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Detection of multiple antibiotic-resistant bacteria from the hospital and non-hospital wastewater sources of a small town in Noakhali, Bangladesh

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ABSTRACT

Multiple antibiotic-resistant (MAR) bacteria present in wastewater cause life-threatening diseases. Using water from unprotected sources where the possibility of entering MAR bacteria from wastewater is high poses a danger to public health. To determine the distribution of MAR bacteria in wastewater, we collected samples from six sites of Maijdee, a small town in the Noakhali district of Bangladesh. We found that 47.62% (30/63) of the isolates were resistant to at least six antibiotics. The predominant MAR bacteria were *Staphylococcus aureus* (20%), *Escherichia coli* (20%), *Enterobacter* spp. (20%), and *Klebsiella* spp. (16.67%). All MAR bacteria were resistant to ampicillin, oxacillin, and cefixime, and 97% (29) of isolates showed resistance to cefotaxime. Altogether, above 50% of the isolates were resistant to cefepime, ciprofloxacin, azithromycin, chloramphenicol, and streptomycin. MAR index values of all but one isolate found as >0.20 implied that they were from high-risk environments. The findings suggested the urgent need to treat hospital wastewater before draining out to the environment properly, and the other wastewater should be reserved in the secluded area.

1. INTRODUCTION

In Maijdee, a small town of Noakhali in Bangladesh, potable water is scarce due to the high salinity and iron level, making underground water unlikely to drink without treatment. Consequently, people have to rely on centrally processed water supplied by the local government. However, it is insufficient to fulfill the large population's requirements. In some areas, people have to use untreated pond water, which is even supplied by the same drinking water pipeline. There is a significant concern regarding the quality of water from these unsafe sources. Moreover, because of the faulty drainage system, these unprotected water ponds are flooded during the rainy season, and there is a distinct possibility that those water could mix up with the surroundings wastewater.

Wastewater from hospital-based or other sources is a reservoir of pathogenic microorganisms, including multiple antibiotic-resistant (MAR) bacteria, and can be a potential risk factor for public health and ecological balance [1]. Drug-resistant diseases cause around 700,000 deaths globally a year, which could increase to 10 million deaths globally per annum by 2050 if no action is taken. Nearly 2.4 million people may die in high-income countries between 2015 and 2050 without a sustained effort to contain antimicrobial resistance [2]. The economic damage of uncontrolled antimicrobial resistance may be like the shocks experienced during the 2008-2009 global financial crisis due to dramatically increased health care expenditures, food and feed production, trade, and livelihoods, and increased poverty and inequality [2]. These MAR organisms can often cause severe and sometimes lethal infections such as enteric diarrheal disease, urinary tract infections (UTIs), sepsis, and meningitis in humans. Drug-resistant bacteria caused infections, resulting in worse clinical outcomes with consuming more health-care resources [3]. Excessive and inappropriate way of using antibiotics is the main reasons which lead to the development of antibiotic-resistant bacteria (ARB). Furthermore, human treatments, excessive use of antibiotics in animal husbandry, and aquaculture can also increase antimicrobial resistance in the environment [4]. Initially, it was believed that antibiotic-resistant bacteria could be found only in care giving settings such as the hospital environment, but now they are everywhere [5].

Hospitals are the source of ARB, but it has been reported that these are potentially passed down to the community by the colonized patient and through hospital waste disposal facilities, especially wastewater [6,7]. Several reports have been focused on that Gram-negative isolates are frequently recovered from hospital wastewater among all pathogens and showed multidrug resistance [6,8,9]. The mentioned Gram-negative ARB are *Escherichia coli, Klebsiella, Pseudomonas, Serratia,* and *Citrobacter* sp. They are usually the most common

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causes of the hospital and community-acquired infections [8,10]. In the Gram-positive group, *S. aureus*, especially methicillin-resistant *Staphylococcus aureus* (MRSA), is one of the leading causes of infections in both hospital and community-acquired cases [11]. The distribution of MAR bacteria is alarmingly high in wastewater, and different human activities like discharging of hospital effluents to the sewer system without prior treatment are the main reason for the dissemination of MAR bacteria in the environment. It has been reported that only few countries recommend or implement the primary treatment of hospital effluents before discharge into the main wastewater flow. However, in developing countries such as Vietnam and Bangladesh, there are no fixed guidelines for hospital effluent discharge [12]. This untreated wastewater gets mixed up with other reservoirs and contributes to MAR bacteria spread in non-hospital-based water [8].

