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PATTERN OF CARBAPENEME RESISTANCE AMONG BACTERIAL POPULATION IN VARIOUS WATER BODIES OF DELHI/NCR

NAMITA BEDI[1]*, ARPIT BANSAL[1] AND RAJNI GIAND[2]

[1]Amity Institute of Biotechnology, Amity University, Sector 125, Noida
[2] Vardhman Mahavir Medical College and Safdarjung Hospital, New Delhi

ABSTRACT

Current investigation was aimed to enhance the knowledge about the prevalence of antibiotic resistance in diverse bacterial genera of varied water bodies of Delhi and NCR. During the period of August to November 2012, samples were collected from various water sources (RO water, tap water, stagnant water, river water and sewage treatment plant water). The most prevalent bacteria were *Enterobacter aerogens* (34.37%) from drinking water, *Klebsiella* (69.23%) from stagnant water, *E.aerogens* (23.52%) from river water and *Klebsiella* (20%) and *Citrobacter diversus* (20%) from sewage treatment plant water. Antimicrobial susceptibility of bacterial isolates was evaluated against 7 antibiotics using Kirby-Bauer disk diffusion method. The highest rate of resistance was observed with carbapeneme (48.38%), Cephalosporin (30.64%) Quinolones (17.74%) and aminoglycosides(3.2%) antibiotics respectively .Carbapenem resistance were detected in 3 isolates of *E.coli* and *Pseudomonas* respectively ,and 1 isolate of *Klebsiella*.

KEYWORDS: *Carbapenem resistance, Enterobacteriaceae, Water bodies*

NAMITA BEDI *
Amity Institute of Biotechnology, Amity University, Sector 125, Noida

***Corresponding Author**

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INTRODUCTION

Water is an essential component of life.[1] Microbial contamination of water especially with pathogens of enteric origin is considered to be a critical factor. The various water bodies are being polluted by extensive agricultural runoff, urban and industrial wastewater effluents and discharge of domestic sewage which affect human health and the biodiversity of the aquatic ecosystem.[2] Faecal contamination in these areas contributes to the spread of these pathogens along with the dissemination of antibiotic- resistance genes .[3] The occurrence and dissemination of antibiotic resistant bacteria have been detected in varied water bodies like rivers, lakes, wastewater and drinking water biofilms.[4,5] The analysis of various water sources in Delhi/NCR were taken to determine the presence of antibiotic resistant microorganisms in drinking water and recreational water. Antimicrobial susceptibility of bacterial isolates were evaluated against 7 antibiotics. Carbapenems (imipenem, meropenem and ertapenem) are beta-lactam antibiotics with an exceptional broad spectrum range of activity. Like all beta-lactam antimicrobial agents, carbapenems bind to penicillin-binding proteins (PBPs), disrupting the growth and structural integrity of bacterial cell-walls. Resistance to carbapenems is developed when bacterium acquire structural changes within their PBPs; when they acquire metallo beta-lactamases that are capable of rapidly degrading carbapenems, or when changes in membrane permeability arises as a result of loss of specific outer membrane porins. The emergence of carbapenemases in *Enterobacteriaceae* provides an added risk of dissemination of antibiotic resistance in the community. These enzymes confer resistance to the other beta-lactam agents as well; including extended spectrum cephalosporins.[6] Infections caused by CRE have limited treatment options and have been associated with high mortality rates. In the previous year, other carbapenemase subtypes, including New Delhi metallo-β-lactamase, have been identified among *Enterobacteriaceae* in the United States.[7]These enzymes are frequently found on mobile genetic elements and have the potential to spread widely. The present study is an attempt to find out the prevalence of carbapeneme resiatance in *Enterobacteriaceae* family from varied water sources of Delhi and NCR, and further examined for resistance to other antibiotics.

