Study of virulence factors of uropathogenic Escherichia coli and its antibiotic susceptibility pattern in a tertiary care hospital

Munilakshmi ponugoti1, M. Venkatakrishna2 Meenakshi3, Sindhura Jhon4

ABSTRACT: Urinary tract infection (UTI) is one of the most common nosocomial infections, caused by Escherichia coli. This study determined the presence of virulence factors in the organism and correlates it with the multi-drug resistance (MDR). Aims: The aim of the following study is to assess the virulence factors of uropathogenic E. coli and antibiotic susceptibility pattern. Materials and Methods: The study was conducted over a period of 1 year in microbiology department, Narayana medical college, Nellore. Urine samples received were processed as per standard microbiological procedures. Virulence factors such as hemolysin, hemagglutination, cell surface hydrophobicity, serum resistance, gelatinase and siderophore production were studied. The antimicrobial susceptibility was done as per Clinical and Laboratory Standard Institute Guidelines. Results: Hemolysin production was seen in 46.8%, hemagglutination in 75.8%, cell surface hydrophobicity, serum resistance, gelatinase and siderophore production in 61.4%, 57%, 69% and 88.5% isolates. Nitrofurantoin was found to be most effective followed by, gatifloxacin and gentamicin. Twenty nine percent (20.62%) isolates were MDR. Conclusions: Therefore, the knowledge of virulence factors of E. coli and their antibiotic susceptibility pattern will help in better understanding of the organism and in the treatment of UTI.

1. INTRODUCTION:

Urinary tract infections (UTIs) are one of the most common bacterial infections affecting humans throughout their life span. [1] They can be symptomatic or asymptomatic.

Escherichia coli is the most common cause of UTIs, accounting for about 85% of community acquired and 50% of hospital-acquired infections, it predominates strongly at most ages. [2] E. coli is a commensal in the human intestinal tract, when enters into unnatural sites, it can cause a variety of infections, e.g., UTIs, sepsis, pyelonephritis etc. Serotypes which lead to UTIs are designated as uropathogenic Escherichia coli (UPEC). [3]

The virulence factors of E. coli are multiple. The common virulence factors include surface hydrophobicity, colonization factor, capsule, serum resistance, resistance to phagocytosis, hemolysin, enterotoxin and siderophore, fimbriae and hemagglutination. [4] These markers of UPEC are expressed with different diseases states ranging from asymptomatic bacteriuria to chronic pyelonephritis.

Moreover, the drug resistance among strains has further aggravated the problem of UTIs. Therefore, the present study was carried out with aim to know the prevalence of various virulence factors in UPEC and to study their antibiotic susceptibility profile.

2. MATERIAL&METHOD:

The study was carried out on a total of 135 E. coli isolates recovered from urine samples of patients with clinically suspected UTIs of all age groups, over a period of 1 year from January to December 2014. E. coli isolates were identified by the standard microbiological procedures. The antibiotic susceptibility testing was performed using the...
standard antimicrobial agents (Hi Media, Mumbai) amoxyclyvalanic acid (30 μg), ceftizoxime (30 μg), cotrimoxazole (25 μg), gatifloxacin (5 μg), gentamicin (10 μg), nitrofurantoin (300 μg), norfloxacin (10 μg), as per Clinical and Laboratory Standard Institute Guidelines. E. coli (ATCC 25922) was used as control strain.

Virulence factors such as hemolysin, hemagglutination, serum resistance, gelatinase test, cell surface hydrophobicity, siderophore production were detected as follows.

3.1. DETECTION OF VIRULENCE FACTORS

1. Hemolysin: The E. coli isolates were inoculated on 5% sheep blood agar and incubated overnight at 37°C. The indicator of hemolysin production was the presence of a zone of complete lysis of erythrocytes around the colony and clearing of the medium.

2. Hemagglutination: The test was carried out as per the direct bacterial hemagglutination test-slide method. One drop of red blood cell (RBC) suspension was added to a drop of broth culture and the slide was rocked at room temperature for 5 min. Presence of clumping was taken as positive for hemagglutination. Mannose-sensitive hemagglutination was detected by the absence of hemagglutination in a parallel set of test in which a drop of 2% W/V D-mannose was added to the red cells and a drop of broth culture. Mannose resistant hemagglutinating (MRHA) was detected by the presence of hemagglutination of 3% 'O' blood group human RBCs in the presence of 2% W/V D-mannose.

