Detection of Multidrug Resistant (MDR) Bacteria in Untreated Hospital Waste Water Samples

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***Abstract*— The emergence of multidrug-resistant (MDR) bacteria in untreated hospital wastewater represents a significant public health concern. The present study aimed to investigate the occurrence and antimicrobial resistance pattern of *Escherichia coli* isolated from untreated hospital wastewater. A total of 35 wastewater samples were analyzed, from which 24 isolates (42.1%) were identified as *E. coli*. Antibiotic susceptibility testing was performed using the disc diffusion method. The results revealed complete resistance (100%) of *E.coli* isolates to antibiotics belonging to the *Penicillin, Cephalosporin, Lincosamide,* and *Glycopeptides* classes. In contrast, the isolates showed 100% sensitivity to *Aminoglycosides* and *Chloroamphenicol*. The multiple antibiotic resistance (MAR) index for *E. coli* isolates was calculated to be 0.58, indicating exposure to high-risk sources of antibiotic contamination. These findings highlight the presence of multidrug-resistant *E. coli* in hospital wastewater and emphasize the need for effective wastewater treatment and continuous monitoring of antimicrobial resistance to reduce the spread of resistant bacteria.**

Keywords*— *Multidrug resistant bacteria, Untreated hospital wastewater, Antibiotic resistance, Multiple antibiotic resistance index (MAR), Public health.

I. INTRODUCTION

Water is essential for the survival of all living organisms, serving as a medium for metabolic reactions and a solvent for biological substances. However, contamination of water with sewage and wastewater promotes the growth and transmission of pathogenic microorganisms. Sewage water consists of untreated effluents originating from domestic, industrial, and hospital sources (Mahesh *et al*., 2017).

Among these sources, hospital wastewater represents a significant public health concern because it carries a high load of pathogenic and antibiotic-resistant microorganisms. Globally, approximately 700,000 deaths occur each year due to antimicrobial-resistant infections, and this number is projected to reach 10 million annually by 2050 if effective control measures are not implemented. In addition to health impacts, antimicrobial resistance may also cause severe economic losses by increasing healthcare expenditure and reducing productivity (Rahman *et al*., 2021; Huemer *et al*., 2020)

Hospital effluents commonly harbor a variety of pathogenic bacteria including *Salmonella*, *Shigella, Staphylococcus aureus, Pseudomonas aeruginosa, Vibrio, Clostridium, Yersinia, Campylobacter, and Leptospira.* Moreover, fecal indicator organisms belonging to the coliform group such as *Serratia, Enterobacter, Klebsiella, and Escherichia coli* are frequently detected in such wastewater (Asfaw *et al*., 2017).

Escherichia coli is widely used as an indicator of fecal contamination because it naturally inhabits the gastrointestinal tract of humans and animals and is commonly present in untreated wastewater. Certain pathogenic strains are capable of causing both intestinal and extra-intestinal infections, including diarrhea, urinary tract infections, septicemia, and neonatal meningitis (Raham *et al*., 2025).

In recent years, *E. coli* has developed resistance to multiple classes of antibiotics through diverse mechanisms. Resistance to β-lactam antibiotics is particularly alarming because these drugs including *penicillins, cephalosporins, carbapenems*, and *monobactams* account for more than 65% of the global antibiotic usage and are widely used as first-line therapies (Raham *et al*., 2025).

Multiple Drug Resistance (MDR) is defined as resistance to at least one antimicrobial agent in three or more antimicrobial categories. MDR significantly reduces treatment efficacy, prolongs illness duration, increases mortality rates, and elevates healthcare costs. The misuse and overuse of antibiotics are considered major contributors to the increasing prevalence of antimicrobial resistance (Aguilar-Salazar *et al*., 2023).

The emergence of Multiple Antibiotic Resistant (MAR) bacteria in aquatic environments further complicates treatment strategies and poses a serious threat to public health. Therefore, monitoring antibiotic resistance in bacteria isolated from hospital wastewater is essential to understand its environmental dissemination and to develop effective control measures (Chatterjee *et al*., 2021).

