ORIGINAL ARTICLE

CHARACTERIZATION AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF UROPATHOGENS FROM KHYBER PAKHTUNKHWA, PAKISTAN

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ABSTRACT

Objective: To characterize different uropathogens and determine their antibiotic susceptibility pattern.

Material and Methods: In the present study, 300 patients with clinical symptoms were investigated from tertiary care hospitals of Khyber Pakhtunkhwa from January 2015 to December 2015. Clean catch midstream urine of patients were collected, leukocyte count was determined and were cultured. The recovered bacteria were identified using different biochemical tests. Susceptibility to antimicrobial agents was determined using disc diffusion method of Kirby-Bauer.

Results: From a total of 300 samples, 28.6% were positive for both bacterial culture and pus cells while 9.6% and 15.6% samples were having only pus cells and bacterial culture respectively. Microorganisms recovered and identified were E. coli (73%) followed by Citrobacter freundii (9%) Staphylococcus aureus (4%), Pseudomonas aeruginosa (4%), Morganella morganii (3%), Proteus, Streptococcus spp. and Candida albicans (1% each), while 4% were identified as normal flora of the urinary tract. The recovered microorganisms were mostly sensitive to tazocin and amikacin while they showed resistance against norfloxacin and tobramycin.

Conclusion: E. coli was the most common uropathogen. There is a strong association between pus cells and UTI. The susceptibility pattern of different uropathogens show increasing resistance to commonly used antimicrobial agents.

Key Words: Urinary tract infection, pus cells, uropathogens.

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INTRODUCTION

Urinary Tract Infection (UTI) is one of the most common bacterial infections[1]. UTI is more frequent in female because of short urethra while in male its occurrence increases with age due to many factors[2]. It may be symptomatic or asymptomatic[3]. The common symptoms include burning micturition, urgency, dysuria, cramping in the lower abdomen, mental changes, back or flank pain, chill, nausea, fever, vomiting, fatigue and weakness etc.[4] UTIs may be complicated or uncomplicated. Uncomplicated UTI mostly occurs in women and may not require antibiotic therapy[5-6] while complicated UTIs are more severe in nature and are difficult to treat[7]. Recurrent UTIs may either be relapse in which the same uropathogen is involved or it may be re-infection in which different uropathogens are involved[8-10].

In most of the cases E. coli is the cause of UTI, other microorganisms involved in UTIs are Klebsiella spp., Mycoplasma spp., Enterobacter spp., and Staphylococcus aureus, Candida albicans, threadworm, fluke etc.[11-13]. Risk factors for UTI vary in men, women and children. In women sexual behavior, pregnancy, contraceptives and menopause increases the risk for UTI[14-16]. In older male, Benign Prostatic Hyperplasia (BPH) increases the risk for UTI in men while circumcision and vesico-ureteric reflux (VUR) are the risk factors specific to children[17-20]. Prolong use of catheters also provide a route for the entry of uropathogens and thus increase the risk for UTI. Certain medical conditions increase the risk for UTI that include diabetes, kidney disorders, immune deficiency, dehydration, unbalanced diet and anatomical abnormalities of the urinary tract[21-23].

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This study was designed to isolate and identify common uropathogens involved in UTI, to find their association with the pus cells in the urine and to determine their susceptibility against different antibiotics in patients visiting tertiary care hospitals in Peshawar.

MATERIAL AND METHODS

In this laboratory based cross-sectional study, which was conducted in tertiary care hospitals of Peshawar from January 2015 to December 2015 a total of 300 patients from different hospitals were included. Clean catch urine samples, consecutive and non-repetitive, were collected from 164 females and 136 males in wide mouth sterile containers. These collected samples were transported to the Microbiology and Pathology laboratory without any delay (within two hours of collection). Collected urine specimens were physically examined for its colour, appearance and odour. For microscopic examination, urine samples were centrifuged in 5ml tubes for 5 minutes at 2000 rpm, pellet at the bottom of the tube was used for the detection of leukocytes and erythrocytes using light microscope.

