Some virulence characteristics of uropathogenic *Escherichia coli* in different patient groups

Rebecca Naveen & Elizabeth Mathai

*Department of Microbiology, Christian Medical College & Hospital, Vellore, India*

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**Background & objectives:** Uropathogenic *Escherichia coli* have virulence properties, that are absent in non pathogenic *E. coli*. The distribution of these markers can vary according to patient populations. Hence, a study was undertaken to describe the presence of virulence factors like P-fimbriae, type 1 fimbriae and haemolysin in *E.coli* causing urinary infections in three groups of patients. Antibiogram was also recorded to determine differences, if any, between the groups.

**Methods:** *E. coli* isolated from three groups of subjects, in counts of $\geq 10^5$ CFU/ml and in pure growth were tested for mannose resistant haemagglutination (MRHA) to indicate P fimbriae and mannose sensitive haemagglutination (MSHA) to indicate type 1 fimbriae. Haemolysin production and antimicrobial susceptibility patterns were also recorded.

**Results:** Significantly more isolates from antenatal and postnatal women possessed P fimbriae compared to groups with urologic abnormalities ($P=0.05$). Haemolysin production was also significantly higher ($P<0.001$) in this group. Greater proportions of isolates from pregnant women were susceptible to commonly used antimicrobials. However, resistance to third generation cephalosporins was present even in these isolates from community infections.

**Interpretation & conclusion:** In patients with urological abnormality, *E. coli* with lower virulence can cause infections. Isolates from these patients exhibited greater drug resistance. In pregnant women and in community acquired infections, simple antimicrobial drugs like nitrofurantoin might still be useful. However, urgent and stringent policies for antimicrobial use and infection control in hospitals are required in India.

**Key words** Adherance - antibiogram - *E. coli* - fimbriae - mannose resistant haemagglutination - urinary tract infection - uropathogenic - virulence

Urinary tract infections (UTI) are probably the most common bacterial infections. Bacteria responsible for UTI, often originate from the faecal and perineal flora$^{1,2}$. Under normal circumstances, these bacteria are cleared from the urinary system by effective protective mechanisms. If, however, they overcome these mechanisms, they can colonize the lower urinary tract. Subsequent progress is determined by the host susceptibility and bacterial virulence factors$^{1,2}$. Manifestations can vary from asymptomatic bacteriuria to symptomatic cystitis, pyelonephritis and blood stream infection$^{1,2}$.
A single bacterial species, *Escherichia coli*, causes majority of UTI. Subsets identifiable using O, K and H antigens were shown to have increased ability to cause symptomatic urinary infections. Thus arose the concept of uropathogenic *E. coli* clones. Recent studies confirm that uropathogenic *E. coli* have several attributes that are lacking in the commensal *E. coli*. They carry chromosomal gene clusters on ‘pathogenicity islands’, encoding adhesins and other virulence factors. The most important amongst these, probably, are the adhesins that help them to adhere to uroepithelium and this property was recognized decades ago. These include type I, S and P fimbriae, and adhesins like Dr. The type 1 fimbriae are widely prevalent and are probably involved in colonization of lower urinary tract. Mannose-sensitive haemagglutination (MSHA) denotes presence of these fimbriae.

The role of P fimbriae in upper UTI is well documented. These are encoded by the *pap* operon and are present in 20 per cent of faecal, 60 per cent of cystitis causing, and 80 per cent of pyelonephritis causing *E. coli* isolates. It is shown that some *pap* positive isolates, especially those isolated form asymptomatic infections, do not express P fimbriae. Phenotypic expression of P fimbriae can be detected by mannose-resistant haemagglutination (MRHA) of human erythrocytes. Attachment of P fimbriae is also associated with increased host inflammatory response.

Other factors associated with uropathogenic *E. coli* include production of haemolysin, serum resistance and release of aerobactin. Haemolysin provides *E. coli* with possible selective advantage by releasing iron from lysed erythrocytes and enhances pathogenicity by destroying phagocytic and epithelial cells.

Measuring a phenotype *in vitro* does not always correlate with *in vivo* expression and may underestimate the presence of a virulence factor *in vivo*. Identifying a genotype, on the other hand, does not mean that it is expressed in the body. However, MRHA can be used for presumptive identification of virulence factors in *E. coli*. The distribution of virulence properties can also vary depending on host characteristics and type of infection. There are however, very few reports in the literature, where, phenotypic expression of virulence factors in *E. coli* and antibiogram have been compared in isolates from different patient groups. The present study was therefore undertaken to determine differences if any, in the presence of phenotypically expressed virulence factors like P-fimbriae, type 1 fimbriae and haemolysin among *E. coli* causing urinary infections in three different groups of patients. Antibiogram was also recorded to determine differences, if any, between the groups.

