


# Antibiotic Resistance Profiles of Bacteria Isolated from Hotspot Environments in Bahir Dar City, Northwestern Ethiopia

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**Background:** Wastes generated from hotspot environments contain a wide range of antibiotics and pathogens that play a significant role in the dissemination of antibiotic-resistant bacteria in the environment. This study was carried out to isolate bacteria from hotspot environments and determine their resistance profiles to commonly used antibiotics in Bahir Dar city, Ethiopia.

**Methods:** A cross-sectional study was conducted from October 2020 to June 2021 in Bahir Dar City. A total of 126 waste and wastewater samples were aseptically collected, transported, and processed for bacteriological isolation and susceptibility testing following standard procedures.

**Results:** A total of 411 bacterial isolates were recovered and the highest value of 122 (29.7%) bacterial isolates were obtained from medical wastewater samples, and the most frequently isolated bacteria were assigned to the species *Escherichia coli* with 82 strains (19.5%). The results revealed that the highest resistance profile of 69 (95.8%) was obtained in *Staphylococcus aureus* against ampicillin and 46 (86.8%) *Citrobacter* spp. against tetracycline. Two hundred and sixteen (52.6%) of bacteria showed multi-drug resistance and the highest multi-drug resistance was observed in *Pseudomonas* spp. 47 (65.3%), followed by *Escherichia coli* 51 (62.2%). The highest resistance profile of 12 (85.7%) and 60 (74.1%) for tetracycline were obtained from beef waste and wastewater and medical wastewater samples. The highest multi-drug resistance was recorded in isolates isolated from beef waste and wastewater samples 11 (64.7%), followed by medical wastewater samples 84 (64.1%). Even though a higher (>0.2) multi-antibiotic resistance index was found in all hotspot environments, the highest multi-antibiotic resistance index (0.477) was recorded in bacteria isolated from medical wastewater.

**Conclusion:** It was concluded that wastes generated from hotspot environments and released in the environment contain large numbers of antibiotic-resistant, multidrug, extensively, and pan-drug-resistant bacteria. Proper waste management strategies should be established.

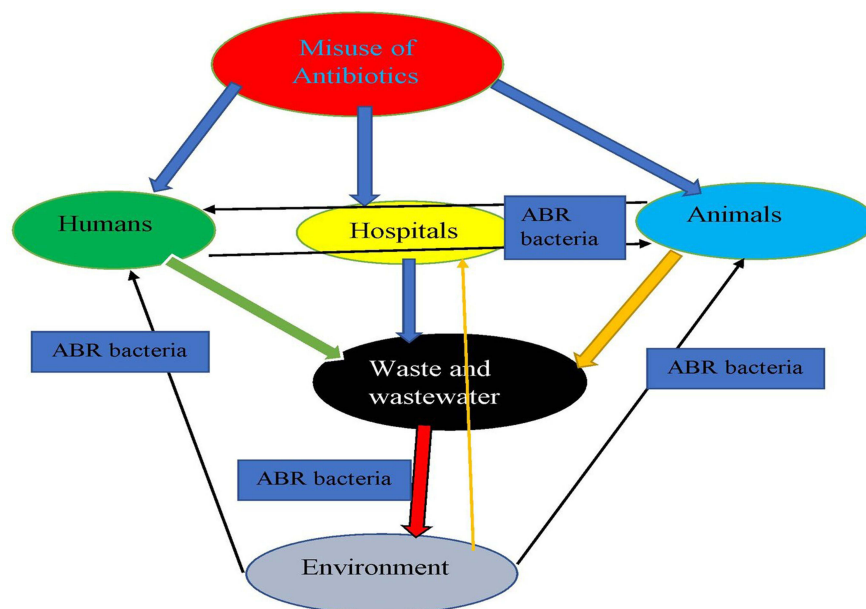
**Keywords:** antibiotic resistance, bacteria, hotspot environments, multidrug resistance, wastewater

## Introduction

Antibiotics are a vital group of therapeutic agents used for the treatment of bacterial infections. However, this wide and intensive use of antibiotics has resulted in their continuous release within the environment<sup>1,2</sup> and a sufficient amount of antibiotics continues to accumulate within the environment in the unmetabolized form. Antibiotic overuse in recent decades has resulted in an explosion of antibiotic-resistant bacteria, which pose a serious public health threat to many people around the world. Antibiotic resistance is most commonly studied in the medical setting, but there is growing concern about the spread of antibiotics into the natural environment.<sup>3</sup>

Untreated wastes generated from humans (municipal wastes), health institutions (medical wastes), and animals are some hotspots that pose the risk of spreading antibiotic-resistant bacteria within the environment. Untreated effluents or the concentrated forms of infectious agents and antibiotic-resistant microbes are shed into communities, leading to water-borne diseases such as cholera typhoid fever, dysentery, and gastroenteritis, which cannot be treated with conventional

## Graphical Abstract



Entry pathways of antibiotic-resistant bacteria into the environment and its transmission

antibiotics.<sup>4</sup> Because of the high densities of nearby bacteria as well as the presence of contaminants in pharmaceuticals and wastewater treatment plants are well-known hotspots for antimicrobial resistance.<sup>5</sup> Chronic pollution of receiving water bodies is caused by wastewater treatment facilities that do not completely remove antibiotics, antibiotic-resistant bacteria, or antibiotic resistance genes.<sup>6</sup>

