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Deciphering antibiotic resistance in bacteria from hospital wastewater drains

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Abstract

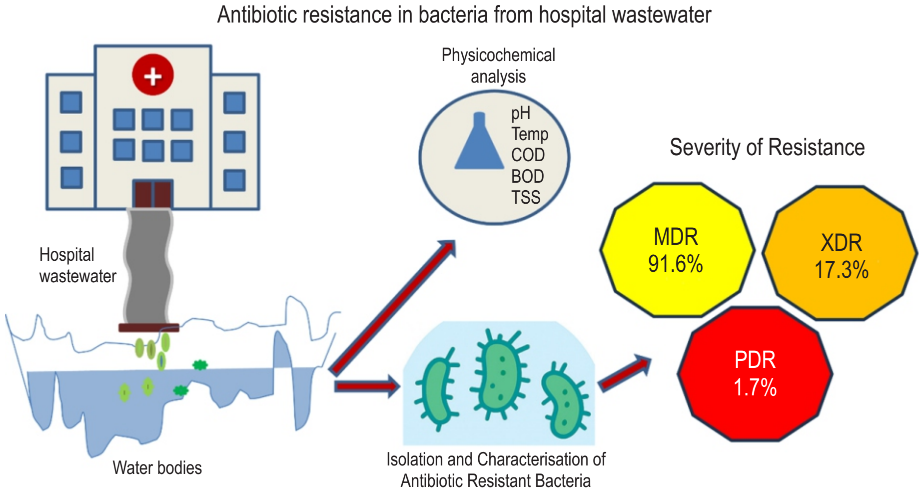
Aim: This study aimed to assess the physico-chemical and microbiological quality of hospital effluents and evaluate the prevalence of multidrug-resistant (MDR), extensively drug-resistant (XDR), and pan-drug-resistant (PDR) bacteria.

Methodology: Wastewater samples were collected from nine hospitals and analysed for physico-chemical and microbiological parameters. Statistical analysis was performed to compare variations among the sites, and antibiotic susceptibility testing of Gram-negative isolates was conducted by disc diffusion method to classify bacterial resistance profiles.

Results: Most of the means and medians of physico-chemical parameters showed no significant variations among the samples ($p > 0.05$), except for BOD and COD. Among 179 bacterial isolates 91.6% were MDR, 17.3% XDR and 1.7% PDR. *Pseudomonas* spp. and over 80% of *Klebsiella, E. coli, Shigella, Salmonella,* and *Proteus* spp. exhibited multidrug resistance. The MAR index of all bacterial species isolated from wastewater was higher than 0.5.

Interpretation: High Multiple Antibiotic Resistance (MAR) indices indicate a substantial risk of horizontal transfer of resistance traits in the aquatic environments. The study underscores the urgent need for effective hospital wastewater management and treatment strategies to prevent environmental dissemination of antibiotic resistance.

Key words: Antibiotics, Enterobacteriaceae, Hospital wastewater, Pan-drug-resistant



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Introduction

The unregulated and disproportionate use of antibiotics in humans and animals leads to an increase in antibiotic resistance, facilitating the spread of resistance genes within environmental reservoirs such as hospital wastewater. These wastewaters create specialized habitats that significantly support the growth of resistant bacterial strains released into the environment (Moges *et al.,* 2014). Recently, Jabbar *et al.* (2025) reported elevated levels of total suspended solids (TSS), biological oxygen demand (BOD), and chemical oxygen demand (COD) that exceed permissible limit of APHA (2017), indicating severe pollution. The rise in antibiotic resistance has caused serious global concern because it reduces the efficiency of current antibiotics against bacterial infections (Le *et al.,* 2016). The emergence of multidrug-resistant (MDR) bacteria, followed by extensively drug-resistant (XDR) bacteria, poses a significant threat to patients with infectious diseases. Recent reports of pan-drug-resistant (PDR) bacteria, resistant to all antibiotic classes, has raised widespread alarm (Moghadam *et al.,* 2020).

