

Original Article

Untreated Liquid Hospital Waste: Potential Source of Multidrug Resistant Bacteria

Md. Kamruzzaman Siddiqui, Nazma Khatoon, Pravas Chandra Roy*

Department of Microbiology, Jessore University of Science and Technology, Jessore-7408, Bangladesh

Antimicrobial resistance in both pathogenic and commensal bacteria is increasing steadily. Failure of antibiotic resistant bacteria containment is responsible for this expansion. Healthcare effluent acts as the store house of harmful infectious pathogens. Potential health risk includes spreading of diseases by these pathogens and wide dissemination of antimicrobial resistance genes. The present study was carried out to investigate the multiple-drug resistance among the bacterial strains that were isolated and identified from the effluents of Jessore Medical College Hospital & Jessore Queen's hospital private limited. Identified bacteria were *E. coli*, *Klebsiella* spp., *Enterobacter* spp., *Proteus vulgaris* and *Salmonella* spp.. Occurrence of *E. coli* and *Enterobacter* spp. were found to have the highest percentages and present in majority of the samples. The identified organisms antibiotic resistant pattern were analyzed by agar disc diffusion method against 6 antibiotics. Results of antibiotic susceptibility test showed that all of the isolates were multi-drug resistant (e⁴). From the study, we observed that 75% of the isolates were resistant to amoxicillin, followed by Ampicillin (64%), Chloramphenicol (31%), Gentamycin (29%), Nitrofurantoin (27%) and least resistant being Ciprofloxacin 23%. Among the isolates *Salmonella* spp. were showed highest rate of resistance against all the used antibiotics. The result denotes that, the identified bacteria have been well exposed to the tested antimicrobials and they have established mechanisms to avoid them. Therefore, proper waste water treatment plant should be established to diminish the risk of disseminating multiple drug resistant microorganisms for the safeguard of public health.

Introduction

Untreated hospital waste water (HWW) possesses the risks of spreading antibiotic resistant bacteria in the environment. In Bangladesh, liquid discharge is directly released into the municipal sewage system and pollutes the surrounding water bodies/rivers¹. HWW can be hazardous to public health since it may contain many kinds of pollutants such as radioactive, chemical and pharmaceutical wastes and pathogenic microorganisms². Discharge of pathogenic microorganisms and un-metabolized antibiotics in low concentrations may also contribute to the development of antibiotic resistance in our natural microflora/environmental microflora^{3,4}. The increasing of antibiotic resistance and the cause of the spread of resistance genes in environmental samples such as HWW is a result of uncontrolled and excessive use of antibiotics⁵. The occurrence of bacteriophages from samples of animals fecal wastes may also act as environmental vectors for the horizontal transfer of antibiotic resistance genes⁶. The main risk for public health is that resistance genes are transferred from environmental bacteria to human pathogen^{7,8}. Although, sewage treatment reduces the number of bacteria in wastewater but the effluent generally contains a large number of both resistant and susceptible bacteria⁹. The basic principle of underlying wastewater management is the strict limit on the discharge of hazardous

liquids into sewers without prior treatment so that living pathogenic organisms are not introduced into the environment. Although the government of Bangladesh has specific rules and regulations regarding the discharge of hospital wastes and its management, however, many hospitals do not strictly follow these rules. In many instances, there is no structured form of medical waste treatment plant and most hospital wastes are dumped in open areas for natural degradation or resold by scavengers.

Untreated hospital liquid waste discharged into the surface water, directly or indirectly, have been causing additional problems and many people die each year due to the cause of waste-related diseases in Bangladesh¹⁰. Again, the un-treated hospital effluents or the concentrated forms of infectious agents and antibiotic resistant microbes, are shed into communities and result in water borne diseases such as cholera, typhoid fever, dysentery and gastroenteritis⁷. Gram-negative bacteria are crucial in this aspect because they are the most common causes of hospital and community acquired infections and they are inherently resistant to many hydrophobic antibiotics.

Sewerage network of Jessore, a town having a number of hospitals and located in the south west of the country, consists of small drains connected with Bhairab river. Therefore, when the hospitals are discharging their healthcare liquid waste into sewerage

*Corresponding author:

Pravas Chandra Roy, Department of Microbiology, Jessore University of Science and Technology, Jessore-7408, Bangladesh. E-mail: pc.roy@just.edu.bd

network, it is mixed with the domestic sewage before coming in contact with surface water without proper treatment. This study was carried out to investigate the antibiotic resistance pattern of gram-negative bacteria isolated from healthcare liquid wastes generated in Jessore town area of Bangladesh.

