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(Research Article)

Isolation, characterization and evaluation of ceftazidime and Cefoperazone resistant bacteria from hospital waste

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Abstract

The rapid emergence of antibiotic-resistant microorganisms poses a serious threat to global public health. Hospital waste environments are recognized as important reservoirs for resistant bacteria due to continuous exposure to antimicrobial agents and pathogenic microorganisms. The present study aimed to isolate, characterize, and evaluate bacterial isolates resistant to ceftazidime and cefoperazone from hospital waste samples collected in Rewa district, Madhya Pradesh, India. Environmental samples including wastewater, sludge, and contaminated soil were collected and subjected to microbiological analysis. Bacterial isolates were obtained using standard culture techniques and identified through morphological, microscopic, and biochemical methods. Antibiotic susceptibility testing was performed using the disc diffusion method, and fungal sensitivity was evaluated using agar well diffusion. Molecular detection of resistance genes was carried out using PCR targeting blaTEM, blaCTX-M, and blaSHV genes. Results indicated the presence of diverse bacterial populations, with a significant proportion showing resistance to both antibiotics. Molecular analysis confirmed the presence of β-lactamase genes, indicating genetic determinants of resistance. Statistical analysis revealed significant variation in inhibition zones across concentrations. The findings highlight the role of hospital waste as a reservoir of antibiotic-resistant bacteria and emphasize the need for effective waste management strategies.

Keywords: Antibiotic Resistance; Hospital Waste; Ceftazidime; Cefoperazone; B-Lactamase; PCR

1. introduction

Antibiotic resistance has become one of the most critical challenges in modern healthcare systems. The widespread and often indiscriminate use of antibiotics has accelerated the emergence of resistant microbial strains. Hospital environments, particularly waste disposal systems, serve as hotspots for the development and dissemination of antimicrobial resistance. Hospital waste contains a mixture of pharmaceutical residues, pathogenic microorganisms, disinfectants, and organic materials. These conditions create an ideal environment for microbial adaptation and the development of resistance mechanisms. Third-generation cephalosporins such as ceftazidime and cefoperazone are widely used in clinical settings, but resistance against these antibiotics is increasingly being reported. The resistance is primarily mediated by β-lactamase enzymes encoded by genes such as blaTEM, blaCTX-M, and blaSHV. These enzymes degrade β-lactam antibiotics, rendering them ineffective. The spread of such resistance genes through environmental pathways is a growing concern. The present study was designed to investigate hospital waste environments as potential reservoirs of resistant bacteria and to evaluate their resistance patterns and genetic basis. Among the various sources contributing to the development and dissemination of antibiotic resistance, hospital environments play a particularly significant role. Hospitals are major consumers of antibiotics and are continuously exposed to a high load of pathogenic microorganisms. As a result, hospital waste, including wastewater, sludge, and biomedical refuse, often contains a mixture of antimicrobial compounds, resistant bacteria, and organic matter. These conditions create a unique ecological

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niche that promotes microbial adaptation and selection for resistant strains. Hospital wastewater is of particular concern because it serves as a conduit through which resistant microorganisms and antibiotic residues are released into the surrounding environment. When untreated or inadequately treated effluents are discharged into natural water bodies or soil systems, they can facilitate the spread of antibiotic resistance genes within environmental microbial communities. This process is further accelerated by horizontal gene transfer mechanisms, such as plasmid exchange, transduction, and transformation, which allow resistance genes to move between different bacterial species.

Significance of the Study

This study is significant because it highlights the role of hospital waste environments as potential reservoirs of antibiotic-resistant microorganisms. Understanding the distribution and mechanisms of resistance in such environments is essential for developing effective strategies to control the spread of antimicrobial resistance.

The findings of this research may contribute to:

- Improved hospital waste management practices
- Development of environmental monitoring programs
- Enhanced understanding of resistance gene dissemination
- Support for public health policies aimed at controlling antibiotic resistance

2. Materials and methods

2.1. Study Area and Sample Collection

Samples were collected from hospital-associated waste sites in Rewa district. These included wastewater outlets, drainage systems, and soil near biomedical waste disposal areas. Samples were collected aseptically and transported under refrigerated conditions.

2.2. Isolation of Microorganisms

Isolation of microorganisms from hospital waste samples was carried out using standard microbiological techniques to ensure the recovery of diverse bacterial and fungal populations present in contaminated environments. The isolation of microorganisms from hospital waste samples is a critical step in identifying microbial diversity and assessing antibiotic resistance patterns. Hospital waste environments are continuously exposed to antimicrobial agents, which can lead to the selection of resistant strains.