**Material & Methods**

Consecutive *E. coli* isolates (n=163) obtained in counts of >10⁵ cfu/ml and in pure growth, from routine urine cultures of the three groups of patients viz., antenatal and postnatal women, patients presenting to urology department, and patients being seen in the rehabilitation unit, between January and December 2002 in Christian Medical College, Vellore were included in the study. These three groups were selected based on the assumptions that the first group is likely to have community acquired UTI while the second group, because of the underlying urological abnormalities is more likely to have complicated and also hospital acquired UTI. The rehabilitation unit has patients with paralysis and so is likely to have infections associated with long-term use of catheter. Identification of isolates was done using standard microbiological techniques. Antimicrobial susceptibility testing was done on Mueller Hinton agar by disc diffusion method using National Committee for Clinical Laboratory Standards (NCCLS) guidelines.

Virulence factors studied were haemolysin production, MRHA of human O group 3 per cent erythrocytes in the presence of 2 per cent mannose to indicate P fimbriae, and MSHA of 3 per cent human erythrocytes to indicate type 1 fimbriae. Haemolysis was defined as clearing of 5 per cent sheep blood agar around or beneath bacterial colonies after over night incubation. Other phenotypic characteristics like production of gas, motility and fermentation of lactose were also noted.

Chi square test was used to analyse the data statistically. Epi Info version 5 was used for this analysis.
Results

Phenotypic characteristics of 163 isolates of *E. coli* isolated from the three groups of patients were studied (Table I). Significantly (*P*=0.05) more isolates from pregnant women exhibited MRHA, indicating the presence of P fimbriae, compared to isolates from other groups. However, MSHA indicating type 1 fimbriae was present equally in all the three groups. Of the 44 isolates from pregnant women, which agglutinated erythrocytes, 25 (56.8%) were indicative of P fimbriae. In contrast, only eight (33.3%) of the 24 isolates from patients from rehabilitation unit on long-term catheters, which haemagglutinated, had mannose resistance. Haemolysin production was also significantly higher in isolates from antenatal and postnatal women (*P*<0.01) as compared to other groups. All haemolytic strains from pregnant and postnatal women agglutinated human erythrocytes indicating presence of either P or type 1 fimbriae. Of these, 58.1 per cent were mannose resistant. There were no significant differences in the other variable characters like motility, lactose fermentation and gas production among the groups.

Among the isolates from pregnant women, about 90 per cent were susceptible to nitrofurantoin and 86.4 per cent to cefuroxime. Resistance to co-trimoxazole was observed in 57.6 per cent of isolates. Resistance rates were high in the other two groups with only 66 and 74 per cent respectively being susceptible to amikacin. Cefotaxime resistance was observed in 10.2 per cent of isolates from antenatal women compared to 50 per cent from those on catheters (Table II).

### Table I. Phenotypic characteristics of *E. coli* isolates

<table>
<thead>
<tr>
<th>Phenotypic characters</th>
<th>Antenatal/postnatal (n=59)</th>
<th>Urology (n=58)</th>
<th>Rehabilitation (n=46)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mannose-resistant haemagglutination</td>
<td>25 (42.4)*</td>
<td>15 (25.9)</td>
<td>8 (17.3)</td>
</tr>
<tr>
<td>Mannose-sensitive haemagglutination</td>
<td>19 (32.2)</td>
<td>16 (27.6)</td>
<td>16 (34.8)</td>
</tr>
<tr>
<td>Haemolysin</td>
<td>24 (40.7)**</td>
<td>3 (5.2)</td>
<td>4 (8.7)</td>
</tr>
<tr>
<td>Motility</td>
<td>43 (72.8)</td>
<td>38 (65.5)</td>
<td>33 (71.7)</td>
</tr>
<tr>
<td>Lactose fermentation</td>
<td>48 (81.4)</td>
<td>47 (81)</td>
<td>36 (78.3)</td>
</tr>
<tr>
<td>Production of gas</td>
<td>54 (91.5)</td>
<td>51 (87.9)</td>
<td>39 (84.8)</td>
</tr>
</tbody>
</table>