MATERIALS AND METHODS

Sample Collection

Sampling was done from different sites located in Delhi/NCR region from August to November 2012. The water samples collected were distributed in four different categories: Drinking water, stagnant water, River water and Sewage treatment plant water. Water samples were collected using sterile glass bottles and transported in an ice- box to the laboratory to be analyzed within 2 hours. Water samples were inoculated on Carbapenem (Imipenem) containing (1µg/ml) MacConkey's agar plates using two methods: Membrane Filtration Assembly method and Spread Plate Method[8].Ten-fold dilutions were plated by spreading 0.1 ml of sample on plate and incubated aerobically at 37^{o} C for 24-48 hrs[9]. After incubation, based on colony morphology representative colonies were picked and sub-cultured on different selective and differential media such as MacConkey agar (Himedia) and Eosin methylen blue agar (Himedia) then identified biochemically following standard methods.[10]

Antimicrobial susceptibility test

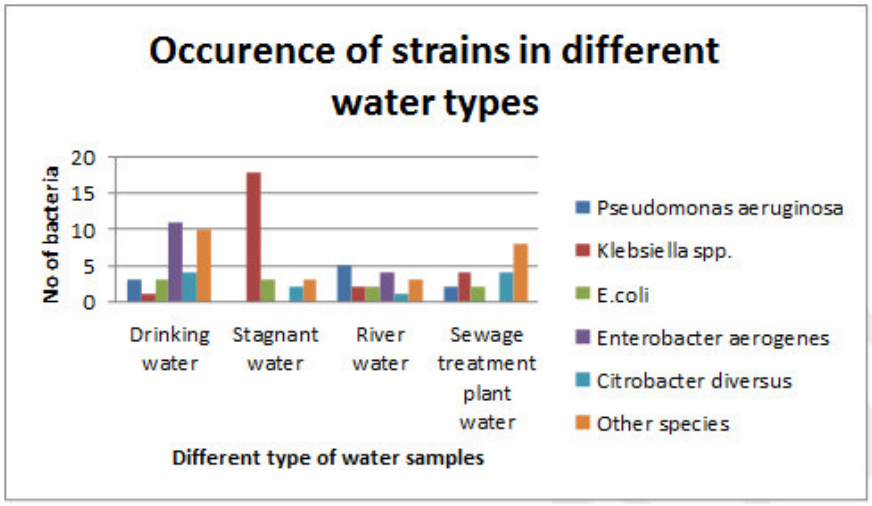
Antibiotic Susceptibility testing using Kirby-Bauer Disc Diffusion method was performed for following drugs Ertapenem (10µg), Meropenem (10µg), Imipenem (10µg), Ciprofloxain (30µg), Ceftazidime (Cephalosporin) (30µg) ,Netillin(Aminoglycoside) (30µg), Amikacin (Aminoglycoside) (10µg), Nalidaxic acid (30 µg) (manufactured By Himedia Laboratories). After 24hrs of incubation at 37^{o}C the zones of inhibition were measured and compared with the control strain of(*Escherchia coli* ATCC 25922).

RESULTS AND INTERPRETATION

The most prevalent bacteria were *Enterobacter aerogens* (34.37%) from drinking water, *Klebsiella* (69.23%) from stagnant water, *E.aerogens* (23.52%) from river water and *Klebsiella* (20%) and *Citrobacter diversus* (20%) from sewage treatment plant water. Presence of carbapeneme resistance isolates of *Enterobacteriacea family and S.aureus from* the drinking water of the public places is alarming for health of humans and animals. This indicates that infection may be acquired by the community through these sources of drinking water. These microbes came in the category of faecal indicator. As well presence of carbapeneme resistant *S.aureus* from sewage treatment plant will result in further spread of antibiotic resistance to water reservoir.. The highest CFU of $6.0x10^5$ cells/ml of *E.coli* and $5.7x10^5$ of *Enterobacter aerogens* from drinking water of crowded area . The lowest and highest CFU were of *klebsiella* i.e$1.0x10^4$ cells/ml from Park located in Sector 36 of Noida and $4.5x10^5$ cells/ml of SardarVallabh lake, Rohini . The highest CFU of $4.8x10^4$ cells/ml of *Enterobacter aerogens* from hindan river water and $1.3x\ 10^4$ cells/ml of *E.coli* from Yamuna river. The microbial density during this study was found to be very high than WHO standard limits. EPA's, 2012 recommended limit of *E. coli* within recreational waters such as swimming and water skiing (full body contact) within recreational waters is equal to or less than 126 cfu/100 ml (colony forming units per 100 milliliters of water) based on a geometric mean or a one-time measurement equal to or less than 235 cfu/100 ml. Present results confirmed high burden of antibiotic resistant bacteria in polluted Yamuna and Hindan River, this may be attributed to discrete discharge of industries, but also the poor sewage system, saturated landfills, human settlements around the river and the agricultural waste that gets washed into it.

