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Original Article

EPIDEMIOLOGICAL STUDIES ON VARYING EXTENDED-SPECTRUM β-LACTAMASES PRODUCING UROPATHOGENIC BACTERIA

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ABSTRACT

Objective: Urinary tract infection (UTI) is the most common serious infection during infancy, adult male and female, as well as at the time of pregnancy. UTI is also the most common nosocomial infection in many hospitals and accounts for approximately 35% of all hospital acquired infections. Hence the present study was aimed to screen the presence of multi-drug resistant bacterial pathogens among the urine samples collected from in and out patients of multi-speciality hospital.

Methods: Standard microbiological laboratory protocols were followed and about 152 samples were processed and screened. Among them, 49 reported positive for the presence of urinary bacterial pathogens.

Results: Among 49 isolates, *Escherichia coli* registered its prevalence in about 44 samples followed by *Klebsiella* spp. (4) and *Pseudomones* spp. (1). The gender wise distribution was found to be more among female patients (42%) than male patients (24%). The age wise distribution of infection among male and female patients was also noticed. Further, the multi-drug resistance of the isolates was done by using 8 antibiotics.

Conclusion: All the isolates exhibited the multiple antibiotic resistance and the isolates showed 27 different antibiotic resistance patterns. This confirms the prevalence of ESBL producers among the urinary pathogens.

Keywords: Uropathogens, *E. coli*, ESBL, Hospital acquired infection.

INTRODUCTION

Urinary tract infection (UTI) is projected to be about 150 million incidences per annum worldwide [1]. More than 8 million patients with urinary tract infections visit urology or gynecology clinics per year in the United States [3]. UTIs develop by either ascending or descending bacterial invasion into the urinary tract. The most common mode of infection is the ascending pathway, where fecal flora gain access to the urinary tract via colonization of the urethra [2]. Descending infections are the result of hematogenous spread of bacteria from a primary source located elsewhere in the body and it's rare. The lower urinary tract infection is known as a simple cystitis (Bladder infection) and the infection in the upper urinary tract or in kidney is known as pyelonephrites [16].

UTI is one of the most recurrent infectious diseases among hospital-acquired group [8]. It also reported to occur by community acquired group. The major causal agents of urinary tract infections by bacterial pathogens were found to be *Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Serratia marcescens,* and *Pseudomonas aeruginosa* [11].

Depending on whether the infection is occurring for the first time or a repeated event, the UTI is classified into two types, uncomplicated and complicated infections. Uncomplicated UTI occurs due to bacterial infection, most often by *E. coli*. Women are frequently affected by uncomplicated UTI than men [26]. Complicated infections, which occur in men and women of any age, are also caused by bacteria, but they tend to be more severe and more difficult to treat [22].

In order to achieve a satisfactory therapeutic effect, it is suggested that local information regarding the antimicrobial resistance of frequent pathogens should be established as a reference for the selection of empirical antimicrobial therapy [10]. Higher multidrug resistance rates were found to be common among hospital acquired UTI pathogens [7]. β-lactam antimicrobial agents are among the most widely used antibiotics to treat those communities and hospital acquired infections [14].

Few authors have studied the risk factors associated with the UTI due to ESBL-producing bacteria in hospitalized patients. Extended-spectrum β-lactamases (ESBLs) are a group of β-lactamases enzymes belongs to group 2, produced by Gram negative Enterobacteriaceae [4]. Due to rapid emergence of ESBL producing uropathogens over the last decade, the antimicrobial susceptibility profile has changed dramatically [19, 28]. Hence, the aim of the present study was to determine the prevalence and antibiotic resistant patterns of ESBL-producing bacterial uropathogens isolated from urine sample of both in and out patients in multi-specialty hospitals around Coimbatore.

