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Research Article

PATTERN OF SERUM RESISTANCE IN UROPATHOGENIC ESCHERICHIA COLI

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ABSTRACT

Serum resistance is an important virulence marker in uropathogenic E. coli (UPEC) but its pattern with respect to commensal gastrointestinal strains is less studied. We compared the serum resistance pattern of UPEC with commensal strains in stool samples from healthy individuals. A total of 40 isolates of UPEC (test) and intestinal E. coli (control) each was obtained from patients of all age groups with symptomatic UTI infections and healthy individuals respectively. Bacterial suspension of E. coli was mixed with serum and incubated at 37°C and the viable count was determined at 0, 1, 2 and 3 hours, followed by surface plating on MacConkey agar. The percentage of strains and the mean viable colony counts in both groups were compared using the χ² and unpaired t – test to ascertain the statistical significance in serum resistance pattern. Sixty percent of the test strains exhibited significant growth at 3 hours incubation respectively with serum compared to 12.5% for the control strains. (p value <0.0001). The mean colony count of the test strains (0.91 ± 0.27 x 10⁵ CFU/mL) was significantly higher than the control strains (0.53 ± 0.35 x 10⁵ CFU/mL) at 3 hours. (p value =0.0015). Serum resistance pattern in UPEC is an important virulence marker and is expressed significantly more commonly compared to commensal strains from gastrointestinal tract.
KEY WORDS

serum resistance, uropathogenic *Escherechia coli*.

INTRODUCTION

*E. coli* is one of the commensals in the human intestinal tract as well as an important pathogen causing infections ranging from diarrhoea, dysentery, urinary tract infections (UTI) and various soft tissue infections to meningitis, peritonitis and septicemia etc. Several virulence factors enable *E. coli* to cause extraintestinal infection and help to evade host immune response in those sites: “serum resistance” being one of them.¹² Many gram negative bacteria are susceptible to the bacteriological action of human and animal sera. Till date, we found only one study reporting the difference in serum resistance between uropathogenic *E. coli* (UPEC) and intestinal commensals.² The main aim of our study was to demonstrate the pattern of serum resistance in UPEC compared to gut *E. coli* commensals.

MATERIAL AND METHODS

A total of 40 isolates of UPEC (test) and intestinal *E. coli* (control) was obtained from patients of all age groups with symptomatic UTI infections (clean catch mid stream urine) and healthy individuals (stool) respectively over a period six months, (February 2008 to July 2008) from various departments at our hospital. The specimen collected were clean catch mid stream urine and fresh stool. The samples were processed immediately by using standard procedures. The isolates were identified by standard conventional methods.

Detection of serum resistance:

A standard bacterial suspension (0.5 McFarland) prepared from fresh overnight cultures of *E. coli* were grown at 37°C on nutrient agar. Serum was obtained from healthy donors on the day of each test. Bacterial suspension of 0.5 mL was mixed with 1.5mL of fresh undiluted serum and incubated at 37°C. The viable count was determined at the beginning of incubation (0 hrs) and after 1, 2 and 3 hours of incubation followed by surface plating on MacConkey agar and further incubation for another 24 hours. Serum resistance was studied by inhibition of growth in the latter. A colony count of >100 was taken as >10^5 colony forming units /mL (CFU/mL) i.e. 100% growth. Like wise a colony count of 60 – 99, 30 – 59 and < 30 was considered as 75 %, 50% and 25% growth respectively. Serum sensitive was defined as no growth after incubation with serum in both test and controls while serum resistance was defined as ≥90 after incubation.³

The mean growth value at 0, 1, 2 and 3 hours was calculated for the test and control strains using the following formula:

\[ \text{Mean growth value at nth hour} = \frac{\sum \text{CFU/mL of all test/control strains at nth hour}}{\text{Number of positive strains}} \]

\[ \text{Mean growth percentage at nth hour} = \frac{\text{Mean growth value}}{10^5 \text{CFU/mL}} \times 100 \]

The results were analysed using \( \chi^2 \) for comparing the serum resistance pattern between the test and control strains and the unpaired t – test to compare the hourly mean viable colony counts. A p value < 0.05 was regarded as significant.

RESULTS

Seventy five percent, 67.5%, and 60% of the test strains exhibited > 100% (> 10^5 CFU/mL) growth at 1, 2 and 3 hours incubation.
respectively with serum compared to 62.5%, 30% and 12.5% at similar intervals for the control strains. (p value 0.42, 0.002 and 0.0001 respectively) (Table 1) The number of strains showing 75%, 50% and 25% growth at 0, 1, 2 and 3 hours are shown in Table 1. No growth was seen in 15%, 22.5% and 17.5% of the test strains compared to 20%, 50% and 70% of the controls strains at 1, 2 and 3 hours respectively. (Table1)

