Current Trends in Biotechnology and Pharmacy
Vol. 17(Supplementary issue - 3A) 1090 - 1096, August 2023, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2023.3s.47

Antibiotic Resistance againt *E. coli* Isolated from City of Lake Bhopal, Madhya Pradesh

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Abstract

E. coli samples from Upper Lake Bhopal were examined in this investigation for different antibiotic resistances. Different integrons and genes were employed to describe MDR isolates. The diffusion method has been utilized to scrutinize 150 *E. coli* by Kirby Bauer disc detaches from nine sample locations in Upper Lake Bhopal (UPB) for phylogenetic relationships and antibiotic susceptibility. The level of resistance was significantly higher in vancomycin (74%) as compared to cefazolin (88%). Out of 150 isolates, 37 (25%) were found to be resistant to ten or more drugs. Additionally, a high prevalence of polymyxin-B and azithromycin predisposition was observed in 90% of *E. Coli* isolates. All the collected test samples exhibited resistance to fluoroquinolones, tetracyclines, chloramphenicol, and beta-lactam antibiotics. It was higher in middle-lake Bhopal *E. coli* isolates than in the upper and lower reaches. *E. coli* samples from Upper Lake Bhopal showed rising trimethoprim/sulphonamide resistance. MDR isolates were present in all samples. MAR scores greater than 0.25 indicated contamination in the upper, middle, and lower Upper Lake. The animal isolates showed resistance to lactams and aminoglycosides, with 80.9% of all isolates being multidrug-resistant. Integrons were found in 85 MDR isolates resistant to at least six chemicals and four antimicrobial types of classes.

Keywords: Upper Lake, *Strain E.coli*, MDR, Antibiotics Resistance, Antimicrobial activity

Introduction

Bhopal heart of Madhya Pradesh and city of Lake famously Known for two artificial lakes that are situated in the central of Bhopal. Surface area of 1.30 km^2 and a catchment area of 9.7 km^2, and a combined catchment area of 363 km^2, is smaller than the main lake i.e., Upper-Lake is a significant resource of drinking water, meeting over 40% of Bhopal's residents' average daily water needs while rerouting was mainly carried out in the Lower Lake. However, the ecological environment of Lake Bhopal has deteriorated in recent years due to the inflow of point and non-point source toxins from the metropolitan area, including sewage, solid waste, as well as dregs and improvements as of the national catchments. This is also a result of lakeshore encroachment. The lake's biological health and water quality have declined as a result of these circumstances.

E. coli is an enteric- bacteria that is oft en discovered in untreated sewage and huma

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n gastrointestinal tracts. It also poses a serious threat to human health since it may cause a number of extra-intestinal diseases such pneumonia, meningitis, urinary tract infections, and bacteremia. Antibiotics are often used to treat these infections effectively. Antibiotic overuse, on the other hand, has resulted in a substantial rise in bacterial resistance. Because of the significant frequency of antibiotic resistance in *E. coli,* the efficacy of treatments such as penicillin, sulfa medicines, fourth-generation cephalosporins, and fluoroquinolones has been greatly reduced over the last several decades. The placement of carbapenem-resistant Enterobacteriaceae, particularly *E. coli*, to (WHO) as global pathogen list of priorities demonstrates that the issue of antibiotic resistance has worsened and necessitates a comprehensive response. According to prior study (1, 2) the rise of multidrug-resistant *E. coli* makes antibiotic therapy ineffective, resulting in a rise in mortality and morbidity around the world.

Escherichia coli is a kind of bacterium found in human as well as in animal intestines even though almost all of *E. coli* strains are safe, a small number may trigger mild to severe illness. (8) When present in high concentrations, *E. coli* can significantly alter the microbial composition of water. (3, 4) It might cause harmful algal blooms and other issues for aquatic ecosystems by changing how other bacteria and microorganisms in the water are balanced. To stop the spread of disease and safeguard the health of people and wildlife, it's critical to keep an eye on *E. coli* levels in water sources, especially those used for recreational pursuits like swimming or fishing. *E. coli* levels in water systems can be decreased with routine testing and proper waste disposal. (5) This is due to the fact that *E. coli* may act as a food source for other microorganisms, increasing their diversity and population in the water. It can also result in a decline in overall microbial diversity and an increase in dangerous bacteria if the amount of *E. coli* is too high. Certain species that can thrive on *E. coli* may find a more hospitable environment in areas with higher concentrations of the pathogen, increasing their diversity and population. (5) If the ecosystem's delicate balance is upset, this could also result in competition for resources and potentially hazardous changes. *E. coli* density has an impact on the microbial composition of water. To ensure that it stays within safe ranges and does not upset the balance of microbial diversity, it is crucial to regularly check the mass of *E. coli* in water systems. (6) This can be accomplished by conducting routine testing and putting policies in place to stop contamination from sources like sewage discharge or agricultural runoff. (7)

