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Antimicrobial resistance profile of bacteria from hospital wastewater at two specialized hospitals in Bahir Dar city, Ethiopia

Kirubel Endalamaw[1], Senait Tadesse[2], Zelalem Asmare[3], Destaw Kebede[1], Mulat Erkihun[4] and Bayeh Abera[2*]

Abstract

Background The high levels of antimicrobial consumption in hospitals contribute to the occurrence of antimicrobial resistant bacteria. Antimicrobial resistant bacteria and sub-lethal concentrations of antimicrobial metabolites can end up in hospital wastewater which can spread to the environment and to the community. However, information on the resistance profile of bacteria isolated from environments is not well studied. Thus, the main aim of this study was to determine the antibiotic resistance profile of bacteria from hospital wastewater in Bahir Dar City, Northwest Ethiopia.

Method A total of 70 hospital wastewater samples were collected from two comprehensive specialized hospitals using a grab-sampling technique. Bacteria were identified using colony morphology, Gram staining, and biochemical tests. The drug susceptibility test was performed using the Kirby-Bauer disc diffusion method on Muller-Hinton agar.

Result The most dominant bacterial isolates from hospital wastewater were *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E. coli*), *Citrobacter* spp., *Acinetobacter* spp., *Enterobacter* spp., *Klebsiella pneumoniae* (*K. pneumoniae*), and *Pseudomonas aeruginosa* (*P. aeruginosa*). All *K. pneumoniae* and 52.4% (11/21) *E. coli* were resistant to ceftazidime (third-generation cephalosporin), while *Citrobacter* spp., *Acinetobacter* spp., and *Enterobacter* spp., were susceptible to ceftazidime. Likewise, S. *aureus* revealed 64% (16/25) resistance to erythromycin. Overall, 22.2% of bacterial isolates were multidrug resistant bacteria to the commonly prescribed antimicrobials.

Conclusion Hospital waste waters contain high prevalence of multiple drug-resistant bacteria, particularly the third-generation cephalosporin resistant *K. pneumoniae* and *E. coli* would be a big concern. Infection prevention and control practices with proper treatment of hospital wastewater before discharging should be in practice to contain the spread of drug-resistant bacteria from hospital to external environment.

Keywords Antibiotic resistant bacteria, Environment, Hospital wastewater

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Background

Antimicrobial resistance (AMR) represents one of the most pressing global health challenges in the healthcare system [1]. The widespread use of antibiotics in clinical settings has led to the emergence and dissemination of resistant bacterial strains, posing severe threats to public health and healthcare systems [2]. The emergence and spread of AMR are global health concerns significantly driven by the misuse or overuse of antibiotics, as introducing sub-lethal doses into the environment can accelerate the development of resistance in hospital settings [3].

An estimated 40–60 L of water per day for every inpatient, leading to a significant amount of wastewater production influenced by factors such as the number of beds, facility type, services provided, and on-site management capacities [4]. Hospital wastewater (HWW), generated from healthcare facilities, encompasses various areas like patient rooms, surgery rooms, laboratories, and disposal of pharmaceutical waste, along with wastewater from administrative units, laundries, kitchens, and other hospital departments [5, 6]. If not properly treated, HWW can release antimicrobial resistant bacteria (ARB) into municipal sewage systems, rivers, and other water bodies, thereby disseminating resistance traits into the environment and human populations [7].

Hospital wastewater, a critical yet often overlooked component in the spread of AMR, serves as a significant reservoir and conduit for antimicrobial-resistant bacteria (ARB) [8]. Hospital wastewater is a hotspot for ARB due to its high concentration of antibiotics and other pharmaceuticals, which significantly contribute for development of antimicrobial resistant pathogenic bacteria, and spreading infections globally [9, 10]. Introducing sub-lethal metabolites of antibiotics into wastewater can accelerate AMR development in hospitals. Hence, hospital wastewater can function as a significant route for antibiotics to enter the environment, containing various biological substances including human wastes with unmetabolized antimicrobials [11]. Hospital wastewater harbors various pathogenic bacteria such as *E. coli*, *P. aeruginosa*, and *Enterococcus* spp., originating from patients, healthcare workers, or contaminated medical equipment [12]. Hospital wastewater is a significant global source of AMR development in pathogenic bacteria according to reports of the World Health Organization (WHO) [13]. For instance, from hospital wastewater a resistance rates of 30.1% in Pakistan [14], 31.3 to 33.3% in Ethiopia were reported [15, 16].

