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RESEARCH ARTICLE

Comparative analysis of multidrug resistance micro-organisms prevalence in hospital waste water and household sewage water

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ABSTRACT

Background: In Bangladesh, antibiotic resistance is a growing health concern, driven by the indiscriminate use of antibiotics without prescriptions or proper dosage. This misuse, along with untreated hospital wastewater (HWW) containing antimicrobial residues, contributes to the spread of antibiotic-resistant bacteria in the environment. Hospital and household sewage can serve as reservoirs for these resistant bacteria, posing a risk to public health. **Objective:** To isolate and identify common bacterial species from hospital and household sewage, assess their antibiotic susceptibility, and compare multidrug resistance between hospital and household wastewater. **Methods:** Sewage samples from hospital and household sources were collected from various locations in the Saver zone over three months. After enrichment, the samples were plated on selective media (MacConkey, EMB, Cetrimate, TCBS, SS agar). Bacterial colonies were identified based on morphology and confirmed through biochemical tests. Antibiotic susceptibility was tested using the Kirby-Bauer disc diffusion method, with 13 antibiotics assessed for multidrug resistance. The process took about three months. **Results:** The prevalence of bacterial isolates was as follows: *E. coli* (15.05% in hospital, 15.22% in household), *Klebsiella spp.* (18.28% in hospital, 17.39% in household), *Salmonella spp.* (16.13% in hospital, 10.87% in household), *Shigella spp.* (12.90% in hospital, 10.87% in household), *Vibrio spp.* (11.83% in hospital, 8.70% in household), *Pseudomonas spp.* (11.83% in hospital, 17.39% in household), and *Staphylococcus spp.* (13.98% in hospital, 19.57% in household). Among the antibiotics tested, *Azithromycin (AZM)*, *Ciprofloxacin (CIP)*, and *Cefixime*

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(CFM) were the most effective. Over 80% resistance was observed for other antibiotics. All isolates were multidrug-resistant, with hospital sewage showing slightly higher resistance levels compared to household sewage. **Conclusion:** The study underscores the role of hospital wastewater in spreading multidrug-resistant bacteria, with higher resistance observed in hospital sewage. Antibiotic misuse is a major factor driving resistance, posing a significant public health threat. Effective antibiotic management and wastewater treatment are urgently needed.

Keywords: multidrug-resistant bacteria; hospital wastewater; antibiotic resistance; sewage contamination; public health threat

1. Introduction

Untreated hospital wastewater (HWW) poses a significant risk to public health and the environment, as it often contains a range of pollutants, including hazardous chemicals, pharmaceuticals, and pathogenic microorganisms[1]. In Bangladesh, HWW is frequently discharged directly into municipal sewage systems, contaminating rivers and surrounding water bodies. This liquid waste can carry antibiotic-resistant bacteria, contributing to the spread of resistance genes within the natural and environmental microflora[2]. The unchecked use of antibiotics, both in healthcare and agriculture, exacerbates the development of antibiotic resistance, leading to resistant strains in hospital and household sewage[3].

In addition to resistant bacteria, bacteriophages found in fecal waste can act as vectors for transferring resistance genes. Though sewage treatment plants reduce bacterial numbers, they are often ineffective at eliminating multidrug-resistant organisms, which may continue to contaminate the environment and pose a risk to public health[4]. Improper disposal of hospital waste, including liquid effluents, can result in waterborne diseases such as cholera, typhoid fever, and gastroenteritis[5]. Moreover, the presence of multidrug-resistant bacteria in hospital wastewater is concerning, as it can contribute to the spread of resistance in aquatic environments, affecting both wildlife and humans[6].

The growing issue of antimicrobial resistance (AMR) has been attributed to the widespread and irrational use of antibiotics in both clinical and agricultural settings, leading to a rise in resistant strains[7]. Hospital wastewater contains higher levels of antibiotic-resistant microorganisms compared to domestic sewage due to the concentrated use of antibiotics in healthcare facilities. Studies show that hospital effluents not only introduce but also selectively propagate resistant bacteria, which then enter the broader environment, posing an ongoing public health threat[8].

