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A Prevalence Study of Uropathogenic *Escherichia coli* and Characterization of Virulence Markers

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A B S T R A C T

Urinary Tract Infections (UTI) account for more than 7 million visit for outpatient consultation. UTI is the second commonest one, after the Respiratory Tract Infection. Prevalence in young women is 30 times more than in men. The prevalence of bacteriuria increases with hospitalization and concurrent diseases. UTI types of complicated, uncomplicated, nosocomial UTI, and especially Uropathogenic *Escherichia coli* (UPEC) are some of important ones. The main aim of the study include to find the prevalence of uropathogenic *E.coli* Strains – UPEC at a Tertiary Care Centre; To Characterize the virulence factors of Uropathogens; To evaluate bacterial isolates contributing to the uropathogens, and its virulence factors; To study the antimicrobial susceptibility pattern of uropathogenic *E.coli*. A cross sectional & analytical study the conducted in a Tertiary Care centre involving Medical, Surgical, Nephrology, Urology, Paediatrics, Paediatrics Medicine, Paediatrics Surgery & Ob- Gyn, Outpatient departments. A Midstream sample of urine collected and by routine methods the uropathogens were isolated, and UPEC's virulence factors determined and antibiogram for the same done. The study was undertaken to find out UPEC strains and virulence factors like Haemolysin, Haemagglunation, Cell surface hydrophobicity, Serum resistance shown by Serum Bactericidal assay and Serotyping were detected. Among 320 urinary samples 138 UPEC identified by the above said virulence factors. Antibiogram done by Disc diffusion method in Muller Hinton agar by following NCCLS guidelines. This study showed incidence of UTI more in female, especially in postmenopausal women, Among children UTI more common in males. UPEC is one is the most important cause of UTI and its important virulence factors are haemolysin, haemagglutination, cell surface hydrophobicity and serum resistance were detected.

Introduction

Urinary tract infection is an inflammatory response of the urothelium to the bacterial invasion, associated with bacteriuria and pyuria[1]. Pasteur (1863) recognized urine as a good culture medium for bacteria, and Roberts (1881) related the presence of bacteria in the urine to symptoms[3]. Kass introduced the term Significant bacteriuria defined as presence of 10^5 or more of the organism per ml of urine. In Symptomatic women with coliform infection,

a bacterial count of 10^5 / ml or more has a specificity of 99%. The incidence of UTI is greatly influenced by age, sex and the predisposing factors which impair the defence mechanisms[15].Concept of low count bacteria described by Stamm and colleagues is on basis of low counts of 10^2 or more organisms per ml, especially in infection with Staphylococcus saprophyticus infections[8].

Generally urinary tract infections defined clinically according to site of origin like Cystitus involving the bladder, Acute pyelonephritis-Acute bacterial infection of kidney[4]. Uncomplicated UTI occurs in neurologically normal urinary tract whereas Complicated UTI refers to infection of urinary tract with abnormalities[9].Nosocomial UTI occurs in hospitalized patients, Community acquired or domiciliary UTI caused by common fecal bacteria. *E. Coli* is by far most common organism in community acquired UTI[2]. 90% of cases derived from fecal flora as per Plos *et al.*, 1995 but hematogenic infections do occur as indicated by Ward & Jone 1991[20]. In addition to host factors (age, sex, sexual activity), Genetic factors-pili associated with pyelonephritis and also virulent[14] traits such as adhesions , toxins , LPS , Iron acquisition , presence of capsules[16] or Serum resistance are important in determining uropathogenicity of E.coli strains according to Salyers and Whitt 1994[18].

Materials and Methods

Study design – A cross sectional and analytical study done at Tertiary care hospital, after getting Institutional Ethical committee clearance

Study period – March 2013 to Sept 2013 [Six month]

Sample size - About 320 patients from departments of Urology, Nephrology, Medicine, Surgery, Ob Gyn, Paediatric medicine and Paediatric surgery with history of patient suffering from fever, chills, burning and frequent micturition were screened. In addition 25 healthy individuals were screened to detect virulence factors in faecal samples.

