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# Presence of Multidrug Resistant Bacteria (*E. coli*) in Hospital Waste Water Regards as a Threat to Public Health in Bangladesh

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**Abstract**

Hospital waste is one of the devastative potential health hazards not only to the health care workers but also general people if these are not maintained right ways. There are a lot of pathogenic microorganisms in hospital wastes and currently, hospital waste recognized as a harbor place for the microorganisms. We aimed to identify *E.coli* from hospital waste water and their antibiotics resistant pattern against commonly used antibiotics. From 10 samples, we identified 10 isolates of *E. coli* and on the basis of antibiotic susceptibility test using 8 antibiotics, it has observed that most of the isolated bacteria became resistant to antibiotics. Among 10 isolates, 100%, 80%, 80%, 70%, 30%, 20%, 20% and 10% isolates were resistant to ampicillin, ceftazidime, cefotaxime, tetracycline, chloramphenicol, gentamycin, ciprofloxacin and azithromycin antibiotics respectively. Even we didn't find any isolate which is sensitive to all antibiotics that we have used. As antibiotics resistance is increasing day by day proper step should be taken to prevent it, as well as waste water treatment plant should be established to dispose off hospital wastes so that these can't be biological weapons for environment contamination.

**Keywords:** *E. coli*; Hospital waste water; Public health; Environment contamination

## Introduction

Although the uncovering of antimicrobials have brought the introduction of expectation but that has been afflicted by the emergence of resistant bacterial strains against antibiotics or therapeutics. 20-50% human uses of antibiotics are inessential and 40-80% agricultural uses of antibiotics are highly suspicious [1]. On account of the use and misuse of antimicrobials in the last few decades, today's clinically important bacteria have been converted from susceptible to single drug resistant and also multiple antibiotics resistant. And these multidrug resistant bacteria are becoming progressive public health hazard all over the world [2].

Hospital wastewater can be risky to public health and ecological balance as it can contain various radioactive, chemical, pharmaceutical wastes and pathogenic microorganisms [3] and, owing to heavy antibiotic use, hospital wastewater contains larger numbers of resistant organisms than domestic waste water [4].

Hospital waste effluents carry pathogenic drug- resistant bacteria and responsible for spreading pathogenic and drug resistant organisms to the environment even if it is treated [5] and about one tenth or more of the world's population consumes food produced through irrigation with recycled wastewater; treated, partially treated and/or untreated hospital waste effluents containing resistant bacteria, and that's why these resistant bacteria also have been entering in our food chain directly. Moreover Hospital waste effluents contaminate aquatic environment and that have very dangerous impact on fish and aquatic life, and others animals like ducts, chickens, human beings etc. In the Southern Netherlands, almost 80 percent of raw chicken was found to be containing multidrug-resistant bacteria and these germs were identical with the specimens collected from hospital patients [6].

Hospital effluent with its high content of multidrug resistant Enterobacter and the presence of enteric pathogens could pose a grave problem for the community [7-9]. The main risk for public health is that resistance genes are transferred from environmental bacteria to human pathogen [10,11]. The bacteriophages in the samples of animal's fecal wastes can be environmental vectors for the horizontal transfer of antibiotic resistance genes [12].

Data on the significance of environmental contamination with antimicrobial-resistant *E. coli* for human health are limited. In Bangladesh especially, in south part of Bangladesh there is no data concerning resistance profiles of *E. coli* (against ampicillin 10μg, tetracycline 30μg, ceftazidime 30μg, cefotaxime 30μg, gentamycin 10μg, azithromycin 15μg, ciprofloxacin 5μg and chloramphenicol 30μg antibiotics) isolated from hospital effluents. This study is therefore, attempt to generate original local data and examine the magnitude of drug resistance E. coli in hospital wastewater in Noakhali, Bangladesh.

## Methods

### Study setting and sampling

A cross-sectional study was conducted from November 2015 to September 2016 at General Hospital, Prime hospital, Good heal hospital, and Royal Hospital in Noakhali. These medical hospital provides health service to over than 3 million inhabitants in South-eastern Bangladesh, and is located 151km South-eastern from the capital city, Dhaka, Bangladesh.

### Hospital waste water samples

Fifteen untreated hospital wastewater samples were collected from General Hospital, Prime hospital, Good heal hospital, and Royal Hospital main drainage system at different sites.

