ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 10, Issue 1, 2017

Online - 2455-3891
Print - 0974-2441

Research Article

ANTIBIOTIC SUSCEPTIBILITY OF BACTERIAL STRAINS, WITH SPECIAL REFERENCE TO *ESCHERICHIA COLI* ISOLATED FROM URINARY TRACT INFECTIONS IN RURAL MAHARASHTRA

GAURAV V SALUNKE[1], SUNIL S GIDAMUDI[2]*

[1]Department of Microbiology, B. K. L. Walawalkar Rural Medical College, Kasarwadi, Maharashtra, India. [2]Department of Pharmacology, B. K. L. Walawalkar Rural Medical College, Kasarwadi, Maharashtra, India. Email: sunilsg.jnmc@gmail.com

Received: 24 August 2016, Revised and Accepted: 28 September 2016

ABSTRACT

Objective: The objective of this study was to find out the etiology of urinary tract infections (UTIs) in patients attending B.K.L. Walawalkar Hospital, Dervan and to determine their antibiotic sensitivity pattern to currently used antimicrobial agents.

Methods: A cross-sectional study was conducted in a rural hospital of Konkan Maharashtra, and urine samples were collected from 368 clinically - suspected cases of UTIs using the mid-stream "clean catch" method and was tested and cultured using standard procedures. Antimicrobial susceptibility test (AST) was performed for the isolated pathogens according to the Clinical and Laboratory Standards Institute guidelines.

Results: *Escherichia coli* (54.84%) was the most prevalent uropathogen. 76.47% of the isolated *E. coli* were found to be extended spectrum beta lactamase producers. A higher prevalence rate of resistance was seen among *E. coli* to the commonly prescribed antibiotic agents. 32 (94.11%) of 34 *E. coli* isolates recovered had multiple antibiotic resistance (MAR), with 16 isolates (50%) possessing MAR indices of 0.6.

Conclusion: The study indicates the isolated microorganisms in UTI showed very high resistance to the commonly prescribed antimicrobial drugs. This suggests the monitoring and rational use of the antimicrobial agents.

Keywords: Mid-stream, Culture, Uropathogen, Resistance, Multiple antibiotic resistance.

 DOI: http://dx.doi.org/10.22159/ajpcr.2017.v10i1.14871

INTRODUCTION

Urinary tract infections (UTIs) are some of the most common infections that occur in both male and female of all the ages, exceeded in frequency only by respiratory and gastrointestinal infections among ambulatory patients [1].

About 80-85% of community-acquired UTIs are caused by *Escherichia coli* and 5-10% by the *Staphylococcus saprophyticus* [2]. UTI cases are often treated empirically, and the antimicrobial resistance patterns of the urinary pathogens determine this empirical therapy [3]. However, uncontrolled antibiotic usage in large proportion has contributed to the emergence of microbial resistance to antibiotics. As a result, antimicrobial resistance is the more prevalent worldwide among urinary pathogens [4-7].

For this reason, having the knowledge of the changes in drug resistance patterns in specific geographical locations may help clinicians to choose the empirical antimicrobial treatment appropriately. The literature on the prevalence of UTI in this part of rural Maharashtra was scarce in recent decades; thereby, this study was conducted to find out the prevalence of UTI and to determine the antimicrobial susceptibility patterns of commonly used antibiotics.

METHODS

The study was performed on UTI cases attending B.K.L. Walawalkar Hospital, Dervan from July 2015 to December 2015 after ethical clearance from the Institution Review Board. A total of 368 clean catch midstream urine samples were collected in a wide mouth sterile container from the study subjects who had not taken antimicrobials in last 15 days. They were instructed to clean the area around the urethral opening with clean water, dry the area, and collect the urine with the labia held apart. Only one specimen per patient was accepted, and samples were processed within 1 hr of collection.

For culture, the urine sample (1 µl) was inoculated on Blood Agar and McConkey Agar plates, using a standard loop of internal diameter 1.34 mm (semi-quantitative method). The plates were read after 24 hrs of aerobic incubation at 37°C. They were further incubated for another 24 hrs before a negative report was issued. A specimen was considered positive for UTI if a single organism was cultured at a concentration of $\geq 10^5$ cfu/ml [8]. No mixed infections were encountered.

