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

GAURAV V SALUNKE1, SUNIL S GIDAMUDI2

1Department of Microbiology, B. K. L. Walawalkar Rural Medical College, Kasarwadi, Maharashtra, India. 2Department of Pharmacology, B. K. L. Walawalkar Rural Medical College, Kasarwadi, Maharashtra, India. Email: sunilsg.jnmcc@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 were 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.

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 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 antibiotics 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 ≥10^4 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 63.3% 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 (56.8%).
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 (4.65) |
| *Citrobacter species* | 6 (4.65) |
| *Proteus mirabilis* | 4 (9.68) |
| *Morganella morganii* | 2 (6.45) |
| Total               | 62 (100.00) |

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               |
| Aminocillin – Clavulanic acid | 17.65        | 33.33              | NR                 | NR                     | 50.00                | 50.00              | 0.00               |
| Ampicillin        | 0.00           | NR                 | NR                 | NR                     | NR                   | 50.00              | 50.00              |
| Cefotaxime        | 5.88           | 33.33              | NR                 | 0.00                   | 0.00                 | 50.00              | 0.00               |
| Cefoxitin         | 64.71          | 66.67              | NR                 | 33.33                  | NR                   | 50.00              | 0.00               |
| Ceftriaxime       | 5.88           | 33.33              | NR                 | 33.33                  | NR                   | 50.00              | 0.00               |
| Cefuroxime        | 5.88           | 33.33              | NR                 | 33.33                  | NR                   | 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                 | 66.67                  | 0.00                 | 50.00              | 0.00               |
| Fosfomycin        | 100.00         | 66.67              | NR                 | NR                     | 50.00                | 50.00              | 0.00               |
| Gentamicin        | 11.76          | 33.33              | 100.00             | 66.67                  | 50.00                | 50.00              | 0.00               |
| Imipenem          | 82.35          | 33.33              | 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         | NR                 | 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       | 4 (12.5)             |
| 0.5       | 16 (50)              |
| 0.6       | 4 (12.5)             |
| 0.7       | 2 (6.25)             |
| 0.8       | 2 (6.25)             |
| 0.9       | 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 rate. Another factor could be the generalized use of fluoroquinolones in animals feed (especially in poultry), and the subsequent transmission of resistant strains to 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 years’ 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, Spotaleri P, Giligi 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):19-9.
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. Haders PE. 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. Tissue distribution 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, Alkhanlili MY, Ghotsasou R, Nahlili B, Nakhlband A. Causative agents and antimicrobial susceptibility patterns of urinary tract infections in the northeast of Iran. Int J Infect Dis 2004;9(3):140-4.
15. Assefa A, Asrat D, Woldeamanuel Y, G/Hiwot Y, Abdella A, Melesse T. Tissue distribution 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.
16. Manjunath GN, Prakash R, Vamsheerudu 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, Gautham R, Subhedar 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, Charett 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. Oluwemi 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):504-7.
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 JNMIC hospital Aligarh, India. Ann Clin Microbiol Antimicrob 2007;6:4.
24. Das RN, Chandrashekhar TS, Joshi HS, Gurung M, Shresta 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):291-5.
25. Yilmaz K, Nyal C, Aysegul G. Co-trimoxazole and quinolone resistance in Escherichia coli isolated from urinary tract infections over the last 10 years. Int J Antimicrob Agents 2005;26(1):75-7.
26. Ehimmida 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, Antonisamy 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.