AETIOLOGY AND ANTIBIOTIC RESISTANCE PATTERN OF UROPATHOGENS IN A TERTIARY CARE HOSPITAL

Sanaga Shanthi Kumari[1], Tupili Ramya[2], Kailashnath Reddy[3], Gundela Swarnalatha[4], Venkata Swapna[5], Basireddy Sreekanth Reddy[6], Ramakrishna[7], Anitha Lavanya[8]

[1]Assistant Professor, Department of Microbiology, KMC, Kurnool.
[2]Senior Resident, Department of Microbiology, NLR.
[3]HOD, Department of Microbiology, Viswabharati Hospital, Kurnool.
[4]HOD, Microbiology, KMC, Kurnool.
[5]Senior Resident, Department of Microbiology, NLR.
[6]Assistant Professor, Department of Microbiology, KMC, Kurnool.
[7]Senior Resident, Department of Microbiology, ANTP.
[8]Assistant Professor, Department of Microbiology, KMC, Kurnool.

ABSTRACT

Urinary tract infections (UTI) continue to be the commonest nosocomial infections. UTI is often treated with different broad-spectrum antibiotics when one with a narrow spectrum of activity may be appropriate. The extensive use of antimicrobial agents have invariably resulted in development of antibiotic resistance, which in recent years has become a major problem worldwide.

AIM AND OBJECTIVES

1) Aim of the study is to isolate bacteria that cause urinary tract infections (UTI). 2) To understand resistance pattern of uropathogens which assist in choosing empirical therapy for urinary tract infections (UTI).

MATERIALS AND METHODS

The present study was conducted on patients with clinically suspected UTI attending our hospital. 500 urine samples were collected from these patients and were tested. All the isolates were identified according to the standard biochemical tests and antibiotic susceptibility testing was done by disc diffusion method.

RESULTS

160 isolates of uropathogens isolated from 500 clinical samples of urine were included in this study. Among 160 isolates (major bacterial isolates from UTI), the predominant isolate was Escherichia coli and the second most common organism was Klebsiella spp. followed by CONS, Candida spp., Pseudomonas spp., Enterococcus spp., Acinetobacter spp., Staphylococcus aureus and Enterobacter. 25.62% of organisms were isolated from male patients and 74.37% were isolated from female patients. This showed a female predominance. Age group between 21-30 showed highest incidence of UTI followed by 1-10, 11-20, 31-40, 41-50, 61-75, 51-60 and ≥75. This showed more incidence of UTI among sexually active population. Escherichia coli predominated in hospital as well as in community patients. The second most common was Klebsiella spp. which predominated in hospitalised patients. Among the community patients, Escherichia coli predominated followed by Klebsiella spp., Enterococcus spp., CONS and Pseudomonas spp. In the present study, it was observed that ESBL production was 91.04% in Escherichia coli, 77.77% in Klebsiella spp., in Pseudomonas spp. it was 50%. MBL production was observed in 2.98% of Escherichia coli, 50% of Pseudomonas spp., 16.66% of Klebsiella spp. In this study, Gram-negative bacilli isolated from UTI were highly sensitive to amikacin and imipenem, and Gram-positive organisms were sensitive to vancomycin and imipenem except Enterococci which showed 100% resistance to vancomycin.

KEYWORDS

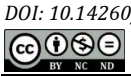
Urinary Tract Infections, Antibiotics, E. Coli, Klebsiella.

HOW TO CITE THIS ARTICLE: Kumari SS, Ramya T, Reddy K, et al. Aetiology and antibiotic resistance pattern of uropathogens in a tertiary care hospital. J. Evolution Med. Dent. Sci. 2016;5(75):5534-5538, DOI: 10.14260/jemds/2016/1250

INTRODUCTION

Urinary tract infection (UTI) remains one of the most common bacterial infections and second most common infectious disease in community practice after respiratory infections. It accounts for one million hospitalisations annually.(1)

Financial or Other, Competing Interest: None.
Submission 10-08-2016, Peer Review 31-08-2016,
Acceptance 09-09-2016, Published 16-09-2016.
Corresponding Author:
Dr. S. Shanthi Kumari,
Assistant Professor,
Kurnool Medical College, Kurnool.
E-mail: s.shanthi03@gmail.com
DOI: 10.14260/jemds/2016/1250



UTI if diagnosed early and with adequate antibiotic coverage is not alarming. However, if inadequately treated, can cause significant morbidity and mortality.(1)

UTI continues to be the commonest nosocomial infection accounting for approximately 40% of all hospital acquired infections. UTI occurs in all age groups from neonates to elderly. More common in boys during the first 3 months, often in association with urologic abnormalities. Once adulthood is reached, prevalence of asymptomatic bacteriuria increases in female population. Female urethra appears to be particularly prone to colonisation with colonic Gram-negative bacilli because of its proximity to anus, its short length and its termination beneath the labia. Sexual intercourse causes introduction of bacteria into the bladder.

