Virulence factors, Serotypes and Antimicrobial Suspectibility Pattern of *Escherichia coli* in Urinary Tract Infections.

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Abstract:

Purpose: To study the virulence factors, serotypes of uropathogenic *Escherichia coli* (UPEC) and antimicrobial suspectibility pattern. **Methods:** A total of 200 *Escherichia coli* from symptomatic cases of urinary tract infections and 50 stool samples from apparently healthy individuals were included. UPEC were screened for virulence factors namely haemolysin, mannose resistant, mannose sensitive haemagglutination (MRHA, MSHA) and serum resistance by recommended methods. Antimicrobial susceptibility testing done by Kirby Bauer disc diffusion method as per NCCLS guidelines. **Results:** Among 200 *Escherichia coli* 42(21%) were haemolytic, 60(30%) showed MRHA and 72(36%) MSHA, 99(49.5%) were serum resistant. In 50 controls 8(16%) were haemolytic, 2(4%) showed MRHA and 4(8%) MSHA, 16(32%) were serum resistant. The difference between cases and controls for MRHA and serum resistance were significant ($p<0.001$ and $p<0.05$) respectively. Majority of uropathogenic *Escherichia coli* belonged to serotype O20, O131, O25 and O101.Most 85%-92% of the isolates were sensitive to Nitrofurantoin and Amikacin. Majority 70%-80% of the isolates were resistant to routinely used antibiotics.**Conclusion:** The present study revealed that 160(80%) *Escherichia coli* uroisolates exhibited one or the other virulence factors. Serotype O20 was isolated with maximum frequency followed by O131, O25 and O101 respectively.

Key words: Uropathogenic *Escherichia coli*, serotype, virulence factor, antimicrobial susceptibility.

Introduction

Urinary tract is among the most common sites of bacterial infection both in community based and hospitalized patients [1].*Escherichia coli* is the commonest cause of urinary tract infection in women and children especially in those with uncomplicated infections [2]. In *Escherichia coli* virulence results from the cumulative impact of several special properties or virulence factors (VFs), which serve to distinguish potential pathogens from harmless intestinal strains [3]. The virulence of individual strains in a given infection is determined by the presence and actual expression of the virulence genes present in them and also by the environmental conditions in the host [4].

It has been traditionally described that certain serotypes of *Escherichia coli* were consistently associated with uropathogenicity and were designated as uropathogenic *Escherichia coli* (UPEC). These markers of UPEC are expressed with different frequencies in different disease states ranging from asymptomatic bacteriuria to chronic pyelonephritis [5].

Virulence factors of UPEC include the ability to adhere to uroepithelial cells, certain specific serotypes O and K antigens, resistance to phagocytosis and to the bactericidal action of normal serum. Other factors known to contribute to the virulence are the production of α haemolysis (AH), colicins, aerobactin, cytotoxic necrotizing factor and cell surface hydrophobicity [6].

This study was undertaken:

1. To know the virulence factors associated with *Escherichia coli* uroisolates.
2. To study the serotypes of uropathogenic *Escherichia coli* in urinary tract infections.
3. To know the antimicrobial susceptibility pattern of *Escherichia coli* uroisolates.

Materials and Methods

Sample: A total of 200 *Escherichia coli* from symptomatic cases of urinary tract infection attending outpatient departments of Karnataka Institute of Medical Sciences (KIMS) Hospital Hubli were studied for detection of virulence markers of *Escherichia coli* in the department of microbiology. Faecal isolates of *Escherichia coli* from 50 healthy individuals were included as controls.

Urine samples were processed as per standard protocol. Lactose fermenting colonies on MacConkey's agar (MA) showing significant bacteriuria were processed and identified as *Escherichia coli* (*E.coli*) by standard biochemical tests [7].The *E.coli* isolates were stored on Nutrient agar (NA) butts and preserved at 4^0C.

Virulence factor Haemolysin production: Haemolysin was detected by the presence of a zone of lysis around the colony on Blood agar plate [5].

Serum resistance: Overnight cultures of *Escherichia coli* grown at 37^0C on Mueller Hinton agar (MHA) were harvested and the cells were suspended in Hank's Balanced Salt Solution (HBSS). The wells of microtitration plate were used for incubation of bacterial suspension with serum. 0.05ml of bacterial suspension and 0.05ml of serum were added to each well. Control wells contained 0.05ml of HBSS instead of serum. The plate was placed on shaker waterbath and rotated for 3hrs at 37^0C. 10µl of each sample was withdrawn and spread on MHA plates simultaneously 10µl of bacterial suspension without serum was spread onto MHA plates and incubated for 18-24hrs at 37^0C and viable count was determined. Strains were termed serum sensitive if the viable count dropped to 1% of the initial value and resistant if >90% of organism survived after 3hrs incubation period [6].

Haemagglutination(HA): *Escherichia coli* grown on MA plates were inoculated into 5ml of phosphate buffered saline pH 7.4(PBS) incubated for 5 days at 37^0C to get fimbriae enriched *E.coli.* The pellicle formed onto surface was noted and subcultured onto Colonization factor antigen (CFA) agar. 5ml of fresh group A positive blood was obtained from blood bank and added to an equal amount of Alsever's solution. This was washed three times and 3% erythrocyte suspension was prepared with PBS. The colonies of *E.coli* growth on CFA were emulsified on a VDRL slide in PBS to form milky white suspension. To this equal volume of 3% suspension of erythrocytes was added and gently mixed with a wooden applicator. The slide was rotated manually for 3-5mins observed for haemagglutination macroscopically within 10mins. To determine mannose resistant haemagglutination, colonies from CFA

were emulsified on a slide in PBS. A drop of 2.5% of mannose was added. To this mixture equal volume of 3% suspension of erythrocytes were added and gently mixed with a wooden applicator. Slide was rotated for 3-5mins and observed for HA. Haemagglutination was designated as mannose resistant haemagglutination(MRHA) if HA was observed with and without mannose to the same degree and mannose sensitive haemagglutination(MSHA) if HA was inhibited in presence of mannose [8]. Following controls were used: ATCC *E.coli* 25922 for MSHA. UPEC serotypes O6 and O11 for MRHA [5].