Antimicrobial resistance is recognized as a major medical challenge that increases the mortality, morbidity, hospital stay durations, costs, and limits treatment options. The World Health Organisation (WHO) lists multi-drug resistance (MDR) among the top ten global health threats. A recent systematic analysis estimated that 4.95 million deaths occurred in 2019 due to bacterial MDR. Another estimate suggests that at least 700000 lives are lost each year due to drug-resistant diseases. By 2030, AMR could push up to 24 million people into extreme poverty, and by 2050, it might cause the loss of 10 million lives annually (Addae-Nuku *et al.,* 2022). Studies show hospital wastewater as a confirmed hotspot for harbouring and spreading MDR, XDR, and PDR bacterial strains, including *E. coli, Proteus, Salmonella, Pseudomonas, Klebsiella, Enterobacter,* and *Shigella* species (Geeta *et al.,* 2022) and (Maidamma *et al.,* 2023). Untreated hospital effluents serve as reservoirs for antimicrobial compounds and resistant bacteria, significantly aiding the spread of antimicrobial resistance (AMR) in natural water bodies (Conforti *et al.,* 2025). The Multiple Antibiotic Resistance (MAR) Index is a key indicator in wastewater microbiology that assesses the risk and extent of antibiotic resistance contamination, particularly in environments exposed to medical, industrial, or agricultural waste. MAR > 0.2 usually indicates bacteria from areas with high antibiotic exposure, highlighting potential hotspots for resistance development (Shakir *et al.,* 2021).

Antibiotic resistance is no longer just a clinical issue but a complex environmental and public health challenge with serious global impacts. Soil, water, wastewater, and agriculture are increasingly seen as key reservoirs and transmission pathways for spreading antibiotic resistance genes and resistant bacteria (ARGs and ARB) (Adeoye *et al.,* 2025). These pathways link human, animal, and ecosystem health—making antibiotic resistance a quintessential 'One Health' issue. The spread of resistance via environmental route lead to treatment failures, fewer available drugs, and disproportionately impacts low- and middle-income countries (Aissi *et al.,* 2025). The availability of antibiotics over the counter (OTC), their misuse by public and industries such as poultry and livestock, is a common factor contributing to the development of drug resistance. The needless prescription of antibiotics for various reasons has been reported in many countries (Zgliczyński *et al.,* 2022). Educating the public about the proper use and potential misuse of antibiotics is essential to prevent the rise of drug-resistant bacteria. In view of the above, this study aimed to explore the incidence of antibiotic resistance in hospital wastewater isolates, which has not previously been reported from Ayodhya district, Uttar Pradesh, India

Materials and Methods

Collection of wastewater sample: Hospital wastewater samples were collected from nine sites viz., Chiranjeevi Hospital (S1), Kishan Hospital (S2), Sanjeevani Hospital (S3), Ayodhya District Hospital (S4), Ayodhya Women's District Hospital (S5), Medical collage-1 (S6), Medical collage-2 (S7), Sri Ram Hospital (S8) and Leprosy Mission Hospital (S9) from different locations in Ayodhya district of Uttar Pradesh during January 2021 to December 2021 by the grab sampling method. The temperature and pH of the samples were recorded on-site. The collected samples were stored in sterile glass bottles and transported on ice to the laboratory, where they were stored at 4°C until further analysis. Additional physico-chemical and microbiological tests were performed within the first 24 hr of sampling.

Analyses of water quality parameters: Turbidity, temperature, and pH were measured on-site at the time of sample collection with a portable water analyser kit 371 (Systronics, India). Physico-chemical parameters such as dissolved oxygen, Biological oxygen demand, Chemical oxygen demand, and Total suspended solids (TSS) were estimated by the standard methods of APHA (2017).Total coliform (TC) and faecal coliform (FC) in the samples were determined using the five-tube fermentation method for most probable number (MPN) and presence-absence (PA) test, respectively, by following the APHA (2017) guidelines. The analyses were conducted in the Department of Microbiology, Dr. Rammanohar Lohia Avadh University (Uttar Pradesh), India.