Medical sewage is a route for antibiotics to enter the environment<sup>7</sup> and most sewages contain a combination of various forms of chemical waste and biological substances, which are highly toxic and dangerous.<sup>8</sup> Human waste (urine and feces) are other constituents of medical effluent which contain unmetabolized antibiotics.<sup>9</sup> According to studies, hospital wastewater is a highly selective environment that contributes to the high rates of resistant bacteria such as *Klebsiella* spp., *Pseudomonas* spp., *E. coli*, *Citrobacter* spp., and *S. aureus* that are discharged into the natural environment.<sup>10,11</sup>

Municipal wastewater contains several contaminants, including pharmaceuticals and private care products from households, and hospital waste with high concentrations of antibiotics and disinfectants, and compounds from industrial activities. Waste discharge from livestock farms is also a hotspot for the spread of antibiotic-resistant bacteria and antibiotics to the environment.<sup>12</sup> In many instances, there is no structured form of waste treatment plants, and most wastes are discharged into the environment for natural degradation. Timely and proper management of waste produced in a particular facility plays a critical role in reducing the emergence and dissemination of AR bacteria in the community.

Antibiotic resistance has been determined in very limited studies in Ethiopia, and most of these studies mainly focused on clinical settings.<sup>13,14</sup> A study also has been conducted to determine the antibiotic resistance patterns of bacteria isolated from poultry litter.<sup>15</sup> Environmental aspects could be taken into consideration in risk assessments associated with antimicrobial resistance<sup>16,17</sup> because the resistance genes are transferred from environmental bacteria to human pathogens, which can be a significant threat to a community. In Ethiopia, particularly in the Amhara region, multi-

antibiotic resistance profiles of bacteria isolated from environmental reservoir (animal, municipal, and hospital) wastes and wastewater as well as the multi antibiotic resistance index of these bacteria are limited. Therefore, this study was carried out to identify bacteria from hotspot environments and to determine their profiles of resistance to commonly used antibiotics in Bahir Dar city, Ethiopia.

## Materials and Methods

### Study Design, Setting, Sample Collection, and Preparation

A cross-sectional study was conducted between October 2020 and June 2021. A total of 126 samples (36 dairy waste and wastewater, 36 poultry waste, 18 beef waste and wastewater, 18 medical wastewater, and 18 municipal wastewater) were collected from 14 sites in Bahir Dar city using sterilized sample bottles in the icebox. Wastewaters were collected from a wastewater treatment pond at the Bahir Dar University Peda Campus, Felege Hiwot Specialized Hospital, dairy, and beef farms from the outermost chambers before being discharged into the environment by following standard procedures of the microbiology laboratory. Solid waste samples from poultry, dairy, and beef farms were collected from deposited waste for fertilizer. These samples were transported to the Bahir Dar University postgraduate Microbiology Laboratory and processed within 6 h of collection following standard procedures.

One gram of solid sample or 1 mL of the liquid sample was measured and transferred to 9 mL of sterile peptone water and homogenized by shaking in an aseptic environment. Serial dilutions from  $10^{-1}$  up to  $10^{-9}$  were prepared by adding 1 mL of a homogenized sample to a sterile test tube containing 9 mL of normal saline and mixing properly.

### Isolation and Identification of Bacteria from Hotspot Environments

From  $10^{-4}$  and  $10^{-5}$  dilutions, 0.1 mL aliquots were evenly spread on Mannitol Salt Agar (HiMedia, India) for *S. aureus*, Eosin Methylene Blue Agar (Hi-Media, India) for *E. coli*, Salmonella-Shigella Agar (Hi-Media, India) for *Salmonella* and *Shigella* spp., Nutrient Agar with sucrose for *Pseudomonas* spp. and MacConkey Agar (Hi-Media, India) for other Enterobacteriaceae. The plates were incubated at 37°C for 24–48h under aerobic conditions.<sup>18</sup> All bacteriological analyses were performed in triplicate, and representative colonies were selected and purified by successive subculturing. Bacteria were identified based on their morphological, Gram staining, cultural and biochemical tests (Methyl Red/Voges-Proskauer, catalase, indole, urease, citrate utilization, triple sugar iron, Sulfide, Indole, Motility (SIM), oxidase, and carbohydrate fermentation) following standard procedures<sup>19</sup> and comparing the characterization of the isolates with Bergey's Manual of Determinative Bacteriology.<sup>20</sup>

### Antimicrobial Susceptibility Test of Isolates

The sensitivity of bacterial isolates to antibiotics was determined using the standardized single disc diffusion method developed by Bauer et al.<sup>21</sup> The culture turbidity was adjusted to a 0.5 McFarland standard (approximately  $1 \times 10^8$  CFU/mL) using sterile normal saline, and the suspension was aseptically swabbed on the surface of Mueller Hinton Agar (HiMedia, India) plates. Antibiotic susceptibility testing was performed against commonly used antibiotics for the treatment of human and animal infections in the study areas. Discs (HiMedia, India) containing erythromycin (15µg), norfloxacin (10µg), tetracycline (30µg), chloramphenicol (30µg), ciprofloxacin (5 µg), ampicillin (30µg), co-trimoxazole (25µg), and gentamicin (10µg) were carefully placed on the surface of Muller-Hinton Agar previously inoculated with a broth culture of the test bacteria and incubated at 37°C for 24 h. The zones of inhibition around each disc were measured using a caliper, and the results were classified as sensitive, resistant, and intermediate. Multidrug-resistant bacteria were selected based on their resistance to over three classes of antibiotics, XDR bacterial isolates remain susceptible to only one or two categories, and PDR bacteria were non-susceptibility to all agents in all tested antimicrobial categories.<sup>22</sup>

The multiple antibiotic resistance index (MARI) for each sampling site was determined using the formula described by Mohanta and Goel and Titilawo et al.<sup>23,24</sup> It was mathematically expressed as  $MARI = A/N (Y)$ , where A is the total number of resistant determinants recorded, N is the number of isolates, and Y is the total number of antibiotics tested.

