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Antibiotic Resistance Pattern in Bacterial Isolates Obtained from Different Water Samples of Delhi Region

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ABSTRACT

A major issue in anti-bacterial therapy is the development of resistance in the otherwise sensitive strains of bacteria. Exposure of bacteria to low doses of an antibiotic naturally selects for the survival of resistant organisms. The spread of these resistant strains of pathogenic bacteria from environment poses problems to treat such infections. In order to understand the ecology and evolution of antibiotic resistance in our immediate environments, the present study was undertaken to isolate, identify the hotspots of antibiotic resistant bacteria and their patterns of antibiotic resistance against a panel of 8 representative antibiotics belonging to different classes. The samples were collected from wastewater treatment plants including inflow (untreated) and outflow (treated), ground water, surface water (Yamuna River and lake) and drinking water from different areas of Delhi. From each water sample, bacterial isolates were analyzed for their antibiotic resistance profile. Among 224 isolates 34 strains were found to be resistant against 3 or more antibiotics (multi drug resistant). The Ampicillin resistance was found in maximum number of isolates and none of the isolates showed resistance to Ofloxacin. A *Pseudomonas* sp strain was isolated which showed resistance to five out of eight antibiotics tested.

Keywords: Multidrug resistance, antibiotic resistance bacteria, wastewater, water sources, *Pseudomonas sp.*

INTRODUCTION

Antibiotics are an important group of therapeutic agents used for the treatment of bacterial infections. The discovery and use of antibiotics led to a revolution in medical biology and an increase in agricultural productivity. However, this wide and intensive use of antibiotics has lead to their continuous release in the environment (1,2). A sufficient amount of the antibiotics also keeps accumulating in the environment in the un-metabolized form. This leads to large residues of antibiotics in the recipient waters.

Paradoxically, this use of antibiotics led to appearance of organisms that can evade them, the so called antibiotic resistant bacteria (ARB) which commonly emerge in areas where antibiotics are commonly used. (3). Antibiotic resistance is a natural phenomenon and represents an evolutionary response to the strong selective pressure resulting from exposure to these compounds. (4). Overtime bacterial resistance to all classes of antibiotics has emerged. Many studies have reported the presence of bacteria which are multi drug resistant strains or MDR (5) especially in the hospital acquired infections (6). The emergence of antibiotic resistant bacteria especially MDR, thus, poses serious health problems to humans and has become a global problem.

The major targets for antibiotics are structural proteins, genes or signaling molecules that are essential for bacterial survival such as cell wall, cell membrane, protein synthesis, folic acid metabolism, DNA and RNA synthesis. Resistant bacteria have evolved various mechanisms such as target modification, efflux, escape from immune surveillance of host, and enzyme catalyzed destruction to escape the effects of antimicrobial agents.

Among the water sources, wastewater treatment plants are one of the major source of antibiotic release into the environment (7, 8).The abundant presence of organic, inorganic matter and living organisms in wastewater reserves (9) favors the spread of antibiotic resistance. Antibiotics help in selection of antibiotic resistance genes (ARGs) (10) and Antibiotic resistant bacteria (ARBs). ARBs have been found to be increasing in aquatic environment (11). The antibiotic resistance can be transferred vertically from a bacterium to its progeny. While some genes can also be transmitted horizontally to other bacteria. The set of genes responsible for horizontal transfer are generally plasmid encoded. The transfer of ARG in the environment via horizontal transfer mainly contributes to evolution and thus emergence of ARB (12). In aquatic systems this cycle includes subsequent transmission of antibiotic resistance to human associated bacteria (13).

This global problem of antibiotic resistance is particularly prevailing in developing countries like India where the bacterial load is high due to poor hygiene. The easy availability of antibiotics sometimes without a medical prescription has caused their widespread misuse. Hence, today there is a strong and serious need to identify the sources/hotspots of antibiotic resistant bacteria in aquatic environment which was the emphasis of the present study. Since many infections are also water borne, local antibiotic resistance surveillance was also done. Isolation of antibiotic resistant bacteria and the extent of their antibiotic resistance was evaluated in this study in water samples from 14 different locations of water sources in Delhi region.

METHODOLOGY

Sample collection: The water samples for the present study were collected from the various locations in Delhi, India viz. treated and untreated sample from Industrial and domestic wastewater Treatment Plants, Ground water, Surface water (from three sites of Yamuna river, lake water), Drinking water (tap water and street vendors selling portable non-bottled water). The bottled water was kept as negative control. Each water sample was collected in screw capped sterile glass bottles. The bottles were labeled with the details of source, date and time of collection. The bottles were carried in icebox and brought to the laboratory and stored in refrigerator until analysis.