Isolation and characterisation of bacterial isolates: The samples collected were serially diluted up to 10^{-6} using sterile n-saline (0.9% NaCl), and an aliquot of 100 µl from each dilution was spread on MacConkey Agar (MA), Eosin Methylene Blue (EMB) Agar, and *Salmonella-Shigella* (SS) Agar (HiMedia®, India), followed by incubation at 37 °C (±2) for 18-24 hrs. Bacteria were isolated from the water using culture-dependent methods (Method no. 9215, APHA, 1992). The inoculated Petri dishes were incubated at 37 °C for 24-48 hrs, after which CFU 100 ml^{-1} were calculated. Distinct colonies were picked based on their colony morphology and streaked on agar media for pure culture. The pure culture colonies were subcultured onto nutrient agar slants and stored at 4°C. The isolates were further examined for cell shape and membrane features using phase-contrast and bright-field microscopy, as described by Kumar *et al.* (2013). Bergey's Manual of Determinative Bacteriology (Holt *et al.,* 1994) was referred for the biochemical identification of bacterial isolates with a Biochemical Test Kit (KB003) obtained from HiMedia Labs.

Antimicrobial susceptibility assay: The antibiotic susceptibility test was performed according to the Kirby-Bauer method (Hudzicki, 2009). Small discs containing antibiotics were placed onto a Mueller-Hinton agar plate seeded with bacteria. The inoculated Petri dishes containing antibiotic discs were incubated upright at 37 °C for 24 hr. The absence of bacterial growth around the antibiotic disc was indicated by a clear zone of inhibition, which was recorded using a zone scale. Antibiotics from different classes used in the sensitivity study included: Aminoglycosides: Gentamycin (GEN-10 µg), Glycopeptide: Vancomycin (VA-30 µg), Macrolides: Erythromycin (E-30 µg), β-lactams: Penicillin (P-30 µg), Quinolones: Norfloxacin (NX-10 µg), Tetracyclines: Tetracycline (TE-30 µg), Cephalosporins: Cefazolin (CZ-30 µg), Fluoroquinolones: Ciprofloxacin (CIP-30 µg), Lincosamides: Clindamycin (CD-2 µg), Carbapenems: Meropenem (MRP-30 µg), Sulfonamides: Co-Trimoxazole (COT-30 µg), and others: Chloramphenicol (C-10 µg). The isolates were categorised as either sensitive or resistant according to the Clinical and Laboratory Standards Institute (CLSI, 2023) recommendations for antimicrobial disk susceptibility tests. The resistance phenotype (MDR, XDR and PDR) was assigned to bacteria following the description provided earlier (Magiorakos *et al.,* 2012). Isolates were classified as multidrug-resistant (MDR) if they were non-susceptible to at least one agent in three or more antimicrobial categories, extensively drug-resistant (XDR) if they were non-susceptible to at least one agent in all but two or fewer categories, and pandrug-resistant (PDR) if they were non-susceptible to all agents in all antibiotic categories.

Multiple antibiotic resistances (MAR) index: The multiple antibiotic resistance (MAR) index was applied to the water sample from which several isolates were obtained. The MAR index of bacterial species was calculated as described previously by Krumperman (1983) and Hinton *et al.* (1984) using the following mathematical equation:

$$MAR\ index = \frac{y}{(n.x)}$$

Where y, is the total antibiotic resistance score of all isolates from the sample, n is the number of antibiotics tested, and x is the number of isolates from the sample. When the MAR index exceeds 0.2, it indicates extensive antibiotic use in that area and suggests a high risk of AMR isolate proliferation.

Statistical Analyses: The data obtained from physico-chemical analyses of samples was subjected to statistical analysis using SPSS software. The analyses involved tests of normality, homogeneity of variances, ANOVA, and the Multiple Comparison Test. To test the normality of data, the Shapiro-Wilk test was applied and found that all the physico-chemical parameters followed a normal distribution, as the p-value for each parameter was greater than 0.05. Since the variables under consideration followed a normal distribution, ANOVA was applied to test the equality of means for these physico-chemical parameters. When ANOVA was rejected, the Tukey HSD Multiple Comparison Test was applied to identify which pairs of physico-chemical parameters were responsible for the rejection.