Methodology

Midstream urine [MSU] samples were collected and processed by the following methods Direct microscopy by Gram's staining and Hanging drop preparation for motility detection.

Conventional culture done by Calibrated loop method in full plate in routine medias including Cysteine Lactose Electrolyte Deficient [CLED] media, a selective and differential media for uropathogens.

A battery of biochemical test were done. The E.coli isolates were motile, Indole +ve, MR +ve, VP negative, Urease negative, glucose +ve Lactose +ve, H2S negative ONPG +ve

Detection of virulence factors like α Haemolysin detected in 5% sheep blood agar, Haemagglutination in presence of d – Mannose in[12] CFA (Colonisation factor agar), Cell surface Hydrophobicity was determined in 1.4 molar concentration of ammonium sulphate. Serum[19] Bactericidal Assay was done according to Siegfried *et al.*, with modifications, such as Mueller Hinton agar plates were used in place of blood agar.

Results and Discussion

In this study the 320 patients were selected based on the history suggestive of urinary tract infection. 25 healthy persons were included in the study as controls. Stool sample collected from this 25 control people were cultured on NA and Maconkey media. Of this 320 patients screened& 138 were UPEC. Bacterial counting done by Colony counter, all urine samples having growth off single morphotype

colony with counts more than 10^5 colony / ml considered as Significant bacteriurial.

According to Ward – Wise distribution (Table 3) 85 were from urology, 45 were from Nephrology, 19 were from Paediatrics medicine, 23 were from Paediatrics surgery, 14 were from Obst & Gynaecology ward, 10 were from STD and 3 were from Other ward (Skin).

Table.1

Age Group	Female	Male
Neonatal & Pre School	8	10
School Going	14	14
Young Adults	20	16
Adults	52	46
Elderly	70	70