## Quality Control and Data Management

The quality of the data was ensured by following the standard procedure at each step of the study. The functionality of the instruments was checked before the process was employed. The quality of the media, reagents, stains, and antibiotic discs were ensured following the manufacturer's instructions. In addition, reference strains *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923) were used as controls to perform the disc diffusion test and biochemical tests according to CLSI recommendations.

## Data Analysis

The collected and recorded data were coded and analyzed using the SPSS Version 23 software. The resistance profile for each antibiotic was used to calculate the percentage of antibiotic resistance in hotspot environments and was compared with the chi-square test. Statistical significance was set at  $p < 0.05$ . Finally, the analyzed data were organized and presented in the appropriate tabular and graphical formats.

## Results

### Isolation of Antibiotic-Resistant Bacteria from Hotspot Environments

A total of 126 waste and wastewater samples were processed for the presence of antibiotic-resistant bacteria. Of these samples, 92.1% were positive for one or more bacterial isolates (Table 1). Among the total samples, 411 bacterial isolates were recovered, and the highest 122 (29.7%) bacterial isolates were obtained from medical wastewater samples. Of the total bacterial isolates obtained from poultry waste samples, the highest bacteria isolate were *E. coli* (29.3%), 33.3% of other bacteria from municipal wastewater, 45% of *Proteus* spp. from medical wastewater, 37.8% of *Salmonella* spp. from dairy waste and wastewater, and 9.3% of *Citrobacter* spp. from beef waste and wastewater samples (Table 2).

Except for other bacterial species, the highest number of isolates was obtained from the medical wastewater samples. The highest number of *Pseudomonas* spp. was also isolated from dairy waste and wastewater samples. *E. coli* 82 (19.5%) was the most frequently isolated bacterium, followed by *S. aureus* and *Pseudomonas* spp. 72 (17.1%) (Table 2).

### Antibiotic Resistance Profiles of Bacteria Isolated from Hotspot Environments

As shown in Table 3, among all bacterial isolates, *Klebsiella* spp. showed the highest resistance pattern to erythromycin 10 (55.6%), ciprofloxacin 10 (55.6%), norfloxacin 7 (38.9%), co-trimoxazole 12 (66.7%) and gentamicin 11 (61.1%). The highest resistance was recorded for *E. coli*, *S. aureus*, and *Citrobacter* spp. against chloramphenicol 35 (42.7%), ampicillin 69 (95.8%), and tetracycline 46 (86.8%), respectively. A lower resistance pattern was observed in *S. aureus* against ciprofloxacin 2 (2.8%) and gentamicin 1 (1.4%), and *Citrobacter* spp. against chloramphenicol 2 (3.8%), ampicillin 12 (22.6%), and co-trimoxazole 16 (30.2%). Moreover, lower resistance 5 (25%) was found in *Proteus* spp. against erythromycin and tetracycline and 1 (1.8%) in other bacterial spp. against norfloxacin. The resistance of bacteria against ampicillin 263 (64%) was higher than other antibiotics, and the results showed that there was a statistically significant difference among bacterial isolates for the resistance to all antibiotics except erythromycin ( $p < 0.05$ ) (Table 3).

**Table 1** Distribution, Number of Positive and Negative Waste Samples Taken from Hotspot Environments, Bahir Dar City, 2021

Hotspot Environments	Total Samples N (%)	Positive Samples N (%)	Negative Samples N (%)
Poultry waste	36 (28.6)	35 (27.8)	1 (0.8)
Municipal wastewater	18 (14.3)	16 (12.7)	2 (1.6)
Medical wastewater	18 (14.3)	18 (14.3)	0 (0)
Dairy waste and wastewater	36 (28.6)	34 (27)	2 (1.6)
Beef waste and wastewater	18 (14.3)	13 (10.3)	5 (3.9)
Total	126 (100)	116 (92.1)	10 (7.9)

**Table 2** Proportion and Types of Bacterial Isolates at Each Hotspot Environment in Bahir Dar City, 2021

Bacterial Isolates	Poultry Waste N (%)	Municipal Wastewater N (%)	Medical Wastewater N (%)	Dairy Waste and Wastewater N (%)	Beef waste and Wastewater N (%)	Total N (%)
<i>S. aureus</i>	13 (16.3)	19 (23.8)	24 (30)	13 (16.3)	3 (3.8)	72 (17.1)
<i>E. coli</i>	24 (29.3)	12 (14.6)	26 (31.7)	18 (22)	2 (2.4)	82 (19.5)
<i>Salmonella</i> spp.	9 (24.3)	8 (21.6)	4 (10.8)	14 (37.8)	2 (5.4)	37 (8.8)
<i>Pseudomonas</i> spp.	7 (9.7)	15 (20.8)	24 (33.3)	24 (33.3)	2 (2.8)	72 (17.1)
<i>Citrobacter</i> spp.	5 (9.3)	10 (18.5)	20 (37)	13 (24.1)	5 (9.3)	53 (12.6)
<i>Klebsiella</i> spp.	5 (27.8)	3 (16.7)	8 (44.4)	2 (11.1)	0 (0)	18 (4.3)
<i>Proteus</i> spp.	5 (25)	6 (30)	9 (45)	0 (0)	0 (0)	20 (4.8)
Others spp.	13 (22.8)	19 (33.3)	7 (12.3)	18 (31.6)	0 (0)	57 (13.6)
Total N (%)	81 (19.7)	92 (22.4)	122 (29.7)	102 (24.8)	14 (3.4)	411 (100)

