

## Original Article

# Isolation of post operative hospital acquired infections in a private hospital of Bangladesh and determination of clonal relatedness of *E. coli* by using Pulsed field gel electrophoresis

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### Abstract

Isolation and identification of post operative hospital acquired infection was carried out from July 2008 to December 2008 in Holy Family Red Crescent Medical College Hospital (private hospital). The major pathogen of wound infection was *E. coli*. A total of 120 samples were collected from the surrounding environment of post operative room like floor, bed sheets, instruments, dressing materials, catheter, nasogastric and endotracheal tube. *E. coli* (40%) was the predominant organism followed by *S. aureus* (24%). DNA fingerprinting analysis using pulsed field gel electrophoresis of *XbaI* restriction digested genomic DNA showed that clonal relatedness between the two clinical and environmental isolates were 100%.

**Key words:** Hospital acquired, *S. aureus*, *E. coli*

### Introduction

Post operative hospital acquired infection constitutes a major problem in surgical patients contributing to morbidity, mortality and increased resource utilization and health care costs. Patients in whom SSI develop have an increased number of associated complications, higher risk of requiring a stay in ICU and 2 to 3 times higher risk of mortality. Their hospital stay is increased by 7 to 12 days and they are 5 times more likely to require readmission<sup>1</sup>.

Pulsed Field Gel Electrophoresis (PFGE) has been shown to be a valuable typing method for epidemiological investigations of several bacterial pathogen and clonality studies. In this finger printing method, chromosomal DNA is digested with a restriction endonuclease that generates large fragments. The restriction fragments are resolved in a pattern of discrete bands. The DNA restriction patterns of the isolates are then compared with one another to determine their relatedness and analysis of chromosomal DNA restriction patterns. Tenover and co-workers (1995)<sup>2</sup> have suggested that

macro restriction profiles of an isolate differing by more than six fragments positions can be considered to represent different strains. Choice of restriction enzyme is an important factor to obtain a reproducible and well discriminatory banding pattern in PFGE. For example, NotI gave the best discriminatory banding pattern for *Vibrio* spp<sup>3</sup>. As it has a long ranged DNA cutting site and cut the DNA infrequently; in this study, *XbaI* endonuclease is used for typing of all *E. coli* isolates.

There are various sources and risk factors which mediate the transmission of post operative surgical site infection. The relationship of these risk factors depends on bacterial characteristics like types and colonization of microorganisms in body surface, surgical site characteristics like pre-operative, operative and post-operative risk factors and patient related factors like diabetes, smoking, immunosuppression, etc. The control of transmission of post operative hospital acquired infection also depends on the management system of different hospitals<sup>4</sup>.

The present study is designed to see the source of bacteria (Clinical and environmental) responsible for post operative hospital acquired infection in Holy Family Red Crescent Medical College Hospital.

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## Material and methods

### Woundsampling

A total of 120 samples were collected from the different wound sites of patients from post operative ward of Holy Family Red Crescent Medical College hospital. Duplicate wound swabs; One for microscopy and other for culture was collected. All specimens were inoculated into Blood Agar, McConkey agar and Mannitol Salt Agar media and incubated 37° overnight. Bacterial isolates were identified by colony morphology, staining and appropriate biochemical tests.

### Environmental sampling

Samples were collected monthly from bed sheets, equipments, dressing material of post operative ward by swabbing (15x15 cm area) eight times with sterile swab stick remoisten with saline water and immediately inoculated onto appropriate culture medium. Similarly swab from floor of post operative room were also streaked on to blood Agar and MacConkey Agar plate and incubated at 37°C for 24 hours.

### Molecular typing of *E. coli* by Pulsed Field Gel Electrophoresis (PFGE)

PFGE was performed to determine the clonal relationship of different strains of *E. coli* collected from clinical and environmental samples.

### Preparation of PFGE agarose plugs from cell suspensions

Cell suspensions were made in 2 ml cell suspension buffer from 14-16 h Gelatinase agar plates. The concentration of the cell suspensions was adjusted with Dade Micro scan turbidity meter with standard procedure<sup>2</sup>. Lysis of cells in agarose plugs and the plugs were then disposed in cell lysis buffer. After cell lysis, agarose plugs were washed in shaken water bath (50°C) for 15 minutes.