Urine samples were streaked out using sterile inoculating loop on Cysteine Lactose Electrolyte Deficient (CLED) agar. The inoculated plates were incubated at 37°C for 24-48 hours. Specimens that showed growth of uropathogens in concentration of $\geq 10^5$ cfu/ml were considered positive for UTI. The isolated uropathogens were identified using different biochemical tests such as Triple Sugar Iron (TSI) test, urease test, citrate test, indole test, catalase test, coagulase test and oxidase test. Kirby and Bauer disc diffusion method was used to determine antibiotic susceptibility of the uropathogens following the guidelines of Clinical and Laboratory Standards Institute (CLSI, 2006). Muller- Hinton agar was used for this process. In the present study different antibiotics discs used with their standard concentrations were: gentamicin 10 μg, amikacin 30 μg, cefipime 30 μg, aztreonam 30 μg, ciprofloxacin 5 μg, imipenem 10 μg, norfloxacin 10 μg, ceftazidime 30 μg, tazocine 100/10 μg, tobramycin 10 μg, cefoparazone 75 μg.

RESULTS

A total of 300 samples from different age groups between 3 to 70 years were collected, 136 from males and 164 from females, out of which 77 females and 43 males showed positive result for UTI by yielding growth.

Every urine sample was processed for the isolation of uropathogens and for the determination of pus cells, in order to compare and correlate the presence of pus cells in the urine to UTI. In the present study, 38.3% samples were positive for pus cells. In these pus cells positive samples, 28.6% yielded growth. The recovered isolates were then identified on the basis of different morphological characteristics and biochemical tests i.e. motility, colony shape & colour, texture, gram staining, changes on triple sugar iron (TSI) agar, oxidase, citrate, indole and urease tests. The frequency of isolated uropathogens was E. coli 73%, C. freundii 9%, S. aureus 4%, P. aeruginosa 4%, M. morganii 3%, Proteus spp. 1%, Streptococcus spp. 1% and C. albicans 1% while 4% isolated was normal flora with no significant growth. The identified microbes were processed for the determination of antibiotics susceptibility using different antimicrobial agents mentioned above. E. coli isolates showed highest sensitivity to cefoparazone 80.2% followed by amikacin 50%, imipenem 42.7% and aztreonam 27.1% while it was more resistant to norfloxacin 84.4%, cefepime 82.2%, tobramycin 81.2% and ciprofloxacin 76%. Citrobacter freundii was more sensitive to imipenem, tazocin 41.6% each, gentamicin and amikacin 33.3% each, while it was more resistant to tobramycin 100% followed by cefoparazone 83.3%, aztreonam, ceftazidime 75% each and cefepime 58.3%. Morganella morganii was more sensitive to tazocin 100% followed by gentamicin, ceftazidime 75% each and amikacin 50% while it was 100% resistant to aztreonam, ciprofloxacin, norfloxacin, tobramycin and cefoparazone. Results showed that Pseudomonas aeruginosa was sensitive to amikacin 60%, gentamicin, cefepime, aztreonam, ciprofloxacin and tobramycin 40% each. Streptococcus spp. showed sensitivity to cefepime 100% while it was resistant to the rest of the tested antibiotics i.e. gentamicin, amikacin, aztreonam, ciprofloxacin, norfloxacin, tobramycin, cefoparazone, Imipenem, tazocine and ceftazidime 100% each. Proteus spp. showed sensitivity to amikacin, aztreonam, cefobid and ceftazidime 50% each. It was 100% resistant to gentamicin, cefepime, ciprofloxacin, norfloxacin, tobramycin, cefoparazone and tazocin. S. aureus was sensitive to gentamicin and amikacin 50% each, while it was resistant to cefepime, aztreonam, ciprofloxacin, norfloxacin, cefoparazone and tazocin 100% each. S. epidermidis was also isolated but its growth was less than the significant growth 105CFU/ml.