Table 2

Sex	Adults	Children
Female	144	20
Male	134	22
Total	**278**	**42**

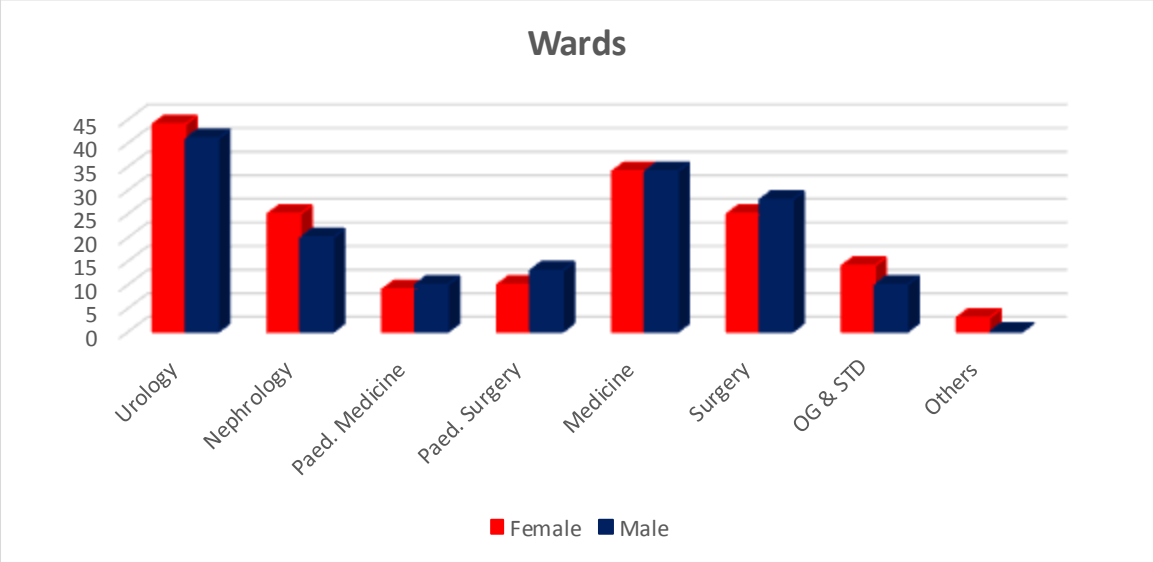
Table 3

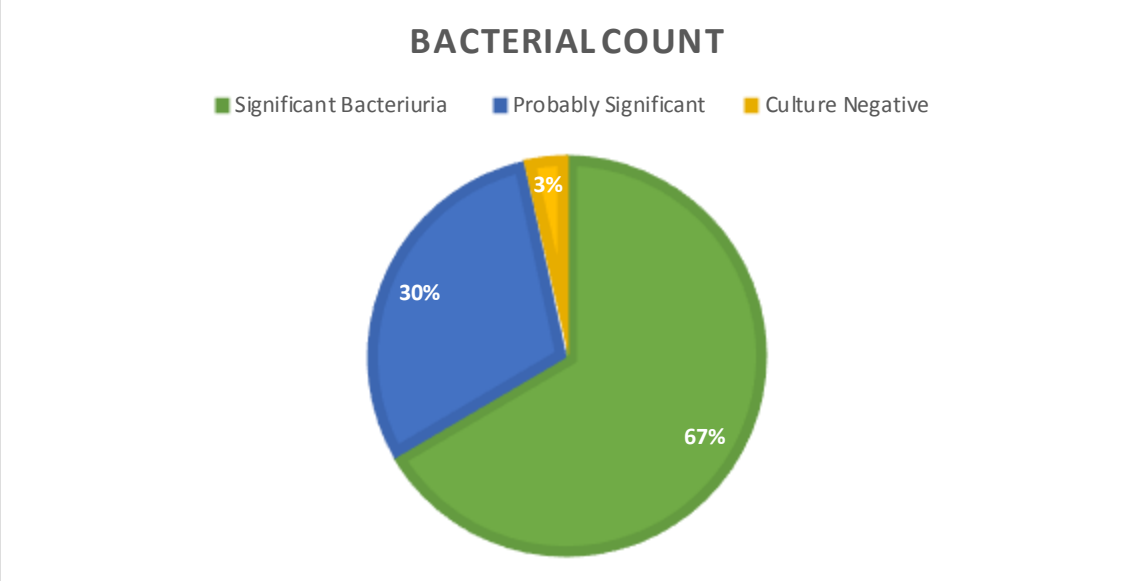
Wards	Female	Male
Urology	44	41
Nephrology	25	20
Paed. Medicine	9	10
Paed. Surgery	10	13
Medicine	34	34
Surgery	25	28
OG & STD	14	10
Others	3	0

