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Article

Isolation and characterization of multiple drug-resistant bacteria from the waste of hospital and non-hospital environment

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Abstract: Antibiotics used in hospitals for patient care which potentially growing antibiotic resistant bacteria in hospital waste and simultaneously transmitting to non-hospital environments by drainage system. Total 20 samples were collected randomly and examined with different bacteriological, biochemical and molecular tests. 55 bacterial isolates were isolated from all samples, among them 32 (58.2%) were from hospital environment and 23 (42.1%) were from non-hospital environment. The result of total viable count showed that maximum countable bacteria (2.20×10^{10}) CFUs/ml that were from MARCH and the minimum number of countable bacteria (1.0×10^{10}) CFUs/ml were isolated from the sample of Kalitola. Among the isolates, *E. coli*, *Pseudomonas* spp, *Klebsiella* spp, *Salmonella* spp, *Staphylococcus* spp and *Vibrio* spp were identified 16 (29%), 12 (21.8%), 9 (16.4%), 8 (14.5%), 5 (9%) and 5 (9%) respectively. Multidrug resistant (MDR) *Pseudomonas aeruginosa* was characterized from hospital wastewater by polymerase chain reaction assays targeting the virulence gene and 16S rRNA gene region was amplified with the universal primers. PCR amplification band was found at 1399 bp. The antibiotic sensitivity study revealed that among the hospital isolates, about (83.3%) were resistant against Ampicillin, followed by Amikacin, Kanamycin and Penicillin (77.8%). On the other hand, non- hospital isolates were resistant against Amoxicillin and Penicillin (66.7%) followed by Ampicillin and Vancomycin (58.3%). Both hospital and non-hospital isolates were sensitive to Gentamycin respectively 72.5% and 75%. The findings of the experiment suggested that hospital wastewater contained more MDR bacteria than non-hospital wastewater which are released into receiving water bodies that may cause a serious threat to public health. Reducing indiscriminate use of antibiotics in both hospital and non-hospital settings and the use of wastewater treatment plant (WTP) in a hospital may reduce this problem.

Keywords: antibiotics; multiple drug resistant; bacteria; wastewater; hospital; environment

1. Introduction

Antibiotics are a class of naturally-occurring, semi-synthetic, and or chemically synthesized compounds with antimicrobial activity. They are widely used in human and veterinary medicine to treat and prevent diseases and as growth promoters in animal intensive industries. The increasing incidence of resistance to a wide range of antibiotics by microorganisms is a major concern facing modern medicine. Clinical infections, disease, and

death caused by resistant bacteria are increasingly common. We know for a fact that antibiotic resistance can be established and propagated in human and animal digestive systems (Launay *et al.,* 2014; Chopra *et al*., 2001). Antibiotic resistance has become a major clinical and public health problem within the lifetime of most people living today (Stuart *et al.,* 2002). Antibiotics exert a selection in favor of resistant bacteria by killing or inhibiting the growth of susceptible bacteria; resistant bacteria can adapt to environmental conditions and serve as vectors for the spread of antibiotic resistance (Wegener *et al*., 1999; Kruse, 1999). The main risk for public health is that resistance genes are transferred from environmental bacteria to the human pathogen (Wegener *et al.,* 1999) Hospital wastewater is considered a hot spot for antibiotic resistance (AR) as a consequence of receiving a cocktail of antibiotic compounds, disinfectants, and inputs of bacterial shadings and metabolized drugs from patient excrement, which potentially contain multidrug-resistant (MDR) pathogens (Chagas *et al*., 2010, 2011; Galvin 2010) As such, hospital wastewaters provide an environment for the exchange of antibiotic resistance genes (ARGs) between clinical pathogens and other environmental bacteria in recipient sewers, which could result in broader epidemiological consequences extending beyond the hospital setting (Bengtsson *et al.,* 2015; Stalder. *et al*., 2014). Survive for long periods in the environment, that contributing to the selection of resistant pathogens disseminated in the environment, as well as in hospitals, industry, and veterinary facilities. These natural reservoirs of resistant genes may contribute to the appearance of resistant bacteria due to gene transfer mechanisms (Aygen*et al.*, 2000, Alp *et al.,* 2002; Sader *et al.,* 1997). The choice of bacterial indicators is thus very important. Bacteria belonging to the *Pseudomonas* genus are extensively present in the environment, such as water soil and sediment. Being known for its innate resistance mechanisms, *Pseudomonas* spp. are capable of staying viable in the aquatic environment for long periods (Spindler *et al*., 2012) which carries the hazard of spreading ARGs and mobile genetic elements and can cause infections in humans (Spindler *et al*.,2012; Quinteira *et al*., 2005). From the above discussion, it seems that drug resistance is now a big threat to our whole ecology, so this problem should not be overlooked at all. Considering all the above facts; the objectives of the current study were; to compare and understand the drug resistance pattern of pathogens from the hospital and non-hospital wastewater. To isolate and identify public health important bacteria from wastewater in hospital and non- hospital environments and molecular characterization of important pathogenic bacteria.