*P*=0.05**<0.001 compared to other 2 groups
Values are denoted as no. (%)

### Table II. Comparison of susceptibility patterns of *E. coli* isolated from different patient groups

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Antenatal/postnatal (n=59) No. (%)</th>
<th>Urology (n=58) No. (%)</th>
<th>Rehabilitation (n=46) No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefuroxime</td>
<td>51 (86.4)</td>
<td>28 (48.2)</td>
<td>23 (50)</td>
</tr>
<tr>
<td>Co-trimoxazole</td>
<td>25 (42.4)</td>
<td>15 (25.9)</td>
<td>20 (43.5)</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>23 (39)</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>53 (89.8)</td>
<td>36 (62.1)</td>
<td>31 (67.4)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>48 (81.4)</td>
<td>26 (44.8)</td>
<td>24 (52.2)</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>46 (78)</td>
<td>6 (10.3)</td>
<td>9 (19.6)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>47 (79.7)</td>
<td>6 (10.3)</td>
<td>9 (19.6)</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>53 (89.8)</td>
<td>nd</td>
<td>23 (50)</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>28 (47.5)</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Amikacin</td>
<td>56 (94.9)</td>
<td>38 (65.5)</td>
<td>34 (73.9)</td>
</tr>
<tr>
<td>nd, not done</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Discussion

Significant numbers of *E. coli* causing UTI in pregnant and postnatal women had P fimbriae and haemolysin compared to those isolated from patients with urologic abnormalities. It is reported that adherence mediated by P fimbriae might be less important when the defenses of the urinary tract were compromised by anatomic abnormality\(^8,9\). However, there are not many studies demonstrating this feature in different populations. Pyelonephritis is most often caused by P fimbriated *E. coli*. These *E. coli* are able to bind to the digalactoside expressed on renal tubular epithelium\(^1,2\). This induces cytokine production and chemotaxis of neutrophils, initiating an inflammatory response. Haemolysins contribute by damaging host cells.

A good proportion of *E. coli* causing UTI in pregnancy are P fimbriated, as was seen in this study also. Hence there is an increased chance for pregnant women to develop pyelonephritis\(^17\). Asymptomatic infection in pregnancy if not treated, progress to pyelonephritis in about 30-50 per cent of cases\(^18\). This has led to the recommendation by some experts that *E. coli* isolated from asymptomatic bacteriuria in pregnant women be tested for virulence factors to identify pregnant women at risk of developing pyelonephritis\(^17\).

Haemolysin, though not essential for the establishment of acute pyelonephritis, might contribute to tissue injury and survival in the renal parenchyma\(^1\). Such injury would also facilitate bacterial entry into the blood stream. Uropathogenic *E. coli* usually have both P fimbriae and haemolysin\(^19\), as was observed in our study also. The prevalence of virulence factors in other studies reported from India was low compared to our data\(^20,21\). This is probably because patients were not differentiated based on host factors predisposing to infection.

Both type 1 and P fimbriae help in adhering to uroepithelial cells in the lower urinary tract\(^1\). Distribution of type 1 fimbriae was almost equal in the three groups studied. These fimbriae are continually expressed in cystitis and are turned off in isolates causing pyelonephritis\(^1\). As expected, other phenotypic characters which have no association with virulence were distributed equally among the three categories.

Antimicrobial susceptibility patterns varied in isolates from different categories of patients. This needs to be considered while developing guidelines for treatment of UTI and while interpreting data from other published studies, which showed high prevalence of antimicrobial resistance among uropathogens\(^20\). From our data, older drugs like nitrofurantoin appeared to be useful and could be considered as a choice for treating uncomplicated lower urinary tract infections. This drug has been recommended as appropriate for use in treating pregnant women with such infections\(^22\). Aminoglycosides appeared to be best suited for complicated infections. The high prevalence of resistance, mostly in complicated UTI, calls for urgent and stringent policies for rational drug use and infection control measures in hospital practice.

In conclusion, *Esch. coli* causing UTI in different patient populations differ in their pathogenic potential and susceptibility to antimicrobials. This has to be taken into account while developing guidelines for management of UTI.

References


*Reprint requests:* Dr Elizabeth Mathai, Professor, Department of Microbiology, Christian Medical College
Ida Scudder Road, Vellore 632004, India
e-mail: mathaim@cmcvellore.ac.in