We have conducted a cross-sectional study to observe the distribution of multiple antibiotic-resistant bacteria in different wastewater sources of Maijdee at Noakhali and determine their resistance pattern to different clinically used antibiotics. Those findings will eventually help local authorities be aware of disseminating resistant bacteria in the water and providing information to implement appropriate guidelines for the proper management of hospital effluents.

2. MATERIALS AND METHODS

2.1. Study Area and Sample Collection

Six sampling sites of wastewater have been selected, including three hospital-based and three non-hospital-based wastewater sources covering around 6 km^2 area located in the Maijdee, a small town of around 12.61 km^2, in Noakhali district, Bangladesh. The hospital-based wastewater sampling sites were Noakhali 250 Bed General Hospital (GH-1), Mamoni General Hospital (Pvt.) Ltd. (MMH-1), and Metro Hospital (Pvt.) Ltd. (MH-1), and other sources were Waste Dump of Krishnarampur (GH-2), Maijdee Housing Estate Dump Pond (MMH-2), and Noakhali Zilla Mosque Dump Pond (MH-2). Wastewater samples collected from each site immediately taken to the lab in a cool bag and processed within 2 h of collection.

2.2. Isolation and Identification of Bacteria

The samples were processed following the American Public Health Association guidelines [13]. The temperature, pH, and odor were measured and documented. Serial dilutions were made up to 10^{-6}. Then, 1 mL of each dilution was plated on different selective and differential media, such as on MacConkey agar, Mannitol salt agar (MSA), Eosin methylene blue (EMB), Cetrimide agar, and Thiosulfate-citrate-bile salts-sucrose (TCBS) agar and incubated them at 37°C for 24 h. After obtaining pure colonies, the single random colonies were picked up and proceeded to identify based on their microscopic, physiological, and biochemical characteristics following the "Bergey's Manual of Determinative Bacteriology," 8th edition [14].

2.3. Screening of Multiple Antibiotic-resistant Bacteria by Antibiotic Susceptibility Test (AST)

Standard Kirby–Bauer disk diffusion method was performed to determine the antimicrobial susceptibility profiles of the isolates following the CLSI-2018 guidelines [15]. The antimicrobial sensitivity testing was performed against 16 antibiotic disks, azithromycin, AZM (15 µg); gentamicin, GEN (10 µg); ampicillin, AMP (25µg); tobramycin, TOB (10 µg); cefepime, FEP (30 µg); doxycycline, DOX (30 µg); nitrofurantoin, NIT (300 µg); netilmicin, NET (30 µg); ciprofloxacin, CIP (5 µg); cefotaxime, CTX (30 µg); chloramphenicol, CHL (30 µg); amikacin AMK (30 µg); oxacillin, OX (1 µg); streptomycin, STR (10 µg); cefixime, CFM (5 µg); and ceftazidime, CAZ (30 µg) for all the isolates, and additional methicillin, MET (5 µg), disk for only *Staphylococcus aureus*. The zones of inhibition for each antibiotic were measured in millimeters. Based on the inhibition zone, the isolates were categorized as resistant (R), intermediate (I), and sensitive (S).

2.4. Multiple Antibiotic-resistance (MAR) Indexing

Isolates showed resistance to three or more than three antibiotics are called multidrug resistant (MDR) or multiple antibiotic resistant (MAR) [16]. MAR index value of all the isolates was determined to evaluate the health risk of the environment. The value was calculated according to the formula: Number of antibiotics to which all isolates were resistant (a)/[number of antibiotics tested (b) × number of isolates recovered from a sample (c)] [8].

2.5. Statistical Analysis

To determine the variation of risk among or between hospital and non-hospital wastewater sample groups, we have performed the two-way analysis of variance (ANOVA) using Statistical Package for the Social Sciences (SPSS) software version 25 (SPSS Inc, Chicago, IL, USA). All statistical test values of $p < 0.05$ were considered statistically significant.

3. RESULTS AND DISCUSSION

Sixty-three bacteria were isolated from wastewater samples of the six selected sites of the Maijdee of the Noakhali district in Bangladesh. The range of pH and temperatures of the wastewater samples was recorded as 6.69 to 8.04 and 28.5 to 36.4°C, respectively. All samples from hospital-based sites were found with malodorous, and the average bacterial load of all sites was found between 3.5×10^8 and 12.8×10^8 CFU/mL [Table 1]. Among the bacterial isolates, we found that 30 (47.62%) isolates were resistant to at least six antibiotics and designated as multiple antibiotic-resistant bacteria (MAR) and thus considered for further analysis in this study. Moreover, among the MAR bacteria, 14 (46.67%) from hospital-based sources, and 16 (53.33%) were from non-hospital-based sources. The predominant MAR bacteria were *Staphylococcus aureus* (n = 6.20%), *Escherichia coli* (n = 6.20%), *Enterobacter* spp. (n = 6.20%), and *Klebsiella* spp. (n = 5, 16.67%). We also found *Pseudomonas* spp. (n = 2, 6.67%), *Proteus* spp. (n = 2, 6.67%), *Vibrio* spp. (n = 1, 3.33%), *Shigella flexneri* (n = 1, 3.33%), and *Staphylococcus epidermidis* (n = 1, 3.33%) in relatively lower abundance [Figure 1].

The data of the antibiotic susceptibility test (AST) revealed that 100% (n = 30) of the MAR bacteria were resistant to ampicillin, oxacillin, and cefixime, and 97% (n = 29) of isolates were resistant to cefotaxime. Altogether, above 50% of the isolates were resistant to cefepime, ciprofloxacin, ceftazidime, azithromycin, chloramphenicol, and streptomycin. If we consider the clinically significant pathogenic gram-negative bacteria separately, then *E. coli, Enterobacter* spp., and *Klebsiella* spp. showed 100% resistance to ampicillin, oxacillin, cefotaxime, and cefixime, whereas over 50% of them found resistant to azithromycin and ciprofloxacin [Table 2].

S. aureus isolates were highly antibiotic-resistant; out of the 17 treated antibiotics 16.67% and 33.37% of the isolates showed resistance to 14 and 13 antibiotics, respectively. In contrast, 50% *Pseudomonas* spp.,