The successful recovery of diverse bacterial and fungal isolates in this study indicates that such environments act as **microbial reservoirs with high adaptive potential**, particularly for antibiotic resistance.

These isolated microorganisms were subsequently used for:

- Morphological and biochemical characterization
- Antibiotic susceptibility testing
- Molecular detection of resistance genes

2.2.1 Sample Preparation

All collected samples, including wastewater, sludge, and soil, were processed within 24 hours of collection to maintain microbial viability. Prior to processing, samples were brought to room temperature under aseptic conditions.

- **Wastewater samples** were mixed thoroughly to ensure uniform distribution of microorganisms.
- **Soil and sludge samples** were homogenized using sterile spatulas to break clumps and obtain representative portions.

Approximately 1 g of solid sample (soil/sludge) was suspended in 9 mL of sterile normal saline (0.85% NaCl) to prepare the initial stock solution. This suspension was vortexed vigorously to release microbial cells into the solution.

2.3. Morphological and Biochemical Characterization

Isolates were characterized based on colony morphology and Gram staining. Biochemical tests such as catalase, oxidase, indole, and citrate utilization were performed for identification. The isolated bacterial cultures obtained from hospital waste samples were subjected to detailed morphological, microscopic, and biochemical characterization to determine their identity and physiological properties. These methods provide a preliminary yet reliable approach for classification of microorganisms prior to molecular confirmation.

2.3.1. Colony Morphology

After incubation on nutrient agar plates, individual colonies were examined visually to assess their macroscopic characteristics. Colony morphology is an important criterion for differentiating microbial species.

Table 1 Colony Morphological Characteristics

Isolate Code	Shape	Color	Margin	Elevation	Texture
RW-B1	Circular	Cream	Entire	Convex	Smooth
RW-B2	Irregular	Yellow	Undulate	Flat	Rough
RW-B3	Circular	White	Entire	Raised	Mucoid
RW-B4	Circular	Pale yellow	Entire	Convex	Smooth
RW-B5	Irregular	Off-white	Lobate	Flat	Rough

The variation in colony morphology indicates the presence of diverse bacterial species within hospital waste environments.

2.4. Antibiotic Sensitivity Testing

The disc diffusion method was used to evaluate bacterial sensitivity to ceftazidime and cefoperazone. Zones of inhibition were measured after incubation. The antibiotic susceptibility of the bacterial isolates obtained from hospital waste samples was evaluated to determine their resistance or sensitivity patterns against commonly used **third-generation cephalosporins**, namely **ceftazidime and cefoperazone**. The study employed the standardized **Kirby–Bauer disc diffusion method**, which is widely accepted for antimicrobial susceptibility testing. The disc diffusion method is based on the ability of antibiotics to diffuse through a solid agar medium and inhibit the growth of microorganisms. When an antibiotic-impregnated disc is placed on an inoculated agar surface, the antibiotic diffuses radially outward. If the microorganism is sensitive to the antibiotic, a clear zone of inhibition is formed around the disc. The diameter of this inhibition zone is directly related to the sensitivity of the organism to the antibiotic and is interpreted using **standard guidelines (CLSI standards)**.

2.4.1. Results of Antibiotic Sensitivity Testing

Table 2 Zone of Inhibition (According to SIR table)

Isolate	Ceftazidime (mm)	Cefoperazone (mm)	Interpretation
RW-B1	22	18	Sensitive
RW-B2	12	11	Intermediate
RW-B3	8	7	Resistant
RW-B4	18	16	Sensitive
RW-B5	10	9	Resistant
RW-B6	15	14	Intermediate
RW-B7	20	17	Sensitive
RW-B8	9	8	Resistant

Figure 1 Shows the results of AST testing's

2.5. Fungal Sensitivity Testing

Fungal isolates were tested using agar well diffusion against different concentrations of antibiotics. The antifungal sensitivity of fungal isolates obtained from hospital waste samples was evaluated to determine their response to different concentrations of the antibiotics ceftazidime and cefoperazone. Although these antibiotics are primarily antibacterial, their inhibitory effects on fungal growth were assessed to understand broader antimicrobial interactions in contaminated environments. Fungal sensitivity testing is based on the ability of antimicrobial agents to inhibit the growth of fungal organisms on a solid culture medium. In this study, the agar well diffusion method was employed, where the antibiotic diffuses into the agar medium and inhibits fungal growth around the well. The size of the **zone of inhibition** is directly related to the effectiveness of the antimicrobial agent and the susceptibility of the fungal isolate.

