ASSESSMENT OF PREVALENCE OF DRUG RESISTANT *Escherichia coli* IN KHAN RIVER, INDORE

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Abstract: Infectious diseases caused by antibiotic resistant bacteria (ARB) have emerged as a huge challenge globally, because of the difficulties associated with the treatments. ARB especially multidrug resistant (MDR) bacteria pose a significant threat to public health due to their high levels of resistance to most available antibiotics. Antibiotic resistance genes (ARGs) carried by bacteria may horizontally transfer to other bacteria. Polluted aquatic environments containing residual antibiotics may create a selective environment for the growth and spread of ARB in the environment. We have investigated the prevalence of antibiotic resistance in *Escherichia coli* population of Khan river, Indore. Water samples were collected from different sites of Khan river. Total heterotrophic bacterial (THB) counts were estimated. The samples were processed using standard protocols for the isolation and identification of *E.coli.* The bacterial isolates were further subjected to antibiotic susceptibility test for 13 different antibiotics by standard Kirby-Bauer disc diffusion method and the results were interpreted using Clinical and Laboratory Standards Institute (CLSI) criteria. The total heterotrophic bacterial (THB) counts were $1.8 \times 10^6 - 1.2 \times 10^{20}$ CFU/ml. Total 24 *E.coli* cultures were isolated from 09 water samples out of which 79.2% showed resistances to ampicillin followed by cefadroxil (58.33%), clarithromycin (54.16%) and cefuroxime (37.5%). The prevalence of resistance to amoxicillin/clavulanic acid, gentamicin, cefotaxime, ciprofloxacin, azithromycin, cefaclor, nalidixic acid and streptomycin was 8.33% to 33.33%. Least resistance was observed against cephoperazon (4.16%).

Key Words: **Antibiotic resistance, Multidrug resistant (MDR), Antibiotic resistance genes (ARGs), Total heterotrophic bacteria (THB).**

I. INTRODUCTION

Emergence of bacteria resistant to multiple antibiotics is a serious matter of concern. Multidrug resistant (MDR) pathogens pose a significant threat to public health due to their high levels of resistance to most available antibiotics. Common microbial infections like urinary tract infections, sexually transmitted infections and respiratory tract infections are becoming untreatable. Also medical procedures such as organ transplantation, general surgeries and post-surgical recovery are becoming much riskier (O'Neill, 2016). Antimicrobial resistance is being studied globally, which has shown that more and more bacteria are getting resistant to multiple antibiotics, including *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Salmonella typhi, Proteus, Shigella, Serratia, Acinetobacter, Vibrio cholerae, Neisseria gonorrhoeae, Staphylococcus aureus, Streptococcus pneumoniae, Mycobacterium tuberculosis* and *Helicobacter pylori* (Ahammad *et al.*, 2014; Bessa *et al.*, 2014; Kaeseberg *et al.*, 2014; Poonia *et al.*, 2014; Kakkar *et al.*, 2017; Tagliabue and Rappuoli, 2018). In some cases, it has been observed that bacteria have become resistant to most of the available antibiotics and are on the edge of becoming untreatable. Carbapenem-resistant Enterobacteriaceae (CRE) is one of such case (Tagliabue and Rappuoli, 2018; Suay-García, 2019). If the problem is not addressed properly, it is estimated that 10 million deaths per year could occur globally due to infections caused by antibiotic resistant microorganisms by 2050 (O'Neill, 2016).

The genes encoding antibiotic resistance are located on the bacterial chromosome and plasmids, and are transmitted to the next generation through vertical gene transfer. Antibiotic resistance genes (ARGs) carried by bacteria may horizontally transfer to other bacteria, when genes are found on mobile genetic elements, such as plasmids, transposons, and integrons. (Davison, 1999; Lipsitch and Samore, 2002).