The current study beheld on the antibiotic's resistance trends of *E. coli* strains recovered from the Upper Lake in Bhopal, Madhya Pradesh. The primary goal of this investigation was to evaluate the incidence of antibiotic-resistant E. coli bacteria in lake water and their resistance patterns to routinely used antibiotics. (9) The findings of this study could provide crucial insights into the potential dangers connected with drinking Upper Lake water and aid in the development of effective ways to prevent the bacterial growth that are resistant to antibiotics.

Materials and Methods

Study site

Isolated bacteria

The study includes looking at 150 *Escherichia coli* samples taken from Upper Lake water as well as 16 isolates previously acquired from faces and sewage. The aquatic *E. coli* samples were collected from several locations in the Upper Lake area of Bhopal, including Kamla Park, Wetland Lake View, Kohe-Fiza, Kaliasot, Kerwa Dam, Bharvan Park, Lake View Point, VIP Road, Van Vihar National Park, Bhadbhada.

This was done using a direct inoculation in enrichment broths was previously used to isolate direct sewage isolates from fecal samples of several types of sewage from Bhopal. These

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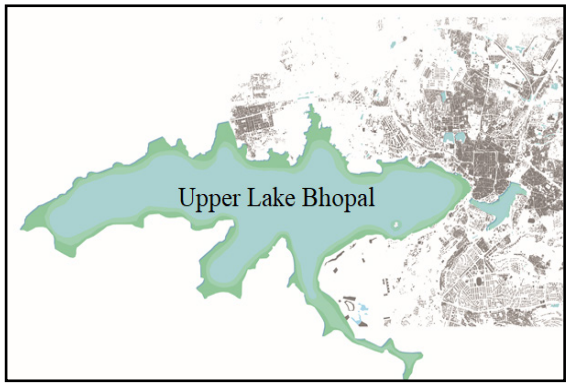
E. coli isolates were all identified using the *E. coli* TM Identification Kit's standard strains. Periodically, the isolates' purity was examined on EMB agar, a selective medium. These isolates' glycerol stocks were created and kept at 4^0C. The strain named "*E. coli ATCC 25922*" was obtained from IMTECH, Chandigarh for reference. Utilizing the Kirby Bauer method, antimicrobial



susceptibility profiling was carried out on all 150 *E. coli* isolates using 19 different antibiotics. (10, 11)

Figure 1. Upper Lake Bhopal, India (GPS Coordinates 23°14'41.532"N , 77°22'37.488 E)

Studies supporting the use of antibiotics

The antibiotic resistance member of enterobacteriaceae family *E. coli* samples were evaluated using the "Kirby Bauer disc diffusion" approach against 19 different drugs (4). To perform the test, medication discs were obtained from HiMedia in Mumbai, India, including Aminoglycosides such as Gentamicin-10 mcg, "Tobramycin and lactams Imepenem-10 mcg, Ceftriaxone-30 mcg, Cefotaxime-30 mcg, Cefazolin-30 mcg, and Cefuroxime-30 mcg nitrofurantoin, linezolid, glycopeptides, polymyxin-B, macrolides, chloramphenicol, tetracycline, fluoroquinolones, and rifampicin. (21) A 0.06 McFarland turbidness average were arranged by mingling 0.6 ml of 1% (H_2SO_4) 99.5 ccs of 1% H_2SO_4, and $BaCl_2$. Then, 6 millilitres of this solution were distributed to bacterial culture tubes for testing, and the turbidness was evaluated using spectrophotometry at 625 nm to ensure an OD between 0.08 and 0.13. The McFarland standard was stored at 4 0C while being wrapped in foil. (11)

The MAR (Multiple Antibiotic Resistance) was used to calculate the total resistance to antibiotics score for the Upper Lake testing sites (New Bhopal). The entire antibiotics confirmed was divided the whole quantity of isolates at that location to arrive at this score. If the resultant index is larger than 0.25, there is a substantial probability of contamination.