Results and Discussion

The hospital wastewater was characterised physico-chemically by measuring the pH, temperature, TSS, DO, BOD, and COD. Results of analysis are presented in Table 1. Data indicated that the pH of wastewater samples ranged between 6.4 ± 0.5 and 7.7 ± 0.2 while temperature of the sampling site fluctuated between 20.3 – 22.0 °C. Similarly, the COD value of all the sites was almost in the same range. Statistical analyses of physico-chemical parameters revealed minor differences in the pH, temperature and COD values of water samples collected from different sites, as indicated by the mean and median values. In contrast, ANOVA result revealed significant differences among the samples from different locations (p = 0.001). The pH and temperature values in the present study were within the acceptable range for wastewater (Amouei *et al.,* 2012; EPA, 2025). Karungamye *et al.* (2023) reported similar results at Benjamin Mkapa Hospital in Dodoma, Tanzania. The comparable pH ranges reported in recent wastewater characterization studies are considered safe for biological activities and aquatic life. Although they promote the solubility and mobility of certain contaminants, but water quality is not just confirmed to pH and temperature, several other physico-chemical parameters are also taken into consideration (Aiwonegbe *et al.,* 2025; Oluwafemi *et al.,* 2026).

Water temperature significantly influences many chemical and physical water properties, including gas solubility, reactivity, toxicity, and microbial activity (Wilson *et al.,* 2021). The temperature of the samples collected ranged from 20.3 °C to 22.0 °C. The test of homogeneity of variances showed minor to no difference in the temperature of the samples collected from different sampling sites. ANOVA revealed a p-value >0.05, indicating no significant difference in temperature among the samples. The samples' temperature was well below the maximum permissible limits of 40 °C which is quite favourable for high microbial activity and play crucial role in maintaining the diversity of aquatic ecosystem (Karungamye *et al.,* 2023; Chauhan *et al.,* 2024; Oluwafemi *et al.,* 2026).

Laboratory physico-chemical analyses showed that the total suspended solids (TSS) ranged from 180.3 to 380.0 mg l^{-1}. The homogeneity of variance test indicated no significant difference in TSS levels among the collected samples. However, the ANOVA indicated significant differences among the samples at 0.05 level of significance. The observed TSS values were 5 times or more than the EPA limits and comparable to previous research reports showing TSS levels of 209.5 mg l^{-1} and 119. 7 mg l^{-1} and

Table 1: Physico-chemical properties of hospital wastewater from hospitals in Ayodhya

Sample ID	Graphical Coordination (DD)		pH	Temp.	TSS	DO	BOD	COD
	Latitude (N)	Longitude (E)		(°C)	(mg l^{-1})	(mg l^{-1})	(mg l^{-1})	(mg l^{-1})
S1	26.762332	82.141769	6.7 ± 0.5	21.0 ± 1.0	197.0 ± 2.7	0.2 ± 0.1	59.3 ± 2.1	204.3 ± 3.8
S2	26.756347	82.140872	7.3 ± 0.3	20.7 ± 1.5	180.3 ± 1.5	0.9 ± 0.1	50.0 ± 2.0	238.5 ± 1.3
S3	26.753385	82.141090	6.9 ± 0.1	21.0 ± 1.0	311.7 ± 1.5	0.5 ± 0.1	54.7 ± 2.5	272.7 ± 2.1
S4	26.778680	82.140672	6.4 ± 0.5	22.0 ± 1.0	380.0 ± 2.0	0.7 ± 0.1	55.3 ± 2.5	272.0 ± 3.0
S5	26.778137	82.141629	7.6 ± 0.3	20.7 ± 2.1	352.0 ± 2.0	1.8 ± 0.1	65.0 ± 3.0	240.0 ± 3.0
S6	26.756655	82.203400	6.5 ± 0.4	21.7 ± 1.5	303.7 ± 2.5	1.2 ± 0.2	54.3 ± 3.1	288.0 ± 3.0
S7	26.756090	82.204161	7.5 ± 0.4	21.0 ± 1.0	375.0 ± 3.6	1.9 ± 0.1	54.7 ± 1.5	255.7 ± 2.5
S8	26.792262	82.199109	7.6 ± 0.4	21.0 ± 1.0	243.3 ± 2.1	0.3 ± 0.1	69.0 ± 3.6	205.0 ± 2.6
S9	26.684212	82.136320	7.7 ± 0.2	20.3 ± 1.5	195.3 ± 3.1	0.4 ± 0.1	60.0 ± 3.0	256.0 ± 3.0
Permissible limit (EPA, 2025)			6 - 9	≤40	30 - 45	5	30 – 45	120 - 250