Evaluation of bacterial load: Enumeration of bacterial colonies was done using spread plate technique. The samples were serially diluted and spread on nutrient agar plates. The number of bacterial colonies obtained were counted after overnight incubation at 37°C. The final results were expressed as colony forming unit (cfu/ml).

Antibiotic resistance determination: Sixteen isolated colonies were randomly picked from spread plates of each water sample tested. Using these colonies master plates were prepared and stored for further analysis. These selected bacterial colonies were screened for their sensitivity/resistance to eight different antibiotics by Kirby-Bauer disk diffusion method (14) on Mueller Hinton Agar. Following eight antibiotics representing different antibiotic classes were used in this study: Amikacin (30mcg), Cefuroxime (30mcg), Co-Trimoxazaole (25mcg), Ampicillin (10mcg), Nitrofurantoin (300mcg), Meropenem (10mcg), Tetracycline (30mcg) and Ofloxacin (5mcg). The antibiotic discs were obtained from Himedia, India. In this method, overnight grown broth culture was swabbed using pre-sterilized swabs on Mueller Hinton Agar plates and antibiotic impregnated disk was placed on it. This was incubated overnight at 37°C. Plates were observed for a clear zone of growth inhibition and results were interpreted using reference table. The bacterial strains which showed resistance to two or more antibiotics (multidrug resistant) were further studied and classified based on their Gram staining character, and biochemical test results.

MAR index calculation. Multiple Antibiotic Resistance or MAR index was determined as the Number of antibiotics to which a isolate is resistant/ total number of antibiotics tested as described by (15).

RESULTS

In the present study, 14 water samples from different sources were assessed for their bacterial load on nutrient agar plates. The ground water sample from the south Delhi region gave the highest cfu/ml value (Table I) of 4.8×10^{12}. Out of these isolates,16 isolates/water sample were randomly picked and characterized for the antibiotic resistance against eight representative antibiotic belonging to different classes (Table II).Out of a total of 224 isolates representing 14 water samples, 76 isolates showed resistance to one antibiotic candidate while 45 isolates showed resistance to two antibiotics (Figure I). A total of 34 isolates showed resistance to three or more antibiotics belonging to different classes i.e., were Multi Drug Resistant (MDR). Among the MDR isolates, the Ampicillin resistance was found in maximum number of isolates (88%) followed by Cefuroxime (85%), Nitrofurantoin (74%) Co-Trimoxazole (50%), tertracycline (9%). Both Meropenem and Amikacin resistance was found in 6% of isolates (Figure II). None of the isolates in the present study showed resistance to Ofloxacin, apparently the emergence of its resistance seems to be the lowest in the samples tested under this limited study. Among the MDR strains, 24 isolates were Gram positive and 11 isolates were Gram negative.

The ground water collected from different sites in West and South Delhi were compared. The different regions showed variation in occurrence of antibiotic resistance. As shown in Figure I, isolate GW 47 was sensitive to all the antibiotics tested. GW44 showed maximum number of isolates showing resistance against one antibiotic. One isolate of ground water sample of south Delhi region was identified as *Pseudomonas sp*. and it showed resistance against 5 antibiotics, Ampicillin, Co-Trimaxozole, Cefuroxime, Nitrofurantoin and tetracycline.

Table I: Enumeration of bacterial load in different samples of water.

NAME OF SAMPLE	Location	Cfu/ml

GWC	Ground Water South Delhi	4.8×10^{12}
GW44	Ground Water West Delhi Site-I	1.3×10^{7}
GW45	Ground Water West Delhi Site -II	9.3×10^{8}
GW 47	Ground Water West Delhi Site -III	5.0×10^{4}
JPR	Lake water Delhi	3.4×10^{4}
YRW-1	Yamuna River Water Site –I	-
YRW-2	Yamuna River Water Site –II	3.8×10^{12}
YWK	Yamuna River Water Site -III	2.2×10^{8}
Tap Water (TWC)	South Delhi	1.4×10^{8}
Machine Water (MW)	VENDOR	2.1×10^{4}
MTP-1 –Untreated	Industrial Waste water, West Delhi	1.5×10^{6}
MTP-2–Untreated	Industrial Waste water, West Delhi	1.5×10^{5}
MTP-3–Chemically treated	Industrial Waste water,, West Delhi	6.3×10^{6}
MTP-4–Physically treated	Industrial Waste water,, West Delhi	1.4×10^{8}
OW	Domestic Waste water,, East Delhi	2.9×10^{8}

Table-II: Mechanism of action of the selected Antibiotics

Name of Antibiotics	Class of Anibiotic	Mechanism of Action
Amikacin	Aminoglycoside	Blocks protein synthesis
Ampicillin	Penicillin	Inhibits cell wall synthesis
Co-Trimaxazole	Sulfonamide	Inhibits folic acid synthesis
Cefuroxime	cephalosporin	Inhibition of cell wall synthesis
Meropenam	Carbapenam	Inhibition of cell wall synthesis
Nitrofurantoin	Nitrofuran	Damages bacterial DNA
Ofloxacin	Quinolone	Inhibits cell division
Tetracycline	Polyketaide	Inhibits protein synthesis

The Physical and chemical treatment of waste water, did not seem to lower the frequency of antibiotic resistance in samples obtained from industrial waste water treatment plants as evident from the results in Table III. Such studies have been reported (16) in the past but still there is lack of information on this aspect. The treated water from domestic wastewater treatment plant (OW) in East Delhi also showed the presence of MDR strains. Both untreated and treated waste water samples showed MDR strains.

