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DETECTION OF DRUG RESISTANT ORGANISMS FROM NATURAL WATER BODIES

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ABSTRACT: Contamination of natural water bodies is increasing drastically and is becoming a major concern. More so, the contaminating organisms are resistant to several antibiotics. Antimicrobial resistance also is getting passed on to several other cohabitating organisms by horizontal gene transfer and leading to emergence of newer resistant strains. Important life-saving drugs have ceased to effective because of the increasing emergence of resistant microbial strains. The current study was an attempt to assess the spread of antimicrobial resistance in organisms in natural water bodies. In this study, water samples from different natural water bodies from in and around Mumbai city were processed to enumerate the microbial load. Also the isolation and identification of microorganisms was carried out. The antibiotic resistance pattern of the isolates was then determined. A total of 13 isolates were identified using conventional biochemical and Vitek method. Antibiotic susceptibility testing of all the isolates was performed using disc diffusion method. 11 out of 13 of the isolated organisms were found to be resistant to multiple antibiotics.

KEYWORDS: Antibiotic resistant organisms, antibiotic resistance, natural water bodies, plasmid coded resistance.

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1.INTRODUCTION

Water resources are sources of water that are useful or potentially useful. Only 3% of earth's water is fresh water. Most of it in icecaps and glaciers (69%) and ground water (30%) while all lakes, rivers and Swamps combined only account for a small fraction (0.3%) of the earth's total fresh water reserves. 97% of the water on the earth is salt water. Water is the natural resource which gets easily polluted by recreational activities and sewage disposal. Studies have been done by various

researchers to measure the chemical and microbial contaminants in the natural water bodies [1]. Water bodies harbor various organisms such *Escherichia coli, Enterobacter spp., Klebsiella spp., Salmonella spp., Pseudomonas aeruginosa, Vibrio parahemolyticus, Aeromonas hydrophila* [2]. Natural water bodies get contaminated due to the discharge of sewage containing human and animal faecal matter. This faecal matter contains a mixture of pathogenic and non-pathogenic organisms. Different drinking water sources have been found to contain antibiotic resistant organisms which can be a major threat to mankind [3]. Antibiotic resistant organisms have been found in natural water bodies which is a major health concern. Antibiotic resistance occurs when bacteria undergo a genetic change that reduces or eliminates the effectiveness of the drugs or other agents designed to cure or prevent infection. Resistant bacterial infections have inevitably followed the widespread use of every new antibiotic introduced [2]. Non-judicious use of antibiotics in animal feeds to boost growth, improper dosages of antibiotics are the two most common reasons for the emergence of antibiotic resistant bacteria and these bacteria often follow the route of sewage into the natural water resources [5]. Hospital wastes along with the pharmaceutical wastes and drug resistant organism, disposal in water have also added to the impact [6]. Resistance spread can worsen the efficacy of medical treatment as has been seen in the case of *Mycobacterium* strains that have started showing multi-drug resistance which is the current problem in the medical treatment of the tuberculosis disease [7]. Antibiotics residues and antibiotic resistant organisms as environmental pollutants have largely been overlooked. Studies have demonstrated that hospital waste water is highly selective environments and that they contribute to high rates of resistant bacteria that are being discharged in the natural environment. Many of the genes coding for antibiotic resistance have been found on extrachromosomal DNA like plasmids which could serve as vectors for horizontal transfer of these genes. Higher numbers of resistant bacteria occur in polluted habitats, indicating that humans have contributed substantially to the increased proportion of resistant bacteria occurring in the environment [4].

2. MATERIALS AND METHODS

2.1 Collection of Water Samples

Clean plastic bottles were used for water sample collection from the site (in and around Mumbai) and the samples were transferred to the laboratory for microbiological analysis [3]. The water samples were processed within 2-4 hours of collections, in case of delay the samples were refrigerated at 4^0C.

2.2 Enumeration of Bacterial Population by Standard Plate Count Method

Bacterial population was determined using standard plate count or viable count method. 10µl of 10^{-3} to 10^{-10}dilution series was plated on Sterile Nutrient Agar plate. Incubation was carried out at 37^0c for 24-48 hours in triplicates [7].

2.3 Identification of Bacterial Isolates– Cultural, Morphological Characters:

Isolation was carried out on MacConkeys agar and XLD agar until pure cultures were obtained. Individual colonies were purified and identified by morphological and biochemical techniques and further identified by biochemical examination and VITEK 2 compact [8][10].