* Data presented is the mean of triplicate reading; ± SD: represent standard deviation

Table 2: Number of Faecal coliforms in wastewater collected from hospital drains as per PA test results

Sample ID	Faecal coliform			
	Combination of positive MPN tubes for PA test			No. of faecal coliforms (100 ml^{-1})
S1	5	5	3	900
S2	5	5	2	500
S3	5	5	4	1600
S4	5	5	3	900
S5	5	5	2	500
S6	5	5	1	300
S7	5	5	4	1600
S8	5	5	3	920
S9	5	5	0	240

339 mg l^{-1}, respectively, from Europe, Asia, and Spain by other researchers worldwide (Suarez *et al.,* 2005; Majumder *et al.,* 2021). TSS is a primary pollutant that worsens the water quality (Verma *et al.,* 2013) and impairs oxygen transfer between air and water (Wei *et al.,* 2020). TSS levels in wastewater have been reported to increase turbidity and sediment levels in receiving waters (Chauhan *et al.,* 2024; Oluwafemi *et al.,* 2026).

Dissolved oxygen (DO) was recorded between 0.20 and 1.87 mg l^{-1}. The analysis of mean and median values revealed significant differences in dissolved oxygen among different samples which is further substanatiated by ANOVA analysis (p-value < 0.05). It is reported that low levels of dissolved oxygen limit the removal of pollutants, including nitrogen (Valipour *et al.,* 2017). Results from this study showed reduced DO levels compared to the standard and indicate high levels of organic pollution, which reduce respiration and are inadequate for aquatic life, according to the Environmental Protection Agency (EPA). Similar results are reported by other researchers from different parts of the worlds and the hypoxic condition is common with the untreated wastewater and polluted surface waters (Placide *et al.,* 2023; Oluwafemi *et al.,* 2026). Biological oxygen demand (BOD) varied from 50.0 to 69. mg l^{-1}, while chemical oxygen demand (COD) ranged from 204.3 to 288.0 mg l^{-1}. The analysis of mean and median values revealed insignificant differences in BOD and COD concentrations among the samples, whereas ANOVA showed significant differences at 0.05 level. Our results showed that BOD and COD levels in this study were lower than those reported in other similar studies, suggesting relatively moderate organic pollution levels in the studied effluents (Majumder *et al.,* 2021; Bader *et al.,* 2022). Furthermore, previously published studies have reported substantially higher COD concentrations in hospital wastewater from different regions of the world. For instance, COD values of 591 mg l^{-1} in Asia, 613 mg l^{-1} in Europe, and 1074 mg l^{-1} in South America have been documented. Even greater COD levels, such as 2464 mg l^{-1} in Spain, 2480 mg l^{-1} in Brazil, and 1142 mg l^{-1} in India, have been reported in earlier investigations (Suarez *et al.*, 2009; Khan *et al.*, 2020; Majumder *et al.*, 2021). The MPN test results showed that all hospital wastewater samples in the present study contained >1.6 × 10^3 coliforms 100 ml^{-1}, exceeding the EPA-permissible limits, while the faecal coliforms count varied between 2.4 x 10^2 to 1.6 x 10^3 cells 100 ml^{-1} (Table 2). The high number of coliforms and faecal coliforms is attributed to the presence of several bacteria, including *Enterococcus* spp., *E. coli, Klebsiella* spp., and Faecal streptococci (NRC, 1985), indicating higher level of contamination in sampled hospital wastwater.