The risk is particularly high with pathogens like *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species, which are notorious for causing healthcare-acquired infections and are resistant to multiple antibiotics[9]. Even after treatment, hospital wastewater often retains high concentrations of resistant bacteria and antimicrobial residues, which can spread resistance through the environment.

Improperly managed healthcare wastes, the practice of self-medication, and substandard antibiotic use contribute to the growing problem of drug-resistant strains in hospitals[10]. The aim of this study is to compare the prevalence of multidrug-resistant microorganisms in hospital wastewater and household sewage, assessing their potential to impact public health and contribute to the spread of antibiotic resistance.

2. Methodology

2.1. Study design and sample collection

A cross-sectional study was conducted from May to September 2018 to assess the prevalence of multidrug-resistant microorganisms in hospital wastewater and household sewage water in the Saver Zone. The study involved the collection of water samples from two primary sources: hospital wastewater and household sewage. A total of 28 water samples were collected, with 14 samples obtained from different hospitals and 14 from various residential areas in the Saver Zone. The hospitals included Habib Hospital, Mumtaz Hospital, Gonoshasthya Somaj Vittik Medical College Hospital, and Shekh Fajilatunnesha Mojib Memorial Hospital. The household samples were collected from residential areas near these hospitals.

All water samples were aseptically collected in sterile containers and transported to the laboratory for analysis within two hours of collection, in accordance with standard procedures. Samples from the hospitals were taken from open surface water flowing near discharge points of untreated wastewater. Samples from residential areas were taken from sewage systems within proximity to the hospitals.

2.2. Sample processing and bacterial isolation

Upon arrival at the laboratory, the samples were processed immediately. Each sample was inoculated into different enrichment media to facilitate the growth of potential pathogens. The enrichment media used included selenate broth, buffer peptone water, and nutrient broth. The samples were incubated at 37°C for 24 hours to allow bacterial growth. Following enrichment, the samples were streaked onto selective and differential agar plates such as MacConkey agar, eosin methylene blue (EMB) agar, salmonella-shigella (SS) agar, cetrimide agar, and thiosulfate-citrate-bile salts-sucrose (TCBS) agar[11].

After incubation, individual colonies were observed based on their morphological characteristics and subcultured onto fresh agar plates for isolation. Pure cultures were obtained from representative colonies. These isolates were then subjected to various tests for bacterial identification.

2.3. Identification of bacterial isolates

Bacterial isolates were identified based on their morphological characteristics and Gram staining. Biochemical tests followed, including the Indole Test (using soybean casein digest media), Methyl Red and Voges-Proskauer Tests (IMViC), Citrate Test (Simmons Citrate Agar), Catalase Test (hydrogen peroxide reaction), and Triple Sugar Iron (TSI) Test to assess sugar fermentation and hydrogen sulfide production[12].

2.4. Antibiotic sensitivity testing

Antibiotic susceptibility was tested using the Kirby-Bauer disk diffusion method. The bacterial isolates were exposed to 12 antibiotics representing various classes, including Aminoglycosides (Gentamicin, Streptomycin), β-lactams (Amoxycillin, Ampicillin, Penicillin, Vancomycin), Cephalosporins (Cefixime), Macrolides (Azithromycin, Erythromycin), Quinolones (Ciprofloxacin), and others like Chloramphenicol, Sulfamethoxazole-trimethoprim, and Tetracycline. The inhibition zones were measured to categorize the isolates as resistant, intermediate, or sensitive according to CLSI standards[13].

2.5. Sampling period and site

The water samples were collected weekly from the designated sampling sites over the five-month study period. Sampling was done both upstream and downstream of the hospital wastewater discharge points. Water samples were collected in sterile containers to ensure no contamination occurred during collection.

2.6. Gram staining procedure

To identify gram-positive and gram-negative bacteria, gram staining was performed. A loopful of bacterial culture was transferred to a clean glass slide with a drop of distilled water. The smear was air-dried, heat-fixed, and stained with crystal violet for 1 minute. The slide was then washed with water, followed by the application of Gram's iodine for 1 minute, followed by washing again. After applying 95% alcohol for 15-20 seconds and rinsing, safranin was applied for 45 seconds. The slide was then washed, air-dried, and observed under a microscope[14].