2.4 Antibiotic Susceptibility Test

Antibiotic Susceptibility testing was performed by Kirby-Bauer test also called as Disc Diffusion test using standard procedure of the Clinical and Laboratory Standards Institute. Antibiotic discs of standard concentrations were placed on agar plates swabbed with isolated organisms and the susceptibilities of the organisms to the antibiotics were determined [9][11]. The antibiotics used were

Amoxycillin (Amx^{30}), Amoxyclav (Amc^{30}), Ampicillin (Amp^{10}), Aztreonam (At^{30}), Carbenicillin (Cb^{100}), Cefotaxime (Ctx^{30}), Ceftriaxone (Cf^{30}), Chloramphenicol (C^{30}), Clindamycin (Cd^{20}), Erythromycin (E^{15}), Gentamycin (Gen^{10}), Imipenem (Imp^{10}), Methicillin (Met^{5}), Nitrofurantoin (Nit^{100}), Oxacillin (Ox^{1}), Penicillin G (P^{10}), Piperacillin (Pi^{100}), Rifampicin (Rif^{5}), Tetracycline (Te^{30}), Ticarcillin (Ti^{35}), Ticarcillin (Ti^{75}), Tobramycin (Tob^{10}), Vancomycin (Va^{30}).

3. RESULTS AND DISCUSSION

Viable count of all the water samples was carried out and the counts obtained are as mentioned in Table 1. As observed in the Table 1, all the water samples showed a very high bacterial contamination. All these water sources were used as potable water even though the water is highly unfit for consumption. Water sample was also streaked on selective media to isolate and then to carry out the identification of the organisms. Thirteen isolates showing varied cultural characteristics were selected to be processed further which were identified. Table 2 shows the results of identification of the organisms. The number of viable organisms found varied from sample to sample owing to the diverse conditions of the locations where the water bodies are situated. The antibiotic resistance pattern was studied using disc diffusion method. Table 3 shows the antibiogram of the thirteen isolates. These isolates were found to be resistant to multiple antibiotics which reflects the current scenario of spread of antimicrobial resistance. The organisms studied were generally gram negative bacteria. These organisms are known to be opportunistic pathogens. The organisms were found to be resistant to beta lactam antibiotics like ampicillin and other widely used antibiotics. Some were also found to be resistant to multiple drugs.

Table 1- Microbial Count of the water samples

SAMPLE NO.	SOURCE	VIABLE COUNT (Cfu/ml)
1.	RAMWADI (VIRAR) Well water	1.29 x 10^6cfu/ml
2.	PIMPLEWADI WELL(VIRAR) Well water	8.70 x 10^3cfu/ml
3.	MUMBAI CENTRAL Well water	1.75 x 10^9cfu/ml
4.	MARINE LINES Well water	2.45 x 10^6cfu/ml
5.	VILE PARLE Well water	3.45 x 10^6cfu/ml
6.	Palghar VAITARNA RIVER	2.10 x 10^6cfu/ml
7.	Thane TANSA RIVER	3.09 x10^5cfu/ml