The consistently high total coliform levels observed in the present study indicate a continuous inflow of organic-rich waste from hospital activities, including patient excreta, laboratory effluents, and clinical waste (Majumder *et al.,* 2021). Similar results are reported in recent studies on hospital wastewater, showing that total coliform concentrations exceeded the maximum or acceptable discharge limits, commonly observed when on-site treatment is absent or inefficient (Liu *et al.,* 2010; Periasamy *et al.,* 2013; Bouchaala *et al.,* 2025). A total of 179 bacterial isolates from nine sites were identified as Gram-negative bacteria. Based on the morphological and biochemical

Table 3: Phenotypic antibiotic resistance profile of bacterial isolates from hospital wastewater

Antibiotics*	**Percentage of antibiotic-resistant bacterial isolates**						
	***Klebsiella* spp.**	***E. coli***	***Proteus* spp.**	***Shigella* spp.**	***Salmonella* spp.**	***Pseudomonas* spp.**	**Others**
P 30	90.6	92.9	76.0	89.3	88.0	100.0	92.9
CZ 30	65.6	76.2	60.0	64.3	76.0	76.9	71.4
MRP 30	21.9	23.8	12.0	3.6	4.0	15.4	14.3
CIP 30	37.5	47.6	36.0	35.7	24.0	38.5	14.3
GEN 10	53.1	42.9	28.0	46.4	36.0	38.5	50.0
VA 30	78.1	83.3	76.0	71.4	84.0	92.3	64.3
E 30	93.8	97.6	88.0	82.1	88.0	100.0	78.6
NX 10	46.9	57.1	40.0	46.4	28.0	53.8	50.0
CD 2	93.8	92.9	84.0	82.1	92.0	92.3	78.6
COT 30	53.1	47.6	48.0	42.9	36.0	38.5	57.1
TE 30	34.4	50.0	24.0	21.4	20.0	46.1	35.7
C 10	31.3	38.1	28.0	32.1	40.0	38.5	14.3

* P: Penicillin; CZ: Cefazolin; MRP: Meropenem; CIP: Ciprofloxacin; GEN: Gentamycin; VA: Vancomycin; E: Erythromycin; NX: Norfloxacin; CD: Clindamycin; COT: Trimoxazole; TE: Tetracycline

characteristics, the isolates were classified as follows: *Klebsiella* spp. (32), *Salmonella* spp. (25), *Proteus* spp. (25), *E. coli* (42), *Shigella* spp. (28), *Pseudomonas* spp. (13), *Serratia* spp. (5), *Enterobacter* spp. (4), *Citrobacter* spp. (2), *Providencia* spp. (1), *Yersinia* spp. (1) and *Hafnia* spp. (1). The high prevalence of *E. coli, Klebsiella, Salmonella, Shigella, Proteus,* and other bacteria in hospital wastewater exhibits a high prevalence of enteric and opportunistic Gram-negative bacteria, reflecting faecal contamination from multiple sources (Adbarzi *et al*., 2020). Other studies from the Middle East, Africa, and Asia have reported *E. coli* and *Klebsiella* as dominant isolates, often accounting for 30-50% of the total bacteria recovered from hospital wastewater (Umar *et al.,* 2025; Lima *et al.,* 2025).

Perusal of data showed that the highest resistance to antibiotics was observed with Penicillin, Erythromycin, and Clindamycin, while the highest sensitivity was observed with Meropenem. The antibiotic sensitivity assay showed that 100 percent isolates of *Pseudomonas* spp. were resistant to Penicillin and Erythromycin, while 92.30% isolates were resistant to Clindamycin. *Klebsiella* spp. (93.75%) were resistant to Erythromycin and Clindamycin, while 90.62% isolates were resistant to Penicillin. Similarly, 97.61% and 92.85% *E. coli* were resistant to Erythromycin and Clindamycin, respectively. Isolates of *Salmonella* spp. (92%) were resistant to Clindamycin, followed by resistance to Penicillin and Erythromycin in 88% isolates (Table 3).