### Restriction digestion of DNA in agarose plugs with *Xba*I

Each of the plugs was immersed in 200 µl of restriction mixer containing 20 µl of Xba I restriction buffer, 2 µl Xba I restriction enzyme, 2 µl of BSA (20U/ml) along with 176 µl filtered deionized water. Then the samples and the control tubes were incubated at 37° C water bath for at least 18 hrs.

### Electrophoresis

PFGE was performed with the contour clamped homogenous electric field (CHEF-DRII) apparatus from the Bio-Rad laboratories (Richmond, CA, USA). The temperature of the running buffer was adjusted to 14°C and the flow rate of the buffer through the electrophoresis cell was maintained approximately at 0.75 liter per minute. The gel was visualized on the UV transilluminator and photographs were taken.

Salmonella serotype Braenderup (H9812) ranging from 20.5 to 1135 kb was used as DNA size standard.

## Results

Environmental samples were collected from different sources of surrounding of patients in post operative room of Holy Family Red Crescent Medical College Hospital. A total of 120 samples were collected from the floor, bed sheets, OT instruments, dressing materials (cotton, gauze), nasogastric and endotracheal tube, catheters. *E. coli* (40%) was the predominant organism followed by *S. aureus* (24%), *Pseudomonas* (12%), *Proteus* (10%) (Table I). The commonly prevalent organisms in floor, bed sheet and trolley were *E. coli* and *S. aureus*. *Pseudomonas* was usually found in sucker and tubes and dressing materials was a good resource of *E. coli* and *S. aureus*. *Proteus*, *Bacillus*, *Citobacter*, *Acinetobacter* and *Candida albicans* was also collected from patients surrounding of post operative room. (Table I)

**Table I. Organisms in patents surroundings of operative and post operative room in private hospital.**

Source (n)	<i>E. coli</i>	<i>S. aureus</i>	<i>Pseudomonas</i>	<i>Proteus</i>	Others*
Floor(15)	3	4	2	-	1
linen bed sheet(15)	5	4	1	2	2
Trolley (15)	4	3	-	3	1
Catheter (15)	6	-	-	2	1
Sucker and tubes(15)	3	2	5	-	2
OT instruments(15)	1	-	-	-	2
Oxygen cylinder (15)	5	3	1	-	1
Dressing materials(15)	6	4	1	1	2
Total(n=120)	33 (40)	20 (24)	10( 12)	8 (10)	12 (14)

\**Bacillus* spp, *haemolytic strep*.spp, *Citrobacter* spp, *Acinetobacter* spp, *Candida* spp. α

A total of 120 samples were collected from the different wound sites of patients admitted in surgical ward in Holy Family Red Crescent Medical College Hospital. *E. coli* (57%) was the predominant organisms followed by *S. aureus* (23%) and *Pseudomonas* (12%). (Table II)

**Table II : Rate of isolation of bacteria from surgical site wound samples (n=120)**

Organisms	No. of organisms (%)
<i>E. coli</i>	68 (57)
<i>S. aureus</i>	27 (23)
<i>Pseudomonas</i>	15 (12)
<i>Proteus</i>	06 (5)
<i>Klebsiella</i>	03 (2)
<i>Streptococcus pyogens</i>	01 (1)
<i>Acinetobacter</i> spp.	00 (0.00)

A dendrogram based on similarities of *Xba*I-digested DNA

PFGE patterns among *E. coli* strains was created with the Bionumerics software using Dice similarity as described by Beutin *et, al* (2000)<sup>5</sup>. PFGE patterns were arranged in two clusters, A and B, which showed 59.74% similarity in their banding patterns (Figure 1). Cluster B again divided into B1 and B2. B1 cluster is again subdivided into B1a and B2a. B1a contains 6 strains (E-02, E-06, E-20, E-04, E-05) of clinical and environmental isolates with clonal relatedness. In B2a, there are 7 strains (E-07, E-11, E-03, E-17, E-14, E-15 and E-12) of clinical and environmental isolates of clonal relatedness. Among them, E-14 of clinical isolate has 100% clonal relationship with environmental isolate E-15. E-14 was isolated from post operative patient of HFRCMCH and environmental sample E-15 was collected from the trolley of the same room. So, may be trolley is the source of that patient's hospital acquired wound infection. B2 is again subdivided into B2a and B2b. B2a contains 4 clinical isolates (E-13, E-16, E-09, E-18) of clonal relatedness. There is no environmental isolates in this group. B2b contains 3 isolates namely (E-10, E-19 and E-01) of clinical and environmental relatedness. The environmental isolates E-10 has 100% clonal similarity with clinical isolates E-19. Environmental isolates (E-10) collected from bed sheet of post operative room of HFRCMCH, which may be the cause of the patient's (E-19) nosocomial manifestation of wound infection. All the isolates were collected from clinical and environmental sources of post operative room and its surroundings of HFRCMCH.