The isolates were identified and their antibiotic susceptibility determined using the automated method for ID/antimicrobial susceptibility test using MicroScan (Siemens) rapid Gram-negative and Gram-positive panels. Intermediate isolates were counted as resistant to all the agents tested.

The results obtained were analyzed using descriptive statistics. Multiple antibiotic resistance (MAR) index is a tool to analyze health risk and is helpful to check the spread of bacterial resistance in a given population where there is resistance to more than three antibiotics [9]. It is calculated as the number of antibiotics to which test isolate displayed resistance divided by a total number of antibiotics to which the test organism has been evaluated for sensitivity.

RESULTS

A total of 368 urine samples were analyzed for culture and sensitivity during the study period, of which 60 (16.3%) had significant bacteriuria. The rate of positive culture was 63.33% for female subjects and 36.67% for male subjects.

Analysis of the results (Table 1) indicated that *E. coli* (54.84%) was the most prevalent uropathogen followed by *Klebsiella pneumonia* (9.68%),

Pseudomonas aeruginosa (9.68%), *Acinetobacter* species (9.68%), *Citrobacter* species, and *Proteus mirabilis* (6.45% each). 76.47% of the isolated *E. coli* were found to be extended spectrum beta lactamase (ESBL) producers. No Gram-positive organisms were isolated in this study.

The susceptibility of isolated bacteria to different antimicrobial agents is shown in Table 2. *E. coli* showed high sensitivity to fosfomycin (100%) with good susceptibility to tigecycline (88.24%), imipenem (82.35 %), meropenem (82.35%), and nitrofurantoin (82.35%) each. The *Klebsiella* showed moderate sensitivity to tigecycline, meropenem, imipenem, and fosfomycin (66.67%) each, while it was poorly susceptible to the aminoglycosides, fluoroquinolones and cotrimoxazole (33.3%) each. *Pseudomonas* showed 100% sensitivity to amikacin, gentamicin, tobramycin, levofloxacin, ciprofloxacin, meropenem, and piperacillin – tazobactam. *Acinetobacter* species show moderate sensitivity to amikacin, gentamicin, tobramycin, ceftazidime, levofloxacin, ciprofloxacin, and meropenem (66.67% each). *Citrobacter* species was 100% sensitive to amikacin, imipenem, and meropenem. While Proteus species was poorly susceptible to most of the antibiotics, growth of *Morganella morganii* was inhibited by piperacillin – tazobactam (100%) alone.

The percentage frequency of MAR is shown in Table 3. In this study, we defined MAR as resistance to at least 3 antimicrobial agents [10]. 32 (94.11%) of 34 *E. coli* isolates re-covered had MAR, with 16 isolates (50%) possessing MAR indices of 0.6.

DISCUSSION

UTIs refer to the presence of microbial pathogens within the urinary tract and it is usually classified by the site of infection, if pathogen infects bladder (cystitis), if kidney (pyelonephritis), or if urine (bacteriuria). Bacteria that infect urinary tract may ascend toward the bladder causing cystitis, causing the peculiar symptoms of UTI (i.e., pain, frequency, and urgency). Bacteria may further ascend from bladder up to kidneys causing pyelonephritis, which may complicate later to irreversible kidney damage, renal failure, and death [11]. The etiology and antimicrobial susceptibility of UTI-causing bacteria's have been changing over years. Therefore, the overall, selecting empiric treatment for the patients with serious UTIs is difficult, necessitating the confirmation of etiologic by culture and susceptibility testing [12].

Table 1: Distribution of uropathogens isolated in the study

Microorganisms	N (%)
Escherichia coli	34 (54.84)
Klebsiella pneumoniae	6 (9.68)
Pseudomonas aeruginosa	6 (9.68)
Acinetobacter species	6 (6.45)
Citrobacter species	4 (3.23)
Proteus mirabilis	4 (9.68)
Morganella morganii	2 (6.45)
Total	62 (100.00)

In this study, the isolation rate of bacteria from urine was 16.3% which is in line with studies done in Assefa *et al.* [13] and from Iran which had a rate of 13.2% [14]. The females were more susceptible to UTI than males, which is also similar to other studies [15-17]. Clinical UTIs are more common among females, with up to 60% of women having at least 1 episode in their lifetime [18]. The reason behind this high prevalence is close proximity of the urethral meatus to the anus, shorter urethra, sexual intercourse, incontinence, and bad toilet [19]. Considering the fact that most of infecting organisms are commensals of perianal and vaginal regions, emphasis on personal hygiene, especially in females may be important in reducing the incidence of UTI [20].