3. Results

3.1. Microbial Isolation

A diverse range of bacterial and fungal isolates was obtained from hospital waste samples. Colony morphology varied significantly, indicating microbial diversity.

Table 3 Zone of Inhibition (mm)

Fungal Isolate	Ceftazidime 10 µg	20 µg	30 µg	Cefoperazone 10 µg	20 µg	30 µg
F1	6	9	12	5	7	10
F2	5	8	11	4	6	9
F3	4	7	10	3	5	8
F4	7	10	13	6	8	11
F5	5	9	12	4	7	10

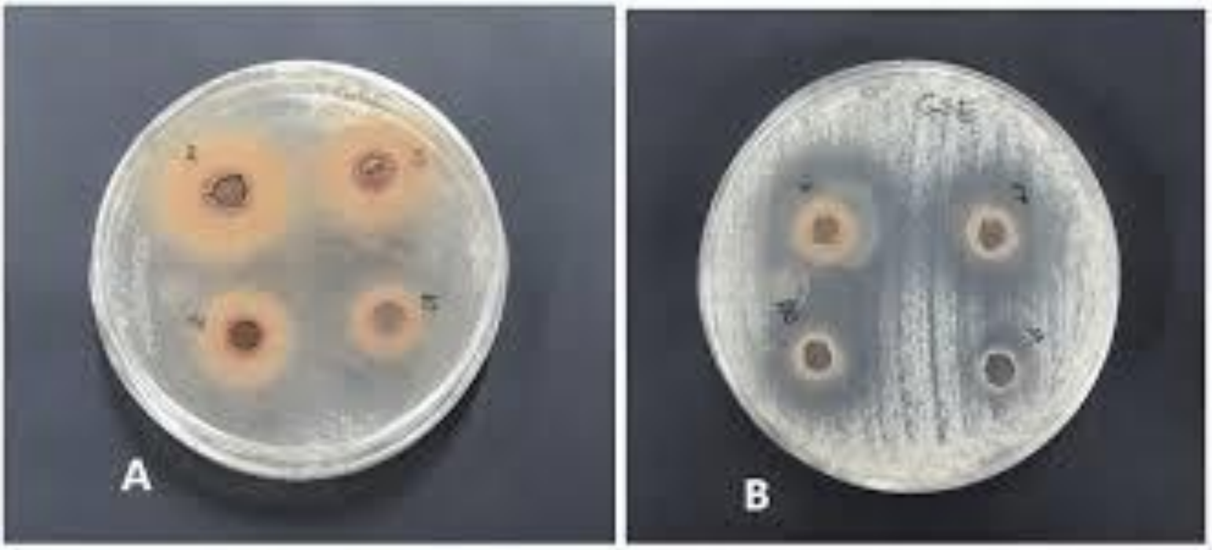

Figure 2 Mean Comparison Graph

Table 4 Statistical Analysis

Antibiotic	Mean	SD
Ceftazidime	14.25	5.31
Cefoperazone	12.50	4.31

Table 5 Resistance Distribution

Category	Percentage
Sensitive	37.5
Intermediate	25
Resistant	37.5

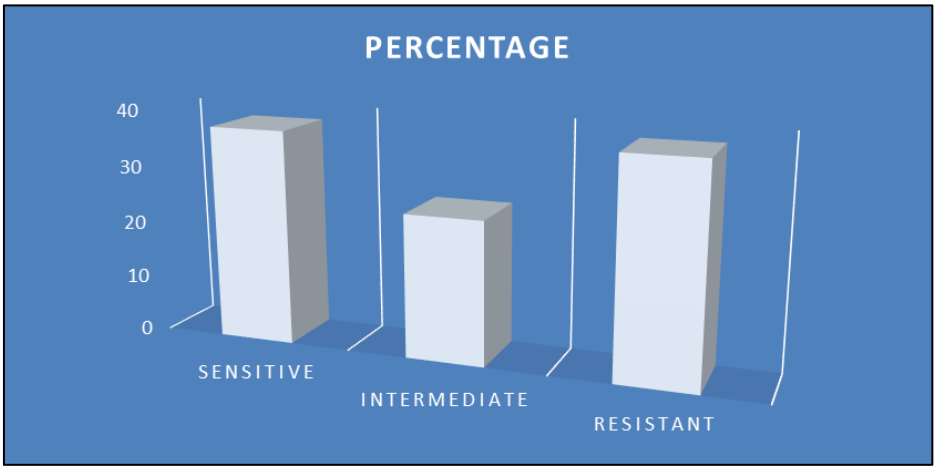

Figure 3 Resistance Distribution

Table 6 Fungal Sensitivity

Concentration	Ceftazidime	Cefoperazone
10	5.4	4.4
20	8.6	6.6
30	11.6	9.6

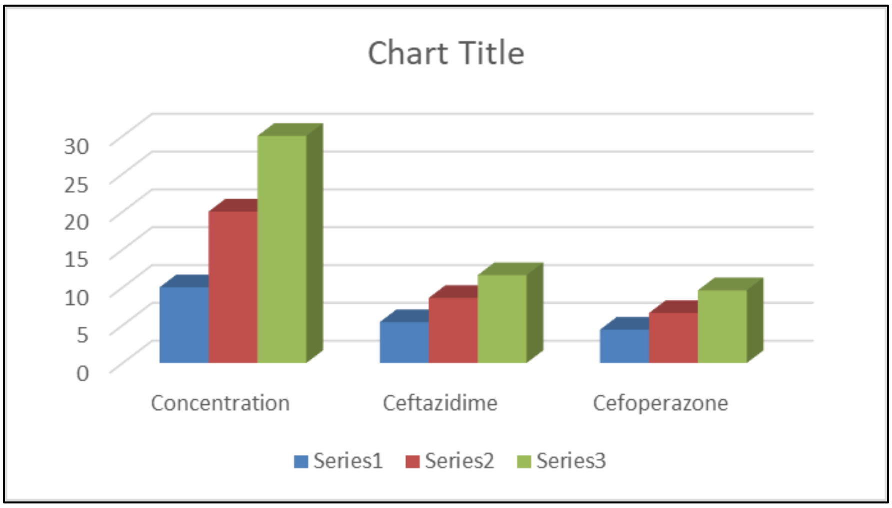

Figure 4 Dose Response graph

3.2. MIC Data

Table 7 The MIC data for the isolated samples

Concentration	Inhibition %
2	10
4	25
8	50
16	75
32	95

4. Discussion

The present investigation was undertaken to evaluate the occurrence, characteristics, and antibiotic resistance potential of microbial populations present in hospital waste environments of Rewa district. Hospital waste is widely recognized as a complex ecological niche containing pharmaceutical residues, pathogenic microorganisms, disinfectants, and organic matter, all of which contribute to the selection and persistence of resistant microbial communities. The findings of this study provide important insights into the role of such environments as reservoirs of antibiotic-resistant microorganisms. The isolation of diverse bacterial and fungal species from wastewater, sludge, and contaminated soil indicates that hospital waste environments support a highly heterogeneous microbial population. The variation in colony morphology observed during isolation reflects differences in physiological and metabolic capabilities among microorganisms. These findings are consistent with