Results and Discussion

In this study, sampling sites covering a total area of 372.35 square kilometres were used, including Kamla Park, Wetland Lake View, Kohe-Fiza, Kaliasot, Kerwa Dam, Bharvan Park, Lake View Point, VIP Road, Van Vihar National Park, Bhadbhada. The maximum height of the tank was 508.65 centi-meter. The study also included nine *E. coli* samples from a sewage source. Nineteen different antibiotics were tested against fifty different *E. coli* strains in all.

Phenotypes of antimicrobial resistance

Susceptibility testing was performed on 155 *E. coli* isolates across 19 different antibiotics from 12 different classes. The evaluation of antibiotic susceptibility was based on the guidelines of CLSI. The resistance, intermediate, or susceptibility status of each isolate against each antibiotic was determined. Out of 124 water *E.*

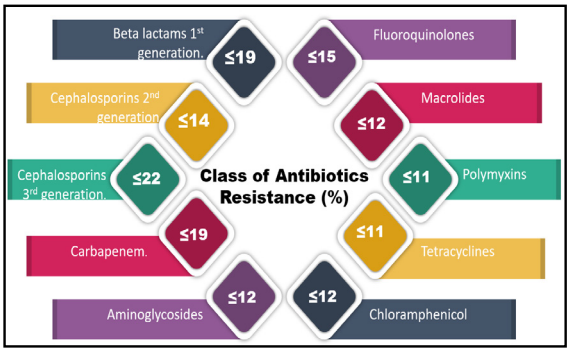


Figure 2. Classes of Antibiotics Resistance

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coli isolates, 80.9% were classified as multi-drug-resistant due to their positive test results for resistance to three different antibiotic classes. Similarly, 26 *E. coli* isolates obtained from direct sewage samples were also found to have MDR features.(23)

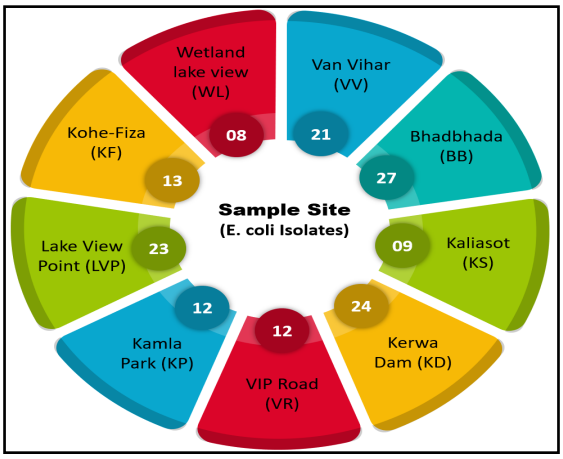

Figure 3. Sampling Sites of E.coli

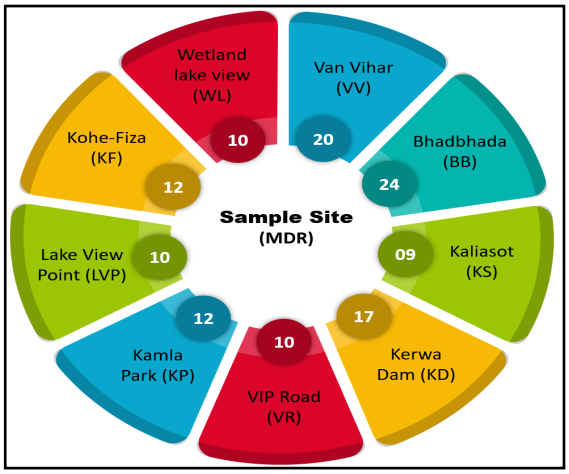

Figure 4. Different location of Upper Lake Bhopal with MDR isolates

Among these various antibiotics tested, cefazolin (88%) and vancomycin (74%) displayed the highest level of resistance. Around one-fourth or 16 out of 150 cells, exhibited resistance to at least ten antibiotics. Azithromycin and polymyxin-B were found to be particularly effective against most of the *E. coli* isolates.