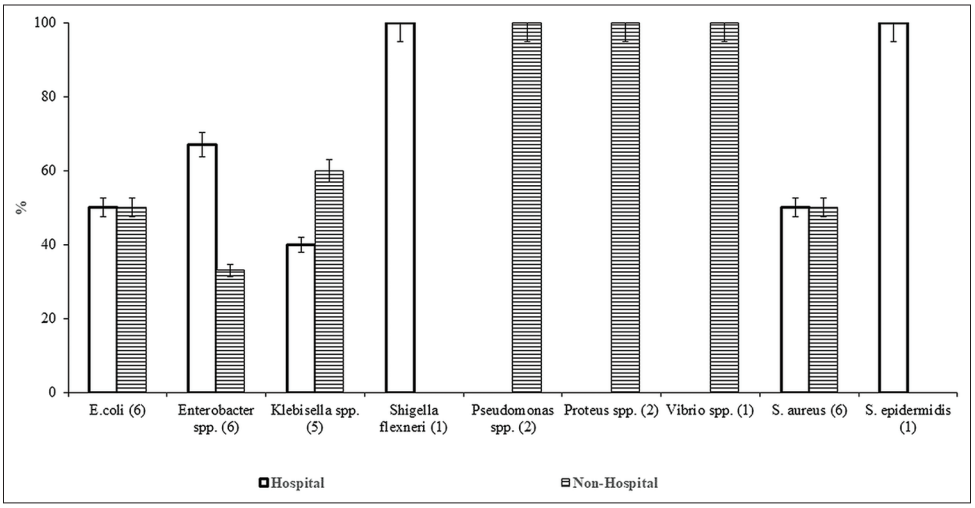

Figure 1: Distribution of multiple antibiotic-resistant bacteria in hospital and non-hospital-based wastewater.

Table 1: The physical and biological properties of wastewater samples were collected from different sites.

Sampling sites	Location name	Wastewater type	Odor	pH	Temperature	Average CFU/ml
GH-1	Noakhali 250 Bed General Hospital	Hospital	Malodorous	7.29	28.5°C	11.8×10^{8}
GH-2	Waste Dump of Krishnarampur	Nonhospital	No odor	6.95	30.3°C	5.4×10^{8}
MMH-1	Mamoni General Hospital (Pvt.) Ltd.	Hospital	Malodorous	6.69	29.7°C	1.16×10^{9}
MMH-2	Maijdee Housing Estate Dump Pond	Nonhospital	No odor	8.04	36.4°C	3.5×10^{8}
MH-1	Metro Hospital (Pvt.) Ltd.	Hospital	Malodorous	6.86	33.1°C	1.28×10^{9}
MH-2	Noakhali Zilla Mosque Dump Pond	Nonhospital	No odor	7.32	33.4°C	3.9×10^{8}

Table 2: High levels of multiple-antibiotic resistance (MAR) by isolated bacteria against 16 commonly used antibiotics.

Isolates	Resistance to Antibiotics (%)															
	AMP	OXA	CHL	DOX	NIT	AZM	CIP	AMK	GEN	NET	STR	TOB	FEP	CAZ	CFM	CTX
Escherichia coli (6)	100	100	100	16.67	0	66.67	83.33	33.33	0	0	83.33	0	100	100	100	100
Enterobacter spp. (6)	100	100	50	0	66.67	83.33	50	16.67	16.67	0	83.33	0	83.3	83.33	100	100
Klebsiella spp. (5)	100	100	40	0	60	80	100	40	60	40	40	20	20	20	100	100
Shigella flexneri (1)	100	100	0	0	0	0	0	0	0	0	100	0	0	100	100	100
Pseudomonas spp. (2)	100	100	50	50	50	50	100	50	0	0	50	0	50	50	100	100
Proteus spp. (2)	100	100	50	100	100	0	50	50	0	0	50	0	100	100	100	50
Vibrio spp. (1)	100	100	0	100	0	0	0	100	100	0	0	0	100	100	100	100
*Staphylococcus aureus** (6)	100	100	66.67	50	33.33	66.67	83.33	33.33	33.33	100	0	50	100	100	100	100
Staphylococcus epidermidis (1)	100	100	0	100	0	100	0	0	0	0	0	0	100	100	100	100

*Additional antibiotic methicillin tested for *Staphylococcus aureus* showed 100% resistance to the antibiotic

50% *Proteus* spp., and 16.67% of *S. aureus* isolates were resistant to 12 antibiotics. Furthermore, 16.67% *E. coli,* 50% *Enterobacter*, 20% *Klebsiella* spp., and 16.67% of *S. aureus* were resistant to 11 antibiotics with different combinations. In contrast, 66.66% of *E. coli*, 20% of *Klebsiella* spp., and 16.67% of *S. aureus* were resistant to 10 antibiotics. Simultaneously, 16.67% of *E. coli* and *Enterobacter* spp., 20% of *Klebsiella* spp., 100% of *Vibrio spp*. and 16.67% of *S. aureus* showed resistance to 9 antibiotics [Figure 2].