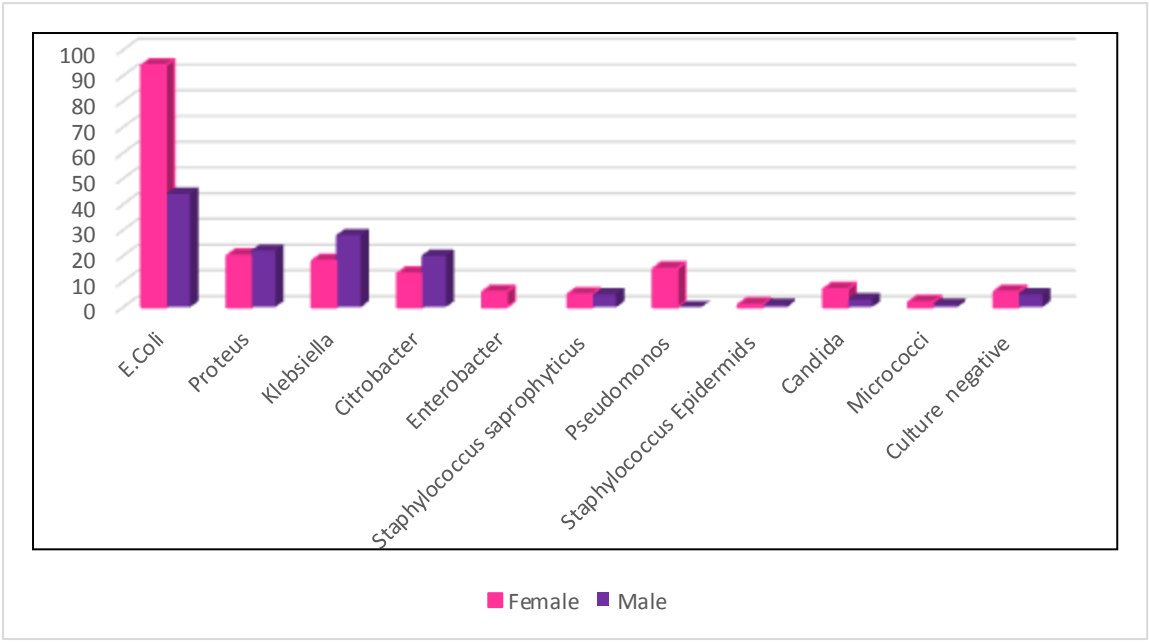
Table 6

One Marker	Cases	Controls
HLY (Lytic)	23	1
MRHA	14	1
CSH	22	0
SR	38	3

Presence of two markers	Presence of three markers
25 uropathogenci E.Coli Showed Lytic & MRHA (Control 1) 10 showed Lytic & CSH (control 0) 12 showed Lytic & SR (control 0) 13 showed MRHA & CSH (control 1) 16 showed MRHA & SR (control 1) 16 showed CSH & SR (control 2)	3 Markers like Lytic + MRHA + CSH were seen in 27 isolated (Control 0) 20 Uropathogenci E. Coli showed Lytic + MRHA + SR (Control 0) 8 Showed Lytic + CSH + SR (Control 1) 3 Showed MRHA +CSH + SR (Control 0)







Among 320 samples E.Coli was isolated in 138 cases (43.13%) as UPEC. Among 138 cases 75 females (23.43%) and 63 males (19.68%)were suffering from UTI due to E.Coli. of the 75 females suffering from UTI 56 (17.18%) were adults and 20 (6.25%) were children. Of the 63 males suffering from UTI 41 (12.81%) were adults and 22 (6.87%) were children. The *E. coli* isolated were tested for the presence of virulence factors to brand them as UPEC.

Among 138 uropathogenic E.Coli, 125 produced haemolytic colonies whereas only 2 in the control produced haemolytic colonies.118 were MRHA positive and 4 in control study showed Haemagglutination. 99 showed aggregation whereas 5 in control study were positive for aggregation. But the aggregation in concentration of 1.54M is considered as optimum (Raksha *et al.,* IJMM). 113 isolates showed resistance to serum bactericidal assay and 6 from the control study showed resistance.

Among 320 Urinary samples 138 were identified as UPEC isolates, by characterising Virulence Factors such,

Haemolysin

The cytolytic protein toxin secreted by most haemolytic E.Coli isolated is known as alpha haemolysin[10]. Haemolysin was detected by determining a zoneof lysis around each colony on 5%sheep blood agar plates after overnight incubation[12].

Haemagglutination

It was detected by clumping of erythrocytes by fimbriae of bacteria in presence of d – Mannose[13]. The method followed was according to siegfred *et al.,* Interpretation – haemagglutination was considered to be mannose resistant when it occurred in presence of D – Mannose and mannose sensitive when it was inhibited by D – Mannose[11].

Cell surface Hydrophobicity

It was determined by Salt aggregation. Strains were considered hydrophobic if they aggregated in concentrations of 1.4 molar concentration of ammonium sulphate. isolated which gave doubtful results were retested.

Serum Bactericidal Assay

It was done according to Siegfried *et al.*, with modifications, such as Mueller Hinton agar plates were used in place of blood agar, and as per the modifications by Taylors method[17]. According to method of Benge were termed serum sensitive if viable count dropped to 1% of initial value and resistant if 90% of organisms survived after 180 minutes.