Graph 1

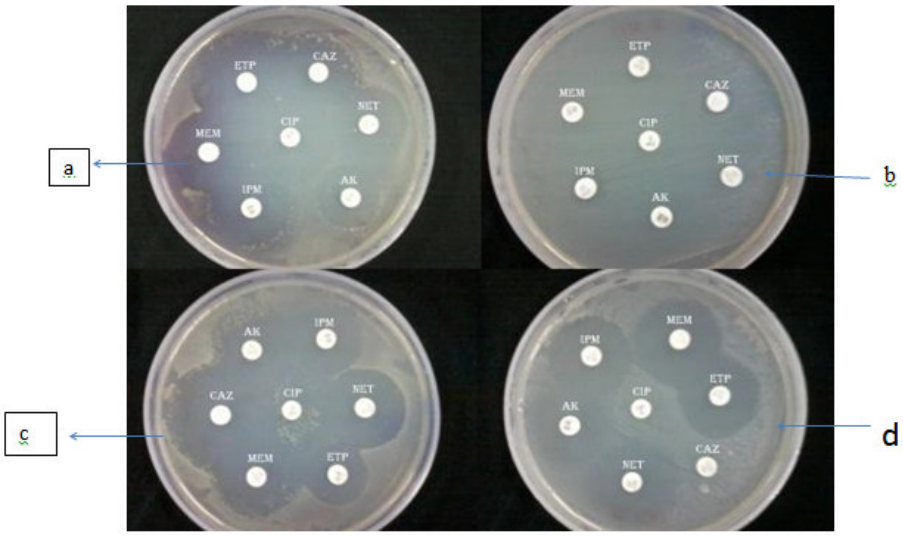
Representing the total number of different type of bacteria in various water bodies of Delhi/NCR



Representation of occurrence of strains in different water samples collected. x axis shows the type of water sample and Y axis shows the number of bacteria isolated from various water samples.

Figure 1

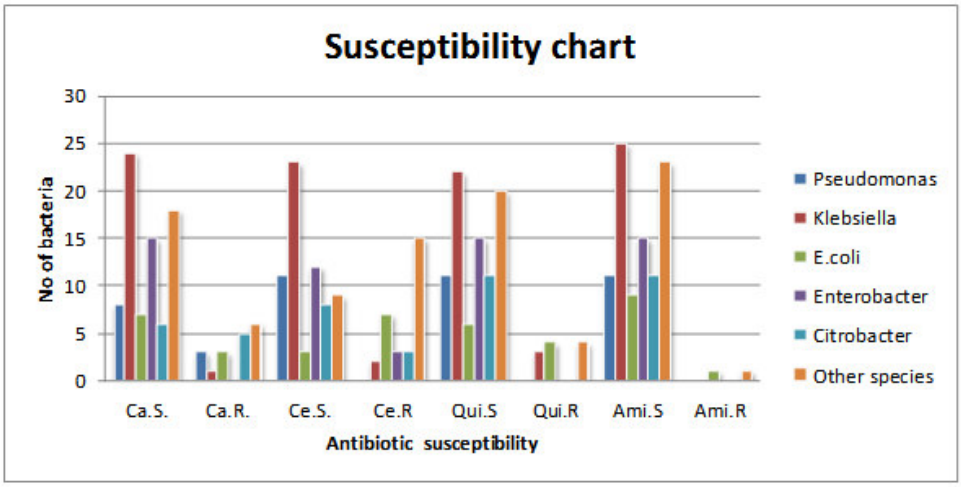
Graphical representation of Susceptibility of microbes towards different drugs



Representing Antibiotic susceptibility test of various bacteria by Kirby-baurer method a)*Pseudomonas aeruginosa*; b) *E.coli* c) *Klebsiella spp.* d) *Pseudomonas aeruginosa .In this th*e antibiotics used were ertapenem(ETP), Meropenem (MEM), imipenem(IPM),ciprofloxacin (CIP),ceftadizime(CAZ),Netillin(NET),

Graph 2

Graphical representation of Susceptibility of isolated bacteria to different antibiotics



Carbapenems, cephalosporin, quinolones and aminoglycoside and R and S is indicating resistance and sensitivity

Graphical representation of Susceptibility of microbes towards different drugs Carbapenem C Cephalosporin Cephalosporin Quinolones Qui Aminoglycosides. Antimicrobial susceptibility of bacterial isolates was evaluated against 7 antibiotics using Kirby-Bauer disk diffusion method. The highest rate of resistance was observed with carbapeneme(48.38%) followed by Cephalosporin(30.64%), Quinolones(17.74%) and aminoglycosides(3.2%) antibiotics respectively .Carbapenem resistance were detected in 3 isolates each of *E.coli* and *Pseudomonas*, and 1 isolate of *Klebsiella* respectively.