DISCUSSION

Urinary Tract Infection (UTI) is a serious health problem affecting both males and females at any age. It is more common in younger people with an age range of 15 - 30 years which is observed in this study. Both the gram positive and gram negative organisms are involved in UTI; however the frequency of gram negative microbes involved is more. It was noted in this study that E. coli is the most common gram negative bacteria involved in UTI. Nearly half of all females and some males experience UTI once in their life, which is treated with antibiotics. The present study shows that 38.3% out of 300 patients have pus cells in their urine. When these urine samples were cultured for growth of

microorganisms, 28.6% yielded growth; the rest 9.6% yielded no growth. This pyuria may be due to some other reasons i.e. kidney stones, some congenital partial obstruction to the urinary flow, ureterovesical reflux or due to some microorganisms e.g. Chlamydia trachomatis infections which cannot be grown on ordinary medium. Chenari et al reported similar results as was found in this study[24]. Another similar study was conducted in south eastern Iran in 2012. Their study reported that the presence of uropathogens and pus cells are the two important markers for the diagnosis of urinary tract infection[25]. Another study was carried out in 2009 in order to find out the relationship between bacterial growth and pus cells in the urine samples of patients having suspected UTI, they tested one thousand urine samples for the presence of pus cells and for the growth of microorganisms. They found 360 urine samples had pus cells and yielded significant growth of microorganisms. About 16% samples showed pus cells but no bacterial growth was obtained (sterile pyuria) and 11% samples had no pus cells but significant bacterial growth was obtained [26]. On the basis of these findings, it becomes clear that those patients having pus cells in their urine samples must be recommended for urine culture and sensitivity. In the present study the most common uropathogen. According to their results the common uropathogens isolated were E. coli, Pseudomonas aeruginosa, Staphylococcus aureus and Proteus[27]. Another study conducted in 2012 in Southwest Ethiopia, the most common uropathogens isolated were E. coli 33.3% followed by Klebsiella spp. 19% and Streptococcus spp. 14.3%. In this study the isolated microorganisms were comparatively more sensitive to gentamycin [28]. A study conducted by Payam Behzadi showed that bacteria were more involved i.e. 93.2% in causing UTI as compared to Candida 6.8% [8]. Similar study was carried out in 2011, in which 399 midstream urine samples were tested. They isolated E. coli 57.5% as the most common uropathogens followed by Staphylococcus aureus 22.5%, Enterococcus spp. 15% and Klebsiella 5.0% 28.

Similarly, E. coli was the most common uropathogen in another study conducted by Allan Ronald. Other uropathogens isolated were Klebsiella spp., Proteus spp., Pseudomonas aeruginosa, Enterococcus spp., and Staphylococcus spp[29]. E. coli is a member of the normal flora of human colon. It can easily invade the urinary tract because of the close proximity of urethra to the anal area especially in female. UTIs are common in females as compared to males, because out of 300 suspected UTI patients 77 female while only 43 male show positive result for UTI. The prevalence of UTI in female were almost double than male, which correlates with the previous studies. Abubakar et al reported that UTI mostly affects females. In that study the female to male ratio was 54.3% females and 45.7% males. The culture and sensitivity was done and isolated E. coli was the most common microorganism involved in UTI which was sensitive to gentamicin (13.26%) as compared to other antibiotics. In our study E. coli was more sensitive (29%) as compared to study carried out by Abubakar [30]. In the present study the prevalence of UTI was more common 40% in the age group 15-30 years. While in the patients aged 40 years and above the prevalence of UTI was 28.3%. Our study showed the prevalence of UTI more in younger age group as compared to older one. Shawn Dason et al., conducted a survey in 2011 on the diagnosis and management of recurrent UTI in women. In the survey it was concluded that 1 out of 3 women experience UTI before the age of 24. Thus their study showed that UTI is more common in young age females but another study conducted in 2009 showed that both young and old age groups are equally affected, the reason being that the elderly women are having low estrogen level and this hormone may have some relation with UTI by supporting/inhibiting growth of different types of microbes[31]. According to the results of the present study the sensitivity pattern of E. coli against the tested antibiotics was cefoparazone, amikacin, imipenem, aztreonam in order of preference. Ali Ghorbani et al., in 2012 conducted a study on UTI pathogens and their susceptibility to antibiotics. The commonly isolated microorganism was E. coli 73.5% which was sensitive to ciprofloxacin 76.9%, amikacin and gentamicin 76.4%, while Enterobacter spp. was sensitive to ciprofloxacin 71.7%. Based on the results obtained, they recommended ciprofloxacin, amikacin and gentamicin as the drugs of choice for E. coli. They also recommended ciprofloxacin 71.7% for Enterobacter spp[32]. The study done by Ali Ghorbani and his team in 2012 in which they recommended ciprofloxacin and amino-glycosides for the treatment of E. coli does not correlate with our study. The contradiction between these results may be because in the present study different antibiotics were used or it may be because of the resistance developed by uropathogens against the indiscriminate and enormous use of these antibiotics against the isolated uropathogens in this region. Neelam Taneja and co- workers conducted a study in for the determination of antibiotics susceptibility of uropathogens. The study revealed that Extended Spectrum β-lactam (ESLB) enzymes producing microorganisms were resistant to aztreonam and cefepime while these microorganisms were sensitive to imipenem 91.8% and tazocin 90.5%. The ESBL non producing microorganisms were resistant to tazocin and imipenem[33]. Seung-Ju-Lee and co-workers, in studied antimicrobial susceptibility of uropathogens. In this study E. coli showed more sensitivity to ciprofloxacin (85.7%) as compared to other antibiotics which showed that E. coli develop quick resistance to ciprofloxacin if used