3. Serum resistance: Overnight culture of E. coli on blood agar plates were suspended in Hank's balanced salt solution. Equal volume of this bacterial suspension and serum (0.05 ML) were incubated at 37°C for 3 h. Then 10 μl of this mixture was inoculated on blood agar plate and incubated at 37°C for 24 h and viable count was determined. It is termed as sensitive when colony count drop to <1% of initial value.

4. Gelatinase test: Gelatinase production was tested using gelatin agar. The plate was inoculated with test organism and incubated at 37°C for 24 h. The plate was then flooded with 1% tannic acid solution. Developments of opacity around colonies were considered as positive for gelatinase.

5. Cell surface hydrophobicity: This test was carried out by salt aggregation test (SAT). One loopful of bacterial suspension in phosphate buffer was mixed with an equal volume of ammonium sulfate solution of different molarity on a glass slide and rotated for 1 min. E. coli strains with SAT value ≤ 1.25 M were considered cell surface hydrophobic.

6. Siderophore production assay: The test was done by using chrome azurole sulfonate agar (CAS) agar diffusion assay. The CAS assay detected color change of CAS-iron complex from blue to orange after chelation of the bound iron by siderophores. A strong ligand was added to a highly colored iron dye complex, when the iron ligand complex was formed, the release of the free dye was accompanied by a color change.

4. RESULTS:

Incidence of UTI was more common in females, i.e., 72 (53.3%) in comparison to males 63 (46.7%) and was more common in sexually active females of age group 21-30 years 37 (27%). These cases were more commonly from gynecology/obstetrics 36 (27%) followed by surgery 34 (25%) and urosurgery 26 (19%).

Among the 135 E. coli isolates, 127 (94%) were sensitive to nitrofurantoin followed by ceftizoxime 88 (65.5%) and gatifloxacin, gentamicin 73 (54%), 29.62% E. coli isolates were multidrug resistance (MDR).
TABLE-1

<table>
<thead>
<tr>
<th>Virulence factors</th>
<th>n</th>
<th>%</th>
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<tbody>
<tr>
<td>Hemolysin</td>
<td>64</td>
<td>(47.4)</td>
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<tr>
<td>Hemagglutination</td>
<td>101</td>
<td>(74.8)</td>
</tr>
<tr>
<td>Serum resistance</td>
<td>79</td>
<td>(59)</td>
</tr>
<tr>
<td>Gelatinase</td>
<td>91</td>
<td>(67.5)</td>
</tr>
<tr>
<td>Cell surface hydrophobicity</td>
<td>82</td>
<td>(61)</td>
</tr>
<tr>
<td>Sierophore production</td>
<td>119</td>
<td>(88)</td>
</tr>
</tbody>
</table>

TABLE-2

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Sensitivity</th>
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</thead>
<tbody>
<tr>
<td>Nitrofurantoin</td>
<td>127(94)</td>
</tr>
<tr>
<td>Ceftoxime</td>
<td>88(65.5)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>73(54)</td>
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<tr>
<td>Gatifloxacin</td>
<td>73(54)</td>
</tr>
<tr>
<td>Cotrimoxazole</td>
<td>40(29.5)</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>31(23)</td>
</tr>
<tr>
<td>Amoxyclavulanic acid</td>
<td>05(4)</td>
</tr>
</tbody>
</table>

Fig 1
Colonies of *E.coli* on macokey’s agar

5. DISCUSSION:

UPEC are the most important group of microorganisms responsible for UTI. UPEC differ from non-pathogenic E. coli by the production of specific virulence factors which enable the bacteria to adhere to uroepithelial cells and to establish UTI. Besides adhesion factors, toxins, modulins, capsules ion uptake system and other bacterial products contributes to virulence of strains.\(^{[11]}\)

Incidence of UTI was more common in females 53.3% than in males in our study. Piatti *et al.*\(^{[12]}\) also reported a higher prevalence of UTI in female (77%). The reasons for the high prevalence of the UTIs in females can be due to the anatomical structure of the urogenital tract having short urethra, presence of normal flora in vagina, menstrual cycle and pregnancy.