### Table 1

**The pattern of serum resistance in uropathogenic versus control strains of E.coli.**

<table>
<thead>
<tr>
<th>Mean Growth Value [x 10^5 CFU/mL] (%)</th>
<th>No. of strains (%)</th>
<th>Growth at 0 hr.</th>
<th>Growth after 1 hr</th>
<th>Growth after 2hrs.</th>
<th>Growth after 3hrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Test</td>
<td>Control</td>
<td>Test</td>
<td>Control</td>
<td>Test</td>
</tr>
<tr>
<td>≥90 (100%)</td>
<td>40 (100)</td>
<td>40 (100)</td>
<td>30 (75)</td>
<td>25 (62.5)</td>
<td>27 (67.5)</td>
</tr>
<tr>
<td>60 – 89 (75%)</td>
<td>0</td>
<td>0</td>
<td>3 (7.5)</td>
<td>4 (10)</td>
<td>3 (7.5)</td>
</tr>
<tr>
<td>30 – 59 (50%)</td>
<td>0</td>
<td>0</td>
<td>1 (2.5)</td>
<td>2 (5)</td>
<td>0 (2.5)</td>
</tr>
<tr>
<td>1 – 30 (25%)</td>
<td>0</td>
<td>0</td>
<td>0 (2.5)</td>
<td>1 (2.5)</td>
<td>1 (2.5)</td>
</tr>
<tr>
<td>No growth</td>
<td>0</td>
<td>0</td>
<td>6 (15)</td>
<td>8 (20)</td>
<td>9 (22.5)</td>
</tr>
</tbody>
</table>

The same has been graphically represented in Figure 1.

The mean growth in the test and control strains at 0, 1, 2 and 3 hours are shown in Table 2. There was no significant difference in the mean colony count at the beginning between the test and control strains and after 1 hour of incubation with serum. (Table 2) However after incubation with serum the mean colony count of the test strains was significantly higher than the control stains i.e. 1.04 ± 0.17 x 10^5, 0.96 ± 0.17 x 10^5 and 0.91 ± 0.27 x 10^5 CFU/mL versus 0.96 ± 0.20 x 10^5, 0.74 ± 0.28 x 10^5 and 0.53 ± 0.35 x 10^5 CFU/mL (p value 0.09, 0.002 and 0.001) at 1, 2 and 3 hours respectively. (Table 2) The
Table 2

Comparison of the mean growth values of uropathogenic versus control strain of *E. coli*.

<table>
<thead>
<tr>
<th>Strains</th>
<th>Mean Growth at 0 hr. ((X \times 10^5 \text{ CFU/mL}))</th>
<th>Mean Growth after 1 hr ((X \times 10^5 \text{ CFU/mL}))</th>
<th>Mean Growth after 2 hrs. ((X \times 10^5 \text{ CFU/mL}))</th>
<th>Mean Growth after 3 hrs. ((X \times 10^5 \text{ CFU/mL}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test</td>
<td>1.21 ± 0.11</td>
<td>1.04 ± 0.17</td>
<td>0.96 ± 0.17</td>
<td>0.91 ± 0.27</td>
</tr>
<tr>
<td>Control</td>
<td>1.18 ± 0.07</td>
<td>0.96 ± 0.20</td>
<td>0.75 ± 0.28</td>
<td>0.53 ± 0.35</td>
</tr>
<tr>
<td>p value</td>
<td>0.2720</td>
<td>0.0907</td>
<td>0.0026</td>
<td>0.0015</td>
</tr>
</tbody>
</table>

Figure 1

Graphic representation of the mean growth values of uropathogenic versus control strain of *E. coli*.

**DISCUSSION**

*E. coli* is the most frequent urinary pathogen isolated from 50% - 90% of all uncomplicated urinary tract infections. It is now recognized that there is a subset of faecal *E. coli* having an array of virulence factors, like serum resistance, *S* fimбриae (hemagglutination), cell surface hydrophobicity and hemolysin production etc which can colonise periurethral area or get hematogenous access to urinary tract and cause symptomatic disease and are currently defined as UPEC. \(^1,^2\)

In our study 60% of the UPEC showed significantly higher serum resistance when compared to controls (12.5%) after incubation for 3 hours. Our results are in stark contrast to Raksha et al.\(^3\) who reported no significant difference between the serum resistance property of UPEC (32.7%) and gut commensals (24%). The difference in sample size may account for such discrepancy. A previous study showed 86.7% of the isolates from urine were
resistant to bactericidal action of serum.\textsuperscript{5} Another study by L. Siegfried et al\textsuperscript{6} reported similar (68\%) serum resistance in UPEC strains expressing mannose resistant hemagglutination which was significantly more common strains expressing mannose sensitive hemagglutination (48\%).

Though there was no significant difference in the pattern of serum resistance, reflected by the mean viable colony count after incubation for 1 hour, the difference was significant after 3 hours of incubation. This probably suggests an inducible genetic mechanism by which the UPEC manifest serum resistance. Taylor\textsuperscript{7} reviewed that bacteria are killed by normal human serum through lytic activity of alternative complement system. Although the basis of serum resistance is not well understood it has been suggested that a number of envelope components such as O side chain moiety of lipopolysaccharides, acidic exopolysaccharides, K antigens and Outer Membrane Protein are able to protect the bacterial cell against complement.\textsuperscript{8,9} Also it has been demonstrated recently that certain plasmid are able to increase the resistance of suitable enterobacteria to the bactericidal action of serum.\textsuperscript{10}

The absence of serotyping of isolates can be considered as a drawback in our study.

**CONCLUSION**

Serum resistance pattern in UPEC is an important virulence marker. Further studies or meta-analysis of all studies comparing the difference in serum resistance pattern between UPEC and gut commensal are required to come to firm conclusions. Elucidation of the genetic basis of serum resistance will help to target such infections in the future. Antibiotic sensitivity pattern of such strains in every region would help in formulating hospital antibiotic policy and prevent emergence of resistance.

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