2.7. Data analysis

The antibiotic resistance profiles were compared between hospital wastewater and household sewage samples to evaluate the prevalence of multidrug-resistant organisms. Statistical analyses were performed to assess the significance of differences in resistance patterns between the two types of wastewaters.

3. Results

A total of 214 bacterial isolates were identified from hospital and household wastewater samples, including *Escherichia coli*, *Klebsiella spp.*, *Salmonella spp.*, *Shigella spp.*, *Vibrio spp.*, *Pseudomonas spp.*, and *Staphylococcus spp.*. The number of isolates obtained from each species were as follows: 25 *E. coli*, 21 *Klebsiella spp.*, 20 *Salmonella spp.*, 17 *Shigella spp.*, 15 *Vibrio spp.*, 15 *Pseudomonas spp.*, and 22 *Staphylococcus spp.*. To differentiate these isolates, cultural characteristics were examined by inoculating the samples onto selective media, allowing identification based on colony morphology, such as color and shape. Additionally, Gram staining revealed that Gram-positive bacteria appeared purple to blue, while Gram-negative bacteria appeared pink to red, corresponding to the identified bacterial species.

The bacterial isolates were identified using various biochemical tests, including the IMVIC test, Triple Sugar Iron (TSI) test, Indole, Methyl Red (MR), Voges-Proskauer (VP), Citrate, and Catalase tests. The results of these tests allowed for differentiation between species, as shown in the **Table 1**.

Table 1. Biochemical test results of identified bacterial species.

Organism	Indole	MR	VP	Citrate	Catalase	TSI Slant	TSI Butt	H_2S	Gas
E. coli	+	+	-	-	+	Yellow	Yellow	-	+
Salmonella spp.	-	+	-	+	+	Red	Yellow	+	+
Klebsiella spp.	-	+	+	+	+	Yellow	Yellow	-	+
Shigella spp.	+	+	-	-	+	Red	Yellow	-	+
Pseudomonas spp.	-	-	-	+	+	Red	Red	-	+
Staphylococcus spp.	-	+	-	-	+	Red	Yellow	-	-
Vibrio spp.	+	-	-	+	+	Yellow	Yellow	-	+

The bacterial species isolated from both hospital and household wastewater samples were compared in terms of their frequency of occurrence. The results showed that hospital wastewater samples contained a higher frequency of bacterial isolates compared to household wastewater, particularly *Salmonella spp.*, *Shigella spp.*, and *Klebsiella spp.*, as indicated in the **Table 2**.

Table 2. Presence of bacterial species in hospital and household wastewater.

Organism	Hospital (n=14)	Household (n=14)
Salmonella spp.	15	5
Shigella spp.	12	5
E. coli	14	7
Klebsiella spp.	17	8
Pseudomonas spp.	11	8
Staphylococcus spp.	13	9
Vibrio spp.	11	4

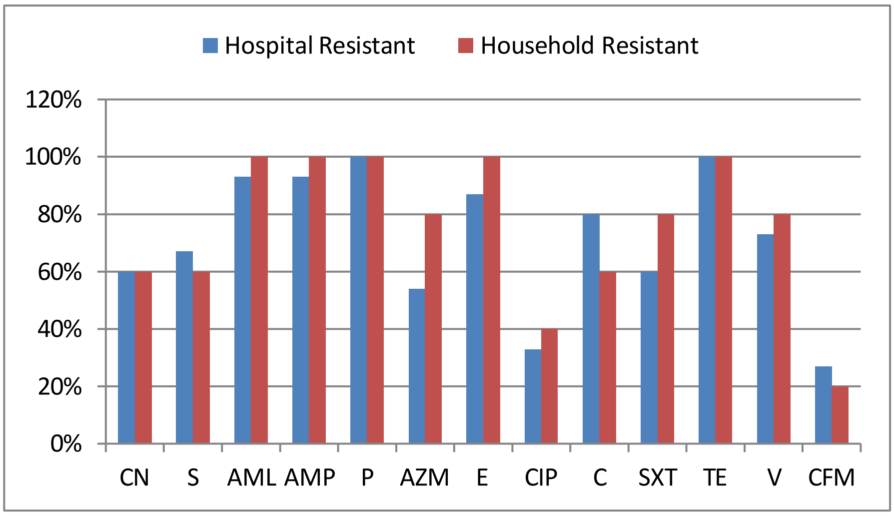

Figure 1. Percentage and comparison of antibiotic-resistant pattern of Salmonella spp. from hospital and residential areas.