MATERIALS AND METHODS

Isolation and identification of uropathogenic bacteria

Collection of samples

With prior ethical clearance from the hospitals for this study, a total of 152 urine samples from multi-specialty hospitals in and around Coimbatore were collected for the isolation of uropathogenic bacteria. One millilitre of the sample was inoculated in Luria Bertani (LB) broth and incubated at 37°C for overnight. After incubation for about 24 h, one loopful of the culture was streaked on selective agar medium. The selective agar medium those were used for the isolation of enteric pathogens is tabulated (Table 1). Further, the isolates were identified by Gram's staining and series of biochemical tests.

Table 1: Selective medium for isolation of pathogens

Selective media	Bacterial genera
Eosine Methylene Blue agar (EMB)	*E. coli*
Mac Conkey Agar	*Klebsiella* spp.
Nutrient Agar (NA)	*Proteus* spp.
King's B medium	*Pseudomonas* spp.

Maintenance of uropathogens

Nutrient agar slants were prepared in the test tubes and pure culture of the uropathogen grown on the selective medium were streaked on it. The tubes were incubated at 37°C for 24 h and refrigerated for preservation. The cultures inoculated on to the Luria Bertani broth tubes were used for further use.

Antibiotic susceptibility testing

Antibiotic sensitivity was tested for all the 49 isolates. The antibiotics that are commonly used for the infection control were selected for the present study.

Test procedure

Antibiotic sensitivity test was carried out by disc diffusion method. Muller Hinton agar (HiMedia, India) were prepared, sterilized and poured onto sterile petriplates. The medium was allowed to solidify. Pure cultures grown in nutrient broth for 6-8 h were swabbed over MHA using sterile cotton swabs. Using antibiotic disc dispenser, discs were placed on the agar surface with sufficient space so as to avoid overlapping of inhibition zones. After 30 min of pre-diffusion time, the plates were incubated at 37°C for 18-24 h. After the incubation period, the diameter of the inhibition zone was measured and compared with the interpretative chart provided by the manufacturer and classified as resistant, intermediate and sensitive.

Multiple antibiotic resistance index

Multiple antibiotic resistance (MAR) index of an isolate is the number of antibiotics to which the test isolate displayed resistance divided by the total number of antibiotics to which the test organism has been evaluated for sensitivity. MAR index value higher than 0.2 is considered that the isolate have originated from high risk source of contamination like human, commercial poultry farms, swine and dairy cattle where antibiotics are very often used. MAR index value less than or equal to 0.2 is considered that the isolate have originated from animals in which antibiotics are used very rarely or never used.

RESULTS

Isolation and identification of uropathogenic bacteria

The urine samples were collected from in and out patient in multispecialty hospitals around Coimbatore from both genders irrespective of age group. Among 152 samples, 133 samples were from in patients and 19 from out patients. The isolates were subjected to gram's staining and streaked on to selective medium. As none of the isolates were found to be gram positive, the screening of gram positive bacteria on selective medium was neglected. A total of 44 isolates exhibited metallic sheen colonies on to EMB agar plates which confirms the presence of *E. coli* in the samples, 4 isolates showed pink colour colonies on Mac Conkey agar confirms the presence of *Klebsiella* spp. And one isolate which produced pyocyanin was further confirmed the King's B medium confirms the presence of *Pseudomonas* spp. None of the isolates exhibited swarming motility on the nutrient agar plates, which shows the absence of *Proteus* spp. Among the samples (Table 2). The isolates obtained from selective medium were further confirmed based on their biochemical profile. The typical biochemical profile of *E. coli, Klebsiella* spp. And *Pseudomonas* spp. Were tabulated (Table 3).

Table 2: Percentage of uropathogenic bacteria

S. No.	Positive samples		Organism isolated	Total no. of isolates	Percentage %
	Male	Female			
1.	16	28	*E. coli*	44	29%
2.	2	2	*Klebsiella* spp.	4	3%
3.	1	-	*Pseudomonas* spp.	1	0.7%

Table 3: Typical biochemical profile of *E. coli, Klebsiella* spp. And *Pseudomonas* spp

Gram's staining	Indole	MR	VP	Urease	TSI	Catalase	Glucose	Lactose	Maltose	Sucrose	motility	Oxidase	Suspected organism
G-ve Rod	-	-	+	-	+	+	-	+	-	-	+	-	*E. coli*
G-ve Rod	-	+	-	+	+	+	+	+	+	+	-	-	*Klebsiella* sp.
G-ve Rod	-	-	-	+	+	+	+	-	+	+	+	+	*Pseudomonas* sp.

(-) Negative (+) Positive

Table 4: Demograhic data of UTI among patients

Category	No. of samples	Male	Female	No. of Positive samples	Percentage %
IP	133	73	60	40	30%
OP	19	8	11	9	47%

Table 5: Gender wise distribution of uropathogenic bacteria in UTI samples

Gender	Total no. of samples	No. of Positive samples	Percentage %
Male	81	19	24%
Female	71	30	42%

Prevalence of uropathogenic bacteria

The percentages of prevalence of uropathogenic isolates were screened. The prevalence was found to be high among out patients (47%) whereas 30% of incidence was noticed among the in patients (Table 4). The gender wise incidence was noticed among the patients.

The highest percentage was noticed among female patients (42%) than male patients (24%), the results are tabulated (Table 5). The age wise prevalence of the pathogens had also registered. The highest prevalence of the UTI was found among the age of 60-69 years of male patients (33%) and 100% of samples from female patients of the age 40-79 showed positive for the presence of bacterial pathogens.

Antibiogram of the isolates

All the 49 isolates obtained were screened for the antibiotic sensitivity against 8 commercially available antibiotics. The highest percentage (100%) of resistance was found against cefotoxime and ceftazidime and least percentage of resistance was found against amikacin (14%). About 98% of isolates showed resistance against cefazolin and cefixime, 96% resistance towards vancomycin, 88% against nalidixic acid and 43% towards gentamicin, respectively. The isolates exhibited about 27 different antibiotic resistance patterns against 8 antibiotics used in this study. *Klebsiella* spp. and *Pseudomona* spp. shares the same antibiotic resistance pattern shown by *E. coli*. This shows that these organisms acquiesced the resistance through transferable elements like 'R' plasmids as these were closely related in their phylogeny.

Multiple antibiotic resistance index

The MAR index value of all the isolates ranged from 0.5 to 1. MAR index of 0.91 to 1 and 0.80 to 0.89 was exhibited by 0.04% and 55% of the isolates. MAR index of 0.70 to 0.79 and 0.60 to 0.69 was exhibited by 35% and 0.04% of the isolates respectively. About, 0.02% isolates exhibited a MAR index value of 0.50 to 0.59. None of the isolates exhibited MAR index less than 0.5. The results serves as an evident that all the isolates were procured from the area were antibiotics are intensively used.

DISCUSSION

Various bacterial pathogens were reported to be involved in UTI. Many reporters had marked their conclusion that *E. coli* and *Klebsiella* spp. were found to be predominant in causing the UTI among both hospital-acquired and community-acquired patients. There are many evidences that these pathogens harbour resistance towards multiple antibiotics by producing varying β-lactamases. Hence the present study was conducted to screen the epidemiology of various bacterial pathogens in the UTI and to determine their antibiotic resistance.

Tankhiwale *et al.* [29] screened a total 217 uropathogens and found that *E. coli* (49.8%) was the most common organisms followed by *Klebsiella pneumoniae* (37.8%), *Pseudomonas aeruginosa* (6.5%). This report strongly supports the present study, as *E. coli* (29%) was found to be more prevail among the patients irrespective of sex and gender followed by *Klebsiella* spp. (3%) and 0.7% of *Pseudomonas* spp. This result also strongly coincides with Jain *et al*. [12].

A large spectrum of organisms has been reported by Jain *et al*. [13] from patients of UTI with *E. coli* (65.95 %) and *Klebsiella* spp. (12.41 %) being the most common in their study. Hasan *et al.* [9] has reported a high incidence of *E. coli* (50.7%) followed by *Klebsiella* spp. (27.6%) in 2436 uropathogens from patients with UTI.