indiscriminately and by self medications [34]. In the present study, the uropathogens showed resistance to ciprofloxacin which may be because of the resistance developed by uropathogens due to the frequent use of this antibiotic in the region. Bashir et al., carried out a study in 2008 on UTI and drug resistant isolates of E. coli. In their study they isolated E. coli 66% as the common uropathogens. Other uropathogens isolated were Enterococci spp. 8.3%, Candida albicans 7.3%, Pseudomonas aeruginosa 7.3%, Enterobacter spp. 2.7%, Proteus and Morganella less than 1%. They stated that the sensitivity pattern of E. coli was different against different antibiotics. According to their results, E. coli was sensitive to imipenem, tazocin, amikacin, ceftazidime, gentamicin in order of preference [35]. Another similar study was carried out in 2013 on the isolation, identification and antimicrobial sensitivity of uropathogens. In the study, E. coli was the common uropathogens. The other isolated uropathogens were Pseudomonas aeruginosa, Klebsiella, Staphylococcus aureus, Proteus and Enterococcus spp. The results revealed that ciprofloxacin was 100% effective against most of the uropathogens while gentamicin was 83% effective [36]. According to the present study the sensitivity pattern of E. coli against the tested antibiotics was cefoparazone, amikacin, imipenem, aztreonam in order of preference. The contradiction between these results may be because in the present study different antibiotics were used or it may be because of the resistance developed by uropathogens against the indiscriminate and enormous use of these antibiotics against the isolated uropathogens in this region of the world.

CONCLUSION

Presence of pus cells in the urine of patients is a strong indication of UTI and the presence of uropathogens. E. coli was the most commonly isolated bacteria involved in UTI. The isolated uropathogens were more sensitive against amikacin and gentamicin while most of the uropathogens showed resistance against tobramycin and ciprofloxacin.

RECOMMENDATIONS

It is strongly recommended that microscopy should be done as microscopy for pus cell will be helpful for the quick diagnosis of suspected UTI cases and for initiating empirical antimicrobial therapy to avoid serious complications. Further studies are needed on larger scale in the country to understand the full picture of UTI in Pakistan.

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AUTHOR'S CONTRIBUTION

Following authors have made substantial contributions to the manuscript as under:

Gul F: Main idea.
Badshah N: Drafting of manuscript.
Ullah Z: Data collection.
Khan SA: Overall supervision.
Amin I: Follow-up

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.