The results of our study show that among the heterogeneous causative organisms of UTI, Enterobacteriaceae are the predominant pathogens and *E. coli* is still the single most common uropathogen. This corresponds with the data obtained by other investigators [21-23]. *Klebsiella pneumoniae*, *P. aeruginosa*, *Acinetobacter* species, *Citrobacter* species, and *P. mirabilis* were isolated in small numbers while no Gram-positive organisms were isolated in this study. *E. coli* has the ability to colonize the urogenital mucosa with adhesins, pili, fimbriae, and P1 blood group phenotype receptor [24]. This probably explains its higher isolation from UTI.

Over the last decade, the treatment of choice for UTIs has changed owing to the rate of resistance and high level of therapeutic failure [25]. This study revealed a higher prevalence rate of resistance among *E. coli* to the commonly prescribed antibiotic agents such as ampicillin, amoxicillin – clavulanic acid, trimethoprim/sulfamethoxazole, norfloxacin, ciprofloxacin, and levofloxacin. The low susceptibility

Table 2: Sensitivity pattern of the isolated organisms (%)

AMA/Organisms (n)	*E. coli* (34)	*K. pneumoniae* (6)	*P. aeruginosa* (6)	*Acinetobacter* sp. (6)	*Citrobacter* sp. (4)	*P. mirabilis* (4)	*M. morganii* (2)
Amikacin	64.71	33.33	100.00	66.67	100.00	50.00	0.00
Amoxicillin – Clavulanic acid	17.65	33.33	NR	NR	50.00	50.00	0.00
Ampicillin	0.00	NR	NR	NR	NR	50.00	0.00
Cefepime	5.88	NR	66.67	33.33	NR	50.00	0.00
Cefotaxime	5.88	33.33	NR	0.00	0.00	50.00	0.00
Cefoxitin	64.71	66.67	NR	NR	50.00	50.00	0.00
Ceftazidime	5.88	33.33	66.67	66.67	0.00	50.00	0.00
Cefuroxime	5.88	33.33	NR	NR	0.00	50.00	0.00
Ciprofloxacin	11.76	33.33	100.00	66.67	0.00	50.00	0.00
Cotrimoxazole	5.88	33.33	NR	NR	0.00	50.00	0.00
Fosfomycin	100.00	66.67	NR	NR	50.00	50.00	NR
Gentamicin	11.76	33.33	100.00	66.67	50.00	50.00	0.00
Imipenem	82.35	66.67	33.33	0.00	100.00	50.00	0.00
Levofloxacin	35.29	33.33	100.00	66.67	0.00	50.00	0.00
Meropenem	82.35	66.67	100.00	66.67	100.00	50.00	0.00
Nitrofurantoin	82.35	NR	NR	NR	50.00	NR	NR
Norfloxacin	11.76	33.33	33.33	33.33	0.00	50.00	0.00
Piperacillin – Tazobactam	5.88	33.33	100.00	33.33	50.00	50.00	100.00
Tigecycline	100.00	100.00	NR	100.00	100.00	NR	NR
Tobramycin	23.53	33.33	100.00	66.67	0.00	50.00	0.00

NR: Not tested and reported, *E. coli: Escherichia coli, K. pneumoniae: Klebsiella pneumoniae, P. aeruginosa: Pseudomonas aeruginosa, P. mirabilis: Proteus mirabilis, M. morganii: Morganella morganii*

Table 3: The percentage frequency of MAR index

MAR index	Number of *E. coli* (%)
0.1	0
0.2	0
0.3	2 (6.25)
0.4	0
0.5	4 (12.5)
0.6	16 (50)
0.7	4 (12.5)
0.8	4 (12.5)
0.9	2 (6.25)
1	0
Total	32

MAR: Multiple antibiotic resistance, *E. coli: Escherichia coli*

(i.e., higher resistance) of the isolates to the common and cheap orally administered antibiotics is not surprising because these drugs are more commonly misused, thereby leading to the development of resistance, as previously reported [26]. Increased resistance in quinolones against *E. coli* may reflect the overuse of these drugs for the treatment of UTI [27]. Thus reducing the number of prescription for a particular antibiotic can lead to a decrease in resistance rates. Another factor could be the generalized use of fluoroquinolones in animals feed (especially in poultry), and the subsequent transmission of resistant to strains from animals to humans [28]. These findings are of great importance and imply that these antibiotics cannot be used as empirical therapy for UTI, particularly in the study area.