Material & Methods

Sample collection

Twenty two waste water samples were collected from different sites including sewage opening of surgery unit & cabin ward of two hospitals located in the heart the city, as well as from the downstream sites of Bhairab River, where the domestic and hospital sewage are released. A minimum of 250 ml samples were collected aseptically in a clean glass sampling bottle.

Isolation and identification of isolates

Samples were transported to the laboratory of the Department of Microbiology, Jessore University of Science & Technology, Jessore, in temperature controlled conditions and processed within two hours of collection. Serially diluted samples were spread over MacConkey agar medium and incubated for 24 hours at 37°C for the isolation of Gram negative enteric bacteria. Distinct colonies were selected from the plates and the pure cultures were subjected to biochemical tests. Following different biochemical and morphological characteristics, the isolates were identified.

Antimicrobial susceptibility testing

Once isolation and identification of the bacteria from collected samples were done, the standard Kirby-Bauer disk diffusion method was used to determine the antimicrobial susceptibility profiles of the isolates¹¹. Bacterial inocula were prepared by suspending the freshly grown bacteria in 4–5 ml sterile normal saline and the turbidity was adjusted to that of a 0.5 McFarland standard. The antimicrobial susceptibility testing was performed using Mueller-Hinton agar medium against Ampicillin (10 µg), Gentamycin (10 µg), Chloramphenicol (30 µg), Amoxicillin (20 µg), Ciprofloxacin (5 µg) and Nitrofurantoin (300 µg). The plates were incubated aerobically at 37°C for 18–24 hours. The zones of inhibition were measured and compared with Clinical Laboratory Standards institute guidelines¹².

Results

Waste water samples were collected from two hospitals, which are main health care service providers of this region. After processing, the isolates were identified and further analyzed for screening of multidrug resistant bacteria. A total of 104 bacterial isolates were recovered from the collected samples and identified following different biochemical and morphological tests. Among the isolates, 32 (30.7%) were *E. coli* and *Klebsiella* spp., 26 (25%) were *Enterobacter* spp., 8 (7.7%) were *Proteus vulgaris* and 6 (5.8%) were *Salmonella* spp., respectively. Significant portion of the isolates were resistant to the commonly used antibiotics (Figure 1). Irrespective of bacterial type, the highest percentage

of resistance was shown against Amoxicillin, where 78 (75%) isolates were found to be resistant, followed by Ampicillin, where 66 (63.5%) isolates were found to be resistant. On the other hand, Ciprofloxacin showed less resistance pattern (23%) in contrast to Amoxicillin and Ampicillin. Other antibiotics showed different degrees of resistance (Figure 1), like Chloramphenicol (30.8%), Gentamycin (28.8%), and Nitrofurantoin (26.9%).

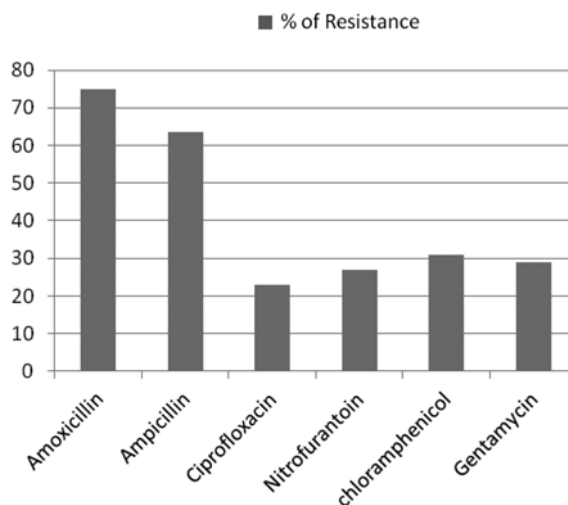


Figure 1. Overall antibiotic resistance pattern of all the isolates collected from two hospitals located in Jessore area.

Among the 6 *Salmonella* isolates, all were resistant to Amoxicillin, Ampicillin and Nitrofurantoin, 4 were resistant to Gentamycin, and two were resistant to Ciprofloxacin and Chloramphenicol (Figure 2). Similarly, among the *E. coli* isolates, 94% were resistant to Amoxicillin, followed by Ampicillin (75%) (Figure 3). All the *E. coli*, *Klebsiella* sp. or *Enterobacter* sp. showed similar resistance pattern against Ampicillin and Amoxicillin (Figure 3, 4 & 5). On the other hand, all *E. coli* isolates were sensitive to Gentamycin and all *Enterobacter* spp. were sensitive to Ciprofloxacin (Figure 3 & 5). Out of total 32 *Klebsiella* spp. isolates, one was resistant