**Table 3** Antibiotic Resistance Profiles of Each Bacterium Isolated from Hotspot Environments Against Tested Antibiotics in Bahir Dar City, 2021

Bacterial Isolates (N)	Antibiotics							
	E N (%)	CIP N (%)	NX N (%)	C N (%)	AMP N (%)	TE N (%)	COT N (%)	GEN N (%)
<i>S. aureus</i> (72)	29 (40.3)	2 (2.8)	4 (5.6)	10 (13.9)	69 (95.8)	41 (56.9)	30 (41.7)	1 (1.4)
<i>E. coli</i> (82)	32 (39)	22 (26.8)	19 (23.2)	35 (42.7)	56 (68.3)	58 (70.7)	51 (62.2)	31 (37.8)
<i>Salmonella</i> spp. (37)	13 (35.1)	5 (13.5)	4 (10.8)	13 (35.1)	32 (86.5)	15 (40.5)	22 (59.5)	2 (5.4)
<i>Pseudomonas</i> spp. (72)	39 (54.2)	39 (54.2)	15 (20.8)	12 (16.7)	26 (36.1)	48 (66.7)	38 (52.8)	42 (58.3)
<i>Citrobacter</i> spp. (53)	20 (37.7)	20 (37.7)	5 (9.4)	2 (3.8)	12 (22.6)	46 (86.8)	16 (30.2)	27 (50.9)
<i>Klebsiella</i> spp. (18)	10 (55.6)	10 (55.6)	7 (38.9)	6 (33.3)	6 (33.3)	15 (83.3)	12 (66.7)	11 (61.1)
<i>Proteus</i> spp. (20)	5 (25)	2 (10)	1 (5)	7 (35)	15 (75)	5 (25)	13 (65)	3 (15)
Other bacteria (57)	18 (31.6)	5 (8.8)	1 (1.8)	14 (24.6)	47 (82.5)	28 (49.1)	23 (40.4)	4 (7)
Total (411)	166 (40.4)	105 (25.5)	56 (13.6)	99 (24.1)	263 (64)	256 (62.3)	205 (49.9)	121 (29.4)
P - value	0.106	0.000	0.000	0.000	0.000	0.000	0.002	0.000

**Abbreviations:** E, erythromycin; CIP, ciprofloxacin; NX, norfloxacin; C, chloramphenicol; AMP, ampicillin; TE, tetracycline; COT, co-trimoxazole; GEN, gentamicin.

Based on the results of the susceptibility test shown in Table 4, the highest resistance (74.1%) profile was obtained from poultry waste for tetracycline, 58.7% from municipal wastewater for ampicillin, 69.7% from medical wastewater for tetracycline, 66.7% from dairy waste and wastewater for ampicillin, and 85.7% from beef waste and wastewater for tetracycline. A lower resistance profile was seen for bacterial isolates isolated from poultry waste (6.2%), municipal wastewater (7.6%), dairy waste and wastewater (0%), and beef waste and wastewater (7.1%) against norfloxacin, and bacterial isolates from medical wastewater showed lower resistance against chloramphenicol (30.3%).

**Table 4** Antibiotic Resistance Profiles of Bacterial Isolates Isolated from Each Hotspot Environment in Bahir Dar City, 2021

Number of Isolates in the Hotspot Environments	E N (%)	CIP N (%)	NX N (%)	C N (%)	AMP N (%)	TE N (%)	COT N (%)	GEN N (%)	MARI
Poultry wastes (n= 81)	22 (27.2)	10 (12.3)	5 (6.2)	25 (30.9)	57 (70.4)	60 (74.1)	40 (49.4)	15 (18.5)	0.353
Municipal wastewater (n=92)	24 (26.1)	14 (15.2)	7 (7.6)	16 (17.4)	54 (58.7)	45 (48.9)	50 (54.3)	21 (22.8)	0.332
Medical wastewater (n=122)	73 (59.8)	54 (44.3)	43 (35.2)	37 (30.3)	76 (62.3)	85 (69.7)	77 (63.1)	55 (45.1)	0.477
Dairy wastes and wastewater (n=102)	38 (37.3)	20 (19.6)	0 (0)	16 (15.7)	68 (66.7)	54 (52.9)	29 (28.4)	25 (24.5)	0.347
Beef wastes and wastewater (n=14)	9 (64.3)	7 (50)	1 (7.1)	5 (35.7)	8 (57.1)	12 (85.7)	9 (64.3)	5 (35.7)	0.412
Total (411)	166 (40.4)	105 (25.5)	56 (13.6)	99 (24.1)	263 (64)	256 (62.3)	205 (49.9)	121 (29.4)	
P	0.000	0.000	0.000	0.018	0.508	0.000	0.000	0.000	

**Abbreviations:** E, erythromycin; NX, norfloxacin; TE, tetracycline; C, chloramphenicol; CIP, ciprofloxacin; AMP, ampicillin; COT, co-trimoxazole; GEN, gentamicin.

Moreover, isolates from beef waste and wastewater showed the highest resistance to erythromycin (64.3%), ciprofloxacin (50%), chloramphenicol (35.7%), and co-trimoxazole (64.3%). The highest percentage resistance was recorded against ampicillin (70.4%) and tetracycline (74.1%) for isolates from poultry wastes, against norfloxacin (35.2%) and gentamicin (45.1%) for isolates from medical wastewater (Table 4).