Figure 1 Represents the percentage and comparison of antibiotic-resistant pattern of ***Salmonella spp*** from hospital and residential source. As the key word of my topic is the antibiotic resistance profile of isolates from hospital and residential isolates.

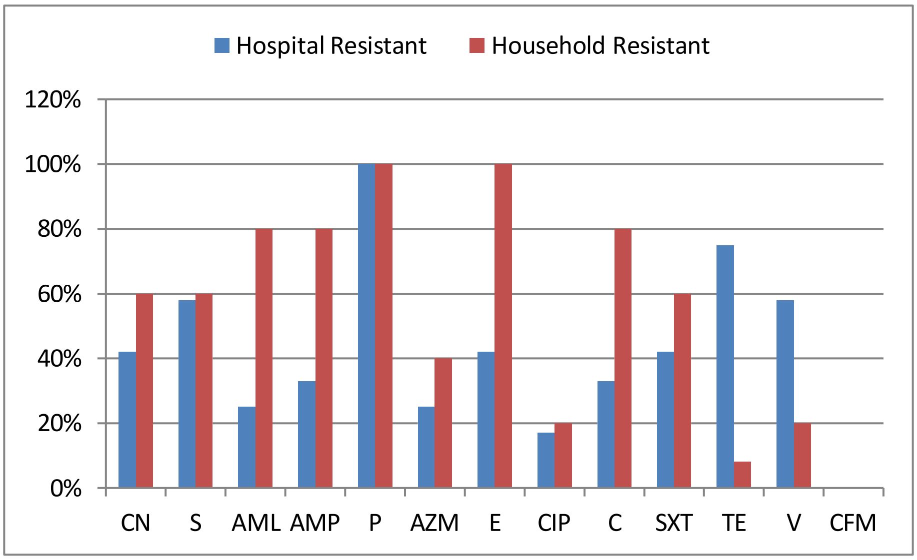

Figure 2. Percentage and comparison of antibiotic resistant pattern of *Shigella spp.* from hospital and residential areas.

Figure 2 illustrates the comparison and relationship of antibiotic resistance patterns of *Shigella spp.* isolates from hospital and residential sources, emphasizing the antibiotic resistance profiles of these isolates.