2. Materials and Methods

2.1. Study area and period

All hospital and non-hospital wastewater samples were collected from different areas at sadar upazila of Dinajpur district and HSTU campus for a period of six months from July to December 2017. All microbiological activity was carried out in microbiology laboratory of Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur.

2.2. Sample collection

Among 20 samples, 10 were collected from different hospitals in the Dinajpur district of which 4 samples were from M. Abdur Rahim Medical College Hospital (MARMCH), 3 from Sadar hospital of Dinajpur (SHD) and 3 samples from Islami bank community hospital, Dinajpur (IBCH). On the other hand, 10 samples were collected from different sites of HSTU campus, namely one from cow farm, one from ostrich farm, and one from poultry farm, two samples from Baserhat bazar, two from Bahadurbazar, two from Lilirmor and one from Kalitola.

2.3. Sample processing and isolation of bacteria

All collected samples were transported to the microbiological laboratory of the Department of Microbiology, HSTU, and Dinajpur, Bangladesh in cool conditions and processed within two hours of collection. To determine the total viable plate count, serial 10-fold dilutions of samples were prepared in physiological saline, and 50 µl (0.05 ml) of aliquot was spread plated on plate count agar (PCA). Plates were incubated for 24 hours at 37ºC before bacteriological counts were done. The number of colonies on each plate having 30–300 colonies was counted by using a digital colony counter. Plates with more than 300 colonies cannot be counted and are designated as too numerous to count-TNTC (Cappuccino, 2005) After that, based on colony morphology representative colonies were picked and sub-cultured on different selective and differential media such as blood agar, MacConkey agar, EMB agar, SS agar, TCBS agar, Cetrimide agar base, etc. After obtaining pure colonies and recording key features such as hemolysis on blood agar isolated organisms were identified biochemically in a systematic way following standard methods (Holt JG *et.al.)*

2.4. Antibiotic susceptibility testing

The standard Kirby-Bauer disk diffusion method was used to determine the antimicrobial susceptibility profiles of the isolates (Bauer, 1999) according to the recommendations of the National Committee for Clinical Laboratory Standards (CLSI-2015). Bacterial inoculums were prepared by suspending the freshly grown bacteria in 4–5 ml sterile nutrient broth and the turbidity was adjusted to that of a 0.5 McFarland standard. The antimicrobial susceptibility testing was performed using Mueller-Hinton medium, all isolates were tested for sensitivities to10 (Ampicillin 25, Amoxicillin 30, Amikacin 30, Chloramphenicol 30, Ciprofloxacin5, Gentamycin10 Kanamycin30, Penicillin10, Tetracycline 30, Vancomycin 30) of routine and practical antibiotics.

2.5. Molecular Technique

2.5.1. Materials used for bacterial genomic DNA isolation

TE buffer, 10% (w\v) Sodium dodecyl sulfate (SDS), 20 mg\ml protinase k (stored in small single-use aliquots at-200C), 3M Sodium Acetate, pH 5.2,25:24:1 Phenol/Chloroform/Isoamyl alcohol Isopropanol, 70% Ethanol, 95% Ethanol & 1.5 ml microcentrifuge tubes.