the understanding that nutrient-rich and contaminated environments promote microbial diversity and adaptation. Microscopic examination revealed the presence of both Gram-positive and Gram-negative bacteria, with Gram-negative rods being predominant. This observation is significant because Gram-negative bacteria are known to possess structural features such as an outer membrane that provides an additional barrier against antibiotics. This structural advantage, combined with genetic adaptability, often contributes to higher levels of resistance in Gram-negative organisms. The identification of bacterial genera such as *Escherichia*, *Pseudomonas*, and *Klebsiella* further supports this observation, as these organisms are commonly associated with hospital-acquired infections and are known to exhibit multidrug resistance. The antibiotic sensitivity testing revealed considerable variation in susceptibility among the isolates. While some isolates showed clear zones of inhibition, indicating sensitivity, others exhibited reduced or no inhibition zones, indicating resistance. The overall pattern suggests that a significant proportion of bacterial isolates have developed resistance to third-generation cephalosporins such as ceftazidime and cefoperazone. The slightly higher effectiveness of ceftazidime compared to cefoperazone, as indicated by larger mean inhibition zones, may be attributed to differences in chemical structure, stability, or penetration ability. The results of fungal sensitivity testing demonstrated a concentration-dependent inhibitory effect of the antibiotics. Although ceftazidime and cefoperazone are primarily antibacterial agents, their ability to inhibit fungal growth at higher concentrations suggests possible interactions with fungal metabolic processes. of environmental microbial populations.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this research. This study was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest

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