Hemolysin production is associated with human pathogenic strains of E. coli, especially those causing more clinically severe forms of UTI.\(^{[11]}\) It is toxic to a range of host cells in ways that probably contribute to inflammation, tissue injury and impaired host defenses.\(^{[13]}\) In the present study, 47.4% *E. coli* isolates produced hemolysin, of these isolates 13 (20.3%) were MDR. In other studies conducted by Raksha *et al.*\(^{[3]}\), Siegfried *et al.*\(^{[7]}\) Hughes *et al.*\(^{[14]}\), Shruthi *et al.*\(^{[15]}\) hemolysin production was detected in 41.36% and 59.6%, 59.7% and 41.9% isolates respectively.
The role of bacterial adherence in the pathogenesis of UTI is that colonization of the urogenital epithelium of susceptible individuals by specific bacteria is associated with successful microbial invasion of the urinary tract. [16] and lead to UTIs. Thus, possession of MRHA by UPEC can be considered as one of the important virulence factor in the pathogenesis of UTIs. This concept has been supported in many researches, e.g., Seigfried et al. [7], Vagarali et al. [17], Raksha et al. [3], Kauser et al. [18] have reported the incidence of MRHA E. coli isolates as 23%, 25%, 30.9%, 30% respectively. In the present study also the rate of MRHA positive E. coli isolates was 45.5% and of these isolates 47.7% showed MDR.

Urinary antibodies resist UTI by preventing the adherence of bacteria to uroepithelial cells. [16] Serum resistance is the property by which the bacteria resist killing by normal human serum due to lytic action of complement system. It is likely that complete resistance to serum results from the accumulation of several distinct components at or near the cell surface. [1,14] In the present study, serum resistant was found in 59% isolates and out of these isolates 57.5% showed MDR pattern. In other studies, Kauser et al. [18] and Sharma et al. [6] have demonstrated the serum resistance in 49.5% and 86.8% of the urinary E. coli isolates. Hughes et al. [1,14] stated that the increased degree of serum resistance is associated with increased virulence of the organisms.

In the present study, gelatinase producing UPEC isolates (67.5%) were found to be associated with MDR (52.75%) to commonly used antibiotics.

The surface hydrophobicity is another important virulence factor, which promotes the adherence of the bacteria to various surfaces like the mucosal epithelial cells. [5] In our study 61% E. coli isolates were hydrophobic, out of these 49% were MDR isolates.

Siderophore production, promotes bacterial growth in the limiting iron concentrations encountered during infection and act as a virulence factor in the pathogenesis of UTI. In our study the siderophore production was seen in 119 (88%) isolates and 46% of these isolates were MDR (statistically insignificant). In other studies, the incidence of siderophore production has been reported to be 76% [19] and 98%, [17] which is in concordance with our study.

A study by Sharma et al., [6] demonstrated that out of 152 E. coli isolates, 36 (23.7%) isolates were hydrophobic, 132 (86.8%) were serum resistant and only 4 were positive for protease. MVF were observed in 6 (44%) of isolates. There was a significant association (P < 0.001) between multiple factors and virulence of E. coli.

Raksha et al. [3] demonstrated among 220 urinary isolates, 91 (41.36%) were hemolytic, 68 (30.9%) showed MRHA, 58 (26.36%) were cell surface hydrophobicity positive and 72 (32.72%) were serum resistant.

Antibiotic susceptibility pattern was studied for all E. coli isolates. These isolates were most commonly resistant to ampicillin, amoxyclovulanic acid and cotrimoxazole. The increasing prevalence of MDR has been reported by other workers as well, which is due to dissemination of MDR strains in hospital settings. In this study, the maximum sensitivity was shown to nitrofurantoin (94%) followed by ceftizoxime (65.5%) and gatifloxacin (54%).

The present study has shown the production of various virulent factors and developing drug resistance in UPEC. Antibiotic resistance may
provide a substantial advantage to the survival of the pathogen. The drug resistance among UPEC is on rise therefore the selection of appropriate antibiotics (after antibiotic susceptibility testing) is must for proper treatment of patients and to avoid emergence of drug resistance.

6. REFERENCES:


