Antibiotic Resistance Bacteria Associated in Waters Discharges of a Hospital in Patna

Vyomesh Vibhaw*1, Kumar Pranay1, Krishnadutt Pratihast1, Bipin Bihari Mishra1 and S. R. Padmadeo2

1 Department of Biochemistry, Patna University, Patna, Bihar, India
2 Department of Botany, Patna University, Patna, Bihar, India

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ABSTRACT

The isolation and screening of antibiotic resistance strains from three samples sites present in and around hospital of Patna resulted in isolation of 300 bacterial isolates. Out of which maximum number of isolates were obtained from site B while site A gave least number of isolates. 89 bacterial strains showed antibiotic resistance. Among these 89 isolates Site B had maximum number of antibiotic resistant isolates (51) followed by site C with 33 isolates. Among the four groups of antibiotic selected for investigation, the isolates showed highest antibiotic resistance against fluoroquinolones with 1, 20 and 14 isolates from site A, site B and site C respectively showing antibiotic resistance. Site B isolates showed significant resistance against group III (aminoglycosides) antibiotic. Site C isolates also showed maximum resistance against Group IV antibiotics followed by Group II, III and Group I. Site A sample showed resistance against Ciprofloxacin, however it showed sensitivity against all other antibiotics. Study showed that there was a dynamic flux in the response of E. coli strains against antibiotic. Antibiotic resistant E. coli was present in wastewater.

Key words: bacterial isolates, antibiotic resistance, E. coli.

INTRODUCTION

Antibiotic represents a fundamental triumph of medical science because it allows effective treatment of bacterial infections. However their widespread use in clinical and agriculture sectors has lead to the fast emergence of resistance. Antibiotics remain unmetabolised in humans and animals and added to the environment via excretion. This contributes to the residues of antibiotics in recipient waters. Pharmaceutical plants and dumping of unused antibiotics also play a role in accumulation of antibiotics in environment [1, 2]. Resistant (R) bacteria enter into the aquatic environment from other sources too [3]. The direct relationship between the presence of antibiotics in the environment and the R bacteria found there is not clearly established. However, the presence of antibiotic residues in the aquatic environment poses a significant to aquatic communities and ultimately to public health [3, 4, 5].

Researches concerning antibiotic residues in hospital effluent and in other environment niches have been conducted mostly in developed nations and there is dearth of research in developing and under developed nations [1, 6, 7, 8]. Very few studies are available on antibiotic prescription in a hospital and potentially R bacteria in the effluent of the same hospital [7, 9, 10]. The present study was designed to isolate, screen and characterize the multidrug resistant bacteria in hospital effluent.

MATERIALS AND METHODS

The study was conducted in the state of Bihar in India. Specially, the setting was the Patna Medical College and Hospital (PMCH) located in Patna, the
capital of Bihar. Three sub studies were conducted (i) Isolation and screening of antibiotic R strains of bacteria (ii) Selection of Multidrug R strain (iii) Biochemical characterization of antibiotic R strains.

**Selection of Antibiotics**

Ten antibiotics from four major antibiotic groups – Amikacin and Gentamycin (Group I, Aminoglycosides), Azithromycin and Erythromycin (Group II, Macrolide-Lincosamide), Chloramphenicol (Group III, Chloramphenicol), Ciprofloxacin, Ofloxacin, Norfloxacin, Pefloxacin, Levofloxacin (Group IV, Floroquinolones) were selected for study. The abovementioned antibiotics were selected on the basis of antibiotic prescription pattern in the wards of the hospital, the rate of metabolism of antibiotics in human body, environmental stability and the environmental impact of the antibiotic.

**Antibiotic Prescription study**

Information regarding the prescribed antibiotics to patients was obtained from the hospital records.

**Selection of sampling sites**

Sampling sites were selected taking into account entry of safe water into the hospital system, its possibility of becoming contaminated with antibiotics in the environment. The three sampling sites were:- (a) Incoming safe water source (Site A), (b) The point of exit of wastewater from wards of the hospital (Site B) and (c) 200 meters downstream from the hospital in subsequent drainage system (Site C).

**Sampling Protocol**

Samples were collected twice at 10:00 hrs and at 17:00 hrs. Sample (250 ml) from each site was collected for analysis of antibiotic susceptibility and was stored in an ice box at 4°C. Samples were collected in sterilized and paper wrapped 300 ml BOD bottles and were transferred to the Department of Biochemistry, Patna University, Patna.

**Isolation of Antibiotic resistant bacterial strains:**

Bacterial colonies were isolated on nutrient agar medium by serial dilution method. Different colonies were purified by streak plate method and were subjected to susceptibility against four groups of antibiotics on Muller-Hinton Agar by disc-diffusion method.

**E. coli antibiotic susceptibility Study**

Most Probable Number (MPN) methodology was used to study the total *Coliform* group and feed *Coliform* group of bacteria in test sample as per Standard multiple tube techniques [18]. The test procedure consisted of three phases, normally presumptive, confirmative and completed phase. The presumptive phase for the drinking water consisted of 20 tubes of 20 ml and 20 tubes containing 10 ml of Lauryl Tryptose Broath medium. For waste water samples dilutions were made with Lauryl tryptose broth medium and 10 tubes of each dilution of 10 ml, 1 ml and 0.1 ml were used. All the tubes were incubated at 37° ± 0.5° C for 24-48 hours. Tubes showing growth (turbidity) with or without gas were selected for confirmatory Phase. All Presumptive tubes showing positive results were shaken gently. Brilliant Green lactose Bile Broth tubes were inoculated with bacterial suspension consisting of 1.67×10^6 cells/ml. Tubes were incubated at 37° ± 0.5° C. Gas formation within 48 hours was taken as positive confirmation. Simultaneously EC Broth tubes were also inoculated for completed phase and incubated at 43.5° C. Tubes showing acid and gas production within 24 hours were taken as positive result indicating presence of *fecal* *coliform* in the sample. *E. coli* were further confirmed by subculturing on MacConkey Agar to obtain pure culture. These isolates were tested for susceptibility using the standard Kirby Bauer disc diffusion method for ten antibiotics according to the standard protocols prescribed by Clinical and Laboratory Standard Institute (CLSI) [19]. The turbidity of the inoculums matching with McFarland 0.5 standard was used to inoculate Muller - Hinton Agar plates using a cotton swab dipped in inoculums. The antibiotics discs were placed on the inoculated plates and then incubated at 37° ± 0.5° C for 18-20 hours. The diameter (in mm) of the zone of inhibition was measured and interpreted as resistant, intermediates or sensitive using CLSI guidelines for the respective antibiotics.

**RESULTS**

Water samples were aseptically procured from three sites namely site A, site B and Site C. The samples were used to isolate antibiotic resistant bacterial strains by serial dilution method. Pure cultures were obtained by streak plate method. 300 isolates were obtained from three sites out of which 127 were isolated from site B while 63 and 110 isolates were selected from site A and site C respectively (Fig. 1.)
Fig. 1. Graphical representation of total number of bacterial strains isolated from samples collected from waters associated with the hospital in Patna.

Site A= Incoming safe water; Site B= At the point of exit of inpatient wards from the hospital; Site C= 200 meters from the hospital in subsequent drains.

Table 1: Antibiotic susceptibility of E. coli found in samples taken from waters associated with a hospital in Patna, Bihar

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Disc Concentration (µg)</th>
<th>Site A*</th>
<th>Site B</th>
<th>Site C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ground Water At 10:00</td>
<td>At 17:00</td>
<td>Ground Water At 10:00</td>
</tr>
<tr>
<td>Amikacin</td>
<td>30</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>30</td>
<td>S</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>Ampoxicillin</td>
<td>30</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>30</td>
<td>R</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>15</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>10</td>
<td>I</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>5</td>
<td>S</td>
<td>S</td>
<td>I</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>10</td>
<td>S</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>5</td>
<td>S</td>
<td>S</td>
<td>I</td>
</tr>
<tr>
<td>Pefloxacin</td>
<td>5</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
</tbody>
</table>

R= Resistant, I=Intermediate, S= Sensitive; Site A= Incoming safe water.*No E. coli was detected in municipal water supply. Site B= At the point of exit of inpatient wards from the hospital; Site C= 200 meters from the hospital in subsequent drains.
The antibiotic susceptibility pattern of all the isolates was done by Kirby disc diffusion method on Muller Hinton Agar. The susceptibility led to selection of 89 isolates showing antibiotic resistance (Fig. 1.). Out of 89 isolates, site B harbored highest number of antibiotic resistance strains (total no=51), while site A had least number of strains showing antibiotic resistance (Total no=5). Site C had 33 isolates showing antibiotic resistance property (Fig.1.). Among the four groups of antibiotic selected for investigation, the isolates showed highest antibiotic resistance against fluoroquinolones with 1, 20 and 14 isolates from site A, site B and site C respectively showing antibiotic resistance (Fig. 2.). Site B isolates showed significant resistance against group III (aminoglycosides) antibiotic (Fig.2.). Site C isolates also showed maximum resistance against Group IV antibiotics followed by Group II, III and Group I (Fig. 2.).

No E. coli was detected in municipal waste supply. Site A sample showed resistance against Ciprofloxacin, however it showed sensitivity against all other antibiotics. Study showed that there was a dynamic flux in the response of E. coli strains selected from the three sites (Table 1). Significant no. of antibiotic resistance E. coli was present in wastewater (Table 1).

**DISCUSSION**

To our Knowledge, very few studies of Patna hospital effluents with regard to presence of antibiotic resistant strains have taken place. From this study, it appeared that many of the antibiotics are reaching nearby aquatic environment. There was a significant increase in the number of antibiotic resistant bacterial strains in the effluent coming out of the hospital. Among the isolates maximum resistance was reported against the fluoroquinolones and similar reports have come out of studies on the effluent of hospitals in different parts of the world [1, 8-11, 22-24]. In our investigation, E. coli resistance against amikacin was not detected in any of the collected samples. E. coli from the wastewater sample showed resistance to azithromycin as well as intermediate behavior. E. coli isolated from the wastewater samples was resistant to Ciprofloxacin but showed intermediate behaviour in three samples. Antibiotics have been found to be present in hospital effluent in high-income countries [3]. The present study has shown that antibiotics also enter the aquatic environment through hospital wastewaters in low-and middle-income countries. This might pose a greater threat in these countries due to potential scarcity of resources for treatment of wastewaters. In India, for example, not much wastewater undergoes any type of treatment and along with other pollutants; antibiotics must also be finding their way into recipient waters like rivers, lakes, reservoirs, etc. In situations where the wastewater is not subjected to any treatment, it is sometimes argued that antibiotics are diluted in the recipient waters in comparison with therapeutic concentrations and may not cause any harm. However, it is widely accepted that exposure to sub-therapeutic concentrations over long periods of time provides suitable conditions for the transfer of resistance genes.

**CONCLUSION**

The present study highlights the fact that hospital effluents are a major source of antibiotic resistance against many broad spectrum antibiotics. The fact that antibiotic resistance was found to be present in drains having the water discharge from the hospitals present a worrisome trend which needs more detailed investigation and highly effective planning.

**REFERENCES**