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Original Research Article

Prevalence of Urinary Tract Infections in Pregnancy and Detection of the Uropathogenic Markers in *E. Coli* Isolates

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ABSTRACT

Background: Urinary Tract Infection is one of the most frequently seen medical complications in pregnancy, and the most common causative organism is *Escherichia coli*. Pregnant women are at increased risk of UTIs, as it possesses complications such as acute and chronic Pyelonephritis, Toxaemia, Anaemia, Hypertension, Intra Uterine Growth Retardation (IUGR) and increased perinatal mortality.

Objectives: The present study was carried out to,

- Study the incidence of bacterial pathogens causing UTI among pregnant women.
- Detect the antimicrobial sensitivity patterns and the Uropathogenic markers.

Materials and Methods: A total of 300 samples were investigated from pregnant women aged between 18 to 35 years, and women with varying gravida and irrespective of all three trimesters were included in a period of one year. The identified pathogens were screened for pathogenic factors namely haemolysin production, Mannose Resistant and Mannose sensitive Haemagglutination (MRHA, MSHA), Cell surface hydrophobicity and Serum resistance by recommended methods.

Results: The prevalence of UTI was found to be 61.0% and the highest predominant uropathogen was *E.coli* (74.3%).

Key Words: Urinary tract infection, Pregnancy, Uropathogen, Uropathogenic Markers.

INTRODUCTION

Urinary Tract Infection is more prevalent in women and is one of the most frequently seen medical complications during pregnancy. [1] In pregnancy urethral compression at the pelvic brim caused by enlarged uterus leads to urinary stasis (stoppage of the flow or discharge of urine, at any level of the urinary tract,) that results in incomplete emptying of the bladder or pooling of urine in diverticula, which is the single most important factor that can initiate the proliferation of organisms. [2] Asymptomatic and Symptomatic bacteriuria can result in temporary or permanent renal damage in the pregnant women, [3,4] and can also affects the foetus in the form of Intra Uterine Growth Retardation, prematurity, increased risk of prenatal death and congenital abnormalities. [5] *E. coli* accounts

for 50-90% of all uncomplicated urinary tract infections. [6] Other gram negative rods such as *Proteus Mirabilis* and *Klebsiella pneumoniae* are also common. [7] Gram positive organisms such as group B *streptococcus spp.* and *Staphylococcus* are less common causes of UTI. [7]

The detection of bacteriuria allows an approach to be made for the prevention of chronic urinary disease and to avoid complications in pregnancy at an early stage.

The present study was designed to determine the prevalence of UTI among pregnant women and the detection of the uropathogenic markers.

Aims and objectives:-

1. Isolation and identification of uropathogens in pregnant women.
2. Detection of the virulence markers of E.col isolates.
3. Antimicrobial sensitivity pattern of the isolated organisms.

MATERIALS AND METHODS

A total of 300 urine samples were collected from pregnant women aged between 18 to 35 years over a period of 1 year.

1. **Collection of Sample:** Subjects were instructed to collect midstream urine sample with all aseptic precautions in sterile urine container. [9]
2. **Processing:**
   a. **Macroscopic:-**The urine sample thus obtained was observed for its altered colour, presence of turbidity, deposit and the findings were recorded. [9]
   b. **Microscopic:-**Wet preparation of fresh uncentrifuged urine was done to identify pus cells (WBCs) RBCs and organisms (Pus cell significant if ≥10 WBC/mm^3 of urine) [9]
   c. **Isolation: -** All the samples were cultured by the semiquantitative method by inoculating into MacConkey's agar, CLED media and blood agar using a calibrated loop to determine Colony Forming Unit (CFU). Identification of the organisms was done using standard microbiological techniques. [8]

The antimicrobial sensitivity was tested by standard disc diffusion method using commercially available antibiotic discs. [8] The degree of sensitivity was noted by measuring the zone of inhibition produced by diffusion of drug from disc into the surrounding medium.

The organisms thus obtained were screened for the pathogenic markers namely:-

i. **Haemolysin:** Detected by determining the presence of a zone of complete lysis of erythrocytes around the colony and clearing of the medium (5% sheep blood agar). [6]

ii. **Haemagglutination (HA):** This was detected by clumping of erythrocytes by fimbriae of bacteria in the presence of D-mannose. The presence of clumping was taken as positive for haemagglutination. HA was considered to be mannose resistant when it occurred in presence of D-mannose and mannose sensitive when it was inhibited by D-mannose. MRHA indicate expression of P. fimbriae and MSHA indicate expression of type 1 fimbriae virulence marker. [6]

iii. **Cell surface hydrophobicity**: Salt aggregation test, in which the bacteria was tested for their hydrophobic property by using different molar concentration of ammonium sulphate. Those which aggregate with salt particles and formed clumps were considered as hydrophobic. [6]

iv. **Serum resistance:** Overnight culture of organism 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 37^0c for 3h. Resistance of bacteria to

serum bactericidal activity was determined by the percentage of bacteria surviving after 180 minutes of incubation with serum in relation to the original count. Bacteria was termed serum sensitive, when viable count drop to 1% of initial value and resistant if >90% organisms survive after 180 minutes. [6]

v. **Gelatinase test**: Gelatinase production was tested using gelatin agar plates inoculated with organism and incubated at 37^0c for 24 h. The plate was then flooded with 1% tannic acid solution. Developments of opacity around colonies were considered as positive for gelatinase. [12]

vi. **Siderophore production assay:** The test was done by using chrome azurol sulfonate (CAS) agar diffusion assay, in which CAS detects colour change of CAS-Iron complex from blue to orange halo, which was taken as positive after chelation of the bound iron by siderophores. [11]

RESULTS

Out of the 300 samples collected, 117(39%) samples were sterile and rest 183 samples (61.0%) showed bacterial growth. *E.coli* was found as the most common pathogen and next in order was *Klebsiella pneumoniae* as the 2nd most bacterial pathogen.