2.5.2. Purification of PCR product and sequencing

Genomic DNA from *Pseudomonas* spp. isolates cultured in a sodium thioglycolate broth was extracted using the chloroform-isoamyl alcohol method. Then PCR product was visualized by 1% agarose gel electrophoresis. PCR Amplification band was found at 1399 bp. PCR bands were cut and DNA extraction was done by EZ-Pure™ Gel extraction Kit ver.2 (Enzynomics, Korea) followed by the manufacturing procedure. Purified PCR products were sent for sequencing.

2.5.3. Materials used for polymerase chain reaction

dNTP, MgCl2, Forward Primer (27F), Reverse Primer (1492R), Nano Pure Water, DNA Taq DNA Polymerase, Final Volume, Thermal Cycler (Thermo cycler, ASTEC, Japan), 0.85% agarose gel, Gel casting tray with gel comb, TAE buffer, Microwave oven, Conical flask, Electrophoresis apparatus (Biometra standard power pack P 2T), Bromphenicol blue of loading buffer, Ethidium bromide (0.5 µg/ml), Distilled water, UV trans-illuminator.

2.5.4. Procedure of polymerase chain reaction

Molecular confirmation of resistant bacteria was done by PCR targeting by the 16s rRNA gene using universal primers, forward primer-27F (5'-AGAGTTTGATCCTGGCTCAG-3'); reverse primer-1492R (5'-TACCTTGTTACGACTT-3'). The PCR reaction was performed in 25 µl reaction scale. The reaction consisted of 12.5 µl of 2x master mix (GENE Amp Fast PCR Master mix (2x)). About 2µl sample (samples were diluted at 50 ng/µl), 0.2 µl Taq DNA polymerase, 0.5µl forward primer, 0.5µl reverse primer were used. 9.3µl molecular grade water was added to make final volume of 25µl.For Mx-Sironi primer samples were subjected to initial denaturation for all 95ºC for 10 minutes; followed by 35 cycles of denaturation at 94ºC for 1 minute; annealing at 53ºC for 1 minute; extension at 72ºC for 1 minute; and a final extension at 72ºC for 10 minutes on Gene Atlas (Model: G02, Japan).

3. Results

3.1. Results of total viable counts

Table 1. Results of total viable counts of samples from each sampling point (dilution10^{-7}).

Sampling Sites	Number of colonies	Result CFUs/ml
M. Abdur Rahim Medical College Hospital, Dinajpur (MARMCH)	110	2.20×10^{10}
Sadar Hospital Dinajpur (SHD)	More than 300	TNTC
Islami Bank Community Hospital, Dinajpur (IBCH)	79	1.58×10^{10}
HSTU campus	51	1.02×10^{10}
Baserhat Bazar	More than 300	TNTC
Bahadurbazar	More than 300	TNTC
Lilirmor	55	1.10×10^{10}
Kalitola	50	1.0×10^{10}

3.2. Distribution of samples taken from hospital environments of Dinajpur

Table 2. Distribution of samples taken from hospital environments of Dinajpur.

Sample sites	Total samples	Sample positive	Total number of bacterial isolates
M. Abdur Rahim Medical College Hospital, Dinajpur (MARMCH)	4	4 (40)	13 (40.6)
Sadar Hospital Dinajpur (SHD)	3	3 (30)	10 (31.2)
Islami Bank Community Hospital, Dinajpur (IBCH)	3	3 (30)	9 (28.1)
Subtotal	10	10 (100)	32 (100)

3.3. Distribution of samples taken from non-hospital environments of Dinajpur

Table 3. Distribution of samples taken from non-hospital environments of Dinajpur.

