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INTEGRATED ONE HEALTH STRATEGIES FOR MITIGATING ANTIMICROBIAL RESISTANCE IN CLINICAL AND ENVIRONMENTAL SETTINGS

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ABSTRACT
Antimicrobial resistance (AMR) is an escalating global health threat that severely impacts families and communities by increasing disease burden, mortality, and healthcare costs. Projections estimate that AMR could cause 10 million deaths annually by 2050, with 1.27 million deaths recorded in 2019. In OECD (The Organisation of Economic and co-operation and development) countries alone, AMR accounts for approximately 79,000 deaths each year, with associated healthcare costs exceeding USD 28.9 billion. A critical but often overlooked driver of AMR is improperly treated hospital wastewater, which can contaminate rivers and streams used for drinking, irrigation, and recreation. These waters often contain antibiotic-resistant bacteria, increasingly common due to excessive antibiotic use in clinical settings. Such bacteria make infections harder to treat and increase the risk of severe, life-threatening illnesses in surrounding communities. Addressing AMR requires a One Health approach, which integrates human, animal, and environmental health. This study explores the role of environmental pathways—particularly hospital effluents—in AMR transmission and emphasizes the need for integrated surveillance, policy reform, and sustainable wastewater management. The findings support global efforts to protect family and community health from the complex and interlinked challenges of AMR.

KEYWORDS: Antimicrobial resistance (AMR), hospital wastewater, OECD, Integrated surveillance.

1. INTRODUCTION

One of the leading worldwide causes of death is infection caused by antimicrobial resistant pathogens. The resistance of pathogens to antimicrobial compounds leads to a lack of treatment options, resulting in increased mortality rates. In fact, previous estimates have determined that 10 million deaths per year could be attributable to antimicrobial resistance by 2050 (Review on Antimicrobial Resistance, 2016). An estimated 79,000 people die each year due to infections caused by antimicrobial-resistant pathogens within 34 OECD (The Organisation of Economic and co-operation and development) countries. Apart from the loss of human lives, antimicrobial resistance in pathogens also has financial consequences since it also results in prolonged hospitalizations and increased treatment costs. Within the 34 OECD and EU/EEA countries, the treatment of the complications associated with infections caused by antimicrobial-resistant pathogens was estimated to cost over USD 28.9 billion per year. The main drivers of the antimicrobial resistance burden are commonly referred as the ESKAPE group (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa and Enterobacter spp.*) and the well-known pathogen *Escherichia coli*. It is also widely known that antibiotic resistance genes can disseminate across different bacteria/ecosystems in natural environments and eventually reach human pathogens [Santajit, S. & Indrawattana, N., 2016]. In this context, the One Health approach, which links the environment, plants, animals and humans, is highly relevant for addressing the antimicrobial resistance crisis as the health of one influence the health of the others. As such, it is important to coordinate different disciplines and sectors to share information and policies in order to help in the prevention and control of different health threats. In fact, recently, the World Health Organization (WHO), the Food and Agriculture Organization (FAO), the World Organisation for Animal Health (WOAH) and the United Nations Environment Programme (UNEP) made a quadripartite collaboration based on the One Health approach to tackle the antimicrobial resistance crisis.

Wastewater treatment plants (WWTPs) have long been considered hotspots for the transmission and selection of antimicrobial resistance genes. Studies have shown the high presence of antibiotic resistance genes in

downstream water environments from WWTP effluent. This is the result of two factors: first, the urban WWTPs are source of antibiotic residues and secondly, they also promote the selection of antibiotic-resistant bacteria. The link between the resistance genes found in clinical isolates and the nearby wastewater treatment plants has also been established. The presence of antibiotic resistance genes/antibiotic-resistant strains in downstream water environments from WWTPs constitutes a risk for public health, and consequently, their surveillance in wastewaters is crucial for tackling the antibiotic resistance crisis under the One Health approach. This surveillance was historically based on culture-based methods but has recently been relying more on culture-independent approaches such as quantitative PCR (qPCR) or metagenomics.

