Frequency of Uropathogens in Different Age Groups and Genders - A Laboratory Based Study in Islamabad

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Abstract
Objective: To find out the frequency of uropathogens in different age groups and gender.

Materials and methods: This cross sectional study was carried out in the Microbiology Department of Islamabad Diagnostic Centre from October 2014 to February 2015 A total of 1434 urine samples were collected in sterile containers from patients both sexes with clinical evidence of urinary tract infection determined by their treating physicians. The patients already on antibiotics, younger than 2 years in age and mixed growth of organisms on culture were excluded from study.

Results: Significant growth was revealed in only 464 patients (32.36%), while mixed growth or no growth /insignificant growth was found in 158 (11.02%) and 812 (56.62%) patients respectively. E. coli was found to be the most common organism isolated in 61.20%, followed by Enterococcus Spp (11.85%) and Klebsiella Spp. (10.35%). E. coli was the most common isolated organism in all age groups and both sexes. Age group 51-70 years has the highest frequency of uropathogens (29.10%) followed by age group 31-50 years (25.65%). Conclusion: that E. coli was found to be the most common uropathogen in all age groups and both sexes.

Key Words: Cysteine Lactose Electrolyte Deficient (CLED) agar, E. coli, Urinary tract infection (UTI), Uropathogen.

Introduction
History of urinary tract infections (UTIs) have been described since ancient times with the first documented description in the Ebers Papyrus dated to c. 1550 BC.1 It was described by the Egyptians as "sending forth heat from the bladder".2 Effective treatment did not occur until the development and availability of antibiotics in the 1930s before which time herbs, bloodletting and rest were recommended.1 UTIs are serious health affecting problems worldwide, and are the most common infections after upper respiratory tract infections.3 It is estimated that 150 million UTIs occur yearly world-wide, accounting for $ 6 billion in health care expenditures.4 Incidence of UTI is higher in women than men. Although normal female anatomical features predispose all women to a degree, for most women, UTIs should not be more than an occasional event.5

The UTI incidence ratio in middle-aged women to men is 30:1; yet, during later decades of life, the ratio of infection in women to men with bacteriuria progressively decreases.6 It has been estimated that at least one-third of all women in the United States are diagnosed with a UTI by the time they reach 24 years of age.7 According to the National Health and Nutrition Examination Survey (NHANES-III), the incidence for UTI is 13,320 per 100,000 adult women per year.7 Among young, healthy women with cystitis, the infection recurs in 25% of women within 6 months after the first urinary tract infection, and the recurrence rate increases with more than 1 prior urinary tract infection.9,10 Urinary Tract Infections may be symptomatic or asymptomatic, and either type of infection can result in serious sequelae if left untreated.12 UTIs are also classified as uncomplicated or complicated. The term uncomplicated UTIs refer to the invasion of a structurally and functionally normal urinary tract by a non-resident infectious organism. A complicated UTI is an
infection associated with a condition, such as structural or functional abnormalities of the genitourinary tract or the presence of an underlying disease, which increases the risk of acquiring an infection or of failing therapy. Acute uncomplicated pyelonephritis is much less common than cystitis (estimated ratio, 1 case of pyelonephritis to 28 cases of cystitis), with a peak annual incidence of 25 cases per 10,000 women 15 – 34 years of age. Although several different microorganisms can cause UTIs, including fungi and viruses, bacteria are the major causative organisms and are responsible for more than 95% of UTI cases. E. coli is the most predominant organism responsible for UTI followed by Enterobacter, Klebsiella, Pseudomonas, and others. Candida albicans is the leading cause of fungal UTI especially in women.

In recent years UTI pathogens have become resistant to most of the therapeutic agents that have been developed against them, the major contributing factor is the overuse of broad spectrum antibiotics which changed the intestinal flora and induced bacterial resistance. Antibiotic resistance has also increased in urinary bacterial pathogens due to improper use of drugs which involve the public (e.g. self-medication), prescriber (e.g. misinformation, absence of diagnostic tools) and dispenser (e.g. over the counter use, inadequate storage, use of expired drugs). There is insufficient epidemiological data regarding the common uropathogens in different gender and age groups in different regions of Pakistan. Our study focuses on frequency of uropathogens in different gender and age groups in Islamabad area.