Sample sites	Total samples	Sample positive N (%)	Total bacterial isolates recovered N (%)
HSTU campus	3	3 (30%)	8 (34.8)
Baser Hat Bazar	2	2 (20%)	4 (17.4)
Bahadur Bazar	2	2 (20%)	4 (17.4)
LilirMor	2	2 (20%)	4 (17.4)
Kalitola	1	1 (10%)	3 (13.0)
Subtotal	**10**	**10 (100%)**	**23(100)**

3.4. Number of bacteria isolated from each sampling points

A total of 20 wastewater samples were processed for the presence of drug-resistant bacterial pathogens. Of these samples, 100% of the samples were positive to one or more isolates. Among the total samples, 55 bacterial isolates were recovered. Among them, 32 (58.2%) were from the hospital environment and 23 (42.1%) were from a non-hospital environment which was shown in Table 4.

Table 4. Number of bacteria isolated at each sampling points from hospital and non-hospital environments of Dinajpur.

Bacterial isolates	Hospital environment No. (%)	Non –hospital environment No. (%)	Total No. (%)
E. coli	10 (31.2)	6 (26.0)	16 (29)
Pseudomonas spp.	7 (21.9)	5 (21.7)	12 (21.8)
Klebsiella spp.	5 (15.6)	4 (17.4)	9 (16.4)
Salmonella spp.	5 (15.6)	3 (13.0)	8 (14.5)
Staphylococcus spp.	3 (9.4)	2 (8.7)	5 (9)
Vibrio spp.	2 (6.3)	3 (13.0)	5 (9)
Total	**32 (100)**	**23 (100)**	**55 (100)**

3.5. Identification of bacteria by different bacteriological methods

The cultural characteristics of *E. coli*, *Klebsiella* spp, *Salmonella* spp, *Vibrio* spp, *Pseudomonas* spp, *and Staphylococcus* spp, on various media, are presented in Table 5.

Table 5. The result of the cultural characteristics of the bacteria isolated from different hospital & non-hospital environments.

Name of bacteria	Name of media	Colony characteristics
E. coli	Nutrient Agar	Large, mucoid, white colony.
	EMB agar	Transmitted light blue-black center with a narrow, clear edge. Blue-green metallic sheen with reflected light.
Pseudomonas spp.	Nutrient agar	Large, smooth, low convex, and greenish pigment with a fruity odor.
	Cetrimide agar	Colonies are greenish

Klebsiella spp.	Nutrient Agar	Large colony.
	EMB agar	Mucoid, no metallic sheen. With transmitted light, gray-brown centers, and pink color with clear edges.
Salmonella spp.	Nutrient agar	Smooth. Opaque, translucent colonies.
	S.S agar	Opaque, smooth, round with black centered colonies.
Staphylococcus spp.	Nutrient Agar	Black color/ non-colour smooth, glistening colonies.
	Mannitol Salt Agar	Yellow colonies.
Vibrio spp.	MacConkey Agar	Colorless colonies
	TCBS Agar	Colonies are large yellow or green

3.6. Results of biochemical tests

Table 6. Result of biochemical tests.

Name of the Test → / Name of the Organisms ↓	Catalase	MR	VP	Indole	Citrate Utilization	MIU	TSI		
							Slant	Butt	H_2S
E. coli	+	+	-	+	-	+	Y	Y	-
Pseudomonas spp.	+	-	-	-	+	+	R	R	-
Klebsiella spp.	+	-	+	-	+	-	Y	Y	-
Salmonella spp.	+	+	+	+/-	+	+	Y	R	+
Staphylococcus spp.	+	+	+	-	+	-	Y	R	-
Vibrio spp.	+	+	+/-	+	+	+	Y	Y	-

[Y= Yellow; R= Red]

3.7. Result of PCR amplification of *Pseudomonas* spp.

Out of 12 *Pseudomonas* isolates only one isolate gave specific amplification (Figure 1). After PCR and sequencing of PCR product results were analyzed by NCBI blast search (www.ncbi.nih.gov/blast). On NCBI Blast search 97% sequence similarity was observed with *Pseudomonas aeruginosa* strain DSM 50071.