DISCUSSION

Resistance to carbapenems is of great concern as they are considered to be antibiotics of the last resort to combat infections by mutli-drug resistant bacteria, especially in ICUs and patient wards. According to a survey by Deshpande et al. 2010, Carbapenem resistance in *Enterobacteriaceae* has increased from 0% in 2006 to 8% in Jan-Aug 2009 in ICU sources[12]. Most cases of carbapenem resistant microorganisms in the community have been reported internationally including Canada, United Kingdom and United States.[13]The prevalent bacteria isolated were *Enterobacter aerogens* (34.37%) from drinking water, *Klebsiella* (69.23%) from stagnant water, *E.aerogens* (23.52%) from river water and *Klebsiella* (20%) and *Citrobacter diversus* (20%) from sewage treatment plant water.The analysis of various water sources in Delhi/NCR shows the presence of various carbapenem resistant microorganisms. In drinking water the presence of pathogens like *Alcaligenes, Escherichia coli, Salmonella, Staphylococcus and Klebsiella spp.* is alarming. This indicates that infection may be acquired by the community through these sources of drinking water. These microorganisms are the microbial indicator for the monitoring of water and causative agents of diarrhea and dysentery [14,15]. Although water is treated at various steps and by different procedures to be free from the microbial contamination, a variety of bacterial microflora continues to persist in it. No matter how efficient the techniques used to eliminate them are, these bacteria are finding ways to enter the human systems. There is a need for new detection tools in drinking water surveillance. It is clear that bacteria will continue to develop resistance to the antibacterial drugs either by mutations or through the process of genetic exchange, because this is what the process of evolution demonstrates [16]. According to our study Imipenem is found to be the most effective carbapenem against the *Entereobacteriaceae* isolates followed by Meropenem and Ertapenem. The 40% of *Klebsiella sp.* and *Escherichia coli* have shown resistance against Quinolones and Cephalosporins. Aminoglycosides has proven to be the effective drug against these Carbapenem resistant strains similar to the study conducted by Bouza E et al.2002[17]. Further study should be required in which antibiotic spectrum analysis need to be done using more drugs.The growing resistance to antimicrobial drugs by bacteria is increasing the pressure on the healthcare sector. It is also leading to complications in treatment and rise in the rate of fatality. It is difficult to outsmart the microorganisms who have evolved over millions of years in the toughest of environments, but it is the need of the hour. Currently, carbapenems are the most advanced form of broad spectrum antibiotics available to humans, and bacterial resistance to them can be a potential nightmare scenario. What can be done is optimizing the dosage and frequency of the usage of drugs, so that the bacteria take time to develop resistance towards them. With sufficient efforts to use antimicrobial agents wisely, thereby preventing the emergence of resistant organisms, and strict attention to infection control guidelines to prevent the spread of resistant organisms when they develop, we need to stay at least one step ahead of them. As well proper care should be taken of rivers, sewage treatment plants, ponds and lakes so that they will not be a prominent mode of transmission of antibiotic resistance bacteria.

CONCLUSION

Water is a potent habitat for the growth of diversified microbial forms. The present study supported the theory that varied type of water bodies are reservoir of emerging antibiotic resistance strains of bacteria. As *E.coli* showed the resistance against all the antibiotics studied in present paper, it (antibiotic resistance) can be further transferred within a genus or a family by horizontal genetic lineage. Future study should emphasize on molecular reasons for the spread of antibiotic resistance to human biome from the environment.

CONFLICT OF INTEREST

Conflict of interest declared none.

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Reviewers of this article

Dr. Arti Goel, M.Sc., Ph.D.

Assistant Pofessor, Amity Institute of Microbial Biotechnology, Amity University, Sector-125
Noida (U.P.), India

G. Bakhya Shree M.S. (Research)

Coordinator and Trainer, Department of Biotechnology and Life Sciences, Dexter Academy, Madurai, Tamilnadu

Prof.Dr.K.Suriaprabha

Asst. Editor , International Journal of Pharma and Bio sciences.

Prof.P.Muthuprasanna

Managing Editor , International Journal of Pharma and Bio sciences.

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