The multiple antibiotic resistance (MAR) index showed that, the MAR values of all the sites were above 0.20 except for the one site (GH-1), which implied that the isolates were from the high-risk environment. The range of MAR index of hospital source GH-1, MH-1, and MMH-1 were 0.16–0.63, 0.28–0.68, and 0.25–0.62, respectively. On the contrary, the range of MAR index values of non-hospital sources of GH-2, MH-2, and MMH-2 was 0.28–0.75, 0.210.68, and 0.31–0.56, respectively [Table 3]. Two-way analysis of variance (ANOVA) of the MAR data did not find any significant difference in environmental risk between or within the hospital and non-hospital-based wastewater.

Globally, antibiotic resistance in bacteria is a serious health threat, particularly in developing countries [17]. Moreover, antibiotic residues and hospital effluents containing antibiotic-resistant bacteria (ARB) are emerging pollutants of the aquatic environment [18]. These pollutants are excreted into the water reservoirs through the hospitals' wastewater discharge process and spread antimicrobial resistance on the ecosystem and human health. In particular, wastewater from hospitals, especially if untreated, could play a role in the ARB

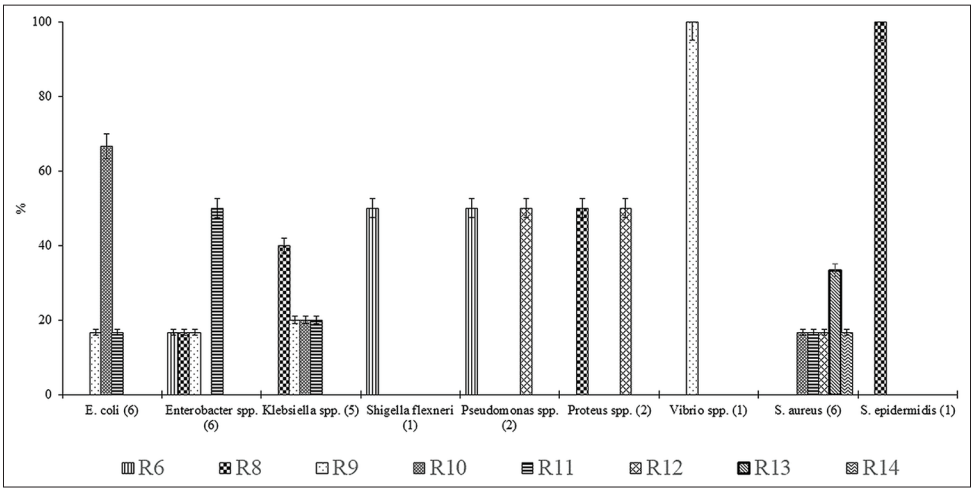

Figure 2: All multiple antibiotic-resistant bacteria from wastewater showed resistance to 6-13 antibiotics (R5, R7, R8, R9, R10, R11, and R12 denoted that the bacteria were resistant to five, seven, eight, nine, ten, eleven, and twelve antibiotics, respectively).

Table 3: MAR index of isolated bacteria.

