Studies on Non-Cathethar Related Urinary Tract Infections from Assam, North-East India

Indu Sharma[a], Ane Laskar[a], Deepjyoti Paul[a].
Department of Microbiology, Assam University, Silchar-788011, Assam, India
Corresponding author:
Indu Sharma

Abstract

A total of 137 mid-stream urine samples were collected and cultured for the presence of bacterial pathogens of which 77 isolates were found to be positive and the other 60 samples were observed to be sterile exhibiting no bacterial growth. Most of the isolates were gram negative bacilli with 71.48% prevalence rate followed by gram positive bacteria with 28.57% prevalence rate. Among all the isolates isolated, *E. coli* (28.57%) was the most predominant organism found to be causing UTI in both male and female patients of different age groups. In the present study, almost 100% sensitivity was observed towards meropenem drug by *K. pneumonia*, *Proteus myxofaciens*, *Edwardsiella tarda*, *Providencia rustigianii*, *Morganella morgani*, *Micrococcus lactis*, *Enterobacter*, *Klebsiella planticola* isolates and about 80-100% sensitivity towards gentamycin by *Proteus vulgaris*, *Citrobacter fruendii*, *K. pneumonia*, *CNS*, *Pseudomonas* sp. and 100% resistance towards methicillin by all the isolated isolates.

KEYWORDS: Urinary Tract Infection, prevalence, antibiotic susceptibility, uropathogens.

Introduction

Urinary tract infections (UTI) are among the most common bacterial infections in humans, both in the community and hospital settings and have been reported in all age groups in both the sexes. Today UTI has become the most common hospital-acquired infection, accounting for as many as 35% of nosocomial infections, and it is the second most common cause of bacteraemia in hospitalized patients (5,6). Women tend to have UTIs more often than men as the bacteria reach the bladder more easily in women. (1,11). Beside this, pregnant women are at increased risk for UTI's starting in 6-24 weeks. The most commonly implicated bacteria in the pathogenesis of UTI and its complications include *Escherichia coli* (2,12,22), *Klebsiella* sp. (2), and *Staphylococcus aureus* (19,7). Current management of UTIs is usually empirical, without the use of a urine culture or susceptibility testing to guide therapy. However, as with many community acquired infections, antimicrobial resistance among the pathogens that cause UTIs is increasing and is a major health problem in the treatment of UTI (19). There is growing concern regarding antimicrobial resistance worldwide, particularly to *E.coli* which is the dominant causative agent of UTI in pregnant women (19,13,11,28). In most developing countries including India, screening for UTI is not considered as an essential part of health care. Therefore, this study was designed to determine the bacterial profile and antibiotic susceptibility pattern of uropathogens among patients attending different hospitals and diagnostic centers of Assam, N.E India, thus to create an awareness among the public for better improvements in health care systems.

Materials and methods

Specimen collection and bacterial isolates

Urine samples were collected from patients attending different hospitals and diagnostic centres of Assam. Early morning mid stream urine samples were collected in sterile containers (19). The sterile containers were distributed to the patients and an instruction on collection of the samples aseptically was demonstrated. A total of 137 (89.5%) samples comprising females and males were collected for this study. The samples were then labeled indicating the name, age and sex of the patients and immediately transported to the laboratory using ice packs for further processing.

Isolation and identification of isolates

The samples were streaked on Cysteine-Lactose Electrolyte Deficient (CLED) agar medium using calibrated loops for semi-quantitative method and incubated in both aerobic and anaerobic conditions for 24 hours at 37°C. Cultures without any colony at the end of 24h incubation were further incubated for 48h. Samples with colony count equal or more than 10^5 cfu/ml were considered positive. The isolates were identified and confirmed using standard microbiological methods including Gram staining, colonial morphology on media, growth on selective media, lactose and mannitol fermentation, H_2S production, catalase, oxidase, coagulase, indole, and citrate utilization, and urease test (4,9). The sterility of culture media were checked by incubating 3-5% of the batch at 35 – 37^{o}C for overnight and observed for bacterial growth. Those media which showed growth were discarded.