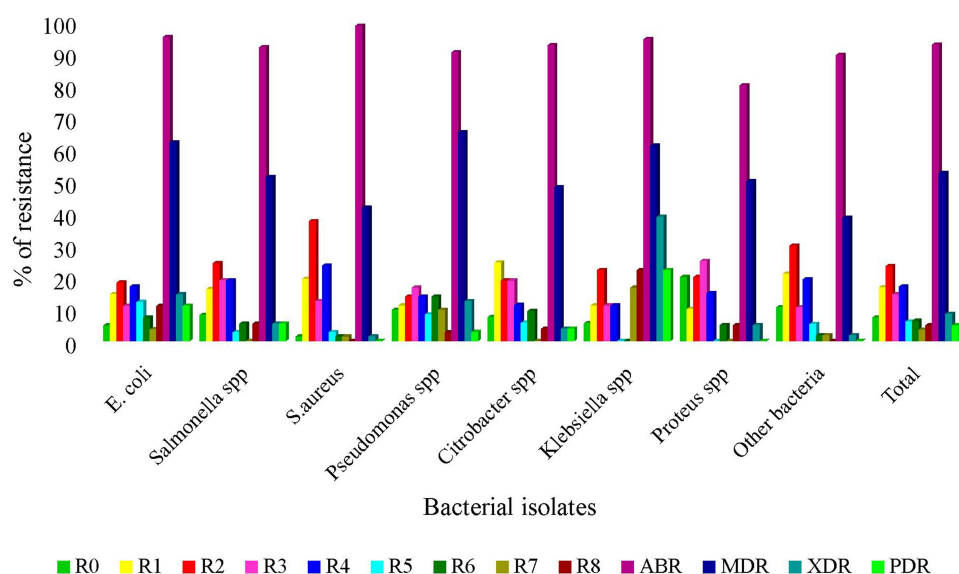
A lower rate of resistance was observed against ciprofloxacin (12.3%) and gentamicin (18.5%) for bacterial isolates isolated from poultry waste, against erythromycin (26.1%), and tetracycline (48.9%) for bacterial isolates isolated from municipal wastewater and against ampicillin (57.1%) from beef waste and wastewater. A lower rate of resistance was also observed against norfloxacin (0), chloramphenicol (15.7%), and co-trimoxazole (28.4%) for bacterial isolates from dairy waste and wastewater (Table 4). Although high ( $>0.2$ ) MARI was found in all hotspot environments, the highest MARI (0.477) was recorded in bacteria isolated from medical wastewater samples. The rate of bacterial resistance to ampicillin (64%) was higher than that of other antibiotics, and the results showed that there was a statistically significant difference among hotspot environments for the resistance rate of all antibiotics except ampicillin ( $P<0.05$ ) (Table 4).

## Multidrug Resistance Profiles of Bacterial Isolates

Among the bacteria isolated from all samples, most isolates of *E. coli* (18.3%), *Salmonella* spp. (24.3%), *S. aureus* (37.5%) and other bacterial isolates (29.8%) were resistant to two antibiotics; 16.7% and 25% of *Pseudomonas* and *Proteus* species were resistant to three antibiotics, respectively. Moreover, 24.5% of *Citrobacter* spp. were resistant to one antibiotic and 22.2% of *Klebsiella* spp. were resistant to two and eight antibiotics, respectively (Figure 1).

Among the isolated bacterial species, 92.7% were resistant to at least one antibiotic. The highest antibiotic resistance was observed in *S. aureus* (98.6%), followed by *E. coli* (95.1%). Only 7.3% of the isolates were susceptible to all the tested antibiotics. Of the bacterial species, 52.6% showed multidrug resistance, and the highest multidrug resistance was observed in *Pseudomonas* spp. (65.3%), followed by *E. coli* (62.2%) (Figure 1). The results also indicated that the highest extensively drug-resistant (XDR) (38.9%) and pan-drug resistant (PDR) (22.2%) were recorded in *Klebsiella* spp., followed by *E. coli* with 14.6% XDR and 11% PDR. Overall, 8.5% and 4.9% of isolates showed XDR and PDR, respectively (Figure 1).

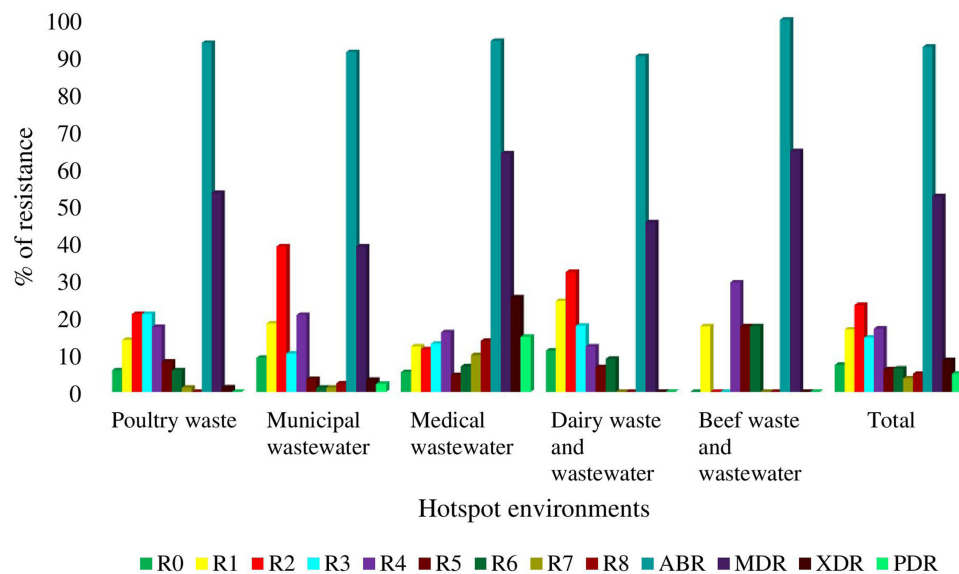
As shown in Figure 2, most bacterial isolates from municipal wastewater (39.1%) and dairy waste and wastewater (32.2%) samples were resistant to two antibiotics, from medical wastewater (16%) and beef waste and wastewater