ARB are distributed in almost all the environments associated with human activities. Presence of antibiotic resistant microorganisms have been reported in aquatic environments, including rivers, hospital and municipal wastewater, surface water and drinking water systems (Thomas Schwartz *et al.*, 2003; Purohit *et al.*, 2020). In present study, occurrence of antibiotic resistant *E.coli* in the Khan river was investigated. Khan river originates from the Vindhya range near village Umaria and flows only about 25 km through the city of Indore. River Saraswati is tributary of Khan river. Saraswati river originates from Hukmakhedi Pond and joins river Khan at Krishnapura Chhatri (Sharma *et al.*, 2012). Khan river receive wastewater from many Nallas including Piliyakhal Nalla, Palasia Nalla, Azad Nagar Nalla, Tulsi Nagar Nalla, Khajarana-Bhamori Nalla and Arvindo College Nalla. These Nallas flow several kilometres through the city, carry industrial, hospital and domestic waste of the area and draining into river Khan (Sharma and Dubey, 2011). Various pollutants including antibiotics, pesticides, insecticides and heavy metals flow into the Khan river through the wastewater discharged from household, hospitals and industries, promoting the emergence and spread of antibiotic resistance. ARB are evolved under the selective pressure created by the presence of residual antibiotics in the polluted aquatic environments, which then grow in large numbers and spread in the environment. Exposure to different antibiotics leads to the development of multidrug resistance (Andersson and Hughes, 2014; Hiltunen *et al.*, 2017). It is required to examine water quality parameters frequently to get essential information that can be used for the efficient water management.

II. METHODS

Sample collection and processing:

Water samples were collected from river Khan in sterilised glass bottles from different sites located between Limbodi and Kabit khedi. Water Samples were aseptically carried to the laboratory in a cold box within three hours from the time of sampling. Samples were processed in the laboratory on the same day of collection. Total aerobic heterotrophic bacterial counts were estimated in each sample as 'colony-forming units (CFUs) per unit volume of sample' by using Standard Plate Count Method. Dilution series were prepared using sterile saline water blanks. Appropriate dilutions were plated on Nutrient agar medium and after incubation colonial counts were determined. The samples were processed on selective and differential media for the isolation of *E.coli* bacteria. Isolates were purified by serial sub-culturing and conformation of *E.coli* isolates were done by staining and standard biochemical tests (APHA, 2005).

Antimicrobial Susceptibility Testing:

The confirmed *E.coli* isolates were further subjected to antibiotic susceptibility test by standard Kirby-Bauer disc diffusion method (Bauer, 1966). Mueller-Hinton agar plates were used to inoculate *E.coli* cultures. Antimicrobial susceptibility of *E.coli* isolates were tested against 13 different antibiotics. Results were interpreted by referring guideline of Clinical and Laboratory Standards Institute. The antibiotic impregnated discs used in the study were ampicillin AMP (10 mcg), cefuroxime CXM (30 mcg), cefadroxil CFR (30 mcg), ciprofloxacin (5 mcg), streptomycin (10 mcg), cefotaxime CTX (30 mcg), cefaclor CF (30mcg), azithromycin AZM 15 mcg), nalidixic acid NA (30µg), cefoperazone CPZ (75 mcg), clarithromycin CLR (15 mcg); gentamicin GEN (10µg) and amoxicillin+clavulanic acid (30 mcg). The isolates that displayed resistance to three different groups of antibiotics were designated as multi-drug resistant (MDR) bacteria.

III. RESULTS AND DISCUSSION

In total 9 water samples were taken from 9 different sites of river Khan between Limbodi and Kabit khedi of Indore city. The total heterotrophic bacterial (THB) counts were 1.8×10^{6} – 1.2×10^{20} CFU/ml with mean value of 1.9×10^{16} CFU/ml. All the water samples were processed for isolation of *E.coli* using selective and differential media. Isolates were purified and confirmed as *E.coli* by performing biochemical tests. Total 24 confirmed *E.coli* isolates were obtained which were used to test the occurrence of antimicrobial resistance against 13 antimicrobials. Results of antibiotic susceptibility test are shown in the Table-1 and Figure-1 below. Out of 24 *E.coli* isolates, 19 showed resistances to ampicillin which comprises 79.2% of the total isolate. Resistance to clarithromycin and cefadroxil was observed in 13 (54.16%) and 14 (58.33%) isolates respectively. Nine (37.5%) isolates showed resistance to cefuroxime. As shown in the table equal number of isolates i.e., 8 (33.33%) exhibited resistance to cefaclor, nalidixic acid and streptomycin. The prevalence of resistance to amoxicillin/clavulanic acid, gentamicin, cefotaxime, ciprofloxacin and azithromycin was 2(8.33%) to 5 (20.83). Least resistance was observed against cephoperazon 1(4.16%).

Table-1: Frequency of antibiotic resistant *E.coli* isolated from Khan river.