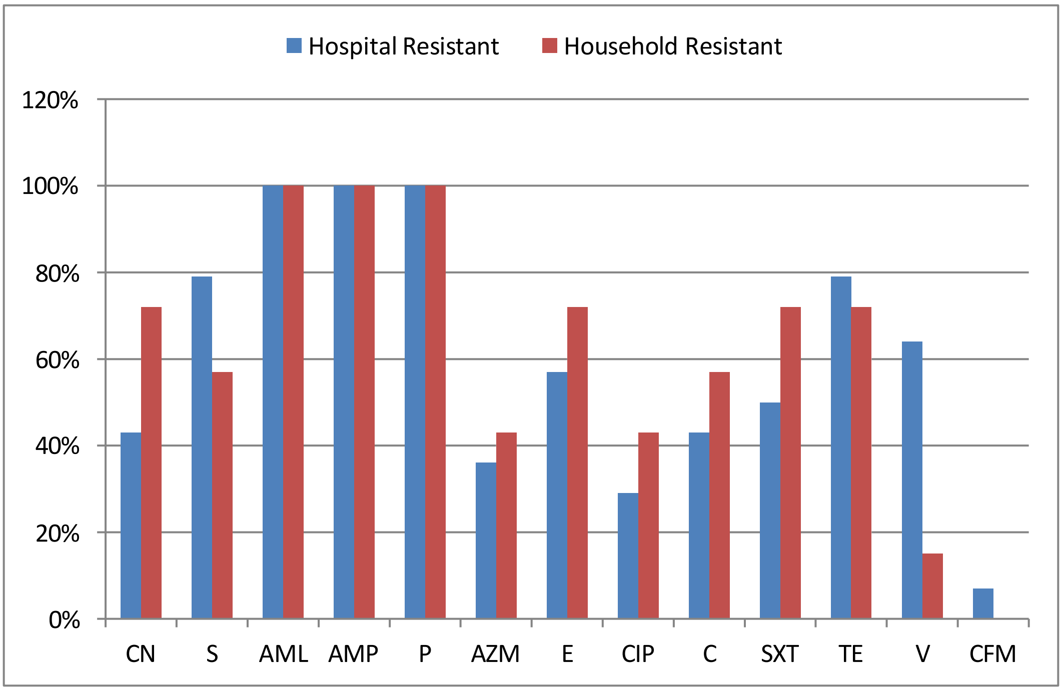

Figure 3. Percentage and comparison of antibiotic resistant pattern of *Klebseila spp.* from hospital and residential areas.

Figure 3 shows the comparison and relationship of antibiotic resistance patterns of *Klebsiella spp.* isolates from hospital and residential sources, highlighting the antibiotic resistance profiles of these isolates.

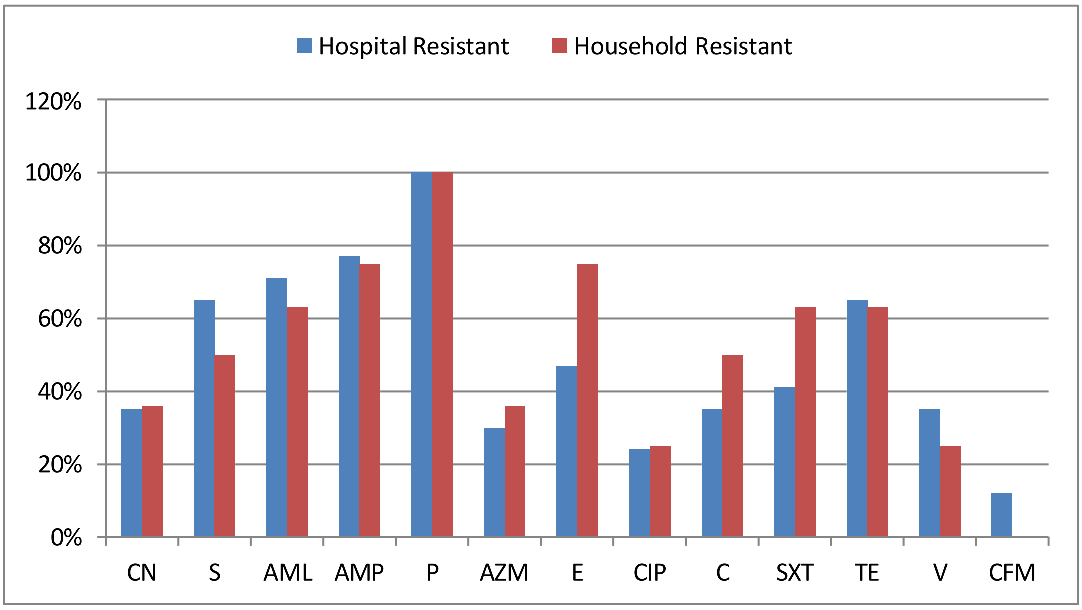

Figure 4. Percentage and comparison of antibiotic resistant pattern of E.coli from hospital and residential areas.

Figure 4 illustrates the comparison and relationship of antibiotic resistance patterns of *E. coli* isolates from hospital and residential sources, emphasizing the antibiotic resistance profiles of these isolates.