1.1. Need for the study

The present study aims to investigate the occurrence of antibiotic-resistant bacteria in hospital wastewater. Bacterial isolates were obtained from samples collected at multiple sites, and their phenotypic antimicrobial susceptibility profiles were systematically evaluated. Improperly treated hospital wastewater has the potential to contaminate surrounding water bodies, such as rivers and streams. Such contaminated water may subsequently be used for drinking, irrigation, or recreational purposes, thereby facilitating the spread of infectious diseases. A critical concern associated with hospital wastewater is the presence of antibiotic-resistant bacteria, whose prevalence is increasing due to the extensive and often inappropriate use of antibiotics in healthcare settings. These resistant strains pose significant public health challenges, as they are more difficult to treat, potentially leading to severe and life-threatening infections.

In light of these concerns, the present study was undertaken with the following aim and objectives:

AIM

To screen hospital wastewater samples for the presence of antibiotic-resistant bacteria.

OBJECTIVES

1.To collect wastewater samples from hospital outlet points using sterile containers.
2.To prepare and sterilize appropriate culture media for bacterial isolation.
3.To prepare different concentrations of selected antibiotics.
4.To assess the presence and resistance profiles of bacterial isolates through phenotypic antimicrobial

2. MATERIALS AND METHODS

2.1. Sample collection

Waste water samples were collected from different hospital outlets located in and around Eluru from the untreated waste water outlet pipe of a selected area Hospital -1, Hospital-2 and Hospital -3 - in a sterile bottle. A volume of 1 litter of wastewater was collected from each site using new first use sterile plastic bottles (sterilized by shaking with 70% ethanol for 3 min followed by three times rinsing with sterile distilled water), preserved on ice, and transported to the laboratory for microbiological analysis.

2.2. Analysis of microbiological parameters

Microbiological parameters were determined for the effluent of hospital waste water. Dilutions of wastewater samples were performed which varied from 10^{-1} to 10^{-6}. For pathogenic bacteria characterization, MacConkey agar medium, (it permits growth of enterococci, staphylococci, and Mycobterium spp.,) Mannitol salt agar medium and nutrient agar medium were used. MacConkey broth was bought from High medium and prepared according to the manufacturer's instructions, then distributed into a screw-capped bottle fitted with an inverted Durham tube for the determination of coliform bacteria. Sterilization is done by autoclaving at 121°C for 15 min under a pressure of 15 lb. per square inch. Serial 10-fold dilutions of wastewater samples were prepared which varied from 10^{-1} to 10^{-6}.

2.3. Detection of coliforms

MPN test was performed to detect the presence of coliforms in water samples as per standard protocol.

2.4. Isolation of bacteria present in waste water samples

0.1 ml of each sample was spread on Nutrient agar, Macconkey agar and Mannitol salt agar plates to isolate bacteria.

2.5. Isolation of antibiotic-resistant bacteria

Isolated colonies were inoculated in to nutrient broth containing different concentrations of antibiotics - Penicillin, Tetracycline and streptomycin.

2.6. Gram staining: The colonies are gram stained as per standard protocol.

3. RESULTS

3.1 Screening of waste water samples for the presence of antibiotic-resistant bacteria

Waste water samples were collected from outlets of different hospitals located in and around Eluru. Three samples were collected each measuring 1litre in sterile bottles and samples were processed.

All the three samples were serially diluted to 10^{-6} dilution 0.1ml of each sample was plated on Nutrient agar, Mannitol salt agar and Macconkey agar the results were noted.

Table 1: Growth on agar plates.

Samples	Nutrient agar	Mannitol salt agar	Macconkey agar
1	++	++	+
2	++	++	+
3	++	++	+

Growth (+) (-) – No growth

Bacterial isolates obtained from mannitol salt agar plates of each hospital wastewater sample were tested for their ability to grow in nutrient broth containing penicillin, tetracycline, or streptomycin at two concentrations (1 mg/mL and 0.1 mg/100 mL). In all cases, visible turbidity was observed after incubation, indicating active bacterial growth despite the presence of antibiotics. This suggests that the isolates were resistant to all three antibiotics at both tested concentrations.