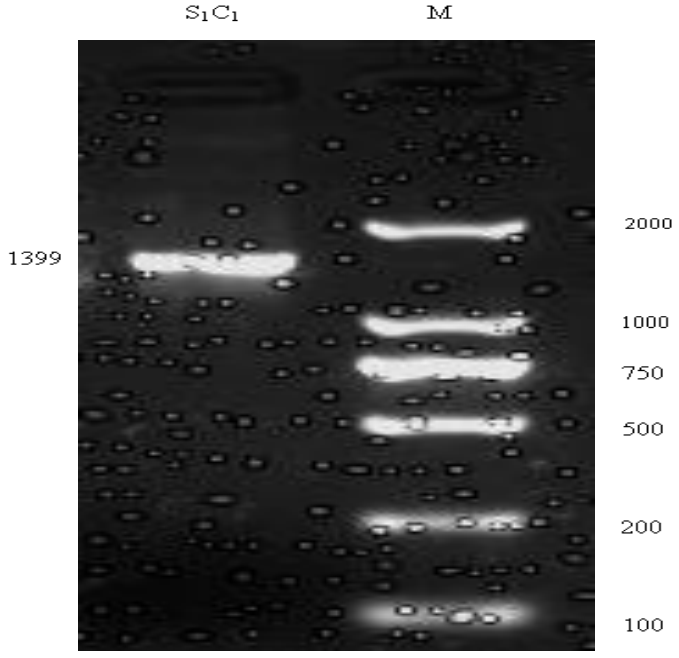

Figure 1. Detection of *Pseudomonas aeruginosa* by PCR. PCR was performed by the using 16S rRNA gene primer. Lane 1: 1399 bp of 16S rRNA gene of *Pseudomonas aeruginosa*; lane 2: DNA ladder.

3.8. Phylogenic tree analysis of *Pseudomonas* spp.

To assess the phylogenetic relationship between our isolate (contig 429) and gene sequences of *Pseudomonas* from NCBI were used to construct phylogenetic tree based on neighbor-joining methods (Figure 2). The phylogenetic tree shows that our isolate was closely related to *Pseudomonas aeruginosa* (strain DSM 50071) and *Pseudomonas* indica (strain NBRC 103045). It was distantly related to *Pseudomonas stutzeri* (strain VKM B-975 and strain DSM 5190).