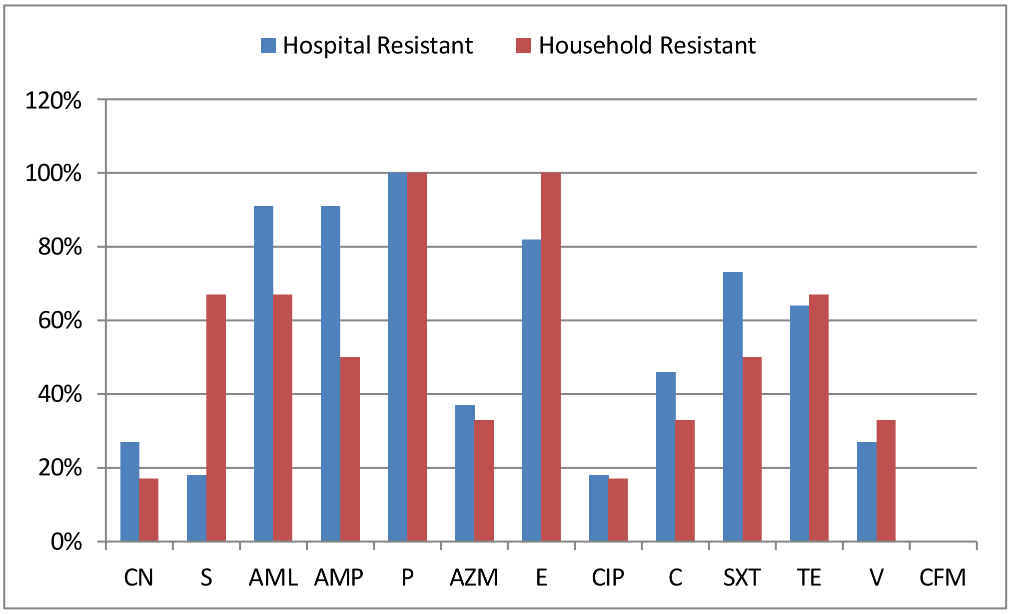

Figure 5. Percentage and comparison of antibiotic resistant pattern of Pseudomonas spp. from hospital and residential areas.

The **Figure 5** shows the comparison and correlation of antibiotic resistance patterns of *Pseudomonas spp.* from hospital and residential sources, focusing on the antibiotic resistance profiles of these isolates.

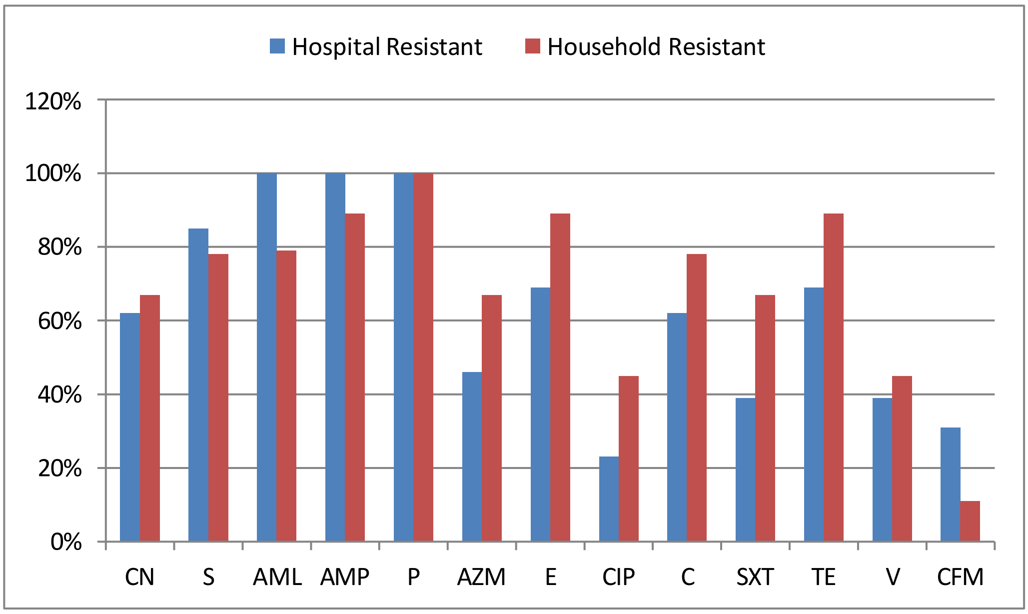

Figure 6: Percentage and comparison of antibiotic resistant pattern of Staphylococcus spp. from hospital and residential areas.

