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RESEARCH ARTICLE

Prevalence and distribution of gram negative bacteria of Enterobacteriaceae causing Urinary tract infections among hospitalized patients

Devanand Prakash* and R.S. Saxena
Dept. of Botany, Meerut College, Meerut, Uttar Pradesh, India
botanicalsmcm@gmail.com*; +91 9456959458

Abstract

Prevalence and frequency distribution in isolated gram negative bacteria causing urinary tract infections (UTI) in patients attending Meerut Kidney Hospital, Meerut city, Uttar Pradesh, India was determined. About 132 clinical samples of urine were collected from the patients and 96 gram negative bacteria were isolated from 64 positive samples of urine. The total prevalence of UTI was found to be 48.48% in the study population. The urinary tract infection was most frequently encountered in females (64.49%) between the age group 26 to 36 years (90.48%) followed by 15-25 years (70.59%). Among the isolated gram negative bacteria, the prevalence of *Escherichia coli* was found highest (42.71%) followed by *Klebsiella pneumoniae* (23.96%), *Proteus* spp. (19.79%) and *Enterobacter* spp. (13.54%).

Keywords: Gram negative bacteria, urinary tract infections, *Escherichia coli*, *Klebsiella pneumoniae.*

Introduction

Among all bacterial infections, UTI is most common which occurs in humans of all age groups in both genders as well as in hospital and community settings (Orret and Davis, 2006; Omoregie *et al.,* 2008). In hospitals and community settings, UTI emerges as a major cause of morbidity (Omigie *et al.,* 2009). It causes many urinary disorders as urosepsis, renal scarring and progressive kidney damage that lead to a high health risk with high mortality, morbidity and economic loss. Infections of urinary tract caused by microbial pathogens are classified primarily on the basis of infection site involved as cystitis in case of bladder, pyelonephritis in case of kidney and bacteriuria in case of urine. Uncomplicated UTI refers to the bacterial infection occurring in humans who have no prior instrumentation and have a normal urinogenital tract, whereas, complicated UTI refers to the bacterial infection in structurally or functionally abnormal urinogenital tract in humans who have instrumentation treatment as indwelling catheters (Gonzalez and Schaeffer, 1999; Stamm and Hooton, 1993).

Globally symptomatic UTI was estimated in 7 million visits to outpatients, 1 million to emergency departments and 10, 000 in hospitalized patients annually (Wilson and Gaido, 2004). It has also been estimated that UTI is one of the major causes of nosocomial infection and a second major cause of bacteremia in hospitalized patients (Weinstein, 1997; Stamm, 2002). *Escherichia coli* is the major enteric pathogen causing UTI, however, the distribution of UTI causing pathogens is changing (Ojiegbe and Nworie, 2000). There are several factors including age and sex (Ojiegbe and Nworie, 2000), hospitalization and obstruction (Epoke *et al.,* 2000) which interfere in the natural resistance to infection of UTI.

Females are more prone to infections with UTI than males except at higher ages (Akinkugbe *et al.,* 1973). The major cause of female UTI is the shorter and wider urethra through which the bacteria, from fecal flora, are readily entered to the bladder and kidney (Jones *et al.,* 2006). The other modes of bacterial entry in female urethra are sexual intercourse as well as during pregnancy and child birth (El-Sweih *et al.,* 2008; Kolawale *et al.,* 2009). A wide range of microorganisms are involved in causing UTI, however, the most common among them is *E. coli* and other members of Enterobacteriaceae family which are estimated approximately 75% of the isolates. *Enterococcus faecalis* and drug-resistant gram negative rods such as *Pseudomonas* spp. are more common in complicated and hospital acquired UTI.

The distribution of UTI causing pathogens depends on age, sex, catheterization and hospitalization of patients (Sefton, 2000). *Escherichia coli* remained the most common pathogen causing uncomplicated UTI and the etiology was about 75-90% of infection (Nakhjavani *et al.,* 2007; Omigie *et al.,* 2009). The other gram negative pathogens causing UTI are *Klebsiella* spp., *Proteus mirabilis* and *Pseudomonas aeruginosa, Enterococci*, whereas, the most frequently found gram positive bacteria in UTI is coagulase negative *Staphylococcus* (Shankel, 2007). The etiology of UTI causing pathogens in community and hospitalized patients has been varied with time and from place to place (Gruneberg, 1980; Gales *et al.,* 2000; Saffar *et al.,* 2008). Hence, the aim of the present investigation is to find out the prevalence of UTI in different sexes and age groups with the distribution frequency of isolated gram negative bacterial pathogens involved in UTI hospitalized patients.

Materials and methods

Study population: The study population was drawn from patients attending Meerut Kidney hospital, Meerut City, Uttar Pradesh, India. One hundred thirty two patients (73 male and 59 female patients) who were not clinically diagnosed for Urinary tract infections were involved in the study. The patients on antibiotic therapy and clinically diagnosed of UTI were excluded from the study.