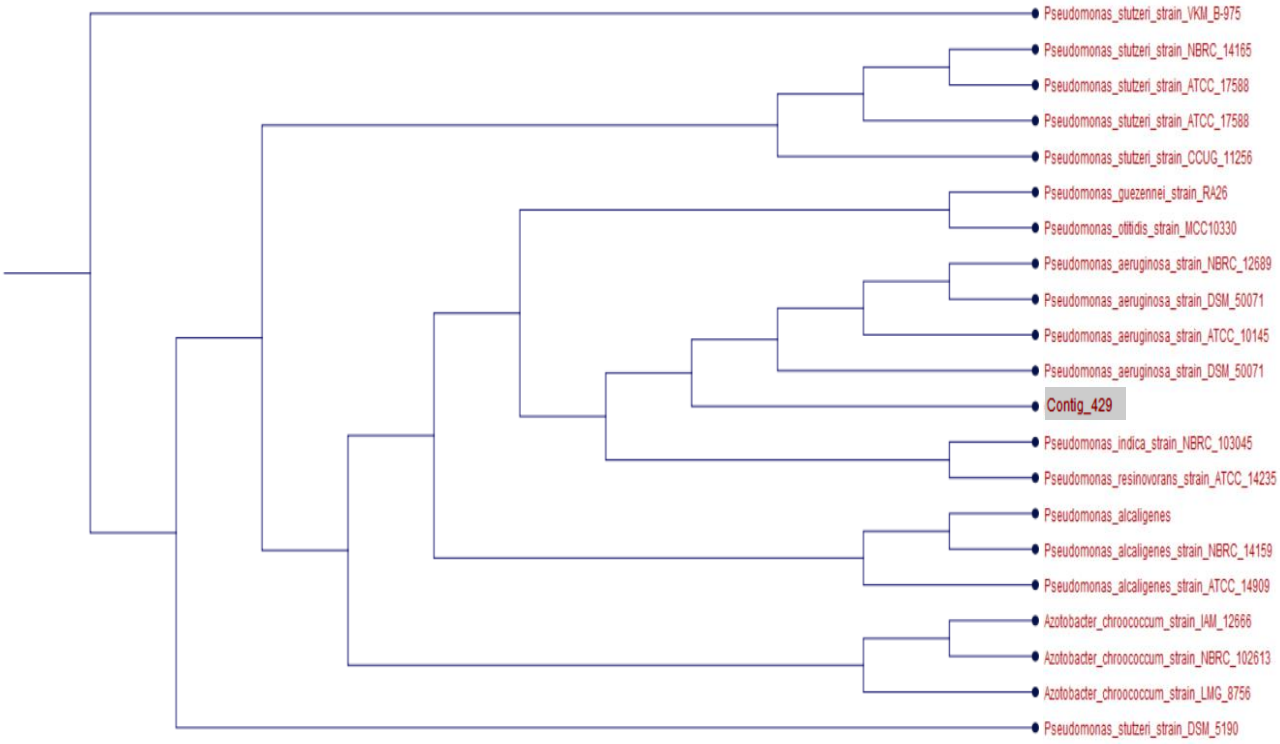

Figure 2. Phylogenic tree analysis of *Pseudomonas* spp.

4.8 Results of antibiotic susceptibility test

4.8.1 Drug resistance pattern of hospital isolates

The antibiotic study also revealed that among the tested hospital isolates; about (83.3%) were resistant against Ampicillin, followed by Amikacin, Kanamycin, and Penicillin (77.8%) which are shown in Table 7.

Table 7. Drug resistance pattern of hospital isolates.

Name of the antibiotic and their disc concentration (µg/disc)	Percentages N (%)						
	E. coli **n=5**	***Pseudomonas*** **spp. n=4**	***Klebsiella*** **spp. n=4**	***Salmonella*** **spp. n=2**	***Staphylococcus*** **spp. n=2**	***Vibrio*** **spp. n=1**	**Total N=18**
Ampicillin (25)	5 (100)	4 (100)	3 (75)	2 (100)	1 (50)	1 (100)	15 (83.3)
Amoxicillin (30)	3 (60)	3 (75)	3 (75)	1 (50)	2 (100)	-	12 (66.7)
Amikacin (30)	4 (80)	4 (100)	2 (50)	1 (50)	2 (100)	1 (100)	14 (77.8)
Chloramphenicol (30)	2 (40)	2 (50)	2 (50)	-	1 (50)	-	7 (38.9)
Ciprofloxacin (5)	2 (40)	3 (75)	2 (50)	1 (50)	1 (50)	-	9 (50)
Gentamycin (10)	1 (20)	2 (50)	1 (25)	-	1 (50)	-	5 (27.8)
Kanamycin (30)	3 (60)	3 (75)	3 (75)	2 (100)	2 (100)	1 (100)	14 (77.8)
Penicillin(10)	4 (80)	4 (100)	3 (75)	1 (50)	1 (50)	1 (100)	14 (77.8)
Tetracycline (30)	1 (20)	3 (75)	2 (50)	1 (50)	1 (50)	1 (100)	9 (50)
Vancomycin (30)	4 (80)	3 (75)	2 (50)	1 (50)	1 (50)	-	11 (61.1)

[Note; (-) =Not Resistant]

4.8.4 Drug resistance pattern of non-hospital isolates

The antibiotic study also revealed that among the tested non-hospital isolates were mostly resistant against amoxicillin and Penicillin (66.7%) which is shown in Table 8.

Table 8. Drug resistance pattern of non-hospital isolates.