This observation aligns with reports that clinically significant Gram-negative bacteria recovered from hospital and municipal wastewater exhibit high levels of antibiotic resistance and β-lactamase production (Irfan *et al.,* 2023; Canan-Rochenbach and Barreiros, 2024; Srivastava *et al.,* 2025; Kusuma *et al.,* 2026). Resistance to penicillin, erythromycin, and clindamycin has also been documented by other researchers (Igwaran and Okoh, 2018; Gavankar, 2024). The sensitivity of isolates to meropenem has reportedly been the lowest in our study, whereas others have reported it to be more effective against Gram-negative pathogens (Igwaran and Okoh, 2018; Gavankar, 2024). Our findings align with other global studies that show hospital wastewater is a significant source of multidrug-resistant bacteria (Davidova-Gerzova *et al.,* 2023). The complete resistance of *Pseudomonas* spp. to penicillin and erythromycin has been attributed to its reduced membrane permeability and multidrug efflux systems (Męcik *et al.,* 2024).

Out of 179 bacterial isolates, *Pseudomonas* spp. (100%), *Klebsiella* spp. (96.87%), *E. coli* (95.23%), *Shigella* spp. (89.28%), *Salmonella* spp. (88%), *Proteus* spp. (84%) and other spp. (85.71%) were screened as multidrug resistant (MDR). While *Klebsiella* spp. (31.25%), *Pseudomonas* spp. (23.08%), *E. coli* (16.67%), *Salmonella* spp. (16%), *Proteus* spp. (12%), *Shigella* spp. (10.71%), and other spp. (7.14%) exhibited extensive drug resistance (XDR) features by resisting antibiotics belonging to >10 different groups. Only three isolates *viz., Proteus* spp. (4%), *Shigella* spp. (3.57%), and *E. coli* (2.38%) exhibited pandrug resistance (PDR) against all antibiotics (Fig. 1). High MDR prevalence among *Pseudomonas* spp., with complete resistance observed in the present study, is supported by reports of 100% multidrug-resistant *P. aeruginosa* isolates recovered from wastewater environments (Adesoji *et al.,* 2023). Similar to the present findings, several studies have reported multidrug-resistant (MDR) *Klebsiella* spp., *Escherichia coli*, *Shigella* spp., *Salmonella* spp., and *Proteus* spp. in hospital wastewater (Lépesová and Olejníková, 2020; Agyarkwa *et al.*, 2022; Irfan *et al.*, 2023; Abdelgalel *et al.*, 2025). However, these studies generally reported lower proportions of MDR, XDR, and PDR isolates than those observed in the present investigation. In contrast, some recent studies have documented increasing antimicrobial resistance among bacterial isolates from hospital effluents, highlighting the growing environmental risk posed by

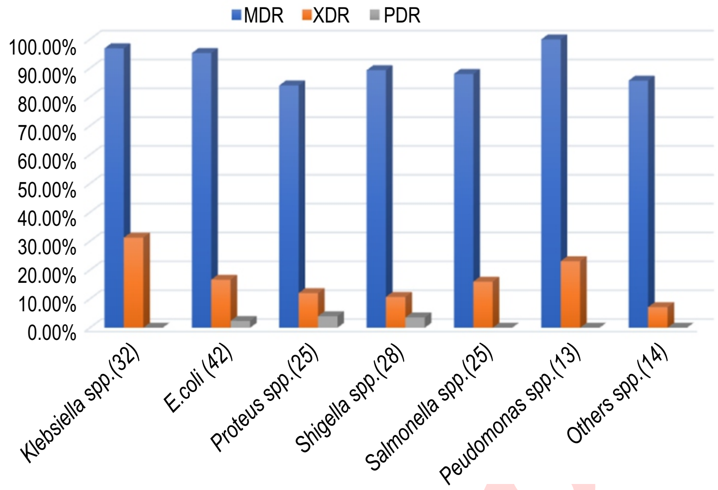

Fig. 1: Percentage of MDR, XDR and PDR isolates of *E. coli.* (42), *Salmonella* spp. (25), *Klebsiella* spp. (32), *Proteus* spp. (25), *Shigella* spp. (28), *Pseudomonas* spp. (13), and other isolates (14).