Table-1: Spectrum of Urinary pathogens isolated from urine samples of pregnant women.

Isolated Organism	No. of cases out of 183	Percentage
Esch. Coli	136	74.3%
Klebsiella Pneumoniae	23	12.5%
Citrobacter Spp.	12	6.5%
Proteus	10	5.4%
Staphylococcus	2	1.09%

As *E. coli* accounted for majority of isolated organisms, those were further evaluated for various pathogenic markers. The most common factor detected was serum resistance in 115(84.5%) cases whereas the cell surface hydrophobicity was obtained as the least common virulence factor.

Most of the isolates were sensitive to Nitrofurantoin followed by Amikacin 79.7%. The antibiotic sensitivity pattern is given in table no. 4

Table-2: Detection of various pathogenic markers in *E. coli* isolates

Pathogenic Markers	n(%)
Haemolysin	57 (41.9%)
Haemagglutination [MRHA] [MSHA]	86 (63.2%)
Cell surface Hydrophobicity	38 (27.9%)
Serum resistance	115 (84.5%)
Gelatinase test	87 (63.9%)
Siderophore production assay	103 (75.7%)

Table-3: Prevalence of MRHA and MSHA E.coli

Total Haemagglutinating isolates	MRHA %	MSHA%
86	42(30.8%)	44(32.3%)

Table-4: The Antibiotic sensitivity pattern of isolates (183)

Antibiotics	Sensitive (%)	Resistant (%)	Intermediate Sensitive (%)
Amikacin (AK)	146 (79.7%)	37 (20.2%)	6 (3.2%)
Nitrofurantoin(NIT)	156(85.2%)	40(22.2%)	2(1.1%)
Norfloxacin (Nx)	115(62.8%)	50(27.3%)	18(9.8%)
Gentamycin(G)	143(77.7%)	38(20.7%)	2(1.1%)
Imipenen (I)	141(76.6%)	21(11.4%)	6(3.2%)
Ceftazidime (caz)	53(28.9%)	110(60.1%)	20(10.9%)
Ampicillin/sulbactam (A/S)	85 (46.4%)	94(51.3%)	4(2.1%)
Piperacillin/Tazobactam(P/T)	140(76.5%)	38(20.7%)	5(2.7%)
Ciprofloxacin (CIP)	102(55.7%)	73(39.8%)	8(4.3%)
Cefuroxime (CXM)	62 (33.8%)	110(60.1%)	11(6.0%)

DISCUSSION

Out of 300 pregnant women, the present research showed that the incidence of UTI was 61%.However this finding is lower than the prevalence rate of 71.6% reported in a similar study by Jellheden et al and 86.6% by Akerele et al. [19,20] This variation can be attributed to factors like the socio-economic status of the group of women studied. [14,15] The present study demonstrated *E. coli* as the main cause of UTI in pregnant women (74.3%) followed by *klebsiella.* Amiri et al. similarly reported

E. coli as the predominant organism causing 83% of UTIs in pregnant women. [16]

A study conducted by Johnson et al, haemolysin was produced by 38% of urinary isolates. [17] In the present study 41.9% strains of *E. coli* produced haemolysin. It has been suggested that colonization with haemolytic strains of *E. coli* is more likely to develop into Urinary Tract Infection.

The high hydrophobicity of the bacterial cell surface promotes their adherence to various surfaces like mucosal epithelial cells, Sharma et al. and coworkers demonstrated 27.6% of the strains were hydrophobic. [13] This research evaluated 27.9% cases as hydrophobic which is consistent with the previous studies. [6,13]

Serum resistance is the property by which the bacteria resist killing by normal human serum due to the lytic action of complement system. In the present study, 84.5% of the isolates were resistant to serum bactericidal activity. [13] A previous study by Sharma et al, showed serum resistance in 86.7% of *E. coli* isolates from urine which is comparable to this result. [13]

The findings of many researchers have been reported the incidence of MRHA in *E. coli* as one of the important virulence factor in the pathogenesis of UTIs. [6,10,11] In the present study, the findings of MRHA positive strains (30.8%) were consistent to the previous researches.

Vagarali and colleagues have reported the incidence of siderophore production to be 98%. [11] Accordingly in the present study siderophore production was found to be 75.7%.

According to Mittal et al, gelatinase producing UPEC isolates were found in 67.5% [10] similar to the present figure of 63.9% *E. coli* isolates showed gelatinase positive.

In a study conducted by Aziz et al, & colleagues, the most of the isolates (88.89%) were sensitive to Nitrofurantoin, [18] in the present study; the result was also in concordance with the previous study in which Nitrofurantoin was the highest sensitive antibiotic against the pathogens (85.2%).

CONCLUSION

This study has revealed that screening for bacteriuria during pregnancy is a useful investigation and the examination of bacteriuria with detection of pathogenic factors, which may help in the prevention of complications associated with UTI in pregnant women. Justified use of antibiotics is important to limit the emergence and spread of antibiotic resistance in bacteria.

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