To ensure appropriate therapy, current knowledge of organisms that cause UTI and their antibiotic susceptibility is mandatory.[2] UTI is often treated with different broad-spectrum antibiotics when one with a narrow spectrum of activity may be appropriate because of concerns about infection with resistant organisms. The extensive use of antimicrobial agents have invariably resulted in development of antibiotic resistance, which in recent years has become a major problem worldwide.[3]

Since the resistance rates to antibiotics differ from region to region, in making an appropriate choice of empiric or definitive therapy for UTI, it is rewarding to have information on prevailing pattern of antimicrobial resistance among common urinary pathogens. This study was therefore carried out to investigate the aetiologic agents of UTI in a tertiary care hospital and to study their susceptibility pattern to different antimicrobial agents.[4]

AIM AND OBJECTIVES

- To isolate bacteria that cause urinary tract infections (UTI).
- To understand resistance pattern of uropathogens which assist in choosing empirical therapy for urinary tract infections (UTI).

MATERIALS AND METHODS

The present study was conducted on patients with clinically suspected UTI attending to our hospital. 500 urine samples were collected from these patients and were tested.

Inclusion Criteria

Both male and female patients having clinically suspected UTI were included in the study. Clinical diagnostic criteria include dysuria, frequency, urgency and fever.

Exclusion Criteria

- Patients on antibiotic therapy prior to or during the investigation.
- Patients on catheterisation.

Specimen Collection

Mid-stream specimen of urine samples were collected from both male and female in a wide mouthed sterile universal container with a secured lid. A proper instruction was given to the patient regarding the method of collection of urine sample. The sample was transported to the laboratory as early as possible and processed.

Wet Film Examination

Observation was also done for presence of pus cells, epithelial cells, red blood cells, bacilli, casts, crystals, parasites, yeasts. All the findings were recorded.

SEMI QUANTITATIVE CULTURE

A calibrated loop that carries 0.01 mL of urine was used to culture urine sample semi-quantitatively. A loop full of urine sample was plated on blood agar and MacConkey's agar.

The plates were incubated at 37^0C for 24 hours. The number of colonies were counted and interpreted as CFU/mL of urine by multiplying the number of colonies grown by 100. Colonies exceeding 10^5 CFU/mL were taken as significant bacteriuria.

Identification and Sensitivity Tests

Identification of uropathogens was carried out by means of standard laboratory tests like. Gram stain, motility, catalase test, nitrate reduction, methyl red test, Voges-Proskauer test, production of indole, H_2S, urease, citrate utilisation, sugar fermentation tests. Decarboxylation of lysine, arginine and ornithine in Moeller's decarboxylation media. Antibiotic susceptibility test was done by Kirby Bauer disc diffusion testing.

TEST FOR MBL PRODUCTION

Imipenem-EDTA Combined Disc Test

Test organisms were inoculated on to the plates with MH agar as recommended by CLSI. Two 10 µg imipenem discs were placed on the plates and appropriate amount of 10 µL of EDTA solution added to one of them to obtain the desired concentration of 750 micrograms.

The inhibition zones were compared after 16-18 hrs. of incubation at 35^0C. In combined disc test, if the increase in inhibition zone was more than 7 mm than the imipenem disc alone it was considered as MBL positive.

TEST FOR ESBL PRODUCTION

Phenotypic Confirmatory Test with Combination Disc

This test requires the use of 3rd gen. cephalosporin antibiotic disc alone and in combination with clavulanic acid. In this study, a disc of ceftazidime 30 micrograms alone and a disc of ceftazidime plus clavulanic acid (30/10 micrograms) were used. Both the discs were placed at least 25 mm apart center to centre on a lawn culture of test isolate on MH agar plate and incubated overnight at 37^0C. Difference in zone diameters with and without clavulanic acid was measured.