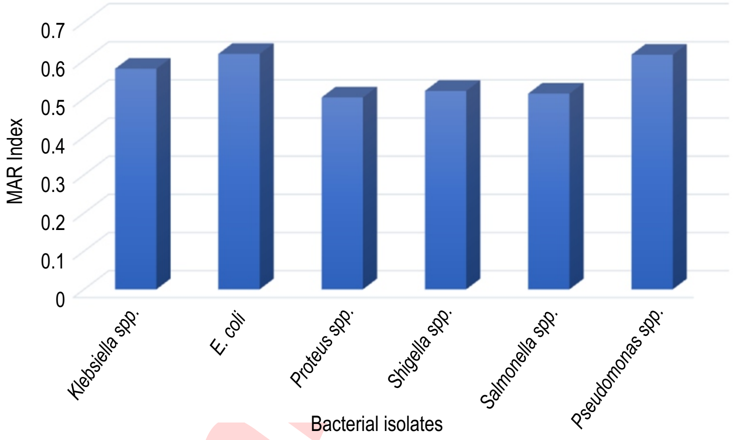

Fig. 2: Multiple Antibiotic Resistance (MAR) index, with each column representing the MAR index of bacterial isolates from hospital wastewater drainage.

highly resistant pathogens (Chimana *et al*., 2025; Chen *et al*., 2025).

Chaudhury *et al.* (2025) reported that the bacterial isolates *Pseudomonas* and *Klebsiella* from wastewater represent XDR community. These organisms can acquire the XDR trait through horizontal gene transfer (HGT) in sewage systems (von Wintersdorff *et al.,* 2016; Manaia *et al.,* 2018; Chaudhury *et al.,* 2025). PDR bacteria exhibit the highest level of resistance phenotype, defined as resistance to all antibiotics tested, thereby severely limiting available treatment options. These isolates pose significant public-health risks because of their potential to serve as sources of resistance genes that can disseminate into the clinical and environmental ecosystems (Manaia *et al.,* 2018; Cortez-Álvarez *et al.,* 2026). MAR index for *Klebsiella* spp. was 0.578, 0.617 for *E. coli,* 0.503 for *Proteus* spp., 0.520 for *Shigella* spp., 0.513 for *Salmonella* spp., and 0.615 for *Pseudomonas* spp. (Fig. 2). The MAR index for all species was greater than 0.2, indicating a high risk of transmission of antibiotic resistance traits to sensitive bacteria in the drains and surrounding environment. Similar high MAR indices have been reported in hospital wastewater in Southern Ethiopia (Mustapha *et al*., 2019), Choba, Nigeria (Osadebe *et al*., 2020), the Oromia region of Ethiopia (Tufa *et al.,* 2025), Odisha India (Pal *et al*., 2024) and northern India (Swain *et al*., 2025). The co-occurrence of AR genes and mobilising elements is rare in the human microbiome but common in wastewater, designating it as a "plausible arena for the initial mobilisation of resistance genes" (Berglund *et al.,* 2023). This promotes horizontal gene transfer (HGT) among taxonomically diverse bacteria, allowing non-pathogenic environmental bacteria to act as intermediate reservoirs that transfer ARGs to opportunistic or pathogenic strains. These genetic exchanges significantly raise the risk of multidrug-resistant pathogens, complicating infection control and limiting treatment options (Hendriksen *et al.,* 2019).

It was found that hospital effluents collected from different locations in Ayodhya district of Uttar Pradesh, India met EPA standards for physico-chemical quality for wastewater reported from similar ecosystem from different studies. They showed little to no variation among sites, although microbial contamination by coliforms and thermotolerant coliforms was evident. A total of 179 bacterial isolates, mainly comprising *E. coli, Klebsiella, Shigella, Proteus, Salmonella,* and *Pseudomonas* spp., were recovered; all exhibited multidrug resistance, including 31 XDR and 3 PDR strains with MAR indices greater than 0.2. The elevated resistance burden suggests a substantial risk of resistance gene spread to aquatic environments, underscoring the importance of improved antibiotic stewardship, public awareness, and behavioural control measures. Therefore, strict monitoring and treatment of these effluents is imperative before release into the natural ecosystem to prevent public and environmental contamination risk.

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