Evaluation of the clonal relationship of *Serratia marcescens* isolates by Pulsed-field gel electrophoresis (PFGE) and identify it’s outbreaks in Government General Hospital at Nagapattinam, Tamilnadu, India

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Abstract

In recent years a significant increases in the incidence of *Serratia marcescens* infections was noted at Government General Hospital, Nagapattinam, India. *S. marcescens* is a well known cause of nosocomial infection and outbreaks, particularly in hospitalized patients with severe underlying disease. Therefore, in 2007-2008, 105 isolates were obtained from patient clinical specimens, 58 isolates were from environmental sources and 59 isolates were obtained from the cockroaches. The pulsed-filed gel electrophoresis is used to examine the isolates to analyses the outbreak strains involved. The two types of *S. marcescens* were confirmed by PFGE fingerprinting pattern. We found that, the isolates from patients, hospital personnels, environmental samples and strains from cockroaches sharing the both type strains (A and B) and proved the source of the outbreaks.

Introduction

*S. marcescens*, a member of the family Enterobactericeae, is recognised as an etiological agent in nosocomial infection. The outbreaks of *S. marcescens* strains are becoming more frequently reported. It cause a wide spectrum of infectious disease, including urinary, respiratory and biliary tract infections, wound infections, intravenous catheter related infections, septic arthritis. The environmental sources of *S. marcescens* infection in the hospital are widely described. In the presence of multiple resistances to antibiotics causes a problem in treating infected patients and controlling outbreaks. The epidemiological marker is an important in attempting to trace the source of contamination or to prevent dissemination of strain among patients. The pulsed-field gel electrophoresis (PFGE) typing is highly effective in epidemiological studies of bacterial isolates at molecular level, and is very accurate to other methods in discriminating among isolates such as *E.coli*, *Staphylococcus aureus* and many other species, the oldest method are often based on phenotypic, bactericin, phage typing, and plasmid typing. PFGE typing can be used to evaluate the clonal relatedness among bacterial isolates and to investigating outbreaks. In this study, we investigate an outbreak of *S. marcescens* infection in Government General Hospital, Nagapattinam, India and identify the source of the outbreaks.

Materials and methods

Sampling

The period of 24 months from 1st January 2007 to 30th December 2008, totally 105 *S. marcescens* were isolated from the clinical specimens like urine, urine from catheter tubes, blood, skin swabs, pus materials, and sputum and ear swabs. About 58 *S. marcescens* isolates were got from the environmental sources like, urinary catheter tube, Mechanical ventilator, transfusion bottle, insulin, adhesive tapes, and hand washing soap and from the healthcare workers, and *S. marcescens* isolates of 59 were obtained from the cockroaches in hospital environment. From all these *S. marcescens* isolates, we took 7 isolates from 7 clinical specimens, 11 isolates of *S. marcescens* 6 from environmental specimen and 5 from healthcare workers, and 4, *S. marcescens* from...
cockroaches were got in four different units in hospital.

**Analysis by PFGE**

**Extraction of genomic DNA**

Isolation of genomic DNA for PFGE was performed using the Genomic DNA Extraction kit-Bacteria (Medox-Bio™). The well isolated colonies from MacConkey agar were inoculated into LB broth (20ml) and incubated for 24 hours at 37°C in shaking incubator. Then took 1.5ml of overnight broth culture into 1.5ml microfuge tube and centrifuged at 10,000 rpm for 15 minutes and removed supernatant, and resuspended the pellet in 0.5ml of solution-I, then centrifuged at 10,000rpm for 10minutes, then discarded the supernatant, and solution-II was added and resuspended and centrifuged at 10,000rpm for 10 minutes and discarded the supernatant (which making the cell wall susceptible to breakage). Then resuspended the pellet in 0.5ml of solution-III (SDS breakdown the cell membranes), then 25µl of lysozyme added and tapped the tubes, then incubated at 37°C for 15 minutes (to lysis and remove protein molecules from the DNA). Then 50µl of solution–IV was added, then tapped (to precipitate the proteins) and heated at 55°C for 10 minutes, and added 250µl of solution-V and mix well (RNase remove RNA), then centrifuged at 10,000rpm for 5minutes. The supernatant was transferred to fresh microfuge tube and added 0.6ml of solution-VI (to precipitate DNA), then centrifuged at 10,000rpm for 10 minutes. Then decanted the supernatant and dried the pellet and 20µl of TE buffer was added to dissolve the DNA.