The uniform growth response across samples and antibiotic types implies a high prevalence of multidrug resistance among the bacterial population present in the hospital wastewater. Such resistance at relatively high antibiotic concentrations (1 mg/mL) is indicative of a strong adaptive capability, likely driven by prolonged exposure to antibiotic residues in the wastewater environment.

Table 2: Growth of bacteria in nutrient broth containing Antibiotics.

Sample	Medium with Pencillin		Medium with Tetracycline		Medium with streptomycin	
	1mg/1 ml	0.1mg/100m	1mg/1 ml	0.1mg/100m	1mg/1 ml	,0.1mg/100m
S1	Growth+	Growth+	Growth+	Growth+	Growth+	Growth+
S2.	Growth+	Growth+	Growth+	Growth+	Growth+	Growth+
S3.	Growth+	Growth+	Growth+	Growth+	Growth+	Growth+

Figure 1 Growth of bacteria in flasks containing antibiotics.

Microscopic and cultural characterization revealed that bacterial isolates grown on Mannitol Salt Agar were Gram-positive cocci, while those obtained from MacConkey Agar were identified as Gram-negative bacilli. This indicates that the hospital wastewater harbors a mixed population of both Gram-positive and Gram-negative bacteria, including potential pathogenic species.

4. DISCUSSION

The present study focused on screening hospital wastewater samples for the presence of antibiotic-resistant bacteria. Three samples were collected from different hospital outlet points and analysed for bacterial isolation and antimicrobial resistance. Bacterial growth was obtained from all three samples, confirming the presence of viable bacteria in the wastewater.

Bacteria from mannitol salt agar were subjected to antibiotic susceptibility testing using three antibiotics: penicillin, tetracycline, and streptomycin. Growth was observed in all antibiotic-containing flasks, indicating resistance to all three agents. These findings suggest the presence of multidrug-resistant (MDR) bacterial strains in the tested wastewater sample.

The results are consistent with the study by Magda M. Mehanni *et al.* (2023), who reported the occurrence of antibiotic-resistant bacteria in hospital wastewater treatment plant effluent and discussed the potential risks associated with its reuse in agricultural irrigation. The detection of such resistant bacteria in untreated hospital wastewater highlights a potential public health hazard, as these microorganisms can disseminate resistance genes into environmental microbial communities through surface water contamination.

5. CONCLUSION

Hospital wastewater contains a complex mixture of chemical contaminants, microbial agents, and cell-free DNA. Many chemical residues are resistant to conventional wastewater treatment processes and can enter surface water bodies, affecting aquatic ecosystems. In addition, hospital effluents often harbor pathogenic microorganisms, including strains resistant to multiple antibiotics. The spread of antimicrobial resistance (AMR) among bacterial populations poses one of the greatest challenges to global public health, contributing to increased morbidity and mortality in both humans and animals.

In light of these concerns, this study was conducted in the Microbiology Laboratory of Ch.S.D.St. Theresa's College for Women (A), Eluru, to investigate the presence of antibiotic-resistant bacteria in hospital wastewater. Bacteria were isolated from all collected samples, and antimicrobial susceptibility testing of Sample 1 revealed resistance to penicillin, tetracycline, and streptomycin. These findings underscore the urgent need for effective wastewater treatment strategies in healthcare facilities to mitigate the environmental dissemination of antibiotic-resistant bacteria.

6. Health Interventions to Address Antimicrobial Resistance

Antibiotic Stewardship Programs(ASP)

Implement hospital-wide ASPs to ensure antibiotics are prescribed only when necessary, at the correct dose, and for the right duration

Education and Training

Provide continuous training for healthcare professionals on rational antibiotic use and AMR risks.

Waste water Treatment Upgrades

Introduce advanced treatment processes in hospital wastewater systems (e.g., membrane filtration, UV disinfection, ozonation) to remove resistant bacteria and antibiotic residues before discharge.

Regular Monitoring

Conduct routine testing of hospital effluents and nearby water bodies for antibiotic residues and resistant organisms.

Safe Waste Disposal

Implement safe disposal methods for expired antibiotics, laboratory cultures, and contaminated clinical waste.

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