. It can be either antibiotics themselves or the pollutants present in the water which are acting on bacteria somewhat like some antibiotics. It was noted that the water systems studied were not polluted with sewage, hospital wastes etc. So the presence of the antibiotics in the environment cannot be suggested as the factor favouring the selective proliferation of multidrug resistant bacteria. However the sites of collection of polluted water samples seemed to be grossly polluted with the industrial wastes which may be containing pollutants including heavy metals.

It is known that in many bacteria, multiple drug resistance is plasmid mediated, and these plasmids can code for heavy metal resistance also. So it can be suspected that the possible presence of heavy metals in the polluted water may be a reason for the selective proliferation of multidrug resistant bacteria. There can be a chance for the presence of some pollutants possibly mimicking the action of some antibiotics. If so the bacterial population sensitive to these pollutants will decline which can eventually result in the selective proliferation of the pollutant resistant population, which can be resistant to some antibiotics also. To analyse the role of these factors in the decline of antibiotic sensitive population and the selective proliferation of multidrug resistant bacteria a detailed study on the effects of various industrial pollutants on bacteria, incidence of heavy metal resistance in the isolates, coexistence of heavy metal resistance and multiple drug resistance in the isolates and the plasmid profiles of these isolates are essential. Detailed studies on these aspects are progressing in our laboratory.

Conclusion

It is concluded that the incidence of multidrug resistant bacteria is higher in the isolates from polluted water compared to the isolates from unpolluted water. It can be suggested that the industrially polluted water bodies can act as reservoirs of multidrug resistant bacteria from where they can spread to the environment raising potential threat of making the other bacteria also antibiotic resistant by the transfer of drug resistance. Hence it is opined that the factors responsible for the higher incidence of multiple drug resistance among bacteria isolated from the industrially polluted water are to be identified and proper measures are to be taken for the remediation during the process of treatment of industrial effluents right before they are discharged to the water bodies.

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Asian Journal of Water, Environment and Pollution



Aims and Scope

Asia, as a whole region, faces severe stress on water availability, primarily due to high population density. Many regions of the continent face severe problems of water pollution on local as well as regional scale and these have to be tackled with a pan-Asian approach. However, the available literature on the subject is generally based on research done in Europe and North America. Therefore, there is an urgent and strong need for an Asian journal with its focus on the region and wherein the region specific problems are addressed in an intelligent manner. In Asia, besides water, there are several other issues related to environment, such as; global warming and its impact; intense land/use and shifting pattern of agriculture; issues related to fertilizer applications and pesticide residues in soil and water; and solid and liquid waste management particularly in industrial and urban areas.

Asia is also a region with intense mining activities whereby serious environmental problems related to land/use, loss of top soil, water pollution and acid mine drainage are faced by various communities.

Essentially, Asians are confronted with environmental problems on many fronts. Many pressing issues in the region interlink various aspects of environmental problems faced by population in this densely habited region in the world. Pollution is one such serious issue for many countries since there are many transnational water bodies that spread the pollutants across the entire region. Water, environment and pollution together constitute a three axial problem that all concerned people in the region would like to focus on.

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