Sampling Site	Type of wastewater	Number of resistant antibiotics (N)	Resistant isolates		Resistance pattern	MAR index
			Number	%		
GH-1	Hospital	5	2	40	AMP, AZM, CTX, OXA, CFM	0.16
					AMP, CTX, OXA, STR, CFM	
		7	1	20	AMP, AZM, CTX, FEP, DOX, OXA, CFM	0.42
		9	1	20	AMP, AZM, CTX, FEP, CIP, CHL, OXA, STR, CAZ	0.56
		10	1	20	AMP, CTX, GEN,TOB, FEP, NET, CIP, CHL, OXA, CFM	0.63
MH-1	Hospital	9	2	50	AZM, AMP, FEP, CIP, CTX, CHL, OXA, STR, CAZ	0.28
					AZM, AMP, NIT, CIP, CTX, CHL, OXA, AMK, STR, CFM,	
		10	1	25	AMP, AZM, CTX,GEN, FEP, NIT, CHL, OXA, STR, CFM	0.62
		11	1	25	AMP, AZM, CTX,TOB, FEP, DOX, NET, CIP, OXA, AMK, CFM	0.68
MMH-1	Hospital	7	1	20	AMP, AZM, FEP, DOX, CTX, OXA, CFM	0.43
		8	2	40	AMP, AZM, CTX, FEP, NIT, STR, OXA, CFM	0.25
					AMP, AZM, CTX, GEN, AMP, NIT, CIP, OXA, CFM	0.25
		9	1	20	AZM, AMP, FEP, CIP, CTX, CHL, OXA, STR, CAZ	0.56
		10	1	20	AMP, AZM, CTX, FEP, NIT, CIP, OXA, AMK, STR, CFM	0.62
GH-2	Non-hospital	5	1	20	AMP, CTX, FEP, CIP, CFM	0.31
		8	1	20	AMP, CTX, FEP, CIP, CHL, STR, OXA, CFM	0.50
		9	2	40	AMP, AZM, CTX, FEP, CIP, CHL, OXA, STR, CAZ	0.28
					AMP, CTX, GEN, FEP, NET, CIP, CTX, STR, CFM,	
		12	1	20	AMP, AZM, CTX, GEN, FEP, DOX, NIT, NET, CHL, OXA, CFM	0.75
MH-2	Non-hospital	8	2	33	AMP, AZM, CTX, GEN, CIP, OXA, CFM, CAZ	0.25
		8			AMP, CTX, GEN, FEP, DOX, AMK. CFM, CAZ	
		10	3	50	AMP, AZM, CTX, FEP, CIP, CHL, OXA, AMK, STR, CAZ	0.21
		10			AZM, TOB, FEP, NET, CIP, CTX, CHL, OXA, AMK, CFM	0.21
		10			AZM, AMP, DOX, NIT, CIP, CTX, CHL, OXA, AMK, STR	0.21
		11	1	17	AZM, AMP, DOX, NIT, CIP, CTX, CHL, AMK, STR, CFM, CAZ	0.68
MMH-2	Non-hospital	5	1	33	AMP, DOX, NIT, CTX, OXA	0.31
		8	1	33	AMP, FEP, DOX, NIT, CTX, CHL, OXA, AMK	0.50
		9	1	34	AMP, FEP, NIT, NET, CIP, CTX, CHL, OXA, CFM	0.56

MAR: Multiple antibiotic resistance

emerging in the environment. A study by Asfaw *et al.* [10] reported that out of 84 identified isolates from untreated wastewater samples, 76.2% (64/84) of them were multi-drug-resistant. Similarly, Moges *et al.* [19] reported that out of 113 identified isolates, the rate of multidrug-resistant bacteria from the hospital and non-hospital sources were 46.9% and 23%, respectively. Our study reported that 47.62% of the recovered isolates as MAR from the hospital and non-hospital sources, and there was no significant difference in MAR isolates from both sources. However, Moges *et al.* [19] reported that the distribution of MAR isolates in the hospital environment was higher than in non-hospital environments. One possible reason behind this, maybe the area was flooded with rain, and both types of wastewater get mixed up.

Furthermore, the higher distribution of such MAR strains in hospital wastewater is due to gram-negative enteric bacteria, which acquires several mechanisms against β-lactams antibiotics [20]. Likewise, two studies in Ethiopia, one in the northwest part of the country, reported *Klebsiella* spp., followed by *Pseudomonas* spp., *E. coli*, *Citrobacter* spp., and *S. aureus* as predominant isolates [19], and another from the southern part reported *Staphylococcus, E. coli, Klebsiella,* and *Shigella* were most frequently detected isolates in hospital wastewater [21]. A study from another developing country, Nigeria, also reported that bacterial genera, *E. coli*, *Klebsiella, Pseudomonas,* and *Shigella* were the most frequently distributed isolates in wastewater [7]. On the other hand, the most frequent MAR bacteria found in this study were *S. aureus* (20%), *E. coli* (20%), *Enterobacter* spp. (20%), and *Klebsiella* spp. (16.67%).