Interpretation

When there is an increase of greater than 5 mm in inhibition zone around combination disc of ceftazidime plus clavulanic acid disc versus the inhibition zone diameter around ceftazidime disc alone, it confirms the ESBL production.

RESULTS

A total of 160 isolates of uropathogens isolated from 500 clinical samples submitted to microbiology department. The remaining 340 samples were either sterile or were showing insignificant growth. The distribution of the different species among these isolates were as follows.

Organisms Isolated	Number	Percentage (%)
Escherichia Coli	67	41.87
Klebsiella Pneumoniae	54	33.75
Coagulase Negative Staphylococcus	10	6.25
Candida spp.	8	5
Pseudomonas spp.	6	3.75
Enterococcus spp.	5	3.12
Acinetobacter spp.	5	3.12
Staphylococcus aureus	4	2.5
Enterobacter spp.	1	3.12
Total	**160**	**100**

Table 1: Frequency of Uropathogens in UTI

Among 160 isolates, major bacterial isolate from UTI was E. coli (41.8%) and the second most common organism was Klebsiella pneumoniae followed by CONS, Candida spp., Pseudomonas spp., Enterococcus spp., Acinetobacter spp., Staphylococcus aureus and Enterobacter.

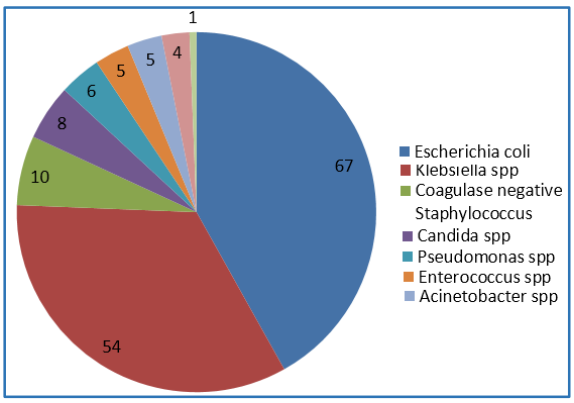


Fig. 1: Frequency of Uropathogens in UTI

Gender	Total Cases (500)	Culture Positive Cases (160)
Male	157(31.4%)	41(25.62%)
Female	343(68.6%)	119(74.37%)

Table 2: Sex wise Distribution of UTI

25.62% of organisms were isolated from male patients and 74.37% were isolated from female patients. This showed a female predominance.

Age Group (Yrs.)	Male	Positive Males	Female	Positive Females	Total Positive Cases
1-10	86	23	52	15	38(23.75%)
11-20	21	7	46	18	25(15.62%)
21-30	11	11	144	42	53(33.12%)
31-40	15	4	46	14	18(11.25%)
41-50	6	4	26	5	9(5.62%)
51-60	9	2	14	4	6(3.75%)
61-75	6	3	13	4	7(4.37%)
>75	3	3	2	1	4(2.5%)

Table 3: Age wise distribution of UTI

Age group between 21-30 showed highest incidence of UTI followed by 1-10, 11-20, 31-40, 41-50, 61-75, 51-60 and ≥75. This showed more incidence of UTI among sexually active population.

Name of Antibiotic	E. Coli N=67	Klebsiella N=54	Pseudomonas N=6	Acinetobacter N=5	Enterococcus N=5	S. Aureus N=4	Cons N=10
AK	20%	39%	33%	20%	80%	0%	100%
LE	36%	23%	67%	20%	60%	25%	50%
CAZ	94%	95%	100%	100%	-	-	-
CIP	73%	81%	83%	-	100%	100%	
CTR	76%	76%	83%	-	-	-	-
NIT	28%	44%	83%	-	80%	25%	30%
COT	79%	70%	100%	60%	-	75%	80%
AMC	75%	85%	100%	100%	100%	100%	-
NA	90%	93%	100%		-	100%	
P/T	12%	52%	33%	20%	40%	-	-
IMP	2.98%	17%	50%	0%	40%	-	-
P					100%	100%	100%
OX						100%	100%
VA					40%	0%	0%
CB			83%				

Table 4: Antibiotic Resistance Pattern of Uropathogens (%)

AK-Amikacin, LE-Levofloxacin, CAZ-Ceftazidime, CIP-Ciprofloxacin,
CTR-Ceftriaxone, NIT-Nitrofurantoin, COT-Cotrimoxazole, AMC-Amoxyclav,
NA-Nalidixic acid, P/T-Piperacillin/Tazobactam, IMP-Imipenem,
P-Penicillin, OX-Oxacillin, VA -Vancomycin, CB-Carbenicillin.