The **Figure 6** shows the comparison and correlation of antibiotic resistance patterns of *Staphylococcus spp.* from hospital and residential sources, highlighting the antibiotic resistance profiles of these isolates.

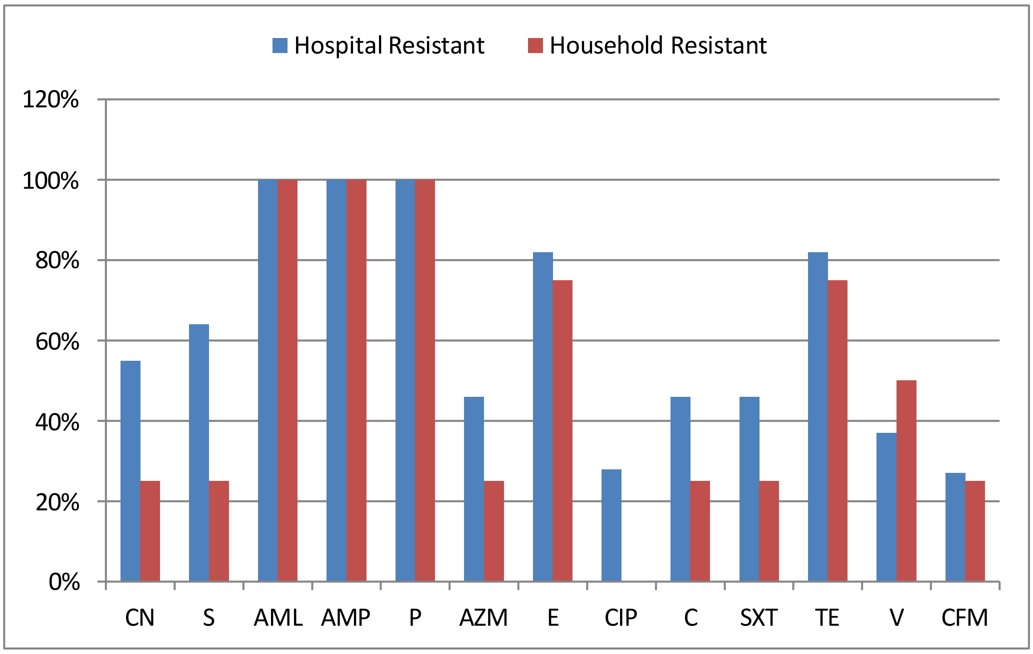

Figure 7. Percentage and comparison of antibiotic resistant pattern of Vibrio spp. from hospital and residential areas.

The **Figure 7** illustrates the comparison and correlation of antibiotic resistance patterns of *Vibrio spp.* from hospital and residential sources, emphasizing the antibiotic resistance profiles of these isolates.

4. Discussion

Over the past six decades, remarkable advancements in the recognition and treatment of infectious diseases have significantly reduced morbidity and mortality rates. These advancements have largely been driven by a better understanding of disease mechanisms and the development of effective antimicrobial treatments. However, the growing global concern of antibiotic resistance, particularly in both common and uncommon bacteria, presents a formidable challenge to public health. The increasing prevalence of multidrug-resistant bacteria emphasizes the urgent need for comprehensive interventions to slow the spread of antimicrobial resistance (AMR), especially given the persistent overuse and misuse of antibiotics.

While multidrug-resistant Gram-positive bacteria such as *Methicillin-resistant Staphylococcus aureus* (MRSA) often dominate discussions around antibiotic resistance, Gram-negative pathogens have emerged as major public health threats. *Escherichia coli*, *Klebsiella spp.*, *Salmonella spp.*, *Shigella spp.*, *Vibrio spp.*, *Pseudomonas spp.*, and *Staphylococcus spp.*, which were selected for this study, have been widely recognized as sources of diverse infections, some of which can be severe or fatal[15]. In particular, resistance in these Gram-negative pathogens has risen substantially, rendering many existing antibiotics ineffective. The dearth of new antibiotics for treating Gram-negative infections further exacerbates the problem, calling for urgent global action to preserve existing treatments and prioritize the development of novel antimicrobials.