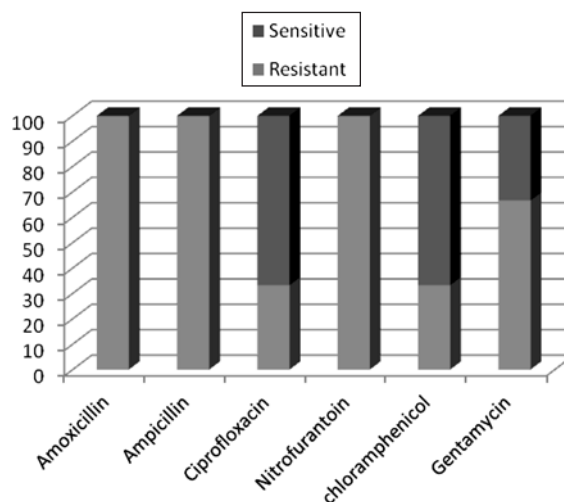


Figure 2. Antibiotic resistant pattern of *Salmonella* spp.

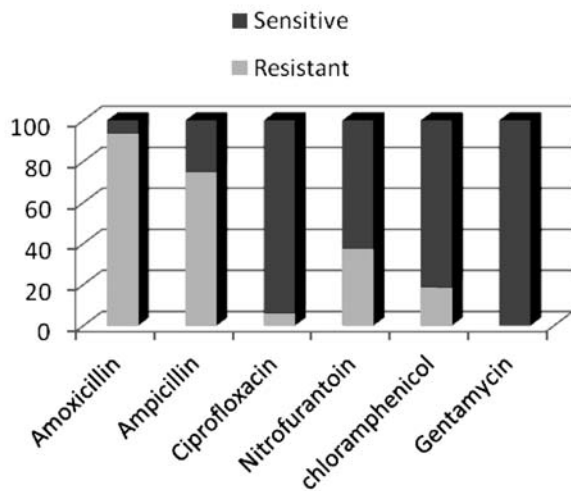


Figure 3. Antibiotic resistant pattern of *E. coli*.

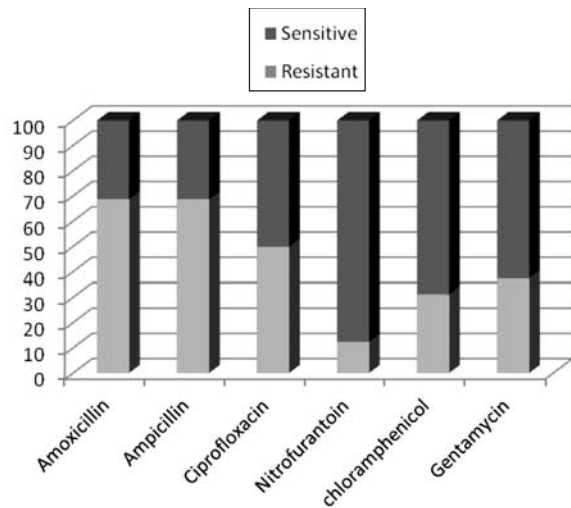


Figure 4. Antibiotic resistant pattern of *Klebsiella* spp.

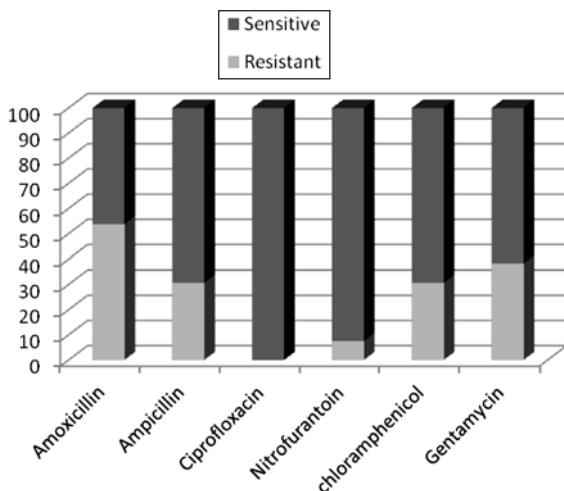


Figure 5. Antibiotic resistant pattern of *Enterobacter* spp.

to all tested antibiotics. Only 13% of *Klebsiella* spp showed lowest resistance against Nitrofurantoin, and 65% showed highest resistance against Amoxicillin and Ampicillin (Figure 4). All *P. vulgaris* was found to be most consistent in term of antibiotic resistance pattern and 75% of the isolates showed resistance against both Ampicillin and Amoxicillin.

In our study, we did not find any significant difference in terms of antibiotic resistance among the bacterial isolates from these two hospitals. From the result it is clear that these organisms have been well exposed to the tested antibiotics and they have developed mechanisms to evade or avoid these antibiotics.