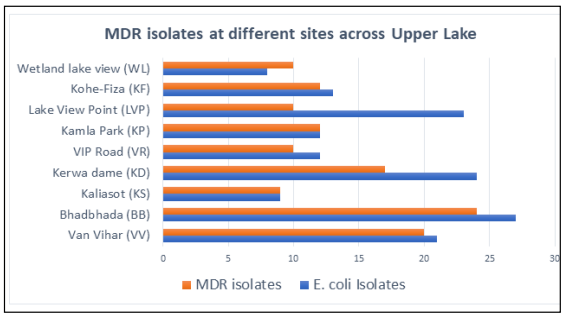

Figure 4. Sample collection site (23°14'41.532"N , 77°22'37.488 E)

Across the study sites, *E. coli* samples responded differently to various antibiotics. The *E. coli* samples taken at VIP Road site, where Upper Lake linked with new Bhopal, were found to be resistant to a variety of antibiotics, together with amino-glycosides and beta-lactams, according to (12, 13). Among the isolates, more than 40% were resistant to beta-lactam antibiotics, while 50% showed (14) resistance to aminoglycosides (gentamicin and tobramycin). (15) Chloramphenicol, however, was found to be highly effective against the isolates. Out of the twenty-one isolates collected from this site, twenty were identified as MDR isolates because they were resistant to four different antibiotic classes.

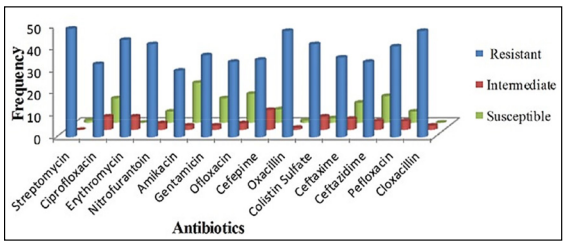

Figure 5. Occurrence of antibiotics compared to intermediate, resistance and susceptible Reprinted with permission (10)

The level of antibiotic resistance displayed by *E. coli* isolates varied across different sampling sites. At the Upper Lake entrance point at VIP Road, a considerable number of water-borne *E. coli* isolates exhibited resistance to multiple antibiotic classes, including amino-glycosides and β-lactams, with 40% and

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50% of the isolates exhibiting struggle to these antibiotic classes, respectively. (16) However, chloramphenicol was able to destroy these isolates, and twenty-one isolates from this site were classified as MDR isolates. At the subsequent upper reaches sampling site VV, *E. coli* isolates showed a sharp rise in resistance to various antibiotics, including fluoroquinolones, β-lactams, tetracycline, and rifampin, with 24 isolates exhibiting high levels of MDR to β-lactam resistance. (17,18) The two isolates, however, did not exhibit drug resistance to any of the examined medications. The internal *E. coli* restrictions from the KP testing location exposed a general increase in resistance in the direction of anti-infection agents, including β -lactams and antibiotic drug bundles, compared to the KD testing site. The middle *E. coli* confines taken from the KP testing location showed a general increase in resistance to anti-infection agents, including -lactams and antibiotic drug bundles, compared to the KD testing site. *E. coli* isolated from the VR region demonstrated the highest level of resistance in the middle reaches, with ten out of twelve isolates (83%) being MDR, and displaying determined confrontation to beta-lactams, chloramphenicol, fluoroquinolones, and tetracyclines, through hundred percentage resistance to the antibiotic's Cefazolin, Cefotaxime, & Cefuroxime. (19, 20) The isolates also showed significant diversity in their antibiotic resistance profile between sample locations located in the middle reaches of Upper Lake.

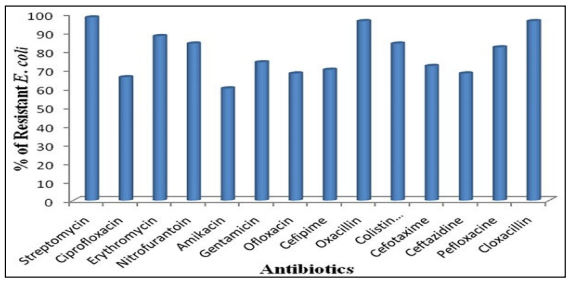

Figure 6. Confrontation Proportion (%) of Antibiotics compared to E. coli. Reprinted with permission (10)