The surface water sample of a lake in Delhi showed 6 MDR strains out of 16 isolates tested. The YRW1 sample obtained from a site of Yamuna river, showed 3 MDR strains, however the CFU/ml could not be evaluated due to excessive growth of Bacilli. YRW2 showed only one MDR isolate. Among Yamuna water samples, the maximum number of MDR isolates were obtained in YWK sample. Other previous studies showed antibiotic resistance status of Yamuna water isolates and in Gomti river (17, 18).

Of the drinking water samples, the tap water sample obtained from site in south Delhi, showed only one MDR isolate. In all there were six isolates showing resistance to one antibiotic. The drinking water sample obtained from vendor, showed 3 MDR isolates. Five isolates showed resistance to one antibiotic and 8 showed resistance to two antibiotics.
MAR index value of all the samples was higher than 0.2 and that of the ground water sample (GWC) was exceptionally higher with a value of 0.625 (Table III). These observations indicate a high risk (19).

Table-III: MAR Index values for the isolates obtained from different water samples

S.NO.	WATER SAMPLE SOURCE	MDR ISOLATE NUMBER	MAR INDEX
	GROUND WATER	GWC/2	0.625
		GWC/4	0.500
		GWC/15	0.375
		GWC/16	0.500
		GW-44/11	0.375
	SURFACE WATER	JPR/1	0.375
		JPR/3	0.375
		JPR/4	0.250
		JPR/6	0.375
		JPR/9	0.375
		JPR/13	0.375
		YRW-2/4	0.375
		YRW-2/5	0.375
		YRW-2/13	0.500
		YRW-2/15	0.375
		YWK/2	0.375
		YWK/3	0.500
		YWK/10	0.375
		YWK/13	0.500
		YWK/14	0.375
	DRINKING WATER	TW/7	0.375
		MW/4	0.375
		MW/5	0.500
		MW/15	0.375
	WASTE WATER	MTP-1/1	0.375
		MTP-1/3	0.375
		MTP-2/6	0.375
		MTP-3/10	0.375
		MTP-3/13	0.375
		MTP-3/15	0.375
		MTP-4/7	0.375
		OW/1	0.375
		OW/5	0.250
		OW/10	0.375