Table 2 - Identification of isolates by Vitek 2

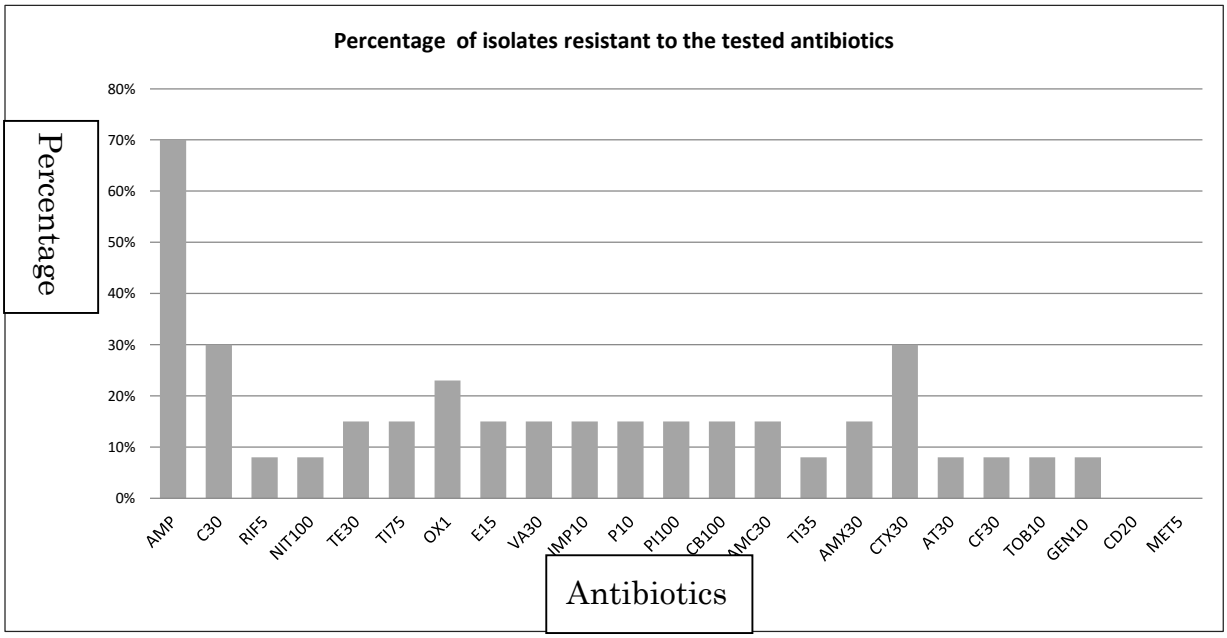
Sample	No. of Isolates Obtained	Identified Organisms By Vitek 2 Compact
1.	**2** (ISOLATE 1,2)	1: *Klebsiella pneumoniae* *2: Acinetobacter* lwoffi
2.	**2** (ISOLATE 3,4)	*3 & 4 :* Strains of *Aeromonas sobria*
3.	**4** (ISOLATE 5,6,7,8)	5,6,7 & 8: Strains of *Ochrobactrum anthropi*
4.	**1** (ISOLATE 9)	9: *Stenotrophomonas maltophilia*
5.	**1** (ISOLATE 10)	10: *Stenotrophomonas maltophilia*
6.	**2** (ISOLATE 11,12)	11: *Brevundimonas diminuta/vesicularis* 12: *Enterobacter cloacae complex*
7.	**1** (ISOLATE 13)	13: *Pseudomonas putida*

The sensitivity was also found to be in the intermediate range for a number of antibiotics for all strains studied, raising concerns that there might be a shift towards resistance in those organisms. Some strains also showed resistance towards third and fourth generation antibiotics. Such organisms pose a grave risk to humans coming in contact with them as an infection caused by such organisms would be virtually untreatable and eventually fatal. This threat is highly magnified by the fact that these organisms are found in natural water bodies that are being continuously used by people for various activities and purposes, many-a-times without any further effective antimicrobial treatment.

Table 3 - Antibiotic resistance pattern of bacterial isolates from water samples

Sr. No.	Organism	Resistance pattern
1.	*Klebsiella pneumoniae*	AMP^{10}, TE^{30}, TI^{75}, OX^{1}, E^{15}, VA^{30}.
2.	*Acinetobacter lwoffii*	AMP^{10}, CTX^{30}, OX^{1}, VA^{30}.
3.	*Aeromonas sobria* (STRAIN 1)	CB^{100}.
4.	*Aeromonas sobria* (STRAIN 2)	IMP^{10}, AMP^{10}, P^{10}, PI^{100}.
5.	*Ochrobactrum anthropi* (STRAIN 1)	CB^{100}, AMP^{10}, PI^{100}.
6.	*Ochrobactrum anthropi* (STRAIN 2)	(AMC^{30}), (TI^{35}), (AMX^{30}).
7.	*Ochrobactrum anthropi (STRAIN 3)*	(AMP^{10}), (C^{30}), (CTX^{30}), (AT^{30}), (CF^{30}), (AMC^{30}), (AMX^{30}), (TOB^{10}), (P^{10}).
8.	*Ochrobactrum anthropi (STRAIN 4)*	(TE^{30}), (AMP^{10}), (CTX^{30}), (GEN^{10}).
9.	*Stenotrophomonas maltophilia (STRAIN 1)*	(AMP^{10}), (TI^{75}), (OX^{1}), (E^{15}).
10.	*Stenotrophomona maltophilia (STRAIN 2)*	_
11.	*Brevundimonas diminuta/vesicularis*	(IMP^{10}), (CTX^{30}), (PI^{100}), (CD^{20}).
12.	*Enterobacter cloacae complex*	(AMP^{10}), (C^{30}), (MET^{5}), (CD^{20}).
13.	*Pseudomonas putida*	(AMP^{10}), (C^{30}), (RIF^{5}), (NIT^{100})