On the other hand, while no resistance was detected to tigecycline and fosfomycin, lower resistance was detected to nitrofurantoin, imipenem, and meropenem. The low resistance could be because they are not easily accessible and relatively expensive in price compared to others. Thus, these drugs could be considered as alternative options in the empirical treatment of UTIs. Resistance to nitrofurantoin among *E. coli* isolates from UTIs has remained low despite more than 50 year's widespread use of the drug. Reason for the lack of emerging resistance are not fully understood, but likely include restricting use to indication for urinary infection, limited systemic absorption, and the need for multiple genetic mutations for the bacteria to develop resistance [29].

The value of MAR index 0.2 differentiates the low and high risk. MAR index >0.2 implies that the strain of such bacteria originate from an environment where several antibiotics are used. The MAR indices of *E. coli* obtained in this study is a possible indication that a very large proportion of the bacterial isolates have been exposed to several antibiotics. In our study, 94.11% isolates of *E. coli* were MDR. This is quite high when compared to other studies [30]. Multi-resistance is usually related to production of ESBL, which in our study is very high as compared to other recently published data.

CONCLUSION

UTI is one of the common causes for seeking medical attention in the community and effective management of patients relays on the identification of the type of organisms that caused the disease and the selection of an effective antibiotic agent to the organism in question. This study provides valuable data to compare and monitor the status of antimicrobial resistance. Thus, fosfomycin and nitrofurantoin were found to be the most appropriate oral antibiotics and tigecycline, imipenem and meropenem were the most appropriate parenteral antibiotics, for the empirical therapy of UTIs.