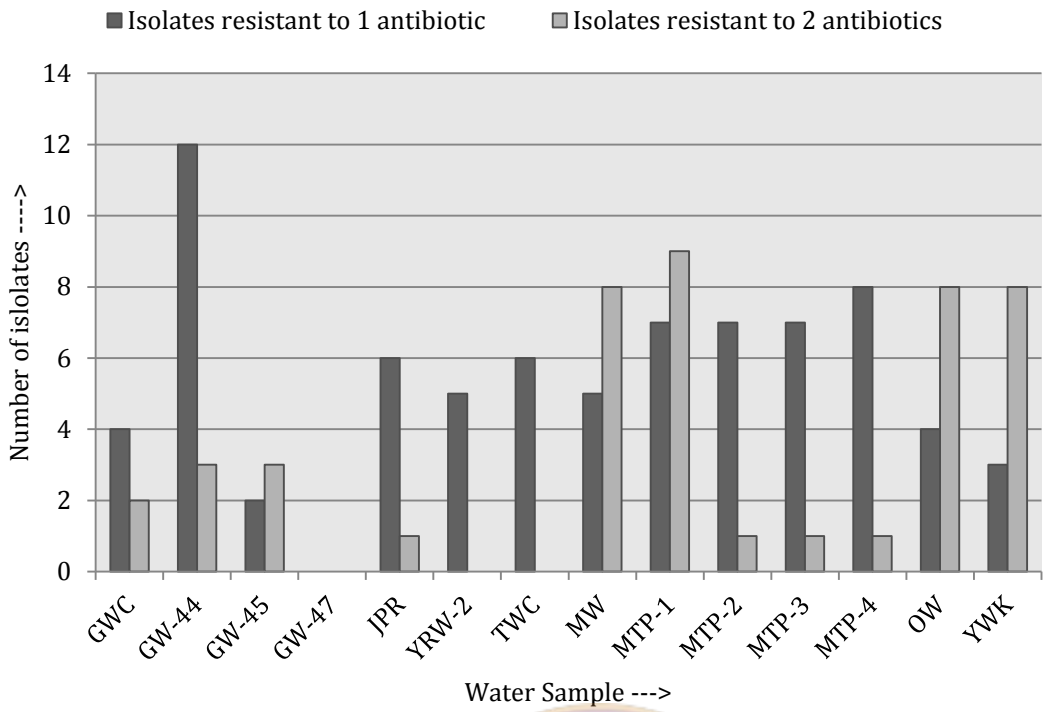

Figure-I: Resistance pattern of bacterial isolates from different water samples

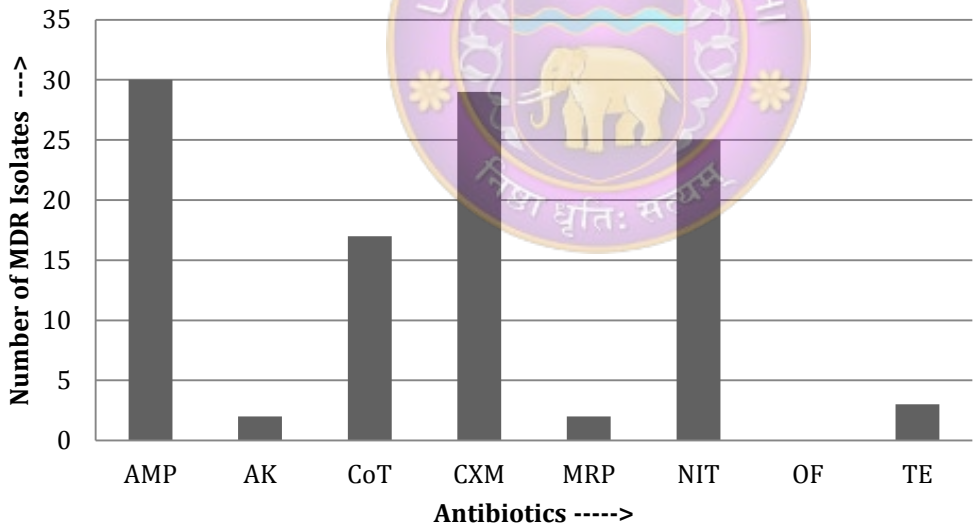

Figure II: Antibiotic Resistance pattern of MDR isolates from different water samples. AMP (Ampicillin), AK(Amikacin), CoT(Co-Trimoxazaole), CXM (Cefuroxime), MRP (Meropenem), NIT(Nitrofurantoin), OF(Ofloxacin) and TE(Tetracycline)

DISCUSSION

Multi drug resistance is increasing at an alarming rate among bacteria. During recent years, the occurrence of antibiotic resistant strains in aquatic environments has been studied by various authors (20, 21). In the current study, we analyzed water samples from different sources of Delhi region. Of the 224 isolates studied, 34 isolates showed resistance to three or more antibiotics. A *Pseudomonas* strain resistant to 5 antibiotics out of 8 antibiotics tested was also

isolated from a ground water sample. Hence, this study clearly establishes how the resistance to different antibiotics is prevalent and is spreading around us in our water sources (22,23,24) even in drinking water samples. The resistance level was seen to be lowest in isolates for Meropenem and Amikacin (25). Amikacin is an aminoglycoside antibiotic used presently to treat severe infections as a last resort when other drugs fail and is quite resistant to inactivation by bacterial enzymes. None of the isolates showed resistance towards Ofloxacin which is a second generation broad spectrum fluoroquinolone antibiotic. A high MAR index observed for all samples indicate that there has been an indiscriminate use of these antibiotics in the area for management of bacterial infections (26).

CONCLUSION

Antibiotic resistance in bacteria has become a major challenge in developing countries like ours. This is because of several reasons including rampant use of antibiotics**,** easy availability of antibiotics – off the shelf, sometimes without medical prescription, Non-compliance – incomplete antibiotic treatment course, poor hygiene which causes rapid microbial growth and spread of pathogens, Poor quality of water, and Poor management of human waste. All such factors have contributed and collectively resulted in a situation today, where there is no antibiotic available that is effective for all bacteria. The emergence of bacterial resistance therefore necessitates the need for a continuous process of seeking, testing, and validating new antibiotics. New antibiotics are now being developed targeting a class of bacteria unlike the earlier agents that used to be broad spectrum. This problem of antibiotic resistance can be managed by conducting such antibiotic surveillance studies, creating awareness in public and by concerted efforts between doctors, research scientists, students, scientists, pharmaceutical industry and policy makers. A systematic approach of monitoring the regulated use of antibiotics in order to study the magnitude of antibiotic resistance should first begin at the local level. All members of society should be made aware of this problem so that we can maintain the effectiveness of currently available antibiotics.

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