Understanding the resistance profiles of bacteria in wastewaters is crucial for developing targeted strategies to reduce the environmental influence of AMR [17]. Despite notable advancements, critical gaps persist to have a comprehensive information about AMR in hospital wastewaters because several studies have concentrated on specific regions, leaving disparities in the comprehension of AMR dynamics across diverse geographic locations including developing countries like Ethiopia. Data on AMR bacteria from hospital wastewater are limited in developing countries including Ethiopia. Furthermore, it was hypothesized that the antibiotic-resistant bacteria profile from hospital wastewater might reflect the magnitude of AMR in hospitals and their infection prevention efficiency. Thus, the principal aim of this research was to determine the antimicrobial resistance profiles of bacteria from hospital wastewaters in Tibebe Ghion Specialized Hospital and Felege Hiwot Comprehensive Specialized Hospital in Bahir Dar City, Northwest Ethiopia.

Methods

Study setting

A cross-sectional study was conducted in Bahir Dar City, Northwest Ethiopia, from May 19 to June 24, 2023. The study was conducted in two government specialized hospitals (Felege Hiwot Comprehensive Specialized Hospital and Tibebe Ghion Specialized Hospital). These hospitals were chosen because of their extensive instrumentation, diverse medical services, higher bed capacity, large patient volume, and comprehensive waste management systems.

Wastewater sample collection

A total of 70 hospital wastewater samples (40 from FHCSH and 30 from TGSH) were collected from seven medical units or wards such as gynecology, pediatrics, clinical laboratory, medical ward, pharmacy services, ophthalmology, and surgery. A "grab sampling technique" was used to collect representative samples following the guidelines of the Environment Protection Authority (EPA) [18] and the American Public Health Association (APHA) [19]. At each hospital, the covers of the manholes were carefully lifted, and 50 ml wastewater samples were taken into sterile plastic containers. Samples were collected twice a day, in the morning at 10:00 A.M and in the afternoon 2:00 P.M within 4-hour interval. The samples were collected near the center of the flow channel, at approximately 10 to 15 cm of water depth. All samples were collected manually and transported with a cold chain within two hours into the Microbiology Laboratory in the College of Medicine and Health Sciences, Bahir Dar University (Ethiopia). Samples were stored in a refrigerator at 4 °C until they are processed.

Bacterial identification

Samples were thoroughly shaken to get a homogeneous mixture, and 10-fold serial dilution were prepared using 0.85% sodium chloride. Then, 0.1 ml of sample was

streaked onto blood agar (Blood agar base, HiMedia, India, with 5% sheep blood) using a sterile inoculating loop, and incubated at 37 °C in atmosphere for 24 h. After that, each bacterial colony with different morphologies and colors were further sub-cultured onto selective media such as Mannitol salt agar (MSA) for *S. aureus* and MacConkey agar (Oxoid, UK). From MSA colonies, S. *aureus* was identified using gram staining, catalase positive and coagulase-positive tests. For Gram negative bacteria the pure colonies of suspected bacteria were identified at the species level using biochemical tests. Biochemical tests such as sugar fermentation, hydrogen sulfide (H_2S) and gas production, citrate utilization, motility tests, indole production, urease, oxidase, catalase, and coagulase tests were used for identification [20].