II. Materials and Methods

A. Sample Collection

The samples were collected using bottles with 1 litre capacity from 35 distinct areas of Amravati city. The period for collecting samples was from January 2024 to March 2024. The autoclave method was utilized to sterilize used bottles. Following Samples were labeled with HWW01 to HWW35. Transporting and collecting samples to the lab were done in an aseptic manner. Date, time and place of the sample collection were recorded. Within 24 hours after sample collection, analysis was completed (Rabbani, *et al*., 2017).

B. Bacterial Isolation and Identification

Bacteria were isolated from untreated hospital wastewater samples using standard microbiological procedures. Then, 1 ml of each dilution was plated on several selective and differential media, including MacConkey agar, EMB agar and incubated at 37℃ for 24 hours. Serial dilutions were prepared up to 10^{-6}. After establishing pure colonies, a single random colony was selected and identified based on its microscopic and biochemical properties using the Ananthanarayan and Paniker's textbook of Microbiology, 10th edition.

C. Antibiotic Sensitivity Testing

All of the bacterial isolates identified were subjected to antibiotic susceptibility testing based on Kirby-Bauer disc diffusion method using Muller Hinton agar (MHA) according to CLSI guidelines. The antimicrobial susceptibility testing was performed against 12 antibiotic disks, *Ampicillin* (10mcg), *Methicillin* (5mcg), *Penicillin* (2 units), *Cefotaxime* (30mcg), *Gentamicin* (10mcg), *Streptomycin* (10mcg), *Tetracycline* (30mcg), *Clindamycin* (2mcg), *Chloramphenicol* (30mcg), *Vancomycin* (10mcg), *Norfloxacin* (10mcg), *Cotrimoxazole* (26mcg) for all isolates. The zones of inhibitions were read in (mm) with the help of Hi-Media zone scale from Hi-Media Laboratories Pvt. Ltd. Mumbai, India. The results were interpreted as per the guidelines of CLSI. Based on zone of inhibition the isolates were categorized as Sensitive (S), Intermediate (I) and Resistant (R).

D. Multiple Antibiotic resistance (MAR) indexing

Isolates showed resistance to three or more than three antibiotics are called multidrug resistant (MDR) or multiple antibiotic resistant (MAR). The values were calculated according to the following formulas (Krumperman, 1983; Tambekar, *et al*., 2006; Deshmukh *et al.,* 2015; Ayandele, *et al*., 2020).

$$\textbf{Multiple antibiotic resistance index for isolates} = \frac{\textbf{No. of antibiotics to which organism is resistant}}{\textbf{Total number of antibiotics tested}}$$

$$\textbf{Multiple antibiotic resistance index for antibiotic} = \frac{\textbf{Number of antibiotics resistant to isolates}}{\textbf{Total number of Antibiotics} \times \textbf{Total number of isolates}}$$

III. Result and Discussion

A total of thirty-five hospital wastewater samples were analyzed, among these, 24 isolates (42.1%) were identified as *Escherichia coli*, indicating that *E.coli* was one of the predominant microorganisms present in hospital wastewater.

The antimicrobial susceptibility of the 24 *E.coli* isolates was evaluated against 12 antibiotics representing nine antibiotic classes:*penicillins*, *cephalosporins*, *aminoglycosides, tetracyclines, lincosamides, chloramphenicol, glycopeptides, fluoroquinolones,* and *trimethoprim*. The susceptibility testing revealed a high level of resistance among the isolates. Complete resistance (100%) was observed to antibiotics belonging to the *penicillin, cephalosporin, lincosamide,* and *glycopeptide* classes. Specifically, all *E.coli* isolates were resistant to *ampicillin, methicillin, penicillin, cefotaxime, tetracycline, clindamycin*, and *vancomycin*. Overall, seven of the twelve antibiotics tested showed resistance against *E.coli* isolates.

In contrast, the isolates exhibited complete sensitivity (100%) to *gentamicin, chloramphenicol, norfloxacin*, and *co-trimoxazole*. The zones of inhibition measured for these antibiotics were 21 mm for *gentamicin*, 25 mm for *chloramphenicol*, 25 mm for *norfloxacin*, and 27 mm for co-*trimoxazole*, indicating strong antimicrobial activity. *Streptomycin* produced an intermediate susceptibility response in all isolates, with a zone of inhibition of 12 mm.