Antibiotic sensitivity test

Antimicrobial susceptibility test was performed on Mueller-Hinton agar (Merck, Germany) using disk diffusion (3) technique according to Clinical and Laboratory Standards Institute (CLSI) guidelines. The antibiotic discs (Hi-media, India) and their concentrations used against gram-positive organisms consisted of Vancomycin (VA) 30µg , Ciprofloxacin (CIP) 5µg, Methicillin (MET) 30µg, Amikacin (AK) 30µg, Ampicillin (AMP) 10µg, Tetracycline (TE) 30µg, Nalidixic acid (NA) 30µg and against gram-negative organisms were Ofloxacin (OF) 2µg Aztreonam (AT) 50µg, Chloramphenicol (C) 50µg, Cefepime (CPM) 50µg, Piperacillin (PIT) 100/10µg, Gentamycin (GEN) 50µg and Tigecycline (TGC) 15µg.

Data analysis:

Data was analysed by performing Z-test for the two sample proportion and values ≤ 0.05 were considered significant.

Results

In this present study, a total of 137 (89.5%) urine samples were collected from UTI infected patients of 153(91%) of total 168 patients attending different hospitals and diagnostic clinics of North-East Assam among which 97 were females and 71 were males. The population of the study comprised of 31(40.25%) males and 46(62.3%) females which were found to be positive. The patients' age under the present study ranged between 1- 80 years.

Observations based upon different age groups showed that the infection frequency by the organisms in both the males and the females were predominantly higher among the age groups of 21-30 years. Further, higher prevalence rate for females were recorded among the age groups of 21-30 years followed by age groups of 31-40, 11-20 and 41-50

years and in the males, higher prevalence was observed among the age groups of 41-50 years followed by 31-40 and 51-60 years (Table 6.1 from thesis to be pasted here).

Of the total 137 samples inoculated, 77 samples were found to be positive i.e. those grown on CLED media and the rest 60 samples were sterile which showed no bacterial growth. Among the 77 isolates, most of them were identified as gram negative organisms (71.48%) and the commonly encountered organisms were *Escherichia coli (*28.57%) followed by *Staphylococcus aureus* (18.18%), *Coagulase negative staphylococcus (9.09%), Pseudomonas* (7.79%), *K. pneumoniae* (6.49%), *Proteus mirabilis(5.19%),Proteus myxofaciens* (5.19%), *Citrobacter fruendii* (3.89%), *Klebsiella sp*. (3.89%), *Edwardsiella tarda* (2.59%), *Providencia rustigianii* (2.59%), followed by *Morganella morganii* (1.29%) *Micrococcus lactis*(1.29%) , *Enterobacter*(1.29%) , *Proteus vulgaris*(1.29%) , *Klebsiella planticola*(1.29%), *Klebsiella sp*. (3.89%), *Edwardsiella tarda* (2.59%), *Providencia rustigianii* (2.59%).

Furthermore, all the isolates identified from both the males and females in this study were screened for antimicrobial susceptibility by Kirby Bauer disc diffusion method with commercially available antibiotic discs (Table 1).

Table 1: Susceptibility percentage of isolates isolated from UTI infected patients of different hospitals of North-East Assam.