Material and Methods

This cross-sectional observational study was carried out in the microbiology department of Islamabad Diagnostic Centre, from October 2014 to February 2015. The urine samples of 1434 patients ≥ 2 years of age, comprised of 955 females and 479 males, who had clinical evidence of urinary tract infection, determined by their treating physicians were initially included in the study. Patients already on antibiotics were not included in the study. Patients having leukocyte count ≤ 5 per high power field on light microscopy of the urine, with mixed growth or insignificant /no growth on culture were also excluded from the study. Finally, 464 patients (325 females and 139 males) with significant growth were studied. A specimen was considered positive for UTI if a single organism was isolated at a concentration of ≥10^5 cfu/mL and > 5 pus cells / HPF were observed on microscopic examination of urine. Organisms were identified on the basis of colony morphology, cultural characteristics, Gram staining, and biochemical test as per standard protocols. All Gram negative rods and enterococci were identified by using conventional biochemicals plus API 20E (Biomeurex) and Oxoid Streptococcal grouping kit respectively.

Data Analysis: Data was categorized according to age and sex. The patients were divided into age groups as 2 – 10 years, 11 – 30 years, 31 – 50 years, 51 – 70 years and > 70 years. Age in years was analyzed as mean, SD and range. Age grouping, gender and culture were analyzed as frequency (number) and relative frequency (%).

Sample Processing: Freshly voided mid stream urine specimens were processed within 30 minutes. A calibrated loop method was used for the isolation of bacterial pathogens from urine samples. The urine was mixed by rotating the container and with sterilized standard calibrated loop, 1 µl (0.001mL) of urine was inoculated on Cysteine Lactose Electrolyte Deficient Agar (CLED Agar) and blood agar. Inoculated plates were incubated aerobically at 37°C for 18 – 24 hours and for 48 hours in negative cases. The number of isolated bacterial colonies was multiplied by 1000 for the estimation of bacterial load per mL of the urine sample. A specimen was considered positive for UTI if a single organism was isolated at a concentration of ≥10^5 cfu/mL or when an organism was grown at a concentration of 10^5 cfu/mL and > 5 pus cells / HPF were observed on microscopic examination of urine.

<p>| Table 1: Distribution of the sample population according to yield of culture (n = 1434) |
|---------------------------------|------------------|------------------|</p>
<table>
<thead>
<tr>
<th>S. No</th>
<th>Culture growth</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No growth / insignificant growth</td>
<td>812</td>
<td>56.62</td>
</tr>
<tr>
<td>2</td>
<td>Mixed growth</td>
<td>158</td>
<td>11.02</td>
</tr>
<tr>
<td>3</td>
<td>Significant growth / isolated organism</td>
<td>464</td>
<td>32.36</td>
</tr>
</tbody>
</table>

Table 2: Age and Gender wise distribution of the sample yielding mixed growth (n 158)
population yielding mixed growth as per sex and age is shown in Table 2. The mixed growth was found in 130 females (82.28%) and 28 males (17.72%). Significant growth was obtained in 464 patients, 324 (69.83%) females with mean age of 43.97 ± 22.66 years and 140 (30.17%) with mean age of 48.61 ± 23.56 years. Gender distribution amongst UTI patients with significant growth is shown in Figure 1. Frequency of uropathogens with significant growth is shown in Table 3. *E. coli* was found to be the most common organism isolated in 61.20% of patients followed by Enterococcus Spp. and Klebsiella Spp. which were 11.85% and 10.35% respectively. Age group distribution of patients yielding significant growth with relative frequency is shown in Table 4. Age group 51-70 years has the highest frequency (29.10%) followed by age group 31-50 years (25.65%). Sex and age group distribution of uropathogens with relative frequency in patients is shown in Table 5. *E. coli* is most common uropathogens in age group 51 – 70 years whereas Enterococcus Spp. in age group 31 – 50 years.

### Table 4: Age group distribution of Patients yielding significant growth (n = 464)

<table>
<thead>
<tr>
<th>S. No</th>
<th>Age group in years</th>
<th>Females (n 130)</th>
<th>Males (n 28)</th>
<th>Total (n 158)</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2 – 10</td>
<td>16</td>
<td>4</td>
<td>20</td>
<td>12.66</td>
</tr>
<tr>
<td>2</td>
<td>11 – 30</td>
<td>41</td>
<td>4</td>
<td>45</td>
<td>28.48</td>
</tr>
<tr>
<td>3</td>
<td>31 – 50</td>
<td>34</td>
<td>8</td>
<td>42</td>
<td>26.58</td>
</tr>
<tr>
<td>4</td>
<td>51 – 70</td>
<td>29</td>
<td>5</td>
<td>34</td>
<td>21.52</td>
</tr>
<tr>
<td>5</td>
<td>&gt; 70 years</td>
<td>10</td>
<td>7</td>
<td>17</td>
<td>10.76</td>
</tr>
</tbody>
</table>