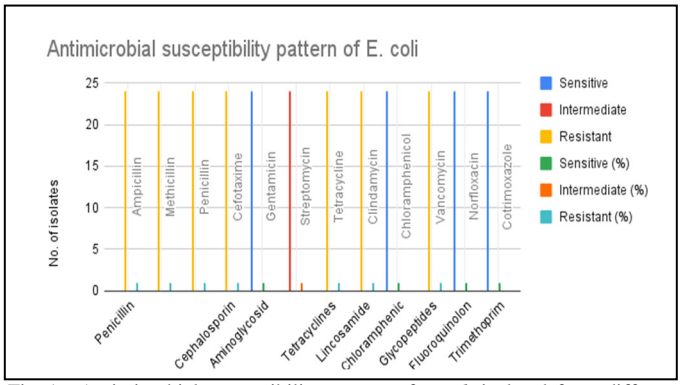

Fig. 1. Antimicrobial susceptibility pattern of *E.coli* isolated from different hospitals waste water samples.

Table no.1 Sensitive, Intermediate and Resistant pattern of isolates towards all antibiotics and Multiple Antibiotic Resistance (MAR) index of isolates.

Isolated Code	No. of antibiotics				
	Sensitive	**Intermediate**	**Resistant**	**Total no. of antibiotics**	**MAR index of isolates**
EC (1-24)	4	1	7	12	0.58

(NOTE: EC- *E.coli*)

The multiple antibiotic resistance (MAR) index calculated for the *E. coli* isolates was 0.58, demonstrating a high level of multidrug resistance. The isolates showed resistance to commonly prescribed antibiotics, including *ampicillin*, *methicillin*, *penicillin*, *cefotaxime*, *clindamycin*, and *vancomycin* (each 100% resistance; MARI 0.083). Moderate resistance levels were observed for *streptomycin* (57%; MARI 0.048), *tetracycline* (66.66%; MARI 0.055), and *norfloxacin* (33%; MARI 0.027). *Gentamicin*, *chloramphenicol*, and *co-trimoxazole* were excluded from the MAR index calculation due to universal sensitivity among the isolates.

Overall, the results indicate that *E.coli* isolates from hospital wastewater exhibited significant multidrug resistance, particularly to *beta-lactam* and related antibiotic classes, while retaining susceptibility to selected *aminoglycosides, fluoroquinolone*, and *trimethoprim* antibiotics.

DISCUSSION

In the present study, *Escherichia coli* was identified as the predominant bacterial isolate from hospital wastewater samples, accounting for 42.1% of the total isolates. This finding is consistent with previous reports by Rahman (2021) and Alam and Imran (2017), who also observed *E. coli* as the most frequently isolated pathogen in hospital effluents. The high prevalence of *E.coli* in wastewater highlights the significant contribution of hospital discharge to environmental contamination and the potential dissemination of antimicrobial-resistant bacteria.

The antibiotic susceptibility pattern observed in this study revealed a high level of resistance among *E.coli* isolates. All isolates exhibited complete (100%) resistance to antibiotics belonging to the *Penicillin*, *Lincosamide*, *Cephalosporin*, and *Glycopeptides* classes. These findings indicate extensive exposure of *E. coli* to commonly used antibiotics in clinical settings, leading to strong selective pressure and the emergence of resistant strains. Comparable trends of high resistance to *beta-lactam* and *tetracycline* antibiotics were reported by Choushette *et al*.,(2022), who documented maximum resistance in *beta-lactams* (85%) and *tetracyclines* (58%).

In the present investigation, *E.coli* isolates demonstrated resistance to *Penicillin*, *Cephalosporin*, and *Tetracycline* classes, while remaining sensitive to *Aminoglycosides* and *Fluoroquinolones*. This pattern partially aligns with the findings of Patel and Deshpande (2020), who reported resistance of *E. coli* to multiple antibiotic classes, including *Aminoglycosides*, *Tetracycline*, *Carbapenems*, *Penicillin*, and *Fluoroquinolones*. The observed differences may be attributed to variations in antibiotic usage patterns, geographic location, and local infection control practices.