**Figure 1** Multidrug resistance profiles of bacterial isolates isolated from hotspot environments in Bahir Dar City, 2021

**Abbreviations:** R0, susceptible to all antibiotics tested; R1, resistant to 1 antibiotic; R2, resistant to 2 antibiotics; R3, resistant to 3 antibiotics; R4, resistant to 4 antibiotics; R5, resistant to 5; R6, resistant to 6; R7, resistant to 7; R8, resistant to 8; ABR, antibiotic resistance; MDR, multidrug resistance; XDR, extensively drug-resistant; PDR, pan-drug resistant.





**Figure 2** Multidrug resistance profiles of bacterial isolates by sample sources in Bahir Dar City, 2021

**Abbreviations:** R0, susceptible to all antibiotics tested; R1, resistance to 1 antibiotic; R2, resistance to 2 antibiotics; R3, resistance to 3 antibiotics; R4, resistance to 4 antibiotics; R5, resistance to 5; R6, resistance to 6; R7, resistance to 7; R8, resistance to 8; ABR, antibiotic resistance; MDR, multidrug resistance; XDR, extensively drug-resistant; PDR, pan-drug resistant.

(29.4%) samples were resistant to four antibiotics. Moreover, 20.9% of the bacterial isolates from poultry waste samples were resistant to two and three antibiotics, respectively. Although higher antibiotic resistance was found in isolates isolated from all hotspot environments, the highest antibiotic resistance was found in isolates from beef waste and wastewater samples (100%), followed by medical wastewater samples (94.3%). The highest multidrug resistance was recorded in isolates isolated from beef waste and wastewater samples (64.7%), followed by medical wastewater samples (64.1%). Moreover, the highest XDR (25.4%) and PDR (14.8%) were recorded for the isolates isolated from medical wastewater samples (Figure 2).

## Discussion

Wastes generated from hotspot environments and released in the environment contain large numbers of antibiotics and antibiotic-resistant bacteria which are the main factors for the spread of antibiotic-resistant bacteria and antibiotics to the environment.<sup>12</sup> In this study, a total of 411 bacterial isolates were recovered, and of the total bacterial isolates isolated from poultry waste samples, the most prevalent bacteria were *E. coli* (29.3%), which is similar to that reported in Nigeria (29.5%),<sup>25</sup> Ethiopia (46%)<sup>15</sup> and Cameroon (59.1%).<sup>26</sup>

In the present study, the most prevalent bacteria isolated from medical wastewater were *Proteus* spp. (45%), followed by *Klebsiella* spp. (44.4%), *Citrobacter* spp. (37%), *Pseudomonas* spp. (33.3%), *E. coli* (31.7%), and *S. aureus* (30%). This result is supported by a study conducted by Moges et al<sup>10</sup> who reported that *Klebsiella* spp. (26.6%) were the most frequently identified bacteria, followed by *Pseudomonas* spp. (16.8%), *E. coli* (11.5%), *Citrobacter* spp. (11.5%), and *S. aureus* (8.2%). A similar study conducted by Hasan et al<sup>27</sup> reported that the most frequently isolated bacteria were *E. coli* (29%), followed by *Pseudomonas* spp. (21.8%), and *Klebsiella* spp. (16.4%). Guessennd et al<sup>28</sup> also reported that the most isolated bacteria were *E. coli*, *K. pneumonia*, *P. aeruginosa*, and *Staphylococcus* spp. from hospital wastewater, and ESBL-producing *Enterobacteriaceae*, *K. pneumonia*, *E. coli*, and *Proteus mirabilis* were predominantly isolated in Gondar.<sup>29</sup>

The current study showed that *Salmonella* spp. (37.8%), *Citrobacter* spp. (9.3%), and other bacteria (33.3%) were the most prevalent bacteria isolated from dairy waste and wastewater, beef waste and wastewater, and municipal wastewater samples, respectively. A study from South Africa indicated that the most frequently identified bacteria from dairy manure were 33 strains of *Salmonella* spp., 30 strains of *E. coli*, 16 strains of *Campylobacter* spp., and 4 strains of *Shigella*

species.<sup>30</sup> *Bacillus* spp. were predominant and found in all types of household waste samples,<sup>31</sup> which is inconsistent with the current findings.

Except for other bacterial species, the highest number of isolates was isolated from the medical wastewater samples. The highest number of *Pseudomonas* spp. was also isolated from the dairy waste and wastewater samples. Samples collected from beef waste and wastewater were devoid of *Klebsiella* spp., *Proteus* spp., and other bacterial species, and dairy waste and wastewater samples were devoid of *Proteus* spp. Overall, the most frequently isolated bacterium was *E. coli*. 82 (19.5%), followed by *S. aureus* and *Pseudomonas* spp. with 72 isolated strains (17.1%).

The current results revealed that *Klebsiella* spp. showed the highest resistance profile to erythromycin 10 (55.6%), ciprofloxacin 10 (55.6%), norfloxacin 7 (38.9%), co-trimoxazole 12 (66.7%) and gentamicin 11 (61.1%). *Klebsiella* spp. were resistant to ampicillin, amoxicillin, and cefuroxime but susceptible to gentamicin, ciprofloxacin, and amikacin.<sup>32</sup> Ayandele et al<sup>33</sup> also reported resistances of 87.5%, 68.75%, and 58.38% for streptomycin, gentamycin, and amikacin, respectively.