Graph 1- Resistance prevalence in the isolates



The observed high frequency of bacterial resistance as seen in Graph 1, may not only result in the therapeutic failure, but also endanger the health of the people who are at risk of infection with these pathogens. There is also a possibility of plasmid transfer of antibiotic resistance to human pathogenic bacteria. Further studies were done on the resistant and intermediate resistant strains of organisms to check whether the resistance conferred upon the organisms was plasmid borne or chromosomal [12]. Many of them showed plasmid borne antibiotic resistance. This can further compound the problem of antibiotic resistance due to the horizontal intra- and inter-genus transfer of plasmids. Amongst the isolates, *Klebsiella pneumoniae* is resistant to a wide range of antibiotics and of particular concern are the Extended Spectrum Beta Lactamase (ESBL) strains of *Klebsiella pnemoniae* [13]. *Acinetobacter lwoffi* is considered to be a part of the normal flora but has been found to be the cause of infections in humans, particularly catheter-associated infections in immune-compromised patients and has also been associated with gastroenteritis cases [14, 15]. *Aeromonas sobria* are water borne organisms that have been implicated repeatedly as the causative agents of clinical illnesses, often serious, ranging from gastrointestinal and wound infections to septicemia. Since they are described as cytotoxic, they are more likely to be virulent and therefore represent increased health risks for humans [16]. *Ochrobactrum anthropi* is an emerging pathogen in immunocompromised patients, particularly in those with dwelling central venous catheters [17]. *Stenotrophomonas maltophilia* is found in aqueous habitats, plant rhizospheres, animals, foods, etc. and is an environmental bacterium. It is another example of a bacteria which is becoming a relevant opportunistic pathogen causing bacteremia, pneumonia and intra-abdominal and muco-cutaneous infections [18, 19]. Enterobacter cloacae are nosocomial pathogens that can cause a range of infections such as bacteremia, lower respiratory tract infections, skin and soft tissue infections, urinary tract infections, endocarditis, intra-abdominal infection, septic arthritis, osteomyelitis and

ophthalmic infections. These bacteria contain beta-lactamase, which is undetectable in-vitro and is highly resistant to antibiotics such as third generation cephalosporins [20]. *Brevundimonas diminuta* shows an intrinsic resistance to fluoroquinolones and is commonly used as a test organism for validation of sterilizing grade membrane filters due to small size of the bacterium [21, 22].

4. CONCLUSION

Multiple Antibiotic resistant bacteria were found in all the water samples. One of the primary concerns of infection control departments is the rise of antibiotic resistant bacterial infections. They have become a ubiquitous problem both in healthcare settings and in the general population. Their causes emerge from natural selection and mutation and are strengthened by human misunderstanding of the purpose and function of antibiotics. Hope for this situation lies in public health campaigns, standardized infection control precautions, research and development of new method sand treatment [23]. It must also be made sure that usage of waters from water bodies suspected of harboring such organisms is avoided as much as possible and if used, is properly treated to destroy the potential antibiotic resistant pathogens present [24]. Organisms such as *Pseudomonas putida* which have been shown to possess biocontrol properties were found to be resistant to multiple antibiotics indicating a worrying trend and can wreak havoc if such biocontrol organisms continue harboring resistance genes [25]. Organisms such as *Acinetobacter lwoffii, Aeromonas sobria* etc. which have been isolated confirm that organisms which were earlier innocuous are now emerging as pathogenic organisms in developing and developed countries [26]. The findings confirm the rampant misuse of our water resources as well as the spread of resistance to antibiotics from nosocomial to environmental settings. Recent studies have also proved that the organisms that acquire resistance to antibiotics also acquire metal resistance which can cause them to be an even more potent pathogen [27]. Population of bacteria with R plasmids have shown a better resistance capacity than those that don't possess the plasmid. Hence the genetic transfer of plasmids play an essential role in the spread of resistance [28]. Patterns of antibiotic resistance in gram-negative bacteria of different genera are found to be correlated well, indicating that bacteria which share a common environment also share a common mode for developing antibiotic resistance [29]. New research pertaining the use of plant extracts to identify the various phytochemicals which can instead be used as drugs are currently underway due to the extreme situation of antibiotic resistance arising amongst pathogenic as well as non-pathogenic bacteria and is a possible new direction of study [30]. Aerobic bacteria, anaerobic bacteria, protozoa etc. have now been found to show various mechanisms for resistance development against the recent drugs as well [31]. If these trends continue, our arsenal of antibiotics would soon become obsolete against pathogens and infections which have so far been treated easily and cured could turn out to be severely debilitating or even fatal. There are still gaps in our knowledge about the role of HGT and its activity in the environmental resistome [32].

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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