### Sample processing, isolation and identification of bacteria

Samples were transported to the Microbiology laboratory of Noakhali Science and Technology University in cool conditions and processed within two hours of collection. A 100mL aliquot of waste water of serial ten-fold dilutions for each sample was filtered through a 0.22μm pore membrane (Millipore, Billerica, MA) which was then placed on plate count agar and incubated aerobically at 37 °C for 24-48 hours. After incubation, based on colony morphology representative colonies were picked and sub-cultured on different selective and differential media as MacConkey agar, EMB agar. After obtaining pure colonies with special colony color in selective and differential agar media, the isolated organism was identified biochemically in a systematic way following standard methods [13].

### Preparation and application of the disc to the plates

The antibiotic sensitivity pattern was determined by the disc diffusion method [14]. For the antibiotic sensitivity pattern test we prepared nutrient broth and a single colony from the pure culture of the isolates were inoculated in the broth and incubated for 2hrs at 35±2 °C. Using sterile cotton with soaked solution, the isolated and identified 10 E. coli were spread on a Mueller-Hinton agar plate by rubbing the cotton bud thoroughly on the surface of the plate. Then 8 different types of antibiotic discs as ampicillin (10μg), ciprofloxacin (5μg), tetracycline (30μg), chloramphenicol (30μg), ceftazidime (30μg), gentamicin (10μg), azithromycin (15μg), cefotaxime (30μg)antibiotics discs (Table 1) were placed on the surface of the inoculated Mueller-Hinton agar plates and incubated for 24hrs at 35±2 °C. The sensitivity pattern was determined by measuring the zones of inhibition with a calibrated ruler and interpreted according to standard guidelines for Clinical Laboratory standards (CLSI) criteria [15]. Then the test organism is reported as 'sensitive' (S), 'intermediate' (I), or 'resistant' (R).

**Table 1:** Antibiotic resistance pattern assessment of isolated E. coli.

| Isolates | Ampicillin | Ceftazidime | Cefotaxime | Tetracycline | Chloramphenicol | Gentamycin | Ciprofloxacin | Azithromycin |
|---|---|---|---|---|---|---|---|---|
| 1 | R | R | R | I | R | S | S | S |
| 2 | R | R | R | S | S | S | S | S |
| 3 | R | S | R | R | R | S | R | S |
| 4 | R | R | R | R | R | R | R | R |
| 5 | R | S | R | R | S | S | S | S |
| 6 | R | R | S | I | S | R | S | I |
| 7 | R | R | R | R | S | S | S | S |
| 8 | R | R | R | R | S | S | S | S |
| 9 | R | R | I | R | S | S | S | S |
| 10 | R | R | R | R | S | S | S | S |

## Results

Total 10 isolates of *E. coli* were confirmed from hospital waste samples by cultural and biochemical tests. Among 10 isolates, 100%, 80%, 80%, 70%, 30%, 20%, 20% and 10% isolates were resistant to ampicillin, ceftazidime, cefotaxime, tetracycline, chloramphenicol, gentamycin, ciprofloxacin and azithromycin antibiotics respectively (Figure 1). And 0%, 20%, 10%, 10%, 70%, 80%, 80% and 80% were sensitive to ampicillin, ceftazidime, cefotaxime, tetracycline, chloramphenicol, gentamycin, ciprofloxacin and azithromycin antibiotics respectively, while 0%, 0%, 10%, 20%, 0%, 0%, 0% and 10% isolates were in intermediate against ampicillin, ceftazidime, cefotaxime, tetracycline, chloramphenicol, gentamycin, ciprofloxacin and azithromycin antibiotics respectively (Figure 1). Isolate 4 was resistant to all antibiotics (Table 1).