Antibiotic susceptibility testing

Antimicrobial susceptibility testing was performed using the Kirby Bauer disk diffusion method based on the guidelines of the Clinical and Laboratory Standards Institute (CLSI) [20]. The tested antimicrobials were erythromycin (15 µg), trimethoprim/sulfamethoxazole (25 µg), ampicillin (10 µg), cefoxitin (30 µg), ceftazidime (30 µg), chloramphenicol (30 µg), and tetracycline (30 µg) (Abtek, UK). A suspension of 3–5 distinct colonies from each isolate was prepared in 5 ml of normal saline, standardized to match 0.5 McFarland standards, and subsequently inoculated onto Mueller Hinton agar (Condalab, Madrid, Spain). After inoculation, antibiotic discs were placed 25 mm apart from each other and 15 mm from the edge of the petri dish, followed by incubation in a normal atmospheric temperature at 37 °C for 18–24 h. The diameter of the inhibition zones around the discs was measured using a ruler and interpreted according to the CLSI guideline [20]. Isolates exhibiting non-susceptibility to at least one agent in three or more antibiotic categories were classified as multi-drug resistant (MDR) [21].

Quality control

Data qualities were ensured by adhering to standard procedures at each study step. Wastewater specimens were collected after sterilizing the manhole with alcohol to prevent contamination. Aseptic techniques were used during sample processing within Biosafety Level 2 cabinets. The sterility of the culture media was verified using indicator tape by incubating 3–5% of each media batch at 37 °C for 24 h. Furthermore, to check the performance of bacterial culture media, and potency of the antimicrobial discs, American Type Culture Collection (ATCC) standard reference strains of *S. aureus* (ATCC 29213), and *E. coli* (ATCC 25922) were used [20].

Data analysis

Data were entered and analyzed using the Statistical Package for the Social Sciences (SPSS) version 27 (IBM Corporation, Armonk, NY, USA). The frequency and percentages of isolates and the antibiotic susceptibility patterns of the isolated bacteria were calculated.

Results

Distribution of bacterial isolates from hospital wastewater

A total of seven different bacterial species were identified from hospital wastewater. The most frequently isolated bacterium was *S. aureus* followed by *E. coli*, *Citrobacter* spp., and *Acinetobacter* spp., (Fig. 1). The highest proportions of bacterial isolates were found from gynecology ward followed by pediatric ward (Table 1). From the pediatric ward, *S. aureus* (76.9%) and *E. coli* (53.8%) were the most frequent. These isolates were also predominant in the gynecology ward. Additionally, *Enterobacter* species were among the predominant bacteria isolated from the gynecology ward, comprising 35.3% of the isolates.

Antimicrobial resistance profiles

Bacterial isolates from hospital wastewater exhibited different levels of antimicrobial resistance. All (100%) *K. pneumoniae* and 52.4% of *E. coli* were resistant to the third-generation cephalosporin (ceftazidime). *E. coli* also showed 52.4% of resistance level to tetracycline. *S. aureus* demonstrated 64% resistance to erythromycin. The detailed resistance profiles of all bacterial isolates are depicted (Table 2). Overall, 22.2% of bacterial isolates from hospital wastewater were MDR (Table 3). Amon these, S. *aureus* was the most prevalent MDR followed by *E. coli* and *K. pneumoniae*. These isolates were resistant to at least three and up to five categories of antimicrobial agents (Table 3).

Discussion

The presence of antimicrobial resistant bacteria from hospital wastewaters showed the spreading of AMR bacteria into the environmental through wastewater. Additionally, the results the present study highlight the importance of infection control measures within hospitals and the prudent antibiotic use to curb the spread of antibacterial resistant bacteria. This finding advocates for the implementation of disinfection of wastewater before discharging to the disposal systems.

The isolated bacteria in hospital wastewater are consistent with previous studies conducted in Ethiopia [15, 22] and in Pakistan [23]. Similarly, a study in Addis Ababa (Ethiopia) reported that *E. coli* and K. *pneumoniae*, were more frequently isolated bacteria [24]. These variations might be due to differences in study period, strategies of the wastewater disposal system and differences in approaches to infection prevention and control measures.