**PFGE-Fingerprint**

For PFGE, we took 100µl of extracted genomic DNA in PCR tubes and added 40U of XbaI Restriction enzyme (Medox-Bio™) and incubated according to the manufacture instruction. Then digested samples were loaded into 1.2% PFGE agarose (sigma) and ran in 0.5x TEB buffer. Then the gels were stained with Ethidium bromide and photographed under UV-Transilluminator (Bionnik Inovation) and compared PFGE band pattern with Marker DNA (Medox-Bio™).

### Table 1. *S. marcescens* strains isolated from patients.

<table>
<thead>
<tr>
<th>Isolate. No</th>
<th>Source PFGE</th>
<th>type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Urine</td>
<td>A</td>
</tr>
<tr>
<td>2</td>
<td>Urine from catheter</td>
<td>A</td>
</tr>
<tr>
<td>3</td>
<td>Blood</td>
<td>B</td>
</tr>
<tr>
<td>4</td>
<td>Skin swabs</td>
<td>B</td>
</tr>
<tr>
<td>5</td>
<td>Pus materials</td>
<td>B</td>
</tr>
<tr>
<td>6</td>
<td>Sputum</td>
<td>A</td>
</tr>
<tr>
<td>7</td>
<td>Ear swabs</td>
<td>A</td>
</tr>
</tbody>
</table>

### Table 2. *S. marcescens* strains isolated from environmental sources

<table>
<thead>
<tr>
<th>Isolate. No</th>
<th>Source PFGE</th>
<th>type</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>Urinary catheter tubes</td>
<td>A</td>
</tr>
<tr>
<td>9</td>
<td>Mechanical ventilator</td>
<td>A</td>
</tr>
<tr>
<td>10</td>
<td>Transfusion bottle</td>
<td>B</td>
</tr>
<tr>
<td>11</td>
<td>Insulin</td>
<td>A</td>
</tr>
<tr>
<td>12</td>
<td>Adhesive tape</td>
<td>B</td>
</tr>
<tr>
<td>13</td>
<td>Hand washing soap</td>
<td>A</td>
</tr>
<tr>
<td>14</td>
<td>HCW(physician)</td>
<td>A</td>
</tr>
<tr>
<td>15</td>
<td>HCW(Nurse)</td>
<td>A</td>
</tr>
<tr>
<td>16</td>
<td>HCW(Students)</td>
<td>A</td>
</tr>
<tr>
<td>17</td>
<td>HCW(Nursing aids)</td>
<td>B</td>
</tr>
<tr>
<td>18</td>
<td>HCW(Maid)</td>
<td>A</td>
</tr>
</tbody>
</table>
**PFGE pattern**

The difference between the DNA band patterns was determined by visual comparisons of DNA fragments. Based on the criterion of Tenover et al.\(^1\), the isolates were considered as identical strains, if their DNA fragments were identical. The one to three bands difference was considered as closely related strains. In the presence of four to six bands difference, it was considered as possibly related.

<table>
<thead>
<tr>
<th>Isolate. No</th>
<th>Source PFGE</th>
<th>type</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>Supply room for surgery room</td>
<td>B</td>
</tr>
<tr>
<td>20</td>
<td>Infirmary</td>
<td>A</td>
</tr>
<tr>
<td>21</td>
<td>Coffee shop</td>
<td>A</td>
</tr>
<tr>
<td>22</td>
<td>Nutrition and Dietetics services</td>
<td>A</td>
</tr>
</tbody>
</table>

**Figure 1 & 2.** PFGE fingerprinting of *S. marcescens* strains (1-7 patients, 8-18 environmental sources and 19-22 cockroaches) isolates in the outbreak. (M: DNA marker).