It is stated that UTI is predominantly a disease of the females due to a short urethra and proximity to the anal opening. The present study also marks its concordance for the early reports as the UTI prevalence was found to be more among female (42%) whereas, in male it's about 24%. In a study, female preponderance for this infection was noted by Jain *et al*. [13]. They had also reported that the *E. coli* and *Klebsiella* spp. Were found to be the common isolates in females. This gives the strong evidence for the present study since, *Pseudomonas* spp. Was found only from male patient.

Jain *et al.* [12] found that 17.3% in females patients with UTI whereas 13.3% in males. In coherence with prior studies, a higher prevalence of UTI among female was noted owing to the presence of anatomical and physical factors showing their increased vulnerability towards UTI was confirmed. The occurrence of UTI predominantly in patients with age ranging between 18-60 years in our study is similar to the findings of studies done in Kuwait and Nigeria [6, 23]. Women in the age group 16-45 years are most likely to suffer from UTI. Among the elderly, after 45 years males became more prone to UTI. The number of male patients increased significantly in the age group 46 years as prostatic gland enlargement and decrease of bacteriostatic prostatic secretions might account for such infections [30]. But in the present study evident for the occurrence was high at the age 40-49 among males and 40-79 among females.

As other several reports, our study has also revealed that *E. coli* is the most predominant pathogen causing UTI in both sexes. On contrary to the findings of various other previous studies which documented *Staphylococcus* spp. as the second most common isolates [18,21], our results showed *Klebsiella* spp. Which is in concurrence with the findings of various authors [1,17,27].

Resistance to several antimicrobial agents was prevalent among the isolates recovered not alone from the hospital and also found in environmental sources. These reports were noticed in many of the research works done world-wide. ESBL-producing Enterobacteriaceae is now evident in the community, especially among adults and is no longer confined only to the healthcare environment. Qi *et al.* [24] described an increase of over 10-fold in the community acquired ESBL *E. coli* incidence among the general population in Chicago, Illinois over a period of only 5 years while screening for urinary pathogens. In the current study, the incidence of uropathogen was noticed high among out patients (47%) than in patients (30%). This might be resulted due to age factor and risk of exposure of a patient.

In the 1970s, multidrug resistance was practically nonexistent and the cause was restricted to mutation of chromosomal genes. However, during the last two decades bacterial resistance mediated by plasmids, which carry resistant gene to a large number of antibiotics, which are rapidly transferred, has worsened the scenario [25]. Due to high levels of resistance found to first choice drugs, it is important to know the resistance aspects of the pathogens in order to help in use of appropriate therapy or else, the empirical therapy became a difficult clinical decision.

In the present study, all most all the isolates showed multidrug resistance. The least percentage of resistance (7%) was noticed against amikacin. *Klebsiella* spp. and *Pseudomonas* spp. Were found to be sensitive towards amikacin. Only *E. coli* was found to be resistant to amikacin. This result coincides with the result of Khan *et al*. [15] who recorded 7.1% of *E. coli* was resistant against amikacin. About 98% of isolates showed resistance against cefazolin in the present study. Moraes *et al*. [20] reported that 99% of *E. coli* was found to be resistant towards cefazolin. But Wu *et al.* [31] reported that cefazolin can be used as an alternate drug for treating urinary pathogens. This difference in susceptibility might be resulted due to non-exposure of the test isolates used by Wu and their co-workers.

The resistance against cefixime and cefotoxime was found to be 90%, 84.3%, 80% and 84.3% according to the reports of Jain *et al*. [13] and Noor *et al.* [30], whereas in the current study it was noticed as 98% and 100%. Jain *et al*. [12] reported that 57% of isolates were found to be resistance towards gentamicin. Dayan *et al*. [5] recorded 50% of resistance towards gentamycin. The result of the current study also more or less coincides with the early reports since, the resistance against gentamicin was found to be 43% in our study.