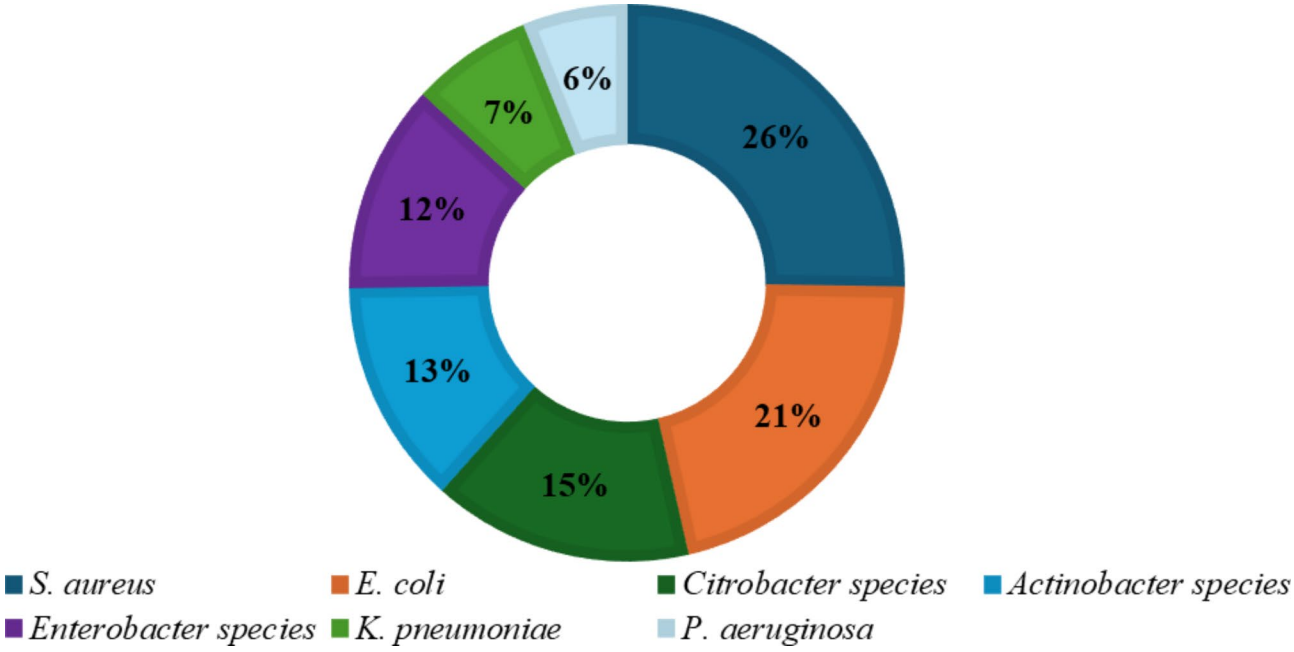


Fig. 1 Proportion of bacteria1 isolates from hospital waste waters in Bahir Dar city in 2023

Table 1 Distribution of bacterial isolates in hospital wastewater from different wards in Bahir Dar City in 2023

Wards/units	Bacterial isolates *n* (%)						
	S. aureus	***E. coli***	***Citrobacter* Spp.,**	***Acinetobacter* Spp.,**	***Enterobacter* Spp.,**	***K. pneumoniae***	***P. aeruginosa***
Gyni (*n* = 17)	5 (29.4)	6 (35.3)	3 (17.6)	4 (23.5)	6 (35.3)	1 (5.9)	3 (17.6)
Pedi (*n* = 13)	10 (76.9)	7 (53.8)	3 (23)	3 (23)	-	1 (7.7)	1 (7.7)
Lab (*n* = 11)	2 (18.2)	3 (27.3)	2 (18.2)	3 (27.3)	2 (18.2)	2 (18.2)	1(9.1)
Med (*n* = 9)	2 (22.2)	3 (33.3)	3 (33.3)	2 (22.2)	-	1 (11.1)	1 (11.1)
Sur (*n* = 11)	4 (36.4)	2 (18.2)	2 (18.2)	-	2 (18.2)	-	-
Phar (*n* = 6)	2 (33.3)	-	2 (33.3)	-	1 (16.7)	2 (33.3)	-
Opt (*n* = 3)	-	-	-	1(33.3)	1(33.3)	-	-
Total	25 (35.7)	21 (30)	15 (21.4)	13 (18.6)	12 (17.1)	7 (10)	6 (8.6)