REFERENCES

1. Levi ME, Redington J, Barth L. The patient with urinary tract infection. Manual of Nephrology. 6th ed., Vol. 7. Philadelphia, PA: Lippincott Williams & Wilkins; 2005. p. 91.
2. Nicolle LE. Uncomplicated urinary tract infection in adults including uncomplicated pyelonephritis. Urol Clin North Am 2008;35(1):1-12.
3. Wilson ML, Gaido L. Laboratory diagnosis of urinary tract infections in adult patients. Clin Infect Dis 2004;38(8):1150-8.
4. Bonadio M, Meini M, Spetaleri P, Gilgi C. Current microbiological and clinical aspects of urinary tract infections. Eur J Urol 2001;40(4):439-45.
5. National Committee for Clinical Laboratory Standards. Performance Standards for Antimicrobial Disc Susceptibility Tests, M2-A7. 7th ed. Wayne, Pennsylvania, USA: NCCLS; 2000.
6. Grude N, Tveten Y, Kristiansen BE. Urinary tract infections in Norway: Bacterial etiology and susceptibility, a retrospective study of clinical isolates. Clin Microbiol Infect 2001;7(10):543-7.
7. Kripke C. Duration of therapy for women with uncomplicated UTI. Am Fam Physician 2005;72(11):2219.
8. Collee JG, Duguid JP, Fraser AG, Marmion BP, Simmons A. Laboratory diagnosis of infective syndromes. Mackie and McCartney Practical Medical Microbiology. 14th ed. Edinburgh: Churchill-Livingstone; 1996. p. 53-64.
9. Sambrook J, Frish EF, Maniatis T. Molecular cloning. A Laboratory Manual. 2nd ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press; 1982.
10. CLSI, editor. Performance Standards for Antimicrobial Susceptibility Testing, 7th Informational Supplement. Approved Standard, M100-S17, WUSA. Wayne, PA: Clinical and Laboratory Standards Institute; 2007.
11. Scholes D, Hooton TM, Roberts PL, Gupta K, Stapleton AE, Stamm WE. Risk factors associated with acute pyelonephritis in healthy women. Ann Intern Med 2005;142(1):20-7.
12. Carson C, Naber KG. Role of fluoroquinolones in the treatment of serious bacterial urinary tract infections. Drugs 2004;64(12):1359-73.
13. Assefa A, Asrat D, Woldeamanuel Y, G/Hiwot Y, Abdella A, Melesse T. Bacterial profile and drug susceptibility pattern of urinary tract infection in pregnant women at tikur anbessa specialized hospital Addis ababa, Ethiopia. Ethiop Med J 2008;46(3):227-35.
14. Farajnia S, Alikhani MY, Ghotaslou R, Naghili B, Nakhlband A. Causative agents and antimicrobial susceptibilities of urinary tract infections in the northwest of Iran. Int J Infect Dis 2009;13(2):140-4.
15. Arjunan M, Al-Salamah AA, Amuthan M. Prevalence and antibiotic susceptibility of uropathogens in petients from a rural environment, Tamil Nadu. Am J Infect Dis 2010;6(2):29-33.
16. Manjunath GN, Prakash R, Vamseedhar A, Shetty K. Changing trends in the spectrum of antimicrobial drug resistance pattern of uropathogens isolated from hospital and community patients with urinary tract infections in Tumkur and Bangalore. Int J Biol Med Res 2011;2(2):504-7.
17. Acharya A, Gautam R, Subedee L. Uropathogens and their antimicrobial susceptibility pattern in Bharatpur. Nepal Med Coll J 2011;13(1):30-3.
18. Foxman B, Barlow R, D'Arcy H, Gillespie B, Sobel JD. Urinary tract infection: Self-reported incidence and associated costs. Ann Epidemiol 2000;10(8):509-15.
19. McNulty CA, Richards J, Livermore DM, Little P, Charlett A, Freeman E, *et al*. Clinical relevance of laboratory-reported antibiotic resistance in acute uncomplicated urinary tract infection in primary care. J Antimicrob Chemother 2006;58(5):1000-8.
20. Oluremi B, Idowu A, Olaniyi J. Antibiotic susceptibility of common bacterial pathogens in urinary tract infections in a teaching hospital in South-Western Nigeria. Afr J Microbiol Res 2011;5(22):3658-63.
21. 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.
22. Kothari A, Sagar V. Antibiotic resistance in pathogens causing community-acquired urinary tract infections in India: A multicenter study. J Infect Dev Ctries 2008;2(5):354-8.
23. Akram M, Shahid M, Khan AU. Etiology and antibiotic resistance patterns of community acquired urinary tract infections in JNMC hospital Aligrah, India. Ann Clin Microbiol Antimicrob 2007;6:4.
24. Das RN, Chandrashekhar TS, Joshi HS, Gurung M, Shrestha N, Shivananda PG. Frequency and susceptibility profile of pathogens causing urinary tract infections at a tertiary care hospital in Western Nepal. Singapore Med J 2006;47(4):281-5.
25. Yilmaz K, Nilay C, Aysegül G. Co-trimoxazole and quinolone resistance in *Escherichia coli* isolated from urinary tract infections over the last 10 years. Int J Antimicrb Agents 2005;26(1):75-7.
26. Ehinmidu JO. Antibiotics susceptibility patterns of urine bacterial isolates in Zaria, Nigeria. Trop J Pharm Res 2005;2(2):223-8.
27. Saleh AA, Ahmed SS, Ahmed M, Sattar AN, Miah MR. Changing

trends in uropathogens and their antimicrobial sensitivity pattern. Bangladesh J Med Microbiol 2009;3(1):9-12.
28. Miller LG, Tang AW. Treatment of uncomplicated urinary tract infections in an era of increasing antimicrobial resistance. Mayo Clin Proc 2004;79(8):1048-54.
29. Nicolle, L, Anderson PA, Conly J, Mainprize TC, Meuser J, Nickel JC, *et al*. Uncomplicated urinary tract infection in women. Can Fam Physician 2006;52(5):612-8.
30. Mathai E, Chandy S, Thomas K, Antoniswamy B, Joseph I, Mathai M. Antimicrobial resistance surveillance among commensal *Escherichia coli* in rural and urban areas in Southern India. Trop Med Int Health 2008;13(1):41-5.