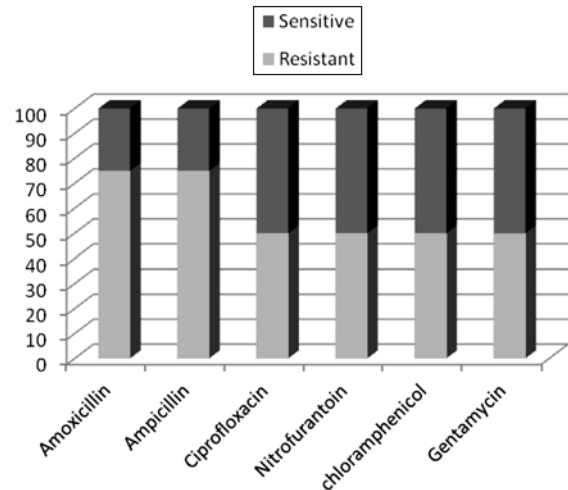


Figure 6. Antibiotic resistant pattern of *Proteus vulgaris*.

Discussion

It has been documented that the antibiotic resistant bacteria in water sources are in a great number throughout the world¹³ and behind the antibiotic resistance of bacteria, main cause is the misuse or following inappropriate strategies of using those antibiotics to control infectious diseases. This crisis is increasing at an unexpected level due to indiscriminate use of antibiotics by the clinical practitioners as well as by the community people without any prescription¹⁴. A patient administered with various antibiotics excretes a small amount of it through urine and other means to the environment¹⁵. As a consequence, the treatment of disease with antibiotic not only kills the pathogen, but also favors the development of resistance in the environment¹⁶.

The result of bacteriological enumeration showed that the total number of bacteria found in different samples varied from sample to sample. But the total number of resistant bacteria was found to decrease with the increase in distance of sample collection site from the two hospitals. In this study, *E. coli* and *Klebsiella* spp. were found to be the most prevalent bacteria followed by *Enterobacter* spp., *P. vulgaris* and *Salmonella* spp. In another study, *Pseudomonas* spp. was found to be most prevalent (27.6%), followed by *E. coli* (24.1%) and *Klebsiella* spp. (20.7%)¹⁷. In this study, highest number of isolates were resistance to Amoxicillin (75%) and same type of result was also shown by another group, where Amoxicillin was the antibiotic to which highest number of resistant bacteria (93.1%) was found¹⁷. In the study, *Salmonella*

spp. were 100% resistant to Ampicillin, followed by *E. coli* (75%), *P. vulgaris* (75%), *Klebsiella* spp. (68.7%) and *Enterobacter* spp. (53.6%). Among all isolates of Gram negative bacteria, 75% of isolates were found to be resistant to Amoxicillin, followed by Ampicillin (63.5%), Chloramphenicol (30.7%), Nitrofurantoin (26.9%), Gentamycin (28.8%) and least being Ciprofloxacin (23.1%). Previously, similar Ciprofloxacin resistant *E. coli* was isolated and characterized from the Chittagong Medical College Hospital & Dhaka Medical college Hospital liquid waste^{1,18}. The resistance pattern of Ciprofloxacin was lower in the present study, although all the bacterial species show resistance against Ciprofloxacin except *Enterobacter* spp. which was fully sensitive. Similar result was found in Northwest Ethiopia where the percentage of resistance to Ciprofloxacin was found to be only 12%¹⁹. However, 100% resistance pattern was noted by another research group conducted in Bangladesh²⁰. Distributions of antibiotic resistant bacteria in the environment are very mysterious and may vary from hospital to hospital and region to region. This variability may be due to the use or exposure of antibiotic, patient type and other environmental factors.

It can be assumed that isolated organisms in this study have been persistently exposed to the antibiotic residues. As a result, they might have developed resistance mechanisms to the antibiotics and have a great possibility of containing multi drug resistant genes present in the hospital effluent of this particular locality to act as a possible source for transferring of these resistant genes to other bacterial population. In this study samples were collected from two different hospitals of Jessore city where hospital sewage lines are connected to the Bhairab river via city sewage line. This connectivity might lead to transferring multidrug resistant gene from hospital sewage bacteria to other strains of city sewage bacteria. So it is clear that the dissemination of multidrug resistant bacteria is increasing day by day and in near future simple infections might become untreatable due to antibiotic resistance. The possible solution to the problem of resistant bacteria in HWW is to kill the same through the establishment of waste water treatment plant and monitoring their functional status regularly so that the microbes are contained and could not be disseminated in the environment. Good safety treatment methods should be implemented before releasing of waste materials to the environment or sewage. Proper regulation and monitoring of an integrated health care liquid waste management practice is essential in order to diminish the risk of disseminating multiple drug resistant microorganisms for safeguarding of public health.

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