Antibiotics	**No. of Resistant n (%)**
Penicillins	
Ampicillin(10µg)	19 (79.2)
Amoxicillin/clavulanic acid(20/10µg)	2 (8.33)
Cephalosporins	
Cefadroxil (30µg)	14 (58.33)
Cefuroxime (30µg)	9 (37.5)
Cefaclor (30µg)	8 (33.33)
Cefotaxime (30µg)	4 (16.66)
Cephoperazon (75µg)	1 (4.16)
Aminoglycoside	
Streptomycin (10µg)	8 (33.33)
Gentamicin (10µg)	3 (12.5)
Macrolides	
Azithromycin (15µg)	5 (20.83)
Clarithromycin (15µg)	13 (54.16)
Quinolones /Fluoroquinolons	
Ciprofloxacin (5µg)	4 (16.66)
Nalidixic acid(30µg)	8 (33.33)

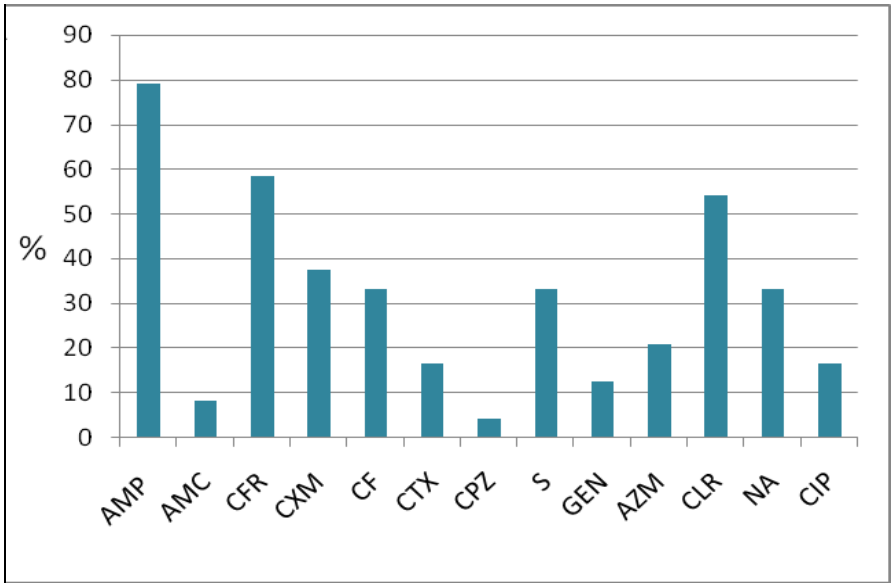

Figure 1: Prevalence of antibiotic resistance in *E.coli* isolates tested against 13 antibiotics.

In total 46% of all *E.coli* isolates were multidrug resistant (MDR), showed resistance to antimicrobials of three different classes of antibiotics. Commonly observed combination of antibiotics against which MDR isolates showed resistance was penicillin, cephalosporin and quinolones. ARB and ARGs have been reported from different rivers of India including Ganges, Yamuna, Cauvery and Kshripa (Biswas *et al.*, 2015; Ahammad *et al.*, 2014; Azam *et al*., 2014; Skariyachan *et al.*, 2015; Purohit *et al*., 2020). In these rivers large numbers of bacteria resistant to different antibiotics including third generation cephalosporin have been reported. In present study, we also found *E.coli* strains resistant to third generation cephalosporin. Phanse *et al.*, (2015) reported fecal contamination and antibiotic resistant bacteria in ground water samples from bore wells present in colonies along the Khan river belt. They suggested that due to seepage of the highly polluted water of the Khan river, ground water has been getting contaminated with antibiotic resistant bacteria. The current study showed the presence of high percentage of MDR bacteria in Khan river which is a serious matter that must be addressed to control the entry and spread of antibiotic resistant microorganisms.

IV. CONCLUSION

In conclusion, results obtained confirmed the existence of *E.coli* population resistant to multiple antibiotics in Khan river. In this study, high values of total heterotrophic bacterial (THB) counts and MDR *E.coli* indicate the possibility of involvement of selection process in the emergence of MDR bacteria in the Khan river, as the evolution can happen in the presence of even very low concentrations of residual antibiotics. The pattern of drug resistant bacteria in Khan river is alarming which may contaminate ground water. It is a very serious matter and must be addressed appropriately. Efforts are required to discourage non-therapeutic use of antibiotics and to design effective methods to eliminate resistant bacteria and residual antibiotics from wastewater. Hospital wastewater treatment plants should be setup to reduce the release of bacteria and antibiotics into the aquatic environment. Further studies are required to evaluate the possible risk associated with resistance dissemination and the role of aquatic environment in the emergence and spread of antibiotic resistance.

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VI. REFERENCES

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