The isolates in this study exhibited 100% resistance to several individual antibiotics, including *Ampicillin*, *Methicillin*, *Penicillin*, *Cefotaxime*, *Tetracycline*, *Clindamycin*, and *Vancomycin*. Similar resistance patterns were reported by Deshmukh *et al*., (2015), who documented high resistance rates to *Ampicillin* (83.58%) and *Tetracycline* (52.23%). Additionally, Alam and Imran (2017) reported resistance of Gram-negative bacterial isolates to *Amoxicillin*, *Methicillin*, *Ampicillin*, *Nalidixic acid*, and *Penicillin*, supporting the widespread occurrence of resistance to commonly prescribed antibiotics.

Interestingly, in the present study, *E. coli* isolates were found to be 100% sensitive to *Co-trimoxazole*, *Gentamicin*, *Chloroamphenicol*, and *Norfloxacin*. This observation contrasts with the findings of Rabbani (2017), who reported resistance of *E.coli* to *Chloroamphenicol* and *Gentamicin*. Such discrepancies may reflect regional differences in antibiotic exposure and stewardship practices, as well as temporal variations in resistance trends.

The Multiple Antibiotic Resistance (MAR) index of *E. coli* isolates in the current study was ranged from 0.027 to 0.083, with an overall value around 0.058. High resistance was particularly observed against widely used antibiotics such as *Vancomycin* (100%, MARI 0.083), *Penicillin* (100%, MARI 0.083), *Cefotaxime* (100%, MARI 0.083), *Tetracycline* (66.66%, MARI 0.055), *Streptomycin* (57%, MARI 0.048), and *Norfloxacin* (33%, MARI 0.027). These findings show partial similarity to the work of Tambekar *et al*. (2006), who reported high resistance to *Vancomycin* and *Ampicillin*. The MAR index range observed in this study is also comparable to that reported by Alam and Imran (2017).

Previous studies by Mustapha and Imir (2019) documented alarming levels of multidrug resistance in *E. coli*, with isolates resistant to up to ten antibiotics and MAR indices ranging from 0.2 to 1.0. Although the MAR indices in the present study are comparatively lower, the detection of multidrug-resistant *E. coli* in hospital wastewater remains a serious public health concern. Hospital effluents may act as reservoirs for resistant bacteria, facilitating their spread into the environment and increasing the risk of transmission to humans and animals.

Overall, the findings of the present study emphasize the significant presence of multidrug-resistant *E. coli* in hospital wastewater. Continuous monitoring of antimicrobial resistance patterns and implementation of effective wastewater treatment and antibiotic stewardship programs are essential to limit the environmental dissemination of resistant strains.

CONCLUSION

The findings of the present study indicate that *Gentamicin*, *Chloroamphenicol, Norfloxacin*, and *Co-trimoxazole* were the most effective antibiotics against *Escherichia coli*, as the isolates showed high sensitivity to these drugs. These antibiotics may therefore be considered as potential therapeutic options for infections caused by *E.coli*. However, the

increasing resistance of *E.coli* isolates to commonly used antibiotics represents a serious challenge in the effective management of bacterial infections. The study demonstrated high resistance to *Ampicillin, Methicillin, Penicillin, Cefotaxime, Clindamycin, Vancomycin, Streptomycin*, and *Tetracycline*. Consequently, the indiscriminate use of these antibiotics should be avoided, and antibiotic therapy should be guided by susceptibility testing. Continuous surveillance of antimicrobial resistance and the implementation of rational antibiotic stewardship programs are essential to prevent further development and spread of resistant *E. coli* strains.

The study highlights the considerable financial costs linked to infections acquired in hospitals. Dealing with hospital acquired infections caused by multidrug resistant organisms involves expensive interventions, such as specific antibiotics, prolonged hospital stays, and infection control measures. Rise in antibiotic resistance of bacteria from hospital wastewater demonstrates the growing problem of antibiotics overuse and misuse. Avoid the burden of resistant bacteria in common, it is important to have strict antibiotic laws, Antimicrobial Resistant (AMR) surveillance programs for multidrug resistant organisms. Understanding as well as community awareness about the importance of proper antibiotic use is crucial; misuse or overuse can lead to antibiotic resistance, rendering previously effective antibiotics ineffective against bacterial infections.

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