Original Research Article

Study of uropathogens and their antibiotics susceptibility pattern in tertiary care hospital in central India

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Abstract

Objective This study was undertaken to determine the spectrum of organism responsible for UTI and their antimicrobial susceptibility pattern in Mahaveer Institute of Medical Sciences and Research (MIMS) located in Central India**. Methods** The present study was carried out in Department of Microbiology from January to December 2021. Patients attending OPD and IPD with any complaint pointing towards UTI were included study. Freshly voided, clean-catch midstream urine was All plates were then incubated at 37°C aerobically for 24 hours. A significant growth is considered if the number of colony is $\geq 10^5$ colony forming unit (cfu)/ml (12). **Results** Most common organism isolated were gram negative organisms.E.coli (51.67%), Klebsiella (14.76%),Enterococcus (11.40).E.coli showed low level resistance for Nitrofurantoin and Amikacin (7.79%).Pseudomonas showed low resistance for Nitrofurantoin, and Amikacin (12.51%)and Meropenem and Gentamicin (37.5%).Enterococcus faecalis showed low level resistance for Nitrofurantoin(11.76%), followed by High level Gentamicin (23.52.MRSA was highly resistant to Amoxycillin (75%). Candida species isolated from various clinical specimens were identified up to species level by standard mycological techniques (77.77 %) non albicans Candida species and (22.22 %) was Candida albicans. **Conclusion** Our study concludes E. coli as most common organism causing urinary tract infection with female predominance. Nitrofurantoin has showed better sensitivity against most organisms causing UTI so it can be used as initial agent for empirical treatment till culture reports are available. There is increasing resistance to commonly used antibiotics due to indiscriminate use of antibiotics without culture and sensitivity report.

Keywords: Epidemiological Investigation, Antimicrobial Susceptibility, Clinical Specimens, Bacteriological Culture

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Introduction

Urinary Tract Infections (UTIs) are one of the most commonly occurring infections in medical practice despite the widespread availability of antibiotics. It is estimated that 150 million people suffer with UTI every year world-wide, costing the global economy 6 billion dollars. It is the most frequent cause of illness in human after respiratory infections. Neonates, girls, young women and older men are most susceptible to UTI. UTI is defined as the presence of growth of more than 10^5 colony forming unit (CFU) of bacteria per ml of urine for asymptomatic individual and 10^3 for symptomatic individual.Most of the UTI are caused by Gram negative bacteria like Escherichia coli, Proteus spp., Klebsiella spp., Pseudomonas aeruginosa, Acinetobacter, Serratia and Morganella morganii. UTI also caused by Gram positive bacteria like Enterococcus, Staphylococcus especially coagulase negative staphylococci and Streptococcus agalactiae[1-7].

Treatment is generally done with broad-spectrum antibiotics due to the concerns of antibiotic resistance. Fluoroquinolones were the most preferred initial agents as a part of the empirical therapy of the infections but are being limited due to high resistance rates and toxicity[8, 9].The extensive use of antibiotics over a period has stemmed from the development of antibiotic resistance, which has now become a problem worldwide.The selection of antimicrobial agents should not be decided by the most likely pathogen, but should rather be decided based on the susceptibility patterns.

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Hence, it is very important to know the patterns of local antimicrobial susceptibility to determine sensible and careful empirical therapy for the treatment of UTIs[8-11].

This study was undertaken to determine the spectrum of organism responsible for UTI and their antimicrobial susceptibility pattern in Mahaveer Institute of Medical Sciences and Research (MIMS) located in Central India.

Materials and methods

Methodology

The present study was carried out in Mahaveer Institute of Medical Sciences and Research, Department of Microbiology from January to December 2021. Patients attending OPD and IPD with any complaint pointing towards UTI were included study. Freshly voided, clean-catch midstream urine was collected from each patient into sterile screw-capped universal container. Specimen in unsterile container, time delayed specimen for culture, which were not kept refrigerated at 4°C and inadequate sample for urine culture were excluded from the study. The specimen was labeled and transported to the microbiology laboratory for processing within 2 hours. Semi quantitative urine culture was done using a calibrated loop. A 4mm loopful of well mixed un-centrifuged urine was inoculated onto the surface of CLED agar media. All plates were then incubated at 37°C aerobically for 24 hours. The plates were then examined for bacterial growth. A significant growth is considered if the number of colony is $\geq 10^5$ colony forming unit (cfu)/ml[12].