DISCUSSION

The similarities and differences in the type and distribution of uropathogens may result from different environmental conditions and host factors, and practices such as healthcare and education programs, socioeconomic standards and hygiene practices in each country. Constant survey of antimicrobial resistance plays a very important role in empiric treatment of UTI.[5] There is an increased emergence of antibiotic resistance in the uropathogens, reasons for this are many like irrational, inappropriate use of antibiotics before the availability of the urine culture results, lack of knowledge of local antimicrobial susceptibility patterns and over-the-counter medications.

In this study, UTI in women (74.37%) vastly outnumbered those in men (25.62%). This correlates to the studies of Gupta et al,[3] Mohammed Akram et al.[6] This is related to factors such as length of urethra, distance of urogenital meatus from anus and the antibacterial properties of prostatic fluid.

Escherichia coli (41.8%) was the most common uropathogen, Klebsiella (33.7%) being the second commonest. This is in correlation with many studies like Tankhiwale et al,[7] Taneja et al[8] where the E. coli and Klebsiella constituted maximum number of isolates in their studies with E. coli topping the list.

Isolate	Tankhiwale et al 2003[7]	Taneja et al 2006[8]	Gupta et al 2007[3]	Present Study 2013
Escherichia coli	49.8%	47.1%	69.9%	41.87%
Klebsiella spp.	37.8%	15.6%	25%	33.75%
CONS	1.84%	-	0.59%	6.25%
Pseudomonas spp.	6.5%	5.9%	0.89%	3.75%
Enterococcus spp.	0.46%	8.7%	0.8%	3.12%
Staphylococcus Aureus	0.9%	1.7%	0.19%	2.5%

Comparison of Percentage of Uropathogens isolated in the Present Study with previous Indian Reports

Other common organisms in our study was coagulase-negative Staphylococcus (6.25%), Pseudomonas spp. (3.75%), Enterococcus spp. (3.12%), Acinetobacter (3.1%), Staphylococcus aureus (2.5%), Enterobacter spp. (0.6%) which also correlate well with the above studies.

Antibiotic resistance is a major clinical problem in treating infections caused by these microorganisms. The resistance to antimicrobials has increased over years. Resistance pattern varies from region to region.

In our study, high level of resistance was seen to ceftazidime. For E. coli it is 94%; for Klebsiella spp. it is 95%; for Pseudomonas spp., Acinetobacter spp. and Enterobacter spp. it is 100%. The resistance rates to ceftriaxone were 76% in Escherichia coli, and 83% in Klebsiella spp. Resistance rate to ciprofloxacin was also high with 73% resistance in E. coli, 81% in Klebsiella spp., 83% in Pseudomonas, 80% resistance in Acinetobacter spp.

In this study, most of the UTIs caused by Gram-negative bacteria were resistant to beta lactams and fluoroquinolones. Higher resistance rates to all antibiotics tested in our study may be explained by high and uncontrolled consumption of these antibiotics during the past decade in our institute. All antimicrobials are available as over-the-counter drugs without requiring the physician's prescriptions in our country.

However, Imipenem showed lowest resistance rates with only 3% resistance in Escherichia coli, 17% in Klebsiella to as high as 50% in Pseudomonas.

Piperacillin/Tazobactam combination also showed less resistance followed by carbapenems with the resistance rate of 12% in Escherichia coli, 52% in Klebsiella, 33% in Pseudomonas.

Resistance rate of cotrimoxazole which is commonly used in urinary tract infections was also high with 75% of Escherichia coli, 70% of Klebsiella spp. being resistant to it. Whereas the conventional urinary antiseptic nitrofurantoin showed 28% resistance in Escherichia coli, and 44% in Klebsiella spp. making this drug an effective drug in 2/3rd of the cases.

Though the Gram-positive organism formed a small subset of the isolates, the resistance rates in them were also very high. All the Staphylococcal isolates were resistant to oxacillin (MRSA & MRCoNS) making all the beta lactam antibiotics ineffective against these infections. Vancomycin remained the best drug against these isolates with 100% sensitivity.