However, the *E. coli* samples from the ITO locations exhibited a higher overall resistance to most antibiotics compared to the KP and LVP sites. In the middle reaches, all *E. coli* separates demonstrated hundred percent resistances with cefazolin. The *E. coli* samples as of the VV sample presented a highest resistance to amino-glycosides, azithromycin, β-lactams, fluoroquinolones, nitrofurans, rifampin with a decrease in resistance to trimethoprim/sulphonamide and tetracycline groups. *E. coli* isolates from the VV site had considerably greater levels of tobramycin and nitrofurantoin resistance. However, at the DND Flyway sampling site, over 40% of *E. coli* displayed resistance to trimethoprim and sulphafurazole. (22) The resistance trend varied significantly between the higher, medium, and lower reaches. The VR (located in the centre) and VV testing locations into industrialized areas had the highest no. of MDR totals. (21) The *E. coli* isolated after the middle assortments exhibited the greatest resistance to six anti-infection classes, including polymyxins, chloramphenicol, fluoroquinolones, nitrofurantoin, and β-lactams. In the lower reaches, *E. coli* isolates displayed a declining level of antibiotic resistance, except for the increasing trend in resistance to sulphonamides-trimethoprim, with the maximum resistance (23, 24) observed at VR -site.

MAR (Multiple Antibiotic Resistance) was calculated for each sampling site, with those above 0.25 indicating a greater contribution to Upper Lake contamination. Damage reports from middle and lower sites were generally higher than those from upper sites. Studies by ecological organizations and water resource authorities (29, 31) ; (30, 32, 35) have highlighted the impact of antibiotic resistance in human, sewage, (25) and environmental sectors. Isolates from VR and VV sites demonstrated the highest MAR indices (0.678 and 0.574, respectively), with high resistance to gentamicin and β-lactam antibiotics such as cefazolin, cefotaxime, and cefuroxime found in direct sewage isolates. Among the sixteen isolates, three were identified as MDR. Amikacin showed the highest sensitivity rate (33.37%), followed by gentamicin and cefepime (22.92%). *E. coli* (26)

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through a Blemish case of 1.01 demonstrated the highest multidrug resistance, and 127 obtainable of 150 isolates i.e., 34% was resilient to all 26-antibiotic tested. Antibiotic residues and resistant bacteria are released into the environment through industrial waste and sewage, contaminating water (23, 27) and food and facilitating the spread of ARB and ARGs. Aquatic environments have been found to harbor antibiotic-resistant microorganisms or genes that confer antibiotic resistance, particularly to aminoglycosides, tetracyclines, and β -lactams. (28, 33) These resistance determinants are often carried by mobile genetic elements, allowing for their rapid spread. (34) In aquatic environments, *E. coli* is frequently used as a biomarker for multiple antibiotic resistance. (10)

Conclusion

Investigating the incidence of MAR-Multiple Antibiotic Resistance in *E. coli* strains from Upper Lake Bhopal was the goal of this investigation. The researchers used integrons and gene cassettes to classify the MDR isolates and examine the phylogenetic relationships among the isolates. They employed the Kirby Bauer diffusion disc technique to assess the antibiotic susceptibility profiles of 157 *E. coli* isolates from Upper Lake and direct sewage samples from the surrounding area. Among the nineteen drugs tested, cefazolin had the highest frequency of resistance (88%), followed by vancomycin (74%). Approximately 25% of the microbiological isolates was resistant to 13 medications, with more than ninety percent *E. coli* isolates being extremely toxic to azithromycin & polymyxin-B. The study found a high level of resistance to antibiotics from the β-lactam class, tetracyclines, fluoroquinolones, nitrofurantoin, and chloramphenicol from *E. coli* isolated water samples of Upper Lake, especially in the middle reaches. However, there was an increasing trend in trimethoprim/sulphonamide resistance throughout Upper Lake, and MDR isolates were present at every sampling site. All sampling locations in the lower middle and upper sections of Upper Lake had MAR indices above 0.25, demonstrating a significant risk of contamination due to the Straight sewage were the samples had high resistance to aminoglycoside and β-lactam antibiotics.

Conflict of Interest

No conflict of interest

Acknowledgements

The author is grateful to the Hon'ble Chancellor Er. B.S. Yadav and Hon'ble Pro-Chancellor Dr. (Prof.) Sunita Singh of the IES University, Bhopal, Madhya Pradesh, India for providing laboratory space and instruments during entire research work.

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