Collection of samples: Freshly voided midstream urine specimens were collected aseptically from 132 patients (73 males and 59 females) with symptoms suggestive of UTIs (Cheesborough, 2006; Savas *et al.,* 2006; Santo *et al.,* 2007). All patients had clinical evidence of urinary tract infections, as determined by the treating physician. Only a single positive culture per patient was included in the analysis. The urine samples were collected into labeled 20 mL calibrated sterile bottles distributed to the patients by the attending physicians suspected to have UTIs. In each container, boric acid (0.2 mg) was added to prevent the growth of bacteria in the urine. All patients were instructed on how to collect the urine samples aseptically and taken to the laboratory immediately for culture. The ethical approval was obtained for the study and subjected to the hospital administration.

Sample processing

Total aerobic plate count: The bacterial load of the urine samples was determined using the surface plating method (Santo *et al.,* 2007). Serial dilutions of the urine samples were carried out by pipetting 1 mL of the urine into 9 mL of peptone water in a sterile test tube. Then 1 mL of this dilution was pipetted into another test tube till 7^{th} test tube was reached in order to obtain countable colonies. One milliliter of the final dilution was spread on sterile 90 mm petri plates and they were then incubated at 35-37°C for 24 h, after which the count was obtained. Only urine samples that yielded ≥10^5 cfu/mL was considered for further analysis. The mean of triplicate results were taken (El-Astal, 2005).

Microscopy: The urine samples were mixed and aliquots centrifuged at 5000 rpm for 5 min. The deposits were examined using both x10 and x40 objectives. Samples with ≥10 white blood cells/mm^3 were regarded as pyuric (Smith *et al.,* 2003).

Bacteriology: In the hospital laboratory, each well mixed urine sample (5 µL) was inoculated on McConkey agar (Oxoid) and Blood agar (Oxoid). The inoculum on the plate was streaked out for discrete colonies with a wire loop following standard procedures (Cheesborough, 2006, Mordi and Erah, 2006). The culture plates were incubated at 35-37°C for 24 h and observed for bacterial growth through formation of colonies. All the bacterial strains were isolated and identified using morphological, microscopy and biochemical tests following standard procedures described by Cowan and Steel (1974) and Cheesborough (2006).

Statistical analysis: All data were analyzed with Statistical Package for Social Sciences (SPSS) software for Windows, version 20.0. The Chi-square test used for statistical comparisons between the groups and a $p<0.05$ was considered as statistically significant at 95% level of confidence.

Results

Totally 132 urine samples from patients (73 males and 59 females) were examined in this study. Out of 132 samples, 64(48.48%) showed significant bacteriuria; 41(69.49%) were females while 23(31.51%) were males (Table 1). Microscopic examination of the centrifuged urine revealed that 62(46.97%) of the urine specimen showed significant pyuria (pus cells of 5 cell/hpf) while 70(53.03%) showed insignificant pyuria (pus cells of 1-2 cells/hpf). Out of the 62 significant pyuric samples, 59(95.16%) yielded significant bacterial growth, 2(3.23%) showed insignificant bacterial growth while 1(1.61%) yielded no bacterial growth (Table 2).

Table 1. Prevalence of UTIs in relation to sex of patients.

Sex	Examined	Positive	Percentage
Male	73	23	31.51%
Female	59	41	69.49%
Total	132	64	48.48%

Table 2. Relationship between significant bacteriuria and pyuria in urine samples of patients.

Pyuria result	Number	Percentage (%)
Significant pyuria	62	46.97%
Insignificant pyuria	70	53.03%
Significant pyuria with growth	59*	95.16%
Significant pyuria without growth	1*	1.61%
Significant pyuria with insignificant growth	2*	3.23%

**From 62 urine samples showing significant pyuria.*

Table 3 shows the prevalence of UTI in relation to age and sex of patients. Results indicate that a high percentage of organisms were isolated from both males and females within the age brackets of 26-36 years (71.43%) followed by 37-47 years (42.31%). Comparatively, however, there were more cases in females than males in all age groups and in total. The highest prevalence of UTIs in females found in the age group between 26 to 36 years (90.48%) followed by 15-25 years (70.59%), 37-47 years (54.55%) and ≥48 years (40%). However, in males, the highest prevalence was found in the age group of 26-36 years (52.38%) followed by 37-47 years (33.33%), ≥48 years (30%) and 15-25 years (14.81%). The female to male ratios for the occurrence of urinary tract infection within the age groups were as follows: 15-25 years (3:1), 26-36 years (1.73:1), 37-47 years (1.2:1) and ≥48 years (1.33:1). Totally 96 isolates of gram negative bacteria were obtained from 64 positive samples of urine.

Table 3. Prevalence of UTIs in relation to age and sex of patients.