ISOLATES	CIP	CPM	GEN	MET	AMP	C	PIT	V	AK	OF	NA	MRP	TGC	AT	TE
E.coli	80.9	71.4	38	0	57.1	52.3	71.4	-	71.5	57.3	61.9	71.5	76.1	48.9	-
S.aureus	1.6	-	23.7	0	46.6	-	-	26.6	66.6	-	33.3	53.3	75	75	46.6
Pseudomonas sp.	24.8	60	88	0	-	100	50	33.3	50	50	66.6	71.4	50	80	-
CNS	80.5	-	100	56	67.9	-	-	42.8	71.4	-	25	75	0	25	75
K.pneumonia	77	43.8	89	0	80	57	80	-	80	25	50	80	80	0	-
Proteus mirabilis	100	54.8	78	24	54.5	100	50	-	20	75	0	75	25	0	-
Proteus myxofaciens	97	56.4	67	0	0	78	25	-	75	0	100	100	0	25	-
Klebsiella sp.	90	23	98	0	-	66	0	-	25	66.6	71.4	0	100	0	-
Citrobacter fruendii	100	51	100	0	-	50	33.3	-	100	66.6	50	80	100	0	-
Edwardsiella tarda	100	66	50	0	-	0	0	-	0	100	100	100	0	100	-
Providencia rustigianii	100	59	64	0	-	0	50	-	100	0	0	100	0	0	-
Morganella morganii	55.6	56	54.9	45	-	0	0	-	100	100	0	100	0	0	-
Micrococcus lactis	40.6	-	0	0	46.8	-	-	100	0	-	100	100	0	100	25
Enterobacter	21.4	0	0	0	-	100	0	-	100	0	100	100	100	0	-
Proteus vulgaris	100	50	100	0	47	0	100	-	100	100	0	0	100	0	-
K.planticola	67	57.5	26	0	0	0	0	-	0	100	100	100	0	100	-

CIP=Ciprofloxacin,CPM=Cefepime,GEN=Gentamycin,MET= Methicillin,AMP= Ampicillin,
C=Chloramphenicol,PIT=Piperacillin,V=Vancomycin,AK=Amikacin,OF=Ofloxacin,NA =Nalidixic acid,MRP=meropenem,TGC=Tigecycline,AT=Aztreonam,TE=Tetracycline.

Discussion

The present study observed that the incidence of UTI was higher among the females (62.8%) than the males (40.25%) and the age group of 21-30 years was recorded as the vulnerable age for infection in females and the age group of 71-80 years exhibited lower rate of infection both in males and females. This result is in also agreement with previous studies which showed that UTI were more common in females than males during adolescence and adulthood (5,7,). Factors such as short urethra and its closeness to the anus as well as sexual activity have been reported to influence the higher prevalence of UTI in females (14,16,1). Isolates were identified by morphological and cultural characteristics of the organism. Most of the isolates were gram negative bacilli with 71.48% prevalence rate followed by gram positive bacteria with 28.57% prevalence rate. The prevalence of gram-negative rod shaped bacteria was found to be higher in this study than the gram-positive cocci bacteria. A total of 54 isolates were found to be gram-negative rods of which 77 isolates were positive and the rest 23 isolates were gram-positive cocci. The most prevalent organism identified in the present study was *E .coli* (27.27%) which is similar with previous works in India (19) and other countries. All the 23 gram positive cocci were tested for coagulase test, among which 7 isolates were *coagulase negative staphylococcus* exhibiting clumping formation. It was also observed that when the rate of isolates of Gram negative and Gram positive bacteria were compared, Gram negative bacteria were found to be the most dominant causative agents of UTI (2,9). The identified uropathogens in the present study are similar to those of studies conducted in India (19) and different countries (6,10).

All the 77 isolated isolates were further subjected to antimicrobial susceptibility screening test by Kirby Bauer disc diffusion method (3) with commercially available antibiotic discs. The present result indicated that *E. coli* was the principal etiological agent of UTI, accounting for 27.27% of the screened cases (9,13,22). Moreover, 95% of Gram negative bacilli which are responsible for UTI. *E. coli* remained dominant causing 80% of UTI followed by *Staphylococcus aureus* (19). Furthermore, *E. coli* was also the most predominant organism found to be causing UTI in both male and female patients of different age groups (19.) Ciprofloxacin as an alternate option in empirical treatment of UTIs has been considered since its multiple mechanisms of action seem to have enabled it to retain potent activity against *E. coli.* (12,15,20) In the present study, Ciprofloxacin showed high levels of activity against *E. coli* in UTI when compared to other commonly used agents such as gentamycin, aztreonam and ampicillin (19,20). Furthermore, *E. coli* also exhibited high susceptibility towards the other following antibiotics, i.e., Cefepime(71.4%), Piperacillin(71.%), Meropenem(71.5%), thus, suggesting that these drugs should be used judiciously so as to ensure that the resistance rates towards these antibiotics for UTIs do not increase. In the present study, almost 100% sensitivity was observed towards meropenem by *K. pneumonia*, *Proteus myxofaciens*, *Edwardsiella tarda*, *Providencia rustigianii*, *Morganella morgani*, *Micrococcus lactis*, *Enterobacter*, *Klebsiella planticola* isolates and about 80-100% sensitivity towards gentamycin by

Proteus vulgaris, Citrobacter fruendii, K. pneumonia, CNS, Pseudomonas sp. However in the current study it was observed that a high level and generalized resistance towards methicillin commonly prescribed in this region, thus reducing the number of prescription for this particular antibiotic can lead to a decrease in resistance rates (7,9).

Studies like the present study are useful in determining local trends and risk factors for (10,14,20) antimicrobial resistance and also there are significant geographic differences in the susceptibility of commonly used antimicrobials against UTIs, therefore it is important for the physicians and other health workers on performing antibiotic susceptibility test before the commencement of any blind antibiotic therapy. Drugs combination use for synergistic effect should be encouraged so as the emergence of resistant strains within any population could be checked and the dissemination of MDR pathogens would be curtailed.

Conclusion

The appropriate treatment for UTI has been a subject of recent research and different studies have shown an alarming increase in resistance of uropathogens in invasive infections like urinary tract infections. In this study it has been found that *E. coli, Pseudomonas, Proteus* sp., *Klebsiella* sp. and *S. aureus* were the most common isolates in which both male and female patients were affected but later varied in their drug sensitivity patterns. Thus to reduce this, health care policies of antibiotics used presently should be revised, extensive health education to the people residing community-wise and improvements in personal hygienic conditions should be strictly implemented and followed regularly for reducing the community antibiotic resistance of commonly occurring pathogens in different populations. Finally, judicial use of antibiotics should be done based on the prevalence of bacterial organisms and its antibiotic sensitive rather than on the guidelines provided.

Acknowledgement

The authors are thankful to the university authorities for their kind help extended towards this work.

References

1. Boye, A., Siakw, P.M., Boampong, N.B., Koffuor, G.A., Dadzie, R.K., Amoateng, E.P., Obodai, G. and Penu, D. (2012). Asymptomatic urinary Tract infections in pregnant women attending antenatal clinic in Cape Coast, Ghana. Journal of Medical Research 1(6): 074-083.
2. Behzadi, P., Behzadi, E., Yazdanbod, H., Aghapour, R., Akbari, C.M., Salehian, O.D. (2010). A survey on urinary tract infections associated with the three most common uropathogenic bacteria, 5(2):111-5.
3. Bauer, A.W., Kirby, W.M., Sherris, J.C., Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol*. 45(4): 493-6.
4. Cruickshank, R., Duguid, J.P., Marmion, B.P. and Swain, R.H.A. Medical Microbiology. 12th Edn. 2. Edinburg, London and New York. Churchill Livingstone 1975.
5. Chukwuocha, U.M., Emerole, C.O., Njokuobi, T.N. and Nwawume, I.C. (2012). Urinary Tract Infections (UTIs) Associated with Diabetic Patients in the Federal Medical Center owerri, Nigeria, Global Advanced Research Journal of Microbiology, 1(5): 062-066.