Name of the antibiotic and their disc concentration (µg/disc)	Percentages N (%)						
	E.coli **n=2**	***Pseudomonas*** **spp. n=2**	***Klebsiella*** **spp. n=2**	***Salmonella*** **spp. n=2**	***Staphylococcus*** **spp. n=2**	***Vibrio*** **spp. n=2**	**Total N=12**
Ampicillin (25)	2 (100)	1 (50)	2 (100)	-	1 (50)	1 (50)	7 (58.3)
Amoxicillin (30)	1 (50)	2 (100)	1 (50)	1 (50)	2 (100)	1 (50)	8 (66.7)
Amikacin (30)	1 (50)	2 (100)	-	2 (100)	-	-	5 (41.7)
Chloramphenicol (30)	1 (50)	-	2 (100)	1 (50)	1 (50)	1 (50)	6 (50)
Ciprofloxacin (5)	2 (100)	1 (50)	1 (50)	-	1 (50)	-	5 (41.7)
Gentamycin (10)	-	-	-	2 (100)	1 (50)	-	3 (25)
Kanamycin (30)	1 (50)	1 (50)	1 (50)	-	1 (50)	2 (100)	6 (50)
Penicillin(10)	2 (100)	1 (50)	1 (50)	1 (50)	2 (100)	1 (50)	8 (66.7)
Tetracycline (30)	1 (50)	1 (50)	1 (150)	-	1 (50)	1 (50)	5 (41.7)
Vancomycin (30)	1 (50)	1 (50)	1 (50)	1 (50)	2 (100)	1 (50)	7 (58.3)

[Note; (-) =Not Resistant]

5. Discussion

Drug resistance in bacteria is a widespread problem throughout the world and is increasing day by day. In this study, six different types of bacteria were isolated and identified. Molecular characterization was done to identify *Pseudomonas* species by16S rRNA Gene Sequencing. The result of total viable count showed that maximum countable bacteria (2.20×10^{11}) CFUs that were from MARMCH Site-2 and a minimum number of countable bacteria (1.0×10^{11}) were isolated from a sample of Kalitola. In the current study, a total of 55 bacterial isolates were isolated. Among them, 32 (58.2%) were from the hospital environment and 23 (42.1%) were from the non-hospital environment. The rate of isolation of bacterial pathogens in the hospital environment was higher than in the non-hospital environment. The finding of this study is almost similar to Moges *et al*. (2014) where he found 65 (57.5%) isolates from the hospital environment and 48 (42.5%) were from non-hospital environments. Six different bacterial isolates *E. coli*, *Pseudomonas* spp., *Klebsiella* spp., *Salmonella* spp., *Staphylococcus* spp*. and Vibrio* spp. were identified. The most frequently isolated bacteria were *E. coli* 16 (29) followed by *Pseudomonas* spp. 12(21.8%) and *Klebsiella* spp. 9(16.4%). A similar result was showed by Onuoha *et al*. (2017) and Elmanama *et al*. (2006). A similar study in Dhaka City, Bangladesh reported that frequently isolated bacteria were *Escherichia coli* and *Klebsiella pneumonia* isolates from two renowned hospitals of Dhaka city Rabbani *et al. (*2017). Guessennd *et al.* (2013) also reported that they mostly isolated *E. coli, K. pneumoniae, P. aeruginosa,* and *Staphylococcus* spp. was from hospital wastewater. Yang *et al.* (2008) also reported that *E. coli* were the leading bacterial isolates in both clinical and sewage samples. Multiple drug resistance was common in Gram-negative isolates to commonly used antibiotics in the study area. *E. coli*, *Pseudomonas* spp*, Salmonella* spp, and *Vibrio* spp were 100% resistant to Ampicillin. This finding is inconsistent from reports in Brazil that the overall resistance rates were low in the isolates of *E. coli*, *P. aeruginosa*, *K. pneumoniae* and *A. baumannii* and the susceptibility pattern of *E. coli* and *Klebsiella* for ampicillin was found40% and 70%, respectively Resende *et al.* (2009). Among all isolates (83.3%) were resistant to Ampicillin, followed by Amikacin, Kanamycin, and Penicillin. This finding agreed with the result of Moges *et al*. (2014). A similar study reported that most isolates were resistant to Ampicillin (73.9 %) Zubair *et al*. (2013), similarly, Krcmery *et al.* (1989) showed as high as 80% Ampicillin- resistant *E. coli* from municipal wastewater. One of the *Pseudomonas* spp isolates was resistant to 8 out of 10 antibiotics that were used in the current study. Another study showed that *Pseudomonas* spp. was resistant to 10 out of 12 antibiotics Moges *et al*. (2014). The resistant pattern of Gram-negative isolates for Ciprofloxacin was moderate (50%) in the present study, this was different from other studies done in Bangladesh where 100 % was resistant Islam *et al*. (2008). Gentamycin was the most effective antibiotic to all of the isolates as it was 72.5% and 75% sensitive to hospital and non-hospital isolates respectively, this result is similar to Ibrahim *et al*. (2010) where he also found Gentamycin as a most effective antibiotic. One of the goals of this current study was to compare drug-resistant