The highest resistance was recorded for *E. coli*, *S. aureus*, and *Citrobacter* spp. against chloramphenicol 35 (42.7%), ampicillin 69 (95.8%), and tetracycline 46 (86.8%), respectively. A study conducted in eastern Ethiopia showed that the highest frequency of resistance was recorded against ampicillin (94.7%) among *E. coli* isolates from hospital wastewater.<sup>34</sup> Osei et al<sup>35</sup> reported that 76.6%, 75%, 48.4%, and 6.3% of *S. aureus* isolates were resistant to erythromycin, tetracycline, chloramphenicol, and tobramycin, respectively. *Citrobacter* isolates were resistant to ampicillin, amoxicillin, and cefuroxime but susceptible to gentamicin, ciprofloxacin, and amikacin.<sup>32</sup> The resistance of bacteria against ampicillin 263 (64%) was higher than other antibiotics, and the results showed that there was a statistically significant difference among bacterial isolates for the resistance to all antibiotics except erythromycin ( $p < 0.05$ ).

The highest antibiotic resistance of 74.1%, 70.4%, and 49.4% were obtained from poultry wastes against tetracycline, ampicillin, and co-trimoxazole respectively and the lowest resistance was found to norfloxacin (6.2%). This finding disagrees with that reported by Eyasu et al<sup>15</sup> reported that high antimicrobial resistance was observed for ampicillin (94.2%), followed by penicillin (92%), tetracycline (73%), and erythromycin (66%), and the lowest resistance was observed for kanamycin (2%). Several bacteria were resistant to cefazolin (86.8%), fusidic acid (84.6%), ampicillin (79.3%), clindamycin (65.5%), and erythromycin (63.7%).<sup>36</sup> Of the bacterial isolates from municipal wastewater, 58.7% were resistant to ampicillin, which is inconsistent with the results of Akter et al<sup>31</sup> who reported that all isolates were resistant to cefuroxime and amoxicillin.

The current results showed that 69.7% of bacterial isolates from medical wastewater were resistant to tetracycline, 63.1% to co-trimoxazole, and 62.3% to ampicillin. According to a study conducted in India, the incidence of antibiotic resistance was found to a maximum of 73.9% of strains were resistant to ampicillin followed by nalidixic acid (72.5%), penicillin (63.8%), co-trimoxazole (55.1%), and resistance to streptomycin, chloramphenicol, nitrofurantoin, tetracycline, and doxycycline was recorded in less than 13% of the strains<sup>37</sup> which disagree with the present results. The current result also disagrees with results reported by Alam et al,<sup>37</sup> Moges et al<sup>10</sup> and Hasan et al<sup>27</sup> reported that most isolates were resistant to ampicillin. The probable reason for this difference may be the type of antibiotic used to test antibiotic susceptibility, the type of bacterial isolates tested, and the antibiotic used in the study areas. According to the reports from studies conducted in Amhara regional state, tetracycline, ampicillin, gentamicin, ciprofloxacin, and cotrimoxazole were the most frequently used groups of antibiotics in animals,<sup>38</sup> and ampicillin, ciprofloxacin, and tetracycline were the most frequently used groups of antibiotics.<sup>39</sup>

Most of the isolates isolated from dairy waste and wastewater (66.7%) and beef waste and wastewater (85.7%) were resistant to ampicillin and tetracycline, respectively, and norfloxacin was the most effective antibiotic for isolates from these waste samples. This finding is inconsistent with the results reported in South Africa, which showed that ciprofloxacin and gentamicin were the most effective and erythromycin was the least effective antibiotic in the control of the bacteria in this study.<sup>30</sup>

Moreover, among the tested antibiotics, erythromycin (64.3%), ciprofloxacin (50%), chloramphenicol (35.7%), and co-trimoxazole (64.3%) showed the highest resistance to isolates from beef waste and wastewater samples. The highest percentage resistance was recorded against ampicillin (70.4%) and tetracycline (74.1%) for isolates from poultry wastes, against norfloxacin (35.2%) and gentamicin (45.1%) for isolates from medical wastewater. A lower rate of resistance was seen against ciprofloxacin (12.3%) and gentamicin (18.5%) for bacterial isolates isolated from poultry wastes, against



erythromycin (26.1%), and tetracycline (48.9%) for bacterial isolates isolated from municipal wastewater and against ampicillin (57.1%) from beef waste and wastewater. A lower rate of resistance was also seen against norfloxacin (0%), chloramphenicol (15.7%), and co-trimoxazole (28.4%) for bacterial isolates from dairy waste and wastewater. The highest resistance was observed with erythromycin (96.97%), followed by kanamycin (90.91%), chloramphenicol (87.88%), vancomycin (87.88%), ciprofloxacin (84.85%), sulfamethoxazole/ trimethoprim (84.85%), tetracycline (78.79%), ampicillin (78.79%), gentamicin (69.70%), amoxicillin-clavulanic acid (57.58%), and cefotaxime (48.5%), with the lowest level of resistance.<sup>40</sup>

Resistance to ampicillin with 263 strains (64%) was higher than that of other antibiotics, and the results showed that there was a statistically significant difference among hotspot environments for the resistance rate of all antibiotics except ampicillin ( $p < 0.05$ ).