Key: Gyni: Gynecology ward, Pedi: Pediatrics ward, Lab: Laboratory unit, Med: Medical ward, Sur: Surgical ward, Phar: Pharmacy unit, Opt: Ophthalmology ward and - = no isolates found

Table 2 Antimicrobial resistance bacteria from hospital waste waters in Bahir Dar City in 2023

Bacteria isolated	Antibiotics tested, *n* (%) resistance						
	AMP	**CHL**	**TTC**	**TSX**	**ERY**	**CAZ**	**CXT**
S. aureus (*n* = 25)	-	1 (4)	11 (44)	6 (24)	16 (64)	1 (4)	13 (52)
E. coli (*n* = 21)	9 (42.9)	1 (4.8)	11 (52.4)	5 (23.8)	-	11 (52.4)	10 (47.6)
K. pneumoniae (*n* = 7)	4 (57.1)	1 (14.3)	1 (14.3)	1 (14.3)	-	7 (100)	1 (14.3)
P. aeruginosa (*n* = 6)	2 (33.3)	2 (33.3)	-	1 (16.7)	-	1 (16.7)	2 (33.3)
*Citrobacter*spp., (*n* = 15)	3 (20)	-	3 (20)	2 (13.3)		-	-
*Acinetobacter*spp., (*n* = 13)	5 (38.5)	1 (7.7)	3 (23.1)	-		-	-
*Enterobacter*spp., (*n* = 12)	3 (25)	1 (8.3)	5 (41.7)	-		-	-

Key: - = not tested, AMP = ampicillin, CHL = chloramphenicol, TTC = tetracycline, TSX = trimethoprim/sulfamethoxazole, ERY = erythromycin, CAZ = ceftazidime and CXT = cefoxitin

Regarding antimicrobial resistance, the isolated bacteria showed high levels of antimicrobial resistance against commonly prescribed drugs. For instance, all *K. pneumoniae* and above 50% of *E. coli* were resistant to third generation cephalosporin (ceftazidime). Likewise, in Addis Ababa governmental hospitals wastewater 43.3% of *E. coli* was resistant to ceftriaxone [24]. This might be due to the production of extended-spectrum beta-lactamases (ESBLs) in *E. coli* and *K. pneumoniae* [25] related to high consumption the third-generation cephalosporins

Table 3 Multi-drug-resistant bacteria from hospital waste water in Bahir Dar City in 2023

Bacteria	Resistance antibiotic combinations	No of antibiotic category	Frequency *n* (%)	MDR *n* (%)
E. coli (*n* = 21)	AMP, SXT, CXT, CHL, TTC, CAZ	5	1 (4.8)	7 (33.3)
	AMP, SXT, CXT, TTC, CAZ	4	1 (4.8)	
	AMP, TTC, CAZ	3	1 (4.8)	
	AMP, CXT, TTC, CAZ	3	4 (19.0)	
S. aureus (*n* = 25)	SXT, CXT, CHL, TTC	4	5 (20)	9 (36)
	ERY, SXT, CXT	3	4 (16)	
K. pneumoniae (*n* = 7)	AMP, SXT, CXT, CHL, CAZ	4	1 (14.3)	2 (28.6)
	AMP, TTC, CAZ	3	1 (14.3)	
Citrobacter spp., (*n* = 15)	AMP, SXT, TTC	3	2 (13.3)	2 (13.3)
Enterobacter spp.,, (*n* = 12)	AMP, ERY, TTC	3	2 (16.7)	2 (16.7)
Total				**22 (22.2)**

Key: AMP = ampicillin, CHL = chloramphenicol, ERY = erythromycin, TTC = tetracycline, CXT = cefoxitin, CAZ = ceftazidime and SXT = trimethoprime/sulfamethoxazole

in the study area. It was also reported that in China, *K. pneumoniae* had the highest resistance against third generation cephalosporin (ceftazidime) [4].