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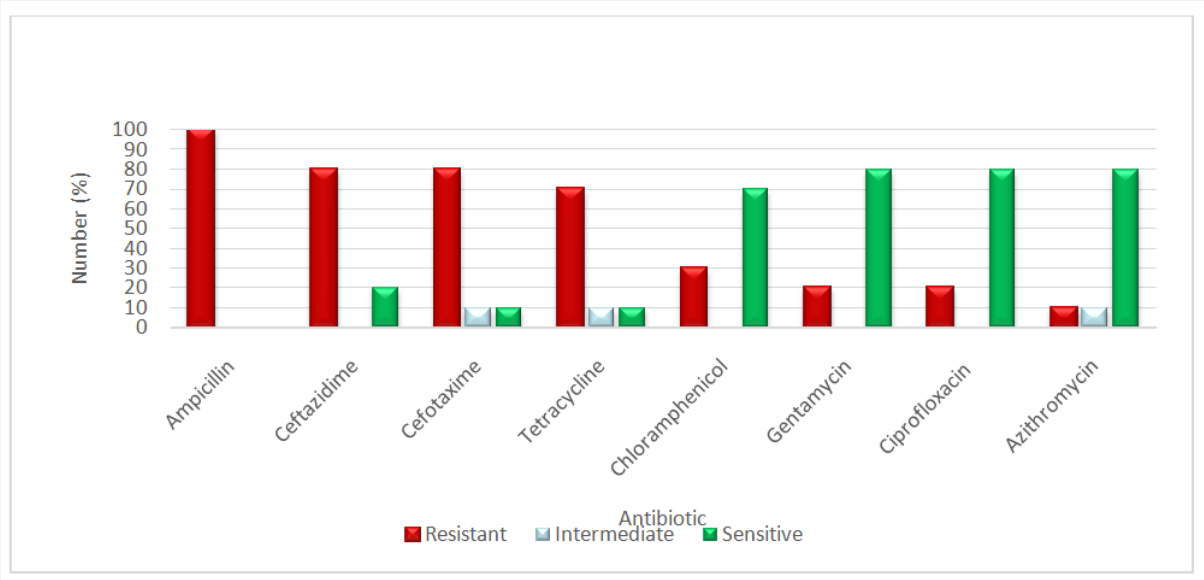


**Figure 1**: Antibiotic sensitivity and resistance pattern chart of isolated microorganism against different antibiotics.

## Discussion

Our study on the isolation, identification and antimicrobial resistance profiles of Escherichia coli from hospital wastes in Noakhali, Bangladesh revealed that antibiotics resistance have been developed vigorously. This indicates it might go to create a devastated health hazard in Noakhali district as well as Bangladesh in near future. The presence of multidrug resistance in *E. coli* was not uncommon now a days and its severity is expanded from town to village broadly everywhere. It was a matter of concern that not a single antibiotic was found which was effective against all isolates. Owing to the use and misuse of antimicrobials bacteria have been converted from susceptible to single drug resistant and also multiple antibiotics resistant. In Bangladesh most of the patients take antibiotics without doctor's prescription, though sometime they take antibiotics according to doctor's prescription but they do not complete the full doses of antibiotics [16]. In our study almost all isolates showed resistance to ampicillin, more than 70% isolates were resistant against ceftazidime, cefotaxime, tetracycline and some isolates also were resistant to chloramphenicol, gentamycin, ciprofloxacin and azithromycin broad spectrum antibiotics. These findings suggest that the hospital waste water has been discharged without treatment. This implies that the multidrug resistant pathogenic organisms might also have been present in such hospital waste water [5].

These water can mix with aquatic environment during the rainy season, as well as these water also have been used to cultivate crops in developing countries like Bangladesh. That's why these resistant bacteria also have been entering in our food chain directly. In the Southern Netherlands, almost 80 percent of raw chicken was found to be containing multidrug-resistant bacteria and these germs were identical with the specimens collected from hospital patients [6]. So, the main risk for public health is that resistance genes are transferred from environmental bacteria to human pathogen [10,11,17]; as the bacteriophages in the samples of animal's fecal wastes as well as hospitals fecal wastes can be environmental vectors for the horizontal transfer of antibiotic resistance genes [12].

To solve the problem proper antibiotics should be used at proper doses to avoid the development of multi-drug resistant bacteria as well as should establish waste water treatment plant and monitoring their functional status regularly so that the microbes are killed and disposed off properly to avoid the spread of multi-drug resistant bacteria in the environment. Otherwise one day a horrible time can come when no antibiotics will not work against bacteria as well as other microorganisms. As this is the first study in Bangladesh especially in Noakhali district about multidrug resistant *E. coli* in hospitals waste water, similar further studies are suggested about multidrug resistant microorganisms in hospital waste water.

## Conclusion

The overall implication of these results is that antibiotics resistance is increasing day by day especially in the hospitals as well as hospitals waste water. If preventive care is not taken, some terrible time will come when these multidrug resistant bacteria will directly mix with our food chain and no dose or level of antibiotic will be effective against bacterial diseases as well as hospital waste water will be vehicles for the transmission of biological weapons.

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