About 100% of isolates showed resistance towards ceftazidime in the present study, Jain *et al*. [13] noticed 82% of resistance. Against nalidixic acid and vancomycin, the test isolates showed 88% and 96% of resistance. This strongly coincides with the report of Jain *et al*. [13] and Noor *et al*. [30] as they reported that their 94% and 82.9% of test isolates showed resistance towards nalidixic acid. In contray, Jain *et al*. [12] has reported only 12% of their test isolates were resistant to vancomycin. These variations in the antibiotic resistance may occur due to the frequency of usage of particular antibiotics.

CONCLUSION

From the present study, we can conclude that *E. coli* was found to be most predominant bacterial uropathogen in UTI cases of the study area. The results of our study give brief information about the multidrug resistance, and also the prevalence of ESBL producing bacteria among the patients reported having community acquired UTI. Further, we also conclude that amikacin can be used in the empirical antibiotic therapy for achieving the better treatment.

CONFLICT OF INTERESTS

Declared None

REFERENCES

1. Akram M, Shaid M, Khan AU. Etiology and antibiotic resistance patterns of community acquired urinary tract infection in JNNC Hospital Aligarh. India Ann Clin Microbiol Antimicrob 2007;6(1):4.
2. Behzadi P, Behzadi E. The microbial agents of urinary tract infections at central laboratory of Shariati Hospital, Tehran, IRAN. Turk Klin Tip Bilim 2008;28:445-9.
3. Burt CW, Schappert SM. Ambulatory care visits to physician offices, hospital outpatients departments, and emergency departments: United States. Vital Health Stat 2004;13:1-70.
4. Bush K, Jacoby GA, Medeiros AA. A functional classification scheme for beta-lactamases and its correlation with molecular structure. Antimicrob Agents Chemother 1995;39:1211-33.
5. Dayan N, Dabbah H, Weissman I, Aga I, Even L, Glikman D. Urinary tract infections caused by community-acquired extended-spectrum β-lactamase-producing and nonproducing bacteria: A comparative study. J Pediatr 2013;163:1417-21.
6. Dimitrov TS, Udo EE, Emara M, Awni F, Passadilla R. Etiology and antibiotic susceptibility patterns of community acquired urinary tract Infections in a Kuwait hospital. Med Pri Prac 2013;13(6):334-9.
7. Fadel R, Dakdouki GK, Kanafani ZA, Araj GF, Kanj SS. Clinical and microbiological profile of urinary tract infection at a tertiary-care center in Lebanon. Infect Control Hosp Epidemiol 2004;25:82-5.
8. Gupta K, Hooton TM, Naber KG, Wullt B, Colgan R, Miller LG, *et al*. International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: a 2010 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. Clin Infect Dis 2011;52:103-20.
9. Hasan AS, Nair D, Kaur J, Baweja G, Deb M, Aggarwal P. Resistance patterns of urinary isolates in a Tertiary Indian Hospital. J Ayub Med Coll Abbottabad 2007;19(1):39-41.
10. Hsueh PR, Hoban DJ, Carmeli Y, Chen SY, Desikan S, Alejandria M, *et al*. Consensus review of the epidemiology and appropriate antimicrobial therapy of complicated urinary tract infections in Asia-Pacific region. J Infect 2011;63:114-23.
11. Ishikawa K, Matsumoto T, Yasuda M, Uehara S, Muratani T, Yagisawa M, *et al*. The nationwide study of bacterial pathogens associated with urinary tract infections conducted by the Japanese Society of Chemotherapy. J Infect Chemother 2011;17:126-38.
12. Jain S, Sharma S, Kumar M, Shree N, Sharma Y. Prevalence of uropathogens in urinary tract infection and their antimicrobial resistance pattern in a North Delhi hospital, India: A nine year study. Int J Microbiol Res 2014;6(1):545-52.
13. Jain S, Soni R, Bhuyar G, Shah H. Prevalence of uropathogens in various age groups and their resistance pattern in a tertiary care hospital in Central India. Nat J Integ Res Med 2011;2(4):7-10.
14. Jalalpoor Sh, Kasra Kermanshahi R, Nouhi AS, Zarkesh E. Survey frequencies of β-lactamase enzyme and antibiotic sensitivity pattern in isolated pathogen bacteria from low and high hospital contact surfaces. Pajuhandeh J 2010;15:77-82.
15. Khan SH, Feroz F, Noor R. Study of extended-spectrum β-lactamase-producing bacteria from urinary tract infections in Bangladesh. Tzu Chi Med J 2013;25:39-42.
16. Kolawole AS, Kolawole OM, Kandaki-Olukemi YT, Babatunde SK, Durowade KA, Kolawole CF. Prevalence of urinary tract infections (UTI) among patients attending. Sci Res Essays 2009;1:163-7.
17. Kothari A, Sagar V. Antibiotic resistance in pathogens causing community-acquired urinary tract infections in India: a multicenter study. J Infect Developing Countries 2008;2:354-8.
18. Manikandan S, Ganesapandian S, Singh M, Kumaraguru AK. Antimicrobial susceptibility pattern of urinary tract infection causing human pathogenic bacteria. Asian J Med Sci 2011;3(2):56-60.
19. Mohammed A, Mohammed S, Asad UK. Etiology and antibiotic resistance patterns of community acquired urinary tract infections in JNMC Hospital, Aligarh, India. Ann Clin Microbiol Antimicrob 2007;6:6.
20. Moraes D, Braoios A, Alves JLB, daCosta RM. Prevalence of uropathogens and antimicrobial susceptibility profile in outpatient from Jataí-GO. J Bras Patol Med Lab 2014;50(3):200-04.
21. Murugan K, Savitha T, Vasanthi S. Retrospective study of antibiotic resistance among uropathogens from rural teaching hospital, Tamilnadu, India. Asian Pac J Trop Dis 2012;2(5):375-80.
22. Naber KG. Use of quinolones in urinary tract infections and prostatitis. Rev Infect Dis 1989;11(5):1321.
23. Omigie O, Okoror L, Umolu P, Ikuuh G. Increasing resistance to quinolones: a four-year prospective study of urinary tract infection pathogens. Int J Gen Med 2009;2:171-5.
24. Qi C, Pilla V, Yu JH, Reed K. Changing prevalence of *Escherichia coli* with CTX-M-type extended-spectrum b-lactamases in outpatient urinary *E. coli* between 2003 and 2008. Diagn Microbiol Infect Dis 2010;67:87-91.
25. Ram S, Gupta R, Gaheer M. Emerging antibiotic resistance among the uropathogens. Indian J Med Sci 2000;54(9):388-94.
26. Raz R, Gennesin Y, Wasser J, Stoler Z, Rosenfeld S, Rottensterich E, *et al*. Recurrent urinary tract infections in postmenopausal women. Clin Infect Dis 2000;30:152-6.
27. Stratchounski LS, Rafalski VV. Antimicrobial susceptibility of pathogens isolated from adult patients with uncomplicated community-acquired urinary tract infections in the Russian Federation: two multicentre studies, UTIAP-1 and UTIAP-2. Int J Antimicrob Agents 2000;28(1):4-9.
28. Taneja N, Rao P, Arora J, Ashok DA. Occurrence of ESBL and Amp-C ß-lactamases and susceptibility to newer antimicrobial agents in complicated UTI. Indian J Med Res 2008;127:85-8.
29. Tankhiwale SS, Jalgaonkar SV, Ahamad S, Hassani U. Evaluation of extended spectrum beta lactamase in urinary isolates. Indian J Med Res 2004;120:553-6.
30. Noor AF, Shams F, Munshi SK, Hassan M, Noor R. Prevalence and antibiogram profile of uropathogens isolated from hospital and community patients with urinary tract infections in Dhaka city. J Bangl Aca Sci 2013;37(1):57-63.
31. Wu Y, Chen P, Hung Y, Ko W. Risk factors and clinical impact of levofloxacin or cefazolin nonsusceptibility or ESBL production among uropathogens in adults with community-onset urinary tract infections. J Microbiol Immunol Infect 2014;47;197-203.