A Comparative Study on Escherichia coli Isolates from Environmental and Clinical Samples

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Pathogenic Escherichia coli remain an important etiological agent of infantile diarrhea in Bangladesh. Present study focused on the features of E. coli strains isolated from clinical and environmental samples. A total of 6 surface water samples and 6 clinical samples were examined. Isolates were analyzed on the basis of colony morphology and biochemical traits. The antibiogram profile of the isolates was determined against 6 commonly used antibiotics. Ten isolates were found to be resistant against more than 1 antibiotic. Both types of isolates showed 50% resistance against trimethoprim-sulfamethoxazole. Clinical isolates showed 100% sensitivity against gentamicin whereas 2 environmental isolates were found to be resistant against the antibiotic. Eighty and sixty five percent of environmental strains were sensitive against ceptriaxone and ciprofloxacin, respectively; while 50% showed resistance against ampicillin and nalidixic acid. In case of clinical strains, 65% isolates were found to be sensitive against ceptriaxone whereas 65% showed resistance against ampicillin and nalidixic acid; and 50% isolates were found to be resistant against ciprofloxacin. The isolates (n=12) were also examined for the presence of plasmids conferring the antibiotic resistance. However, no such plasmid was observed.

Key words: E. coli, clinical strain, environmental strain, antibiotic resistance plasmid.

Introduction

Diarrhea is a world-wide health problem which ranges from a trivial nuisance for travelers to a fatal illness, particularly among children in underdeveloped countries¹. It is one of the world’s leading causes of morbidity and mortality, accounting for around two million deaths each year². Escherichia coli strains belong to the coliform group of microorganisms which are a common part of the normal facultative anaerobic microflora of the intestinal tracts of most mammals including humans.³ E. coli, belonging to the Enterobacteriaceae family, is a commensal bacterium in the intestinal tracts of warm-blooded animals; and some strains have acquired virulence causing intestinal or extra intestinal infections.³

Pathogenic forms of E. coli associated with human and animal diseases are remarkably diverse.⁴ Six E. coli viotypes have been identified so far: enterohemorrhagic E. coli (EHEC) or verocytotoxigenic E. coli (VTEC), enterotoxigenic E. coli (ETEC), enteroinvasive E. coli (EIEC), enteropathogenic E. coli (EPEC), enteroaggregative E. coli (EAEC) and diffusely adherent E. coli (DAEC)⁴. Currently, antibiotic resistance is another vital issue in E. coli pathogenesis. E. coli is a prime candidate as a species in which new multi-drug resistant plasmids may evolve⁵. It has a very wide natural distribution⁵ and a propensity for plasmid carriage⁵. Horizontal gene transfer is a recognized process that allows the rapid spread of antibiotic resistance genes inside and outside the hospitals, thereby impeding antimicrobial chemotherapy. Resistance determinants are readily acquired and disseminated within bacterial populations by conjugation, and hence resulting in a new phenotype⁷. R-plasmids are established to be promiscuously transferred among different enteric bacteria⁸. Typically, 10-20% of gut E. coli of healthy people (1960-1970) has R-plasmids and is found to be antibiotic resistant. Bacteria have been reported to produce colicins, lethal compounds that kill other bacteria which are specified by transmissible plasmids similar to F plasmid, for instance, ColE, ColV plasmids⁹.

Water is another important factor for E. coli pathogenesis. Surface waters in tropical countries have been found to harbor pathogenic E. coli strains and their transmission can occur while bathing and/or using water for cooking⁴. These forms of transmission are common in areas where it is endemic both in the local populations and among the international travelers across these areas. Contaminated food and water sources both contribute to the seasonal outbreaks which affect tourists⁴.

In the line of these evidences and facts, present study attempted to conduct the isolation and identification of E. coli strains from ponds, lakes, drains and stool samples. Antibiogram patterns of the isolates were also examined. In addition, presence of any drug resistance conferring plasmid was tested.

Materials and Methods

Samples and Sampling Sites

A total of 12 samples, 6 environmental and 6 clinical, were included in this study. Environmental samples were collected...
from ponds, lakes, and drain water (areas of Dhaka University, Gulisthan, Dhanmondi and Siddeswari). For clinical samples, stool specimens were collected from suspected patients admitted to Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM) hospital. Sample collection was carried out in between February 2009 and March 2009.

**Sample collection**

The environmental samples were collected aseptically in sterile polyethylene terephthalate (PET) bottles. For clinical samples, stool specimens from suspected diarrheal patients were collected aseptically in sterile containers. Samples were transported to the laboratory as quick as possible. Environmental samples were kept at room temperature and the clinical samples were preserved at 4 °C.

**Isolation of escherichia coli**

Fifty microliter of each sample was inoculated onto MacConkey agar medium by spread plate method. Two or three loopful of the samples were also inoculated onto MacConkey agar by streak plate method. Culture plates were then incubated at 37 °C for 24 h. Presumptive *E. coli* colonies were subcultured onto MacConkey agar to obtain pure cultures. The pure colonies of isolates were again grown on Eosin Methylene Blue (EMB) agar and the characteristic greenish metallic sheen was produced. Xylose Lysine Deoxycholate (XLD) agar were also used to observe the characteristic yellow colonies.

**Identification of escherichia coli isolates**

According to Cappuccino and Shernan (1996), confirmative biochemical tests including the sugar fermentation, citrate utilization, motility indole urease and oxidase tests were performed to identify *E. coli* isolates. Microscopic observation of the isolates was also performed.

**Determination of antimicrobial susceptibility by disc-diffusion method**

Susceptibility of environmental isolates against different antimicrobial agents was determined in vitro by employing the standardized agar disc-diffusion method, more commonly known as the ‘Kirby-Bauer Method’. A suspension of the test organism was prepared by adjusting the turbidity of the broth in phosphate buffer saline by comparing with that of 0.5 McFarland solution. By means of a sterile cotton swab, a uniform lawn of bacterial growth (10^8 cfu/ml) was prepared on Muller-Hinton agar plates (pH 7.0). Before streaking, the swab was passed against the wall of the tube containing the suspension to let the excess fluid drain out. Commercially available antimicrobial discs (Oxoid, UK) were used for the test. Ampicillin (AMP), gentamicin (CN), trimethoprim-sulfamethoxazole (SXT), nalidixic acid (NA), ciprofloxacin (CIP) and ceftiapone (CRO) were tested at a concentration of 10 μg, 10 μg, 25 μg, 30 μg, 5 μg, and 30 μg, consecutively against the isolates. Antibiotic discs were applied aseptically on the surface of the inoculated plates at appropriate spatial arrangement by means of sterile forceps. Plates were then inverted and incubated at 37°C for 24 h. Susceptibility against the specific drug was interpreted by the presence of clear zone around the disc. The zone diameters for individual antimicrobial agent were translated into susceptible, intermediate and resistant according to the interpretations described by Barry and Thornberry (1985).

**Observation of plasmid profile**

Plasmid extraction was carried out according to the modified method of Birnboim and Doly (1979). Fresh *E. coli* suspension (1.5 ml) from the log phase culture was taken in eppendorf tubes and was spun for 10 min at 13,000 rpm in a micro-centrifuge (NF 800R, Niwe). The supernatant was aspirated and the cells were resuspended in 500 μl solution I (2 mg/ml lysozyme, 50 mM glucose, 10 mM cyclohexane diamine tetracetate and 25 mM Tris-HC1, pH 8.0) by recentrifugation. The supernatants were removed and the pellets were completely resuspended in 100 μl of solution I. The tubes were then kept at 30 °C for 30 minutes. 200 μl solution II (0.2 N NaOH and 1% sodium dodecyl sulfate) was added to the tubes and was mixed gently by sample inversion following incubation on ice for 5 min. 150 μl of 3 M sodium acetate (pH 4.8) was added immediately and was mixed gently by inversion until the DNA precipitated. The mixtures were kept on ice for 60 min. Afterwards, the mixtures were centrifuged for 10 min at 13,000 rpm to precipitate the chromosomal DNA. The clear supernatants (400 μl) were transferred to sterile eppendorf tubes. Ice-cold (∼20 °C) 95% ethanol (1 ml) was mixed with the samples, then kept on ice for 30 min following centrifugation for 10 min at 13,000 rpm. The supernatants were removed and the pellets were washed with 70% alcohol and dried for 1-2 min. DNA samples were then resuspended in 25 μl of Tris-Borate (TB) buffer, and the DNA suspension was kept at 20°C until use. Presence of plasmid DNA was checked by 1% agarose gel electrophoresis.

Agarose gel was prepared by dissolving 1% agarose in 40 ml Tris-Borate-EDTA (TBE) buffer. Electrophoresis was carried out at 60 volts for 1 h, gel was placed in a container containing 10 l of ethidium bromide in 100 ml of distilled water for 30 min. Then the gel was observed under a UV-transilluminator for the presence of plasmid bands at 260 nm.

**Results**

**Phenotypic and biochemical characterization of the escherichia coli isolates**

Since the isolation and identification of *E. coli* is very crucial for the culture-based detection purpose, our study used MacConkey agar for primary isolation. Bright pink or red lactose fermenting colonies were selected as presumptive *E. coli*. Presumptive colonies were grown on EMB agar and production of greenish metallic sheen was observed. Typical
circular yellow colonies of *E. coli* was observed on XLD media. Thus, all the 12 isolates were selected as *E. coli* strains for further biochemical analysis.

Detailed biochemical study revealed that all the strains had the biochemical properties typical for *E. coli*. Both environmental and clinical isolates showed the similar biochemical properties (Tables 1 and 2). All the isolates fermented glucose with acidic reactions and produced gas in the tubes of triple sugar iron (TSI) agar. However, it was observed that more gas was produced in cases of the clinical isolates compared to that of the environmental isolates. All the strains exhibited motility and were found to be negative for citrate and urease test.

**Table 1. Biochemical characteristics of environmental *E. coli* isolates.**

<table>
<thead>
<tr>
<th>Strain ID</th>
<th>TSI</th>
<th>MIU</th>
<th>Citrate</th>
<th>Oxidase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Slant</td>
<td>Butt</td>
</tr>
<tr>
<td>C-1</td>
<td>A</td>
<td>AG</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>C-2</td>
<td>A</td>
<td>AG</td>
<td>–</td>
<td>+</td>
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<td>C-3</td>
<td>A</td>
<td>AG</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>C-4</td>
<td>A</td>
<td>AG</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>C-5</td>
<td>A</td>
<td>AG</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>C-6</td>
<td>A</td>
<td>AG</td>
<td>–</td>
<td>+</td>
</tr>
</tbody>
</table>

TSI: Triple Sugar Iron test, MIU: Motility Iodole Urease test
Mot: Motility
Ind: Iodole
K: Alkaline reaction
A: Acidic reaction
AG: Acidic reaction and gas
+ : Positive
–: Negative
C: Clinical isolates

**Table 2. Biochemical characteristics of clinical *E. coli* isolates.**

<table>
<thead>
<tr>
<th>Strain ID</th>
<th>TSI</th>
<th>MIU</th>
<th>Citrate</th>
<th>Oxidase</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Slant</td>
<td>Butt</td>
</tr>
<tr>
<td>E-1</td>
<td>A</td>
<td>AG</td>
<td>–</td>
<td>+</td>
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<tr>
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<td>AG</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>E-6</td>
<td>A</td>
<td>AG</td>
<td>–</td>
<td>+</td>
</tr>
</tbody>
</table>

TSI: Triple Sugar Iron test, MIU: Motility Iodole Urease test
Mot: Motility
Ind: Iodole
K: Alkaline reaction
A: Acidic reaction
AG: Acidic reaction and gas
+ : Positive
–: Negative
C: Clinical isolates

**Antibiotic susceptibility pattern of the isolates**

All *E. coli* strains were tested for antibiotic susceptibility against 6 commonly used antibiotics. The isolates showed variations in the susceptibility pattern against the antibiotics used in the study (Tables 3 and 4). In case of environmental isolates, 5 (80%) were susceptible against ceptriaxone (CRO), 4 were sensitive (65%) against ciprofloxacin (CIP) and gentamicin (CN), 3 were resistant (50%) against ampicillin (AMP), trimethoprim-sulfamethoxazole (STX) and nalidixic acid (NA) (Table 3 and Figure 1A). On the other hand, clinical isolates showed 100% sensitivity against CN, 4 isolates were resistant (65%) against AMP and NA, while 4 were found to be sensitive (65%) against CRO and 3 were resistant (50%) against CIP and SXT (Table 4 and Figure 1B). A total of 10 isolates (83%) were found to be resistant against more than one drug (Table 3 and 4). The antibiogram pattern of the isolates of clinical and environmental origins varied significantly (Figure 1).

**Table 3. Resistance/ Susceptibility pattern of environmental *E. coli* isolates against different antibiotics.**

<table>
<thead>
<tr>
<th>Strain ID</th>
<th>Antibiotic Susceptibility</th>
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<tbody>
<tr>
<td></td>
<td>AMP (10 µg)</td>
</tr>
<tr>
<td>E-1</td>
<td>R</td>
</tr>
<tr>
<td>E-2</td>
<td>R</td>
</tr>
<tr>
<td>E-3</td>
<td>S</td>
</tr>
<tr>
<td>E-4</td>
<td>S</td>
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<tr>
<td>E-5</td>
<td>S</td>
</tr>
<tr>
<td>E-6</td>
<td>R</td>
</tr>
</tbody>
</table>

AMP: Ampicillin
CN: Gentamicin
SXT: Trimethoprim-Sulfamethoxazole
NA: Nalidixic acid
CIP: Ciprofloxacin
CRO: Ceptriaxone
S: Sensitive
R: Resistant
C: Clinical isolates

**Table 4. Resistance/ Susceptibility pattern of clinical *E. coli* isolates against different antibiotics.**

<table>
<thead>
<tr>
<th>Strain ID</th>
<th>Antibiotic Susceptibility</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AMP (10 µg)</td>
</tr>
<tr>
<td>C-1</td>
<td>R</td>
</tr>
<tr>
<td>C-2</td>
<td>S</td>
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<tr>
<td>C-3</td>
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<td>C-4</td>
<td>R</td>
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<tr>
<td>C-5</td>
<td>S</td>
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<tr>
<td>C-6</td>
<td>R</td>
</tr>
</tbody>
</table>

AMP: Ampicillin
CN: Gentamicin
SXT: Trimethoprim-Sulfamethoxazole
NA: Nalidixic acid
CIP: Ciprofloxacin
CRO: Ceptriaxone
S: Sensitive
R: Resistant
C: Clinical isolate
Absence of plasmid bearing the drug-resistance gene

The modified method of Birnboim and Doly (1979) was applied to screen antibiotic resistant plasmids. Seven randomly selected isolates were analyzed for this purpose. However, after performing the 1% agarose gel electrophoresis, no plasmid band was visualized (data not shown).

Discussion

The presence of virulent strains of *E. coli* in the environment may be a potential source of contamination of food and the water supply, hence posing the public health risk. These strains may comprise a potential reservoir of virulence genes acquired from different sources (e.g. bacteriophages and plasmids). *E. coli* strains in aquatic environment may be subjected to a sort of genetic exchange through the lateral gene transfer. Being a competent organism, it has the capacity of horizontal gene transfer to increase the genetic diversity, and under certain circumstances, this can lead to the emergence of new pathogenic strains. According to the previous suggestive data, the present study dealt with drug resistance/sensitivity patterns of the environmental and clinical *E. coli* strains as well with the probable existence of their drug resistance plasmids.

From our study, *E. coli*, both from environmental and clinical samples, exhibited nearly similar morphological and biological traits. However, their antibiogram profile showed some variations. *E. coli* recovered in this study expressed a high level of resistance against antimicrobials that are commonly used in clinical medicine. This could contribute to the spread and persistence of the antimicrobial-resistant bacteria. Surprisingly, in the most developing countries, without identifying the pathogen first, diarrheal diseases are treated by an inadequate quantity of antimicrobials. Thus, the present study is of significance regarding the treatment of enteric diseases in Bangladesh. The emergence of resistance (mostly due to irrational use of antibiotics and inappropriate self-medication) and the decreasing levels of susceptibility of *E. coli* to a wide spectrum of antimicrobials are noteworthy since they may limit the availability of antimicrobials for clinical management of waterborne outbreaks in the future.

Some earlier studies reported that the multi-drug resistant *E. coli* isolates from surface waters were found to be positive for virulence determinants of enterohaemorrhagic *E. coli* (EHEC). Some other studies have shown that the clinical and surface water isolates of *E. coli* resistant to ciprofloxacin were multi-drug resistant. The resistance against nalidixic acid was significantly associated to cephalothin and tetracycline. Webster et al. (2004) reported that *E. coli* isolates from urban areas/point sources had resistance against more antimicrobials than those from rural/non–point sources, possibly because of frequent exposure to antimicrobials. In accordance with these reports, we also extended the investigation towards the screening of drug-resistant plasmid.

As plasmids play a major role in transferring this resistance among different strains, first we aimed to detect the presence of such plasmids. However, no plasmid could be detected in our study and hence, no further investigation on the presence of the drug-resistance gene was carried out. The possible reasons for the absence of antibiotic resistance conferring plasmid might be that the isolates might have possessed small sized plasmid or due to the plasmid instability or incompatibility. Extensive study using molecular detection methods on such plasmid might unveil the drug resistance in *E. coli*.

References