Antibiogram

Antibiogram was done for 318 E.Coli isolates. Among this 105 were susceptible to Gentamycin and Nitrofurantoin. Amoxycillin Sensitivity was seen only in 29 isolates. Amikacin, Ofoxacin sensitivity was seen in 33 isolates. More resistnace pattern was seen for Amoxycillin. Cephalexin Ciprofloxacin, norfloxain, Cefotaxine and Streptomycin. Among 20 isolates from the stool samples were all sensitive to Garamycin, Cefotaxine, Ciprofloxacin, Norfloxacin and cephalexin. Resistance pattern was seen in 5 isolates only.

Serotyping of uropathogenic E. coli

138 isolated were inoculated in Nutrient Agar stab culture, preserved at 4^0C in refrigerator were sent in batches for O antigen serotyping to the National Salmonella and E.Coli Center, Central Research Institute, Kasauli, Himachal Pradesh. Among 138-2 Were non viable, 24 belonged O1, 20 belonged to O4, 18 belong to O8, 17 belonged to O2, 14 belonged to O9,12 belonged to O18, 10 belonged to O11, 8 belonged to O39.The E.Coli isolated from 25 Control indiuals were also sent in Nutrient agar stab culture to CRI, Kasauli for serotyping. Among 25 isolates – 8 belonged to O1, 6 belonged to O2, 7 belonged to O3 and 4 belonged to O4.

It is concluded from this study, the incidence of UTI is more in females, especially in post menopausal women, which is due to attachment of uropathogens of large numbers to uroepithelial cells. Among children, UTI is found more in male children, which is due to the anatomical factors and intact foreskin in male children.

UPEC is the most important cause of UTI, Important virulence factors are α haemolysin, haemagglutination, cell surface hydrophobicity and serum resistance[16].

Majority of UPEC has 3 Virulence factors α Haemolysin is the most common virulence factor found in UPEC. Presence of α haemolysin in the control E. Coli strains (Faecal) denote that faecal strains also has virulence factors and cause Ascending infection[14].

The haemagglutinating activity of E. Coli against the human red cells is due to the presence of P.Pili, otherwise called as MRHA adhesin of UPEC & those P.Pili that do not bind digalactoside binding specificity are X adhesins. So MRHA adhesins and X adhesins are important virulence factors.

Hydrophobicity is a recently developed novel virulence mechanism by UPEC. Young cultures of E.Coli and GNB are more readily killed by Serum Bacterial assay. But serum resistance seen in young cultures of UPEC. which is considered as one of the virulence factors. The prevalence of E. Coli Virulence markers in UPEC occur as a single or double or three markers.

UPEC and Gut strains belong to early O antigenic serotypes, hence the role of host and presence of virulence factor should be considered in UTI before commencing the treatment.

UPEC strains are more sensitive to Gentamicin and Nitrofurantoin. The resistance to Amoxycillin, Cephalexine, Ciprofloxacin, Norfloxacin, Cefotaxime and

Ofloxacin is seen in UPEC.

Suggestion

Early O antigenic serotypes of faecal E.coli have virulence markers same as that of UPEC. When stool culture done and virulence factors are detected, the patients are more prone to get Ascending UTI.

Apart from UPEC virulence factors and importance should be given to the Host factors and Genetic Susceptibility.

As UPEC the second important cause for nosocominal infections and the drug resistance is more in the nosocomial stains, the role of Clinicians, Microbiologists, and Para medical health care workers is crucial in reducing the incidence of UTI especially in nosocomial infections.

Blocking the primary stages of infection namely Bacterial attachment to host cell receptors and colonization of the mucosal surface may be the most effective strategy to prevent bacterial infection.

As Human Leucocyte Antigen studies have shown that HLA A3 may be associated with recurrent UTI, with recent advances prophylactic vaccination with Adhesin should be the aim in near future[21].

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