Gram-negative bacterial isolates from the hospital and non-hospital water sources were found to be highly resistant to ampicillin (100%), cefixime (100%), oxacillin (100%), cefotaxime (94%), and ceftazidime (84%). Previously, Moges *et al.* [19] also reported that maximum isolates (97%) of the study showing high resistance to ampicillin from hospital wastewater sources and from non-hospital water sources. Similar results were seen as highly ampicillin, amoxicillin/clavulanic acid-resistant Enterobacteriaceae were reported by Galler *et al.* [22]. Simachew Dires (2018) also found a higher proportion of ampicillin-resistant *Salmonella*, *E. coli, Shigella*, and *Klebsiella* from hospital wastewater samples [21].

The MAR bacteria in this study showed a high level of resistance to third-generation cephalosporins. Maximum isolates except *Proteus* spp. were resistant to cefixime, ceftazidime and cefotaxime, whereas *Proteus spp*. showed total resistance to all but cefotaxime (50%). Surprisingly, 100% of *E. coli, Proteus* spp., *Vibrio* spp., *S. aureus*, and *S. epidermidis* were even resistant to a fourth-generation cephalosporin (cefepime). Similar to our findings, a study by Rabbani *et al.* [23] reported a high level of cefotaxime, ceftazidime resistance in *E. coli* and *Klebsiella* isolated from two hospital wastewater sources located in Bangladesh. Similar results were seen from non-hospital water sources as cefuroxime, cefotaxime, and ceftazidime resistant Enterobacteriaceae reported by Galler *et al.* [22]. Undoubtedly, the most successful antibiotic group against which most bacteria established their resistance is β-lactam derivatives such as penicillin, cephalosporin, and ampicillin [24]. Over the time bacteria acquires resistance by several mechanisms such as producing β lactamase enzyme, efflux pump, and producing an altered PBP (penicillin-binding protein) with a lower affinity β-lactam antibiotics [24,25].

Among the aminoglycosides group, the highest resistance was observed by *Klebsiella* against amikacin (40%) and gentamicin (60%). Interestingly, all *E. coli* isolates were sensitive against gentamicin. In contrast, Fekadu *et al.* [26] from Ethiopia reported that *E. coli* isolated from wastewater showed maximum resistance against gentamicin. However, low levels of gentamicin resistance were also reported in hospital wastewater of India as they noticed that all the Gram-negative isolates from different hospital wastewater sources were less resistant toward gentamicin; the range varied from 2% to 20% [8]. Dires *et al*. [21] and Ferreira *et al.* [27] also reported relatively lower resistance among bacterial isolates to gentamicin. Furthermore, against streptomycin, *E. coli*, and *Enterobacter* (83.33%) showed high resistance in this study. Similarly, another study in Ethiopia also reported *Enterobacter* (30%), *Klebsiella* (29%), and *E. coli* (8%) isolates showing resistance against streptomycin [19]. Thompson *et al.* [28] also reported gentamicin and amikacin-resistant *S. aureus* isolated from two different hospital wastewater sources.

In the case of macrolides (azithromycin), *E. coli* (66.67%), *S. aureus* (66.67%), *Enterobacter* spp. (83%), *Klebsiella* spp. (80%), *Pseudomonas* spp. (50%), and *S. epidermidis* (100%) were found to be resistant. A study conducted in Ethiopia by Simachew Dires [21] reported more than 40% of *E. coli, Shigella, Klebsiella*, and *Salmonella* to be erythromycin-resistant where *Salmonella* showed maximum resistance (75%). In contrast, Gram-positive *Staphylococcus* spp. showed the lowest resistance (20%) against the drug in our study.

On the other hand, our study's maximum number of isolates showed resistance toward fluoroquinolones (ciprofloxacin) such as *E. coli* (83.33%), *Enterobacter* spp. (50%), and *Klebsiella* spp. (100%). Several studies in other parts of Bangladesh also reported ciprofloxacin-resistant *E. coli* and *K. pneumoniae* from hospital wastewater [23,29,30]. Similar findings were also reported by a study from Portugal that the prevalence of ciprofloxacin resistance was significantly higher in the hospital effluents [31].

Against chloramphenicol, all *E. coli* isolates showed resistance, whereas more than 50% of *Enterobacter* spp., *Pseudomonas* spp., *Proteus* spp., *S. aureus,* and 40% of *Klebsiella* spp. showed resistance to the antibiotic. Dires *et al.* [21] also reported the isolation of chloramphenicol resistant Enterobacteriaceae from wastewater sources, whereas Alam *et al.* [8] reported low levels of resistance against chloramphenicol by hospital wastewater isolate. In non-hospital wastewater isolates, Everage *et al.* [32] reported high level of chloramphenicol resistance among *E. coli, S, aureus,* and *E. cloacae* isolated from six different sewage samples collected from the City of Thibodaux (USA) Sewage Treatment Plant.

Among Gram-positive bacteria, *S. aureus* was predominating isolate in this study. Thompson *et al.* [28] reported the presence of methicillin-resistant *S. aureus* (MRSA) (68%, 131/192) from hospital wastewater and water from sewage treatment plants. The study also mentioned MRSA dissemination possibilities from the sewage treatment plant to normal water sources if sewage water was left untreated. Moges *et al.* [19] also reported *S. aureus* from wastewater resistant to 11 antimicrobials, including ampicillin (100%) and methicillin (100%). Our data also showed that all MAR *S. aureus* were resistant to methicillin and oxacillin, which illustrated that the wastewater could be spreading MRSA into the normal water sources. Although, we did not perform further tests to distinguished MRSA or MSSA (methicillin-resistant *Staphylococcus aureus*).

Multiple antibiotic resistance (MAR) index can indicate a contamination source, especially from high-risk environments such as hospital environments, human, commercial poultry farms, swine, and dairy cattle [8,33]. The MAR value above 0.20 is said to have originated from high-risk areas and originate from an environment

where several antibiotics are used [33]. The MAR index of this study showed that all of the isolates were from high-risk environments. These highly resistant isolates in the environment might work as a potential reservoir for transferring resistant genes into other highly infectious pathogens present in the wastewater [23]. Irrational use of antibiotics facilitated the increasing rate of drug resistance among bacteria. The situation is getting worse, especially in developing countries like Bangladesh [17], where all liquid waste originated from hospitals, veterinaries, and other sources directly or through municipality drainage system is discharged into ecological water bodies resulting serious pollutions of the environment with resistant bacteria [34]. The reduction of selective pressure by regulating antibiotic use is a key step to control the spread of resistance in hospital-associated wastewater, not to favor resistant strains [35].

4. CONCLUSION

The study's findings point out the troubling increase of multidrug resistance in the area's environmental bacteria. Reducing the risk of mortality, morbidity, and healthcare-associated expense of drug-resistant diseases, we strongly suggest that the hospital wastewater required to be treated appropriately before drain out to the environment, and the other wastewater should be drained in a secluded area and must not go to irrigation field, ponds, canals or waterways directly.

5. ACKNOWLEDGMENT

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6. DISCLOSURE STATEMENT

None.

7. FUNDING

None.

8. CONTRIBUTION OF AUTHORS

Rahman MM: Designing the study, laboratory works, and data analysis, writing and critical review of the manuscript. Devnath P: Laboratory works, data analysis, and writing manuscript. Jahan R: Laboratory works, data analysis, and writing manuscript. Talukder A: Writing and critical review of the manuscript.

9. CONFLICTS OF INTEREST

The authors of the study declare that they have no conflicts of interest.

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