The findings of our study align with the growing body of research highlighting the prevalence of antimicrobial resistance in hospital and household wastewater. Our study reveals the prevalence of *E. coli* (15.05% in hospital, 15.22% in household), *Klebsiella spp.* (18.28% in hospital, 17.39% in household), *Salmonella spp.* (16.13% in hospital, 10.87% in household), *Shigella spp.* (12.90% in hospital, 10.87% in household), *Vibrio spp.* (11.83% in hospital, 8.70% in household), *Pseudomonas spp.* (11.83% in hospital, 17.39% in household), and *Staphylococcus spp.* (13.98% in hospital, 19.57% in household) in both hospital and household wastewater. These findings corroborate previous studies that have reported high levels of antimicrobial resistance in wastewater samples from healthcare and community settings, which serve as a significant reservoir for resistant bacteria and resistance genes[16]. The continuous influx of antibiotics, along with the absence or limited treatment of wastewater, creates an environment conducive to the selection and spread of resistant microorganisms.

In terms of antibiotic susceptibility, we found that Azithromycin (AZM), Ciprofloxacin (CIP), and Cefixime (CFM) were the most effective against a majority of bacterial isolates. This result is consistent with previous studies that observed these antibiotics generally retain effectiveness against a range of common pathogens. However, the study also showed higher resistance rates for other antibiotics, particularly in isolates from hospital wastewater, which aligns with findings from other studies demonstrating that hospital wastewater typically harbors higher levels of resistant bacteria compared to community settings. The accumulation of antibiotics in hospital effluents and their potential to exert selective pressure on bacteria contributes to the widespread emergence of resistance in hospital-associated pathogens, which, as our study shows, often exhibit resistance to multiple classes of antibiotics[17].

A key finding of this study is the high prevalence of multidrug-resistant isolates, with many exhibiting resistance to more than two classes of antibiotics. This is particularly concerning given that nearly all bacterial isolates from both hospital and household wastewater demonstrated resistance to at least one antibiotic. Similar patterns of multidrug resistance have been observed in other studies[18], where resistance to multiple antibiotics was frequently noted in hospital-associated pathogens. The transmission of resistance genes among bacterial isolates is a key concern, as the potential for gene transfer between hospital and environmental bacteria increases the spread of resistance into broader populations, further complicating treatment options. The high levels of resistance in *E. coli* isolates, in particular, which were resistant to 29 antibiotics, reinforce the role of hospital effluent as a significant contributor to the dissemination of resistant strains.

While some have suggested that antibiotics in untreated wastewater may be diluted to sub-therapeutic concentrations, thereby reducing their potential to cause harm, it is well-established that prolonged exposure to these sub-therapeutic levels creates ideal conditions for the selection of resistant strains[19]. Numerous studies have shown that even low concentrations of antibiotics in the environment can foster the development of resistance over time[20]. In Bangladesh, where wastewater treatment is often minimal or absent, the risk of antibiotic resistance in environmental water bodies such as rivers, lakes, and reservoirs is exacerbated. Our study underscores the pressing need for effective wastewater treatment strategies to mitigate the spread of antibiotic-resistant bacteria into natural ecosystems and prevent further contamination of water sources.

These findings are supported by studies indicating that untreated hospital wastewater serves as a major contributor to the spread of antibiotic-resistant bacteria into the environment. In countries with limited resources and inadequate wastewater infrastructure, such as Bangladesh, untreated wastewater can become a key vehicle for transferring resistant pathogens and their genes into natural reservoirs, where they may potentially infect humans or other animals. Our research highlights the need for urgent intervention to control antibiotic use and improve wastewater treatment processes in these regions to prevent further exacerbation of the global antibiotic resistance crisis.

5. Conclusion

In conclusion, our results demonstrate that the development of multidrug resistance in hospital sewage is largely attributed to the disposal of untreated hospital waste. To address this issue, efforts must be made to prevent the release of antibiotics into the environment via sewer systems, ensuring the complete destruction of antibiotics before disposal. We hope that the findings of this study will provide a foundation for further scientific investigation in Bangladesh and other countries, focusing on the presence of emerging contaminants like antibiotics in the environment and their impact on public health.

Conflict of interest

The authors declare no conflict of interest.

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