Age group (Years)	Male examined	Male negative (%)	Male positive (%)	Female examined	Female negative (%)	Female positive (%)	Total No. of positive (%)
15-25	27	23(85.19%)	4(14.81%)	17	5(29.41%)	12(70.59%)	16(36.36%)
26-36	21	10(47.62%)	11(52.38%)	21	2(9.52%)	19(90.48%)	30(71.43%)
37-47	15	10(66.67%)	5(33.33%)	11	5(45.45%)	6(54.55%)	11(42.31%)
≥48	10	7(70%)	3(30%)	10	6(60%)	4(40%)	7(35%)
Total	73	50(68.49%)	23(31.51%)	59	18(30.51%)	41(69.49%)	64(48.48%)

significant at p<0.05

Table 4. Frequency distribution of isolated gram negative microorganisms in relation to sex of patients.

Gram negative bacteria	Total number isolated		Proportion			
			Males		Females	
	Number	%	Number	%	Number	%
Escherichia coli	41	42.71	14	42.42	27	42.85
Klebsiella pneumonia	23	23.96	8	24.24	15	23.81
Proteus spp.	19	19.79	7	21.21	12	19.04
Enterobacter spp.	13	13.54	4	12.12	9	14.29
Total	96	100	33	100	63	100

Out of these 96 isolates, 33(34.38%) were obtained from male urinary samples and 63(65.62%) were obtained from female urinary samples. Among all 96 isolates, *E. coli* showed the high prevalence 41(42.71%) in total followed by *Klebsiella pneumoniae* 23(23.96%), *Proteus* spp. 19(19.79%) and *Enterobacter* spp. 13(13.54%) (Table 4). The statistically significant variations were observed ($p<0.05$) at 95% level of confidence for the variables such as sex, age groups ($\chi^2=8.918$; $p=0.030$; degree of freedom=3), male positive and negative urine samples ($\chi^2=9.986$; $p=0.002$; degree of freedom=1) and female positive and negative urinary samples ($\chi^2=8.966$; $p=0.003$; degree of freedom=1).

Discussion

The prevalence of UTI was found to be 48.48% in this study and this rate of prevalence is higher than the other studies which accounts 25.6% (Nedolisa, 1998), 22% (Ekweozor and Onyemenen, 1996), 38.6% (Akinyemi *et al.,* 1997), 35.5% (Ebie *et al.,* 2001) and 36.68% (Mehta *et al.,* 2013). The UTI was found high in females (69.49%) than males (31.51%). Females of the age group 26-36 years were found more susceptible (90.48%) to UTI followed by 15-25 years (70.59%). This study revealed that the female to male ratio, was highest (3:1) in age group of 15-25 years, in respect of incidence of infection. These findings correlate with other reports which showed that females are more prone to UTIs than males during adolescence and adulthood (Orret and Shurland, 1998; Gales *et al.,* 2000; Tambekar *et al.,* 2006; Theodore, 2007; Adedeji and Abdulkadir, 2009; Kebira *et al.,* 2009; Kolawole, 2009). The main cause of high prevalence of female UTIs is the shorter urethra, its close proximity to anus and sexual activity (Adedeji and Abdulkadir, 2009). Highest incidence of UTIs among female to male ratio was found in the age group of 15-25 years (3:1) followed by 26-36 years (1.73:1), ≥48 years (1.33:1) and 37-47 years (1.2:1).

These findings differ from other reports (Kalantar *et al.,* 2008; Kebira *et al.,* 2009) in which female to male ratio in respect of causing UTIs was lower in neonates and young children. The prevalence rate of UTI in boys depends on many factors including congenital malformations and uncircumcised genitalia which often contaminated (Kebira *et al.,* 2009). In this study, the incidence of UTI was found highest in young age patients of age groups 26-36 years (71.43%) followed by 37-47 years (42.31%), 15-25 years (36.36%) and ≥48 years (35%). These results corroborates with other reports where highest incidence of UTI, 63.43% (Dimitrov *et al.,* 2004) and 74.7% (Omigie *et al.,* 2009) was recorded in the age group between 20 to 50 years. *Escherichia coli* (42.71%) was found as the most prevalent bacterial strain followed by *Klebsiella pneumonia* (23.96%) causing UTIs. Similar findings were also reported by several authors (Grunerberg, 1980; Orret and Shurland, 1998; Daza *et al.,* 2001; Dimitrov *et al.,* 2004; Inabo and Obanibi, 2006; Abubakar, 2009; Omigie *et al.,* 2009) but differed in which *Pseudomonas aeruginosa* recorded highest prevalence followed by *Klebsiella* spp. (Ehinmidu, 2003; Aboderin *et al.,* 2009). *Klebsiella* spp. was the second most common UTI causing bacteria in other reports (Gales *et al.,* 2000; Al-Sweih *et al.,* 2005; Uwaezuoke and Ogbulie, 2006; Haghi-Asteiani *et al.,* 2007; Abubakar, 2009) which correlates our study.

Conclusion

It may be concluded that the urinary tract infections affects a large proportion of the population in the study area and is more prevalent in females between the age group of 26 to 36 years. It is also concluded that *E. coli* is the most frequent gram negative bacteria causing UTIs. Finally, since the hospital environment is a sort of collection agency for many pathogenic microorganisms by virtue of the many seriously ill patients who passes through it.

To conclude, it is extremely important for the hospital management to do everything possible to minimize the spread of these organisms to other patients.

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