Serotyping: Serotyping of *E.coli* isolates were done at Central Research Institute Kasauli. Antimicrobial susceptibility testing done on Mueller Hinton Agar by Kirby Bauer disc diffusion method as per NCCLS guidelines.

Results and Discussion

A total of 200 *Escherichia coli* isolates were selected from symptomatic cases of urinary tract infection. These were investigated for their serotype and possession of virulence factors. Faecal isolates of *Escherichia coli* from 50 healthy individuals were included as controls. Out of 200 *Escherichia coli* isolates a total of 160(80%) isolates had one or the other virulence factors.

Haemolysin production was observed in 42 (21%) of uroisolates and 8(16%) of control strains. Though number of faecal isolates showing haemolysin is less than that of uroisolate the difference is statistically not significant ($p>0.05$) shown in Table 1.

Table-1: Virulence markers of UPEC obtained from cases and controls

Sl.no	Virulence markers	Cases n=200	Controls n=50	p value
1.	Haemolysin production	42(21%)	8(16%)	Not significant
2.	Haemagglutination activity. a) MRHA b) MSHA c) NO HA	 60(30%) 72(%) 68(34%)	 2(4%) 4(8%) 44(88%)	Highly significant ($p<0.001$)
3.	a) Serum resistance b) Serum sensitive	99(49.5%) 101(50.5%)	16(32%) 34(68%)	Significant ($p<0.05$)

Agglutination of erythrocyte is an indirect evidence of the presence of fimbriae and it provides a simple indirect method of virulence testing. In the present study 60(30%) isolates were MRHA positive, 72(36%) MSHA positive whereas in controls 2(4%) were MRHA positive 4(8%) were MSHA positive. Highly significant difference was observed in the haemagglutination property of uropathogenic and faecal strains ($p<0.001$) observed in Table 1. Several studies have reported haemolysin positive in the range of 16.6%-41%, MRHA ranging between 16.6%-43.45% & MSHA between 13.6 %-20% [5, 8 - 11].

Normal serum possesses bactericidal activity against a wide range of gram-negative bacteria. It has been suggested that capsular antigen of *E.coli* plays an important role in virulence of bacteria conferring serum resistance and inhibiting phagocytosis.

Bacteria are killed by normal serum through the lytic activity of the complement system [6]. In our study group 99(49.5%) isolates were serum resistant. 16(32%) were serum resistant among controls strains. The difference is statistically significant ($p<0.05$) as seen in Table 1.Certain common groups are significantly more prevalent among urinary than among faecal strains suggesting that UTI associated O groups are particularly more virulent.In our study as many as 25 different serotypes (O20,O131,O25,O101,O60,O8,O9,O156,O6,O153,O128,O2,O115,O136,O141,O89, O54,O21,O132,O12,O13,O15,O79,O152) were isolated from UTI. Twenty isolates were untypable. Majority of isolates 46 belonged to O20, 30 to O131, 27 to O25 and 12 to O101 serotype.

*Correlation between virulence factors***:** In the present study 21(10.5%) *E.coli* isolates showed the presence of all the three virulence factors. Presence of two virulence factors was noted in 71(35.5%) of isolates. Only single factor that is serum resistance was observed in 18(9%), Haemolysin production in 6(3%), MRHA in 18(9%) and MSHA in 26(13%). Isolates from pyelonephritis and renal failure cases possessed all the three virulence factors. Strains from pyelonephritic cases belonged to O20, O25 and O132 serotype. Renal failure isolates were belonging to O2O, 025, O60 and O101 serotype.

Antimicrobial susceptibility pattern: Majority of the isolates 184(92%) were sensitive to Amikacin & 170(85%) were sensitive to Nitrofurantoin. 56(29%)-90(45%) were sensitive Cotrimoxazole, Gentamicin, Ceftazidime, Cefpodoxime, Cefixime, Cephotaxime & Netilimicin. Three (1.5%) isolates were resistant to most antibiotics and were sensitive only to Cefdinir, Cefepime & Ceftizoxime. Maximum resistance was recorded for Ampicillin(91.5%) & Nalidixic acid(93%) shown in Table 2. Multidrug resistant *Escherichia coli* uroisolates has been reported by several studies [12-14].

Table 2: Antibiotic susceptibility pattern

Antibiotics	**Sensitive**	
	Number	Percentage
Ak(Amikacin)	184	92.0
Nf(Nitrafurantoin)	170	85.0
Nt(Netilimicin)	90	45.0
Ce(Cephotaxime)	81	40.5
Cfx(Cefixime)	65	32.5
Cep(Cefpodoxime)	58	29.0
Ca(Ceftazidime)	58	29.0
G(Gentamicin)	58	29.0
Co(Cotrimoxazole)	56	28.0
Cp(Cephalexin)	49	24.5
Cu(Cefuroxime)	35	17.5
Cf(Ciprofloxacin)	34	17.0
Of(Ofloxacin)	34	17.0
Nx(Norfloxacin)	30	15.0
A(Ampicillin)	17	8.5
Na(Nalidixic acid)	14	7.0

Conclusion

Periodic review and formulation of antiobiotic policy are needed for control of acquisition of drug resistance. Further studies on better understanding of interaction of different virulence factors at molecular level are necessary as most urovirulent strain express multiple virulence factors simultaneously.

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