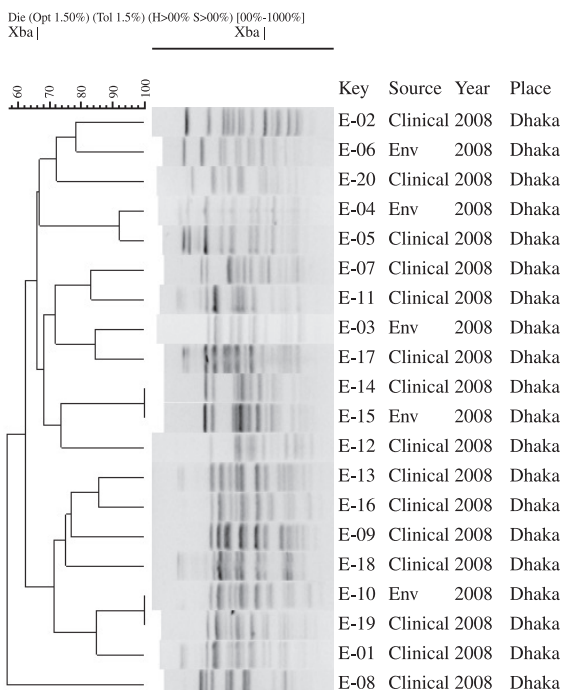


Figure 1: Dendrogram showing clonal relatedness among *E. coli* strains.

## Discussion

*E. coli* was the most frequently isolated organism from wound infection. Almost similar rate was reported by India<sup>6</sup>. Higher prevalence of *E. coli* in this study might be due to its frequent presence in hospital environment<sup>7</sup>. In this study, the rate of *Pseudomonas* in wound infection is 12%. A lower rate of *Pseudomonas* has also been reported by other studies<sup>8,9</sup>.

The importance of airborne transmission as a source of infection in operating theatre is controversial but there is evident to support the view that airborne organisms can cause post operative wound infection<sup>10</sup>. Organisms were found in surroundings of patients of post operative and operative room. Linen bed sheet, Trolley, Sucker, Oxygen cylinder, dressing materials were investigated for the source of nosocomial infection. Previous studies revealed that operative device was a good source of nosocomial infection<sup>11,12</sup>. *E. coli* and *S. aureus* were the dominant pathogens along with *Bacillus* spp, *Klebsiella*, *Acinetobacter* spp etc.

The development of DNA fingerprinting schemes for the typing of bacteria has in part been stimulated by the view that phage typing or Serotyping no longer occupies the "gold standard" position as a high-resolution discriminator between strains. Thus, for *E. coli*, PFGE of genomic DNA digested by rare cutter restriction enzymes has been widely used as a DNA fingerprinting method and has shown itself to have advantages over other DNA-based methods, such as plasmid analysis, which in essence tracks plasmids rather than their hosts; conventional RFLP analysis, which requires the analysis of complex banding patterns; and ribotyping, which is labor-intensive and slow<sup>13</sup>. PFGE profiles of the *E. coli* strains obtained with *Xba*I restriction enzyme showed a variety of patterns. Other strains of different locality and period displayed different but closely related restriction profiles. For example, strain E-14 and E-15 had similar restriction patterns. The other strains were distinguishable from each other and have been placed in different clusters. Clinical samples of *E. coli* (E-14 and E-19) were similar in term of PFGE pattern of environmental samples (E-15 and E-10) However, the strain, E-10 displayed closely related restriction fingerprint patterns with E-19, which is a clinical clone and was included in this study for genomic comparison. So, it can be assumed that the clinical isolate E-19 might have originated from related clone to that environmental E-10 strain.

Advances in infection control practices include improved operating room ventilation, sterilization methods, barriers, surgical technique, and availability of antimicrobial prophylaxis. Despite these activities, post operative infections remain a substantial cause of morbidity and mortality among

hospitalized patients<sup>14</sup>. Thus, to reduce the risk of post operative surgical site infection a systematic but realistic approach must be applied with the awareness that this risk is influenced by characteristics of the patient, operation, personnel and hospital.

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