Untreated Liquid Hospital Waste: Potential Source of Multidrug Resistant Bacteria

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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 (e^4). 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.

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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 37ºC 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
Discussion

It has been documented that the antibiotic resistant bacteria in water sources are in a great number throughout the world 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 prescription. A patient administered with various antibiotics excretes a small amount of it through urine and other means to the environment. As a consequence, the treatment of disease with antibiotic not only kills the pathogen, but also favors the development of resistance in the environment.

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 waste1, 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 Bangladesh 20. 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 genes 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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References