Similar resistance pattern was noted by various workers from different parts of India.[9-14] In Somasekara et al[13] study, highest resistance of E. coli was seen towards ciprofloxacin and ceftazidime (72% and 58% respectively). Cotrimoxazole resistance was also 68.8% whereas only 8% were imipenem resistant. In Mandal et al[14] study, 73% of E. coli were resistant to ciprofloxacin. Meropenem resistance in E. coli and Klebsiella was 9.8% and 18% respectively which is similar to our study. But in their study methicillin resistance in Staphylococcus was only 45% which is lesser when compared to our study whereas vancomycin was the best drug with highest sensitivity which is similar to our study.

Alarming resistance to such agents requires periodic monitoring in order to take appropriate decisions when prescribing such antibiotics. A good infection control and antibiotic policy will certainly help in delaying the era of unabated microorganisms for which no antibiotic is going to be effective.

CONCLUSION

Gram-negative bacteria continues to be the most common aetiological agent of Urinary tract infections with E. coli topping the list. With the increased resistance rates among these bacterial agents, there are only few drugs which have retained their susceptibility and can be used against these agents. Continuous surveillance for the changing resistance patterns is of utmost importance in finding the appropriate empirical therapy as well as definitive therapy against these infections.

REFERENCES

1. Sheryll L, Magalit MD, Tarcela M, et al. Increasing antimicrobial resistance patterns of community and nosocomial uropathogens in Makati Medical Center. Phil J Microbial Infect Dis 2004;33(4):143-8.
2. Manchanda V, Singh NP. Occurrence and detection of amp c beta-lactamases among gram negative clinical isolates using a modified three-dimensional test at Guru Teg Bahadur Hospital, Delhi, India. Journal of Antimicrobial Chemotherapy 2003;51(2):415-8.
3. Gupta V, Yadav A, Joshi RM. Antibiotic resistance pattern in uropathogens. Indian Journal of Medical Microbiology 2002;20(2):96-8.
4. Anbumani N, Mallika M. Antibiotic resistance pattern in uropathogens in a tertiary care hospital. Indian Journal for the Practicing Doctor 2007;4(1):3-4.
5. Martinez-Martinez L, Conejo MC, Pascual A, et al. Activities of imipenem and cephalosporins against clonally related strains of Escherichia coli hyperproducing chromosomal beta-lactamase and showing altered porin profiles. Antimicrobial Agents and Chemotherapy 2000;44(9):2534-6.
6. Akram M, Shahid M, Khan AU. Etiology and antibiotic resistance patterns of community-acquired urinary tract infections in JNMC Hospital Aligarh, India. Annals of Clinical Microbiology and Antimicrobials 2007;6(4).
7. Tankhiwale SS, Jalgaonkar SV, Ahamad S, et al. Evaluation of extended spectrum beta lactamase in urinary isolates. Indian J Med Res 2004;120(6):553-6.
8. Taneja N, Maharwal S, Sharma M. Imipenem resistance in nonfermenters causing nosocomial urinary tract infections. Indian J Med Sci 2003;57(7):294-9.
9. Hasan AS, Nair D, Kaur J, et al. Resistance patterns of urinary isolates in a tertiary Indian hospital. J Ayub Med Coll Abbottabad 2007;19(1):39-41.

10. Gupta N, Kundra S, Sharma A, et al. Antimicrobial susceptibility of uropathogens in India. J Infect Dis Antimicrob Agents 2007;24(1):13-18.
11. Manjunath GN, Prakash R, Annam V, et al. Changing trends in the spectrum of antimicrobial drug resistance pattern of uropathogens isolated from hospitals and community patients with urinary tract infections in Tumkur and Bangalore. Int J Biol Med Res 2011;2(2):504-7.
12. 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.
13. Somashekara SC, Deepalaxmi S, Jagannath N, et al. Retrospective analysis of antibiotic resistance pattern to urinary pathogens in a tertiary care hospital in south India. J Basic Clin Pharma 2014;5(4):105-8.
14. Mandal J, Acharya NS, Buddhapriya D, et al. Antibiotic resistance pattern among common bacterial uropathogens with a special reference to ciprofloxacin resistant Escherichia coli. Indian J Med Res 2012;136(5):842-9.