### Table 5: Sex and age group distribution of uropathogens with relative frequency in Patients with Urinary Tract Infections (n = 464)

<table>
<thead>
<tr>
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<th></th>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>2 - 10</td>
<td>F</td>
<td>23</td>
<td>3</td>
<td>1</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>34</td>
<td>16</td>
<td>7.33 %</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>5</td>
<td>2</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>12</td>
<td>8</td>
<td>2.58 %</td>
</tr>
<tr>
<td>11 - 30</td>
<td>F</td>
<td>36</td>
<td>11</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>9</td>
<td>71</td>
<td>15.30 %</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>11</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>24</td>
<td>5.17 %</td>
</tr>
<tr>
<td>31 - 50</td>
<td>F</td>
<td>57</td>
<td>11</td>
<td>0</td>
<td>9</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>4</td>
<td>85</td>
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<tr>
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<td>M</td>
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<td>2</td>
<td>2</td>
<td>4</td>
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<td>0</td>
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<td>1</td>
<td>34</td>
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<td>66</td>
<td>7</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>4</td>
<td>90</td>
<td>19.4 %</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>21</td>
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<td>1</td>
<td>10</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>45</td>
<td>9.70 %</td>
</tr>
<tr>
<td>&gt; 70</td>
<td>F</td>
<td>33</td>
<td>6</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>45</td>
<td>9.70 %</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>11</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>24</td>
<td>5.17 %</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>284</td>
<td>55</td>
<td>6</td>
<td>48</td>
<td>5</td>
<td>6</td>
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<td>27</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td>23</td>
<td>464</td>
<td>100 %</td>
</tr>
</tbody>
</table>
Discussion

UTI is the most common types of infectious disease worldwide and also in our community. In this study of 1434 patients presenting with clinical features of UTI, only 32.36% had a urinary tract infection. This is possibly because UTI symptoms are not a reliable indicator of infection and in younger children are non-specific. In our study most of the subjects had been referred by general practitioners not specialists. These results indicate that urine culture is mandatory for the definitive diagnosis of UTI, the empirical therapy should only be done by specialist physicians in cases where it is necessary. Our results show a lower UTI rate of 30.17% in males. The reason for this may be due to the higher number of women than men in this study, and because males are less prone to UTIs, possibly because of their longer urethra and the presence of antibacterial substances in prostate secretions. Ghazi Khan et al and Mehr MT also reported a low incidence of UTI in males of 22.80% and 37.31% respectively.21,22

Our study revealed statistically predominance of UTI in females (69.83%). This result is more or less similar to those reported from different studies in Pakistan.19,20,21 Higher prevalence of UTIs in females is attributed to anatomical predisposition (short urethra), close approximation of urethra and vagina and sexually active life during reproductive age group. In our study members of family enterobacteriaceae were major contributors to UTI patients. E. coli was found to be the most prevalent organism isolated in 61.20% followed by Enterococcus Spp. in 11.85%, Klebsiella Spp. in 10.34%, Pseudomonas Spp. in 5.8% and Candida Spp. in 4.9 % of patients. Most of the studies in Pakistan also found that E. coli is the most common uropathogens.22-25 In our study the second most common uropathogens was Enterococcus Spp. where as in other studies it is either Klebsiella Spp. or Staph. Aureus.13,19 The difference and similarity in distribution and type of microorganism may result from different host factors, environment conditions, health care practices, socioeconomic standards, hygiene practices and education programs in each region.26

In this study, the prevalence of UTI was highest for the age group of 50 – 70 years (29.10%) followed by age group 31-50 years (25.65%) and age group 11 – 30 years (20.47%). It was less common in age group 2-10 years (9.91%) and age group > 70 years (14.87%). In all age groups and both sexes E. coli was the most common organism isolated. After E. coli, Enterococcus Spp. and Klebsiella Spp. were more prevalent in females while Pseudomonas Spp. was in males. Our findings are comparable with other studies conducted in Pakistan which also revealed highest prevalence of uropathogens in age groups 21–60 years.19 27 In our study, Enterococcus Spp. was the most common gram-positive uropathogens (11.85%). Yasir Se al reported 8.0% prevalence of Enterococcus in her study.13

Conclusion

It is concluded that gram-negative organisms were the statistically significant uropathogens. E. coli was the most common uropathogen isolated from all age groups and both sexes. Our findings are at par with other studies conducted in Pakistan. Enterococcus was the most common gram-positive organism responsible for urinary tract infections.

Conflict of interest

This study has no conflict of interest as declared by any author.

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