This can indicate the inappropriate disposal of antimicrobials or a lack of awareness about the proper use of antimicrobials [26]. Moreover, it might be due to introducing of sub-lethal doses of antibiotics used in hospitals into the environment, which could exert selective pressure to the susceptible bacteria in the environment. Thus, hospital wastewater contributes to the spread of antibiotic-resistant bacteria in the hospital environment. The increased level of *S. aureus* resistance to erythromycin can indicate the frequent uses of erythromycin. Similarly, high levels of erythromycin resistant *S. aureus* were reported in Ethiopia and Africa [27].

The significant number of bacteria from hospital wastewater demonstrated multiple drug resistance (MDR) against commonly used antibiotics. Namely, *S. aureus*, *E. coli*, *K. pneumoniae* and *Citrobacter* spp., were common bacteria showing MDR. Overall, 22.2% of hospital wastewater bacteria showed multidrug resistance. This is comparable with other previous studies conducted in Ethiopia, which reported 29.5–33.3% MDR bacteria [16, 27], and 30.1% in Pakistan [14]. However, higher levels of MDR bacteria (above 90% of MDR) were reported in other parts of the world such as Nigeria [28], China [4], and Nepal [29].

Limitation of the study

The limitation of this was that antimicrobial resistant bacteria were determined by phenotypic methods and unable to perform Minimum Inhibition Concentration (MIC) for better assessment of Microbial susceptibility tests. Limited numbers of antimicrobial agents were tested against the isolated bacteria because some of them were not available during the study period. Moreover, this study would provide comprehensive information if private hospital wastewaters had been included in Bahir Dar City.

Conclusion

This study highlights the high magnitude of antibacterial resistant bacteria in hospital wastewater, notably the third-generation cephalosporin resistant bacteria K. *pneumoniae* and *E. coli*. Hospital wastewater bacteria showed resistance to multiple classes of antibiotics (MDR). Thus, measures are needed to enhance antimicrobial stewardship, infection prevention and control to contain the spread of antibacterial resistant bacteria.

Recommendation

The presence of high MDR bacteria in hospital wastewater may impose public health challenges because they can transmit resistance traits to other enteric pathogenic bacteria in the community. Thus, wastewater effluents must be disinfected to minimize the microbial burden at each respective ward or unit. A further study is needed to assess factors that could be associated with bacterial contamination of the sewage system.

Abbreviations

AMR	Antimicrobial resistance
ARB	Antibiotic resistant bacteria
CLSI	Clinical and laboratory standards institute
FHCSH	Felege Hiwot comprehensive specialized hospital
HWW	Hospital wastewater
MDR	Multiple drug resistance
TGSTH	Tibebe Ghion specialized teaching hospital

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Author contributions

Kirubel Endalamaw conceptualized, designed, analyzed, and interpreted the study, and also prepared the manuscript. Bayeh Abera and Senait Tadesse involved in conceptualization and designing the study provided supervision through critical review of the overall process of the study and participated in the manuscript preparation. Destaw Kebede, Zelalem Asmare, and

Mulat Erkihun contributed to the technical laboratory work and involved in the manuscript preparation. All authors reviewed and approved the final manuscript for publication.

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Data availability
All data generated or analyzed during this study are included in this manuscript.

Declarations

Ethical considerations
Ethical approval was obtained from the institutional review board of the College of Medicine and Health Science, Bahir Dar University (Protocol number 746/2023), and permission was also obtained from FHCSH and TGSTH. Written informed consent from specialized hospitals was obtained after explaining the purpose and benefits of the study and the confidentiality of the information in the study. The names of hospitals were anonymized and coded before being stored in a locked cabinet and documented on a password-protected personal computer to ensure the confidentiality of the results.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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