**Table 3.** *S. marcescens* strains isolated from cockroaches

**Results**

The characteristics of the 7, *S. marcescens* isolated from patient clinical specimens PFGE typing (Table 1). Table 2. Show the characteristics of 11 outbreak-related *S. marcescens* isolated from environmental sources. The Table 3. Show 4 outbreak-related *S. marcescens* from cockroaches.

Figures 1 and 2 show the PFGE typing of *S. marcescens* outbreak strains isolated from patients, environmental sources and cockroaches. The isolate numbers 1 to 7 of patients and the environmental sources isolate numbers 8 to 18 and the isolate numbers 19 to 22 of cockroaches from the hospital environment. The PFGE fingerprinting was determined by visual comparison of DNA fragments.
fragments. The isolate numbers 1,2,6,7 of
patient clinical specimen, the isolate
8,9,11,13,14,15,16,18 of environmental sources
and 20,21,22 isolate of cockroaches were
showed all the XbaI digest had identical DNA
fragments at maximum numbers and they were
designated as Type-A. The isolate numbers of
patients 3,4,5 and 10,12,17 isolate numbers of
environmental sources and isolate number 19
of cockroach, the XbaI digest showed two extra
bands, one band at above 196kb and another
band at 290kb, when compared the second
group strains with PFGE type-A, it showed two
extra bands, so it was designated as Type-B and
it was considered as closely relates strains. The
marker DNA (M) used to know the size of
unknown fragment size.

Discussion

*S. marcescens* cause severe illness, generally
in the infants, and in immunocompromised
patients. The phenotypic methods like
antibiogram help the microbiologist to monitor
an outbreak. Even though, these techniques
could not confirm the genetic relationship
among outbreak strains. The PFGE typing
method is based on fingerprinting of bacterial
genome, it help microbiologists to detect
the origin, route of hospital outbreaks,
edemics and a step to control and elimination
of infection. *S. marcescens* strain, 4
isolates from the patients, 8 isolates from the
environmental source and 3 isolates from
cockroaches were genetically identical (PFGE
type A), and that this clones were responsible for more
frequent outbreak in Government General
Hospital, Nagapattinam, India. The anther
(PFGE type B) *S. marcescens* outbreak strain
were totally 7, which were less outbreak than
type-A. In this study, the attempt was made to
identify the environmental sources such as
urinary catheter tubes, mechanical ventilator,
transfusion bottle, insulin and adhesive tapes
were carried the *S. marcescens* and outbreak it
to the Susceptible hospitalized patients. So, the
adhesive tapes outbreak the nosocomial
infection. *S. marcescens* among the health
workers in the hospital were carried out and
isolated from them; they may spread the
organisms to patients by hand to hand in
hospital. Different infection control measures
like strict hand washing, and wearing of
gloves by personnel between their handing of
patients. The usage of multi-vial insulin
solution and sharing of drugs in the hospital
may contribute the spread of the *S. marcescens*
among the patients in hospital.
The cockroach (*Periplaneta americana*) are
naturally live in sewage pipes, latrines, garbage
and filthy place which carry germs and
contaminate sterilized materials, equipments
and non-contaminated food in hospital. The
cockroaches at this hospital underline the
importance of developing adequate monitoring
the hygiene, controlling and optimizing food
handling in hospital. The implementation of
a pest control program may control the
cockroaches in hospital environment.

Conclusion

The Pulsed-field gel electrophoresis typing was
found to be more effectively discriminatory
and reproducible for the epidemiological
investigation of *S. marcescens* infection in
hospital environment. PFGE typing can
facilitate the reliable evaluation of the clonal
relationship of common source of outbreaks
and important to type suspected epidemic strain
in order to understand the natural history of
*S. marcescens* outbreak and to educate
the healthcare workers about cross transmission
of the infection. This study shows the clinical
evaluation and microbiological testing can help to identify outbreak and infection
control.
REFERENCES