

Research Article

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Multidrug Resistant *E. coli* in Hospital Waste Water: A Potential Concern for Public Health



Sharmin Akther¹, Tonmoy Debnath^{1,2} and Mohammed Mehadi Hassan Chowdhury^{1*}

¹Department of Microbiology, Noakhali Science and Technology University, Bangladesh

²Department of Public Health and Institute of Public Health, Chung Shan Medical University, Taiwan

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*Corresponding author: Mohammed Mehadi Hassan Chowdhury, Department of Microbiology, Noakhali Science and Technology University, Noakhali-3814, Bangladesh, Email: mehadi@nstu.edu.bd

Abstract

Hospital waste is not only a devastating hazard in environment, but also a potential risk to general people, if the waste is not managed in the right ways. Currently, hospital waste is recognized as a harbour of pathogenic microorganisms. We aimed to identify *E. coli* from hospital waste water and their antibiotic resistant pattern against commonly used antibiotics. From 10 samples, we identified 10 isolates of *E. coli*. We have used 8 commonly used antibiotics against the isolated bacteria, and finally it has observed that most of the isolated bacteria showed resistance to antibiotics. Among the 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. In addition, we didn't find any isolate which was sensitive to all of the antibiotics that we had used. As resistance of these microorganisms is evolving day by day, proper steps should be taken to prevent it. Moreover, waste water treatment plan should be implemented to dispose of hospital wastes, so that these can't be microbiological hazard for environmental contamination.

Keywords: *E. coli*; Hospital waste water; Public health; Environment contamination; Multi-drug resistant bacteria

Introduction

Although the discovery of antimicrobials leads to various expectations, it has been influenced by the emergence of resistant bacterial strains against antibiotics or therapeutics. 20-50% of humans are using antibiotics unnecessarily and 40-80% agricultural usages of antibiotics are highly suspicious [1]. On account of irrational use of antimicrobials in the last few decades, present clinically important bacteria have been converted from susceptible to single and multiple antibiotics resistant which lead a threat in public health sector in Bangladesh [2,3] as well as whole world in general [4].

Hospital waste water can be a potential risk factor for public health and ecological balance, since it contains various hazardous components including pathogenic microorganisms [5]. Moreover, owing to heavy antibiotic use, hospital waste waters contain high numbers of antibiotic resistant microorganisms than domestic wastewaters [6]. Hospital waste effluents carry pathogenic multidrug resistant microorganisms and are responsible for the spread of these organisms to the environment. Sometimes, a treated hospital wastewater can also spread multidrug resistant microorganisms [7]. Hospital waste effluents contaminate aquatic environments causing fish

and other sea creatures dangerously effected. In one research, it was found that almost 80 percent of raw chickens contained multidrug-resistant bacteria and these were identical with the specimens collected from hospital patients [8]. Moreover, it has been reported that, the irrigation water system also has been contaminated by these multidrug resistant bacteria which have a chance to enter in our food chain directly.

The presence of multidrug resistant enterobacter and enteric pathogens has been regarded as a serious problem for a community [9-11]. In some research, it had showed that the resistance genes were transferred from environmental bacteria to human pathogen [12,13] that can be a major threat for a community. Sometimes, the animals' fecal wastes containing bacteriophages can act as the vectors for the horizontal transfer of antibiotic resistance genes [14].

However, the data about environmental contamination with antimicrobial resistant *E. coli* for human health are limited. In Bangladesh, especially in south part of Bangladesh, multidrug resistance profile of *E. coli* against antibiotics is not available specially which are isolated from hospital effluents. Therefore, this study is an attempt to get a scenario on antimicrobials

resistance pattern of *E. coli* isolated from hospital wastewater in Noakhali, Bangladesh.

Methods

Preparation and sampling

This cross-sectional study was conducted for a period of 6 months examining the waste water of different hospitals in Noakhali, Bangladesh. Ten untreated hospital waste water samples were collected from over-mentioned hospitals' main drainage systems at several different locations.

Sample processing, isolation and identification of bacteria

The waste water samples were inoculated on selective and differentiate media including MacConkey agar, EMB agar for the presumptive isolation and identification of *E. coli*. Then incubate the plates with samples at 35 °C±2 for 24 hours. The obtaining selective colonies on selective and differential agar media were then applied for different biochemical tests including indole test,

urease test, triple sugar iron (TSI) test, catalase test, citrate test, coagulase test and oxidase test according to the guideline of the Bergery's Manual of Determinative Bacteriology [15].

Preparation and application of the disc to the plates

The antibiotic sensitivity pattern was examined by the disc diffusion method [16]. The isolated and identified 10 *E. coli* isolates were spread on a Mueller-Hinton agar plate by rubbing the cotton bud thoroughly on the surface of the plate. 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 then placed on the inoculated Mueller-Hinton agar plates and were then incubated for 24 hrs at 35 °C. The sensitivity pattern of the isolates was determined by measuring the zones of inhibition with a calibrated ruler, also interpreted according to Clinical Laboratory standards (CLSI) criteria [17]. The tested organisms were then organized into 'sensitive' (S), 'intermediate' (I), or 'resistant' (R).