6. Das, R.N., Chandrashekhar, T.S., Joshi, H.S., Gurung, M., Shrestha, N. and Shivananda, P.G. (2006). Frequency and susceptibility profile of pathogens causing Urinary tract infections at a tertiary care hospital in western Nepal. Singapore Med J 47(4): 281-5.
7. Grude, N., Tveten, Y. and Kristiansen, B. E. (2001). Urinary tract infections in Norway: bacterial etiology and susceptibility. A retrospective study of clinical isolates. Clinical Microbiology and Infection, 7(10): 543–547.
8. John, G.H. and Baltimore, M.D. *Bergey's Manual of Systematic Bacteriology* 1st Edition Published in 4 volumes: Volume 1 (1984) Gram-negative *Bacteria* of general, medical, or industrial importance ISBN 0-683-04108-8; Volume 2 (1986) Gram-positive *Bacteria* other than *Actinomycetes* ISBN 0-683-07893-3; Volume 3 (1989) *Archaeobacteria*, *Cyanobacteria*, and remaining Gram-negative *Bacteria*. ISBN 0-683-07908-5.
9. Jha, N. and Bapat, S.K. (2005). A study of sensitivity and resistance of pathogenic microorganisms causing UTI in Kathmandu valley, Kathmandu Univ Med J (KUMJ), 3(2):123-9.
10. Jombo, G.T.A., Emanghe, U., Amefule, E.N. and Damen, J.G. (2011). Urinary tract infections at a Nigerian university hospital: Causes, patterns and antimicrobial susceptibility profile. Journal of Microbiology and Antimicrobials, 3(6): 153-159.
11. Kalsoom, B., Khan, J., Rifat, H.B., Munir, S., Akbar, N.U., Ansari, J.A. and Anees, M. (2012). Patterns of antibiotic sensitivity of bacterial pathogens among urinary tract infections (UTI) patients in a Pakistani population. African Journal of Microbiology Research Vol. 6(2): 414-420.
12. Kebira, A.N., Ochola, P. and Khamadi, S.A. (2009). Isolation and antimicrobial susceptibility testing of *Escherichia coli* causing urinary tract infections; Journal of Applied Biosciences 22: 1320–1325.
13. Nakhjavani, F.A, Mirsalehian, A., Hamidian, M., Kazemi, B., Mirafshar, M. and Jabalameti, F. (2007). Antimicrobial susceptibility testing for Escherichia coli strains to fluoroquinolones in urinary tract infections. Iran. J. Public Health, 36(1): 89-92
14. Ogun, A.A., Ejembi, J. and Faleye, T.O.C. (2009). Incidence of urinary tract infections (UTIs) among pregnant women in Akwa metropolis,Southeastern Nigeria. Scientific Research and Essay 4: 820-824.
15. Oluremi, B. B., Idowu, A. O. and Olaniyi J. F. (2011). Antibiotic susceptibility of common bacterial pathogens in urinary tract infections in a Teaching hospital in Southwestern Nigeria; African Journal of Microbiology Research Vol. 5(22): 3658-3663.
16. Obirikorang, C., Quaye, L., Bio, F.Y., Amidu, N., Acheampong, I. and Addo, K. (2012). Asymptomatic Bacteriuria among Pregnant Women Attending Ante-natal Clinic at the University Hospital, Kumasi, Ghana. Journal of Medical and Biomedical Sciences, 1(1): 38-44.
17. Orrett, F.A. and Shurland, S.M. (2001). Bacteriuria in elderly population in a developing country, J. Natl. Med. Assoc. 93(7-8): 238–242.

18. Ronald, P. O., Lizzie, J. H. and Keith S. K. (2009). Antibiotic resistance in urinary isolates of *E.coli* from college women with urinary tract infections; Antimicrob Agents Chemother, 53(3):1285–1286, doi: 10.1128/AAC.01188-08.
19. Sharma, I. and Paul, D. (2012). Prevalence of community acquired urinary tract infection in Silchar Medical College, Assam, India and its antimicrobial susceptibility profile, Indian Journal of Medical Sciences, 66:273-9.
20. Silva, A.A. and Hoffer E. (1993). Resistance to antibiotics and heavy metals in *E. coli* from marine environment; Environ Toxicol Wat Qual, 8:1-11.
21. Tambekar, D.H., Dhanorkar, D.V., Gulhane, S.R., Khandelwal, V.K. and Dudhane, M.N. (2006). Antibacterial susceptibility of some urinary tract pathogens to commonly used antibiotics, African Journal of Biotechnology 5(17): 1562-1565.
22. Zahera, M., Rastogi, C., Singh, P., Iram, S., Khalid, S. and Kushwaha, A. (2011). Isolation, Identification and Characterization of *Escherichia coli* from Urine Samples and their Antibiotic Sensitivity Pattern; European Journal of Experimental Biology, 1(2):118-124.