Among the isolated bacterial species, 92.7% were resistant to at least one antibiotic, and the highest antibiotic resistance was observed in *S. aureus* (98.6%), followed by *E. coli* (95.1%). Only 7.3% of the total isolates were susceptible to all tested antibiotics, which is higher than the result reported by Moges et al<sup>10</sup> who reported that among 113 isolates, 0.9% were susceptible to all antibiotics tested, and only 10.18% of bacterial isolates were sensitive to all antibiotics tested.<sup>36</sup> Of the bacterial species, 52.6% showed multidrug resistance, and the highest multidrug resistance was observed in *Pseudomonas* spp. (65.3%), followed by *E. coli* (62.2%), which was lower than that reported by Moges et al<sup>10</sup> reported that the rate of multiple-drug resistance from 2 to 12 antibiotics was 69.9%. The results also indicated that the highest XDR (38.9%) and PDR (22.2%) were recorded in *Klebsiella* spp., followed by *E. coli* with 14.6% XDR and 11% PDR. Overall, 8.5% and 4.9% of isolates showed XDR and PDR, respectively.

Most bacterial isolates from municipal wastewater (39.1%) and dairy waste and wastewater (32.2%) samples were resistant to two antibiotics, and from medical wastewater (16%) and beef waste and wastewater (29.4%) samples were resistant to four antibiotics. Among the 113 isolates, 29.2% were resistant to only one antibiotic, 19.5% were resistant to two antibiotics, 7.9% were resistant to three antibiotics, 12.4% were resistant to four antibiotics, and 30.1% were resistant to five or more antibiotics.<sup>10</sup> Moreover, 20.9% of the bacterial isolates from poultry waste samples were resistant to two and three antibiotics, respectively. 32 bacterial isolates were resistant to at least 10 antibiotics, four of which were resistant to 16 antibiotics, and 65.4% were resistant to multiple antibiotics.<sup>25</sup> More than half (58.2%) of the 511 total bacteria had MAR.<sup>36</sup> Of all the bacterial species isolated, 37.5% (15) showed multidrug resistance to three antibiotics from dairy manure<sup>30</sup> and multidrug resistance was detected in 58% of isolated strains in municipal wastewater.<sup>41</sup>

Although higher antibiotic resistance was found in isolates isolated from all hotspot environments, the highest antibiotic resistance was found in isolates from beef waste and wastewater samples (100%), followed by medical wastewater samples (94.3%). The highest multidrug resistance was recorded in isolates from beef waste and wastewater samples (64.7%), followed by medical wastewater samples (64.1%). Moreover, the highest XDR (25.4%) and PDR (14.8%) were recorded in isolates from medical wastewater samples, which is in line with the results of Yang et al<sup>40</sup> who reported that 27.27% and 18.18% of isolates were resistant to 10 (XDR) and all 11 tested antibiotics (PDR), respectively. Olonitola et al<sup>25</sup> also reported that four of them showed resistance to 16 antibiotics (XDR).

The probable reason for the presence of the highest antibiotic and multidrug resistance in isolates from beef waste and wastewater samples may be the inappropriate use of antibiotics as a growth promotion on the farm. Medical wastewater also contains the highest antibiotic-resistant, MDR, XDR, and PDR bacteria because hospitals are known to discharge pathogenic bacteria, most of which could be carrying resistance determinants into their wastewater, and traces of antibiotics in urine, feces, and spilled and expired drugs, which are improperly discarded into washbasins, are all channeled to the wastewater systems.

Even though higher ( $>0.2$ ) MARI was found in all hotspot environments, the highest MARI (0.477) was recorded in bacteria isolated from medical wastewater samples, indicating that the isolates were exposed to high-risk sources of contamination originating from humans or animals where antibiotics are often used. When isolates were exposed to high-risk sources of contamination originating from humans or animals, a MARI value higher than 0.2 is observed. When antibiotics are seldom or never used, a MARI value less than or equal to 0.2 is observed. A MARI value of 0.2 indicates a high-risk environment where antibiotics are often used.<sup>24,42</sup> The value of MARI of the current result is inconsistent with

the result of Veloo et al<sup>36</sup> who reported that 54.01% of isolates from the soil and effluent samples had a MARI value higher than 0.2 and 49.5% had MARI values of more than 0.40 and 10.89% had extreme MARI values of more than 0.79.

## Conclusion

In this study, we identified medically important bacteria from hotspot environments, which indicate that these hotspot environments are a potential source of resistant bacteria. Resistance profiles of bacteria varied from isolate to isolate and the highest antibiotic resistance of 71 (98.6%) was observed in *S. aureus*. Resistance profiles of bacteria were also varied among hotspot environments and the maximum resistant bacteria were found in medical wastewater. It was concluded that wastes generated from hotspot environments and released in the environment contain large numbers of antibiotic-resistant, multidrug, extensively drug-resistant, and pan-drug resistant bacteria. Therefore, raising awareness and education regarding proper waste management strategies are needed. Waste should be well treated before being released into the environment, and continuous monitoring and evaluation of waste quality should be developed. Future work will focus on the physicochemical characterization of waste samples and the molecular identification of resistance isolates, including resistance genes.

## Abbreviations

AR, antibiotic-resistant; MARI, Multiple Antibiotic Resistance Index; CLSI, Clinical and Laboratory Standards Institute; XDR, extensively drug-resistant; PDR, pan-drug resistant.

## Data Sharing Statement

All data generated or analyzed during this study are included in this published article.

## Acknowledgments

We would like to thank Habtamu M., Lameneu F., Dr. Baye S., and Misganaw L. for their support during sample collection and laboratory work. The authors also thank the Department of Biology, BDU, for financial support in conducting this research.

## Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis, and interpretation, or in all these areas; took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted, and agree to be accountable for all aspects of the work.

## Disclosure

The authors declare no competing interests in this work.

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