Results

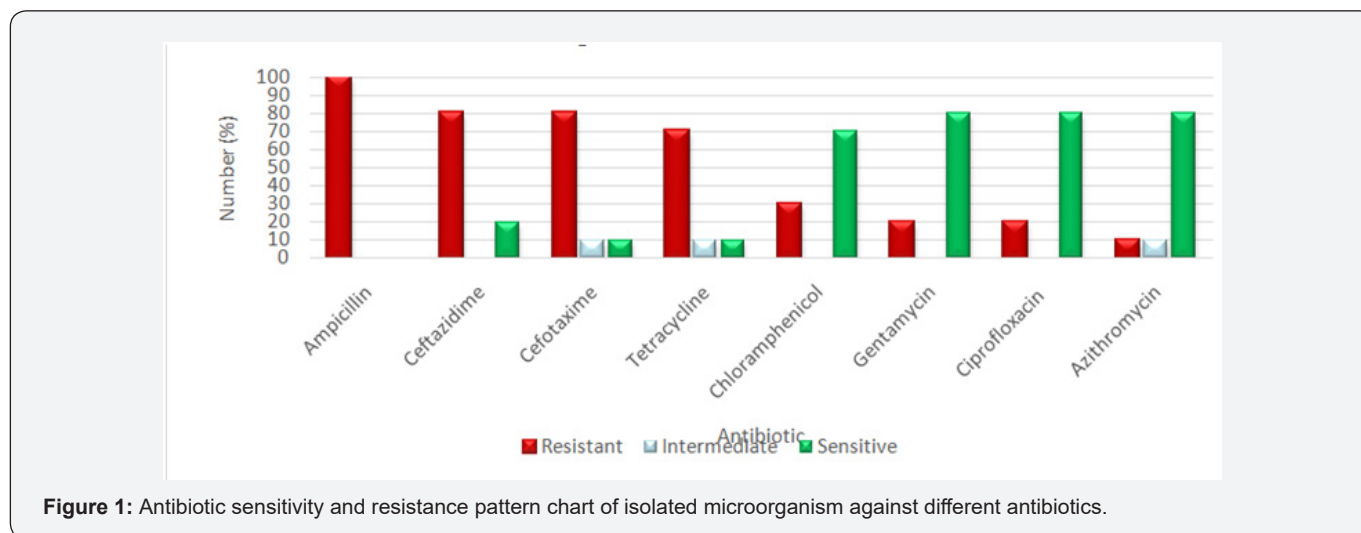


Figure 1: Antibiotic sensitivity and resistance pattern chart of isolated microorganism against different antibiotics.

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

Isolates	Ampicillin	Ceftazidime	Cefotaxime	Tetracycline	Chloramphenicol	Gentamicin	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

All 10 isolates of *E. coli* were confirmed from hospital waste samples by cultural and biochemical tests. Among the isolated *E. coli*; the 100%, 80%, 80%, 70%, 30%, 20%, 20% and 10%

isolates were resistant to ampicillin, ceftazidime, cefotaxime, tetracycline, chloramphenicol, gentamicin, ciprofloxacin and azithromycin antibiotics respectively (Figure 1). The 0%,

20%, 10%, 10%, 70%, 80%, 80% and 80% isolates were sensitive to ampicillin, ceftazidime, cefotaxime, tetracycline, chloramphenicol, gentamycin, ciprofloxacin and azithromycin antibiotics respectively, while, the 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 numbered 4 was resistant to all antibiotics (Table 1).

Discussion

Since gram negative bacteria can cause serious public health problem [18,19], it is important to conduct more research in order to create public awareness. Due to irrational apply of antibiotics in last few years, the number of antibiotic resistant bacteria is increasing day by day and the condition is becoming worse. Developing countries [20,21], such as Bangladesh, are especially being affected. Although environmental microorganisms can be clinical microorganisms when they infect animals or humans [8], studies about antibiotics resistance patterns of environmental microorganisms are limited.

In this study, the resistance patterns of *E. coli* isolates all had different results. Only one isolate showed resistance against all of used antibiotics. We claimed that these highly resistant isolates of *E. coli* might be responsible for transfer their resistant genes into other highly infectious pathogens present in the waste water [12,13,22]. In addition, the bacteriophages of animal's fecal wastes samples as well as the fecal wastes in hospitals can become environmental vectors for the horizontal transfer of antibiotic resistance genes [14]. The rapid development of antibiotic resistant pattern of *E. coli* as well as other microorganisms might create a devastating health problem [2,8], especially in the Noakhali district as well as in Bangladesh in near future.

The presence of multidrug resistance in *E. coli* is not uncommon recently, and its severity has been expanded from town to village and village to everywhere very rapidly. In previous studies, ampicillin, ceftazidime, cefotaxime, tetracycline, chloramphenicol, gentamycin, ciprofloxacin and azithromycin are commonly used in hospitals as treatment. In contrast, one study was conducted in Mexico on hospitalized diarrheal patients revealed that 73% of *E. coli* isolates were resistant to ampicillin [23] whereas, we estimated that 100% of *E. coli* isolates were resistant to ampicillin while 80% isolates were resistant to ceftazidime and cefotaxime. This shows us that the rate of bacteria's resistance to antibiotics is developing rapidly. This fluctuation may be due to different places and different sources of samples. However, it is thought that the isolates from sewages of waste disposal systems have higher chance to be resistant than other sources.

Another study conducted in Pakistan wherein the *E. coli* was resistant to cefotaxime (89.7%), ceftazidime (73.8%), gentamycin (59.8%), ciprofloxacin (54.2%), imipenem (43.3%),

streptomycin (30%) and kanamycin (19.9%) [24] and our research in accordance with that study with little fluctuations.

Islam et al. [25] reported that all of isolates of *E. coli* from the liquid hospital waste water were resistant to more than 3 kinds of antibiotics and in our research we also found similar results [25]. This study was revealed a new concern for focusing on a new site of contamination. Large sample volume and molecular techniques are needed to find out more significant result on multidrug resistant *E. coli* isolated from hospital waste water in future.

Conclusion

The overall implication of these results is that antibiotics resistance of environmental isolates is increasing day by day especially in hospitals waste water. If preventive measures are not implemented urgently, there will be a time when these multidrug resistant environmental isolates will be act as a vector for transferring their resistant genes to other potential infectious pathogens and will lead a microbial outbreak.

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