bacterial isolates from the hospital and non-hospital wastewater; in this case, the result of this study showed that hospital isolate was more resistant to most of the antibiotic which was used. As the antibiotic study revealed that among the tested hospital isolates; about 83.3%, was resistant against Ampicillin, followed by Amikacin, Kanamycin, and Penicillin, all were 77.8% resistant. On the other hand, antibiotic study result revealed that among the tested non- hospital isolates were mostly resistant against Amoxicillin and Penicillin (66.7%) followed by Ampicillin and Vancomycin (58.3%). The result of molecular characterization revealed that isolated multidrug-resistant *Pseudomonas spp* is the *Pseudomonas aeruginosa.* A similar kind of multidrug-resistant *Pseudomonas aeruginosa* was identified from hospital wastewater by Tumeo *et al.* (2008), but he said that there was a difference between *Pseudomonas aeruginosa* that were collected from hospitalized patients and wastewater. So the current study result suggests that multidrug-resistant *Pseudomonas aeruginosa* is predominant in hospital wastewater. One study carried out in Bangladesh in 2008 found out that the resistance development was directly related to the use of antibiotics Islam *et al.* (2008) The results further suggested that the multi-drug resistant bacteria & plasmid containing multidrug-resistant genes present in the hospital waste might act as a possible source of transfer of these highly resistant genes to the bacterial population. The bacterial isolates from hospital environments were less resistant to Gentamycin (27.8% resistant) and Chloramphenicol (38.9% resistant) but resistant to other antibiotics must not have been grown. The number of multidrug-resistant (MDR) bacteria was still alarmingly high for the effluent samples from hospitals. More distressing was the pattern of MDR. Simultaneous resistance for most of the antibiotics including Penicillin (77.8%), Kanamycin (77.8%), Vancomycin (61.1%) MDR pattern for hospital isolates. This pattern of antimicrobial resistance in bacteria is highly consistent with the results of the study carried out in India Chitnis *et al.* (2000). The pattern was almost the same for the various genera grown from the effluent samples. The MDR pattern seen in the bacterial isolates from hospital effluent samples included many of the antibiotics being currently used in the treatment of infectious diseases. From the results, it is clear that hospital wastewater is full of drug-resistant pathogens that are mainly resistant against commonly used antibiotics, which suggested a selection pressure is present that induces the organisms to become resistant. Untreated hospital waste in the study area may be a possible cause to increase drug resistance in the common wastewater isolates to become pathogenic bacteria.

6. Conclusions

The present study demonstrated that untreated hospital waste disposal could contribute to the development of antibiotic resistance in environmental organisms. Resistance pattern varied from isolates to isolates but maximum resistance was observed in one *Pseudomonas* spp. isolates that were resistant up to 8 antibiotics out of 10 antibiotics tested and molecular characterization revealed that it was *Pseudomonas aeruginosa*. From this research work, it can be concluded that there is an urgent need for raising awareness and education on medical waste issues. Proper waste management strategy is needed to ensure health and environmental safety. It is, therefore, advised that all stakeholders and the health sector authorities should look after this issue seriously and takes effective ways to control the spreading of the resistant gene in the environment.

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Conflict of interest

None to declare.

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