Uropathogens were identified on the basis of Gram's reaction, colony morphology and standard biochemical tests. Antibiotic susceptibility test was carried out for bacterial isolates by Kirby Baur disc diffusion technique (as per CLSI guideline[13,14]. Antibiotics against which sensitivity was tested in the present study included Ampicillin

(10µg),Amoxiclav, Ciprofloxacin(1µg) , Norfloxacin(10µg) , Cotrimoxazole(1.2µg /23.8µg), Gentamicin(10µg) , Amikacin(30µg), Nitrofurantoin(300µg),Ceftazidime(10µg), Meropenem(10µg), Piperacillin + Tazobactam(30µg+ 6µg) The control strains used were E. coli ATCC 25922, P. aeruginosa ATCC 27853, and S. aureus ATCC 25923.

Observation Chart

Table 1: Age And Sex Wise Distribution Of Culture Positive Patients

Age group in years	Male	Female	Total
0-10 Yrs	0	2	2
11-20	2	7	9
21-30	27	49	76
31-40	8	15	23
41-50	9	17	26
>50	4	9	13
Total	50 (33.55%)	99 (66.44%)	149 (100%)

Table 2: Organisms Causing UTI

Organism	Number	Percentage (%)
Escherichia coli	77	51.67
Klebsiella species	22	14.76
Enterococcus faecalis	17	11.40
Candida species	9	6.04
Pseudomonas aeruginosa	8	5.36
Citrobacter species	6	4.02
MRSA	4	2.68
Enterobacter species	3	2.01
Proteus mirabilis	3	2.01
Total	149	

Table 3: In Vitro Antibiotic Sensitivity Pattern Of Gram Negative Bacteria

	E.coli 77		Klebsiella Pneumoniae 22		Pseudomonas aeruginosa 8		Citrobacter Species 6		Enterobacter 3		Proteus 3	
	S	R	S	R	S	R	S	R	S	R	S	R
AMP	20 (25.97%)	57 (74.02 %)	ND	ND	ND	ND	4 (66.66 %)	2 (33.33 %)	2 (66.66 %)	1 (33.33 %)	2 (66.66 %)	1 (33.33%)
AMC	25 (32.46%)	52 (67.53 %)	6 (27.27%)	16 (72.72 %)	ND	ND	4 (66.66 %)	2 (33.33 %)	2 (66.66 %)	1 (33.33 %)	1	2 (66.66%)
COT	24 (31.16%)	53 (68.83 %)	7 (31.81%)	15 (68.18 %)	ND	ND	2 (33.33 %)	4 (66.66 %)	1 (33.33 %)	2 (66.66 %)	2 (66.66 %)	1 (33.33%)
GEN	53 (68.83%)	24 (31.16 %)	17 (77.27%)	5 (22.72 %)	5 (62.5 %)	3 (37.5 %)	4 (66.66 %)	2 (33.33 %)	2 (66.66 %)	1 (33.33 %)	2 (66.66 %)	1 (33.33%)
AK	71 (22.07%)	6 (7.79%)	18 (81.81%)	4 (18.18 %)	7 (87.5 %)	1 (12.5 %)	5 (83.33 %)	1 (16.66 %)	2 (66.66 %)	1 (33.33 %)	3 (100%)	0
CIP	28(36.36 %)	49 (63.63 %)	8(36.36 %)	14 (63.63 %)	3 (37.5 %)	5 (62.5 %)	2 (33.33 %)	4 (66.66 %)	2 (66.66 %)	1 (33.33 %)	2 (66.66 %)	1 (33.33%)
NX	70 (90.90%)	7 (9.09%)	18 (81.81%)	4 (18.18 %)	6 (75%)	2 (25%)	5 (83.33 %)	1 (16.66 %)	2 (66.66 %)	1 (33.33 %)	2 (66.66 %)	1 (33.33%)
NIT	72 (93.50%)	6 (7.79%)	19 (86.36%)	3 (13.63 %)	7 (87.5 %)	1 (12.5 %)	4 (66.66 %)	2 (33.33 %)	2 (66.66 %)	1 (33.33 %)	ND	ND
CAZ	67 (87.01%)	10 (12.98 %)	18 (81.81%)	4 (18.18 %)	6 (75%)	2 (25%)	4 (66.66 %)	2 (33.33 %)	3 (100%)	0	2 (66.66 %)	1 (33.33%)
MRP	50 (64.93%)	22 (28.57 %)	14 (63.63%)	8 (36.36 %)	5 (62.5 %)	3 (37.5 %)	2 (33.33 %)	4 (66.66 %)	2 (66.66 %)	1 (33.33 %)	2 (66.66 %)	1 (33.33%)
PIT	38 (49.35%)	39 (50.64 %)	11 (50.00%)	10 (45.45 %)	5 (62.5 %)	3 (37.5 %)	4 (66.66 %)	2 (33.33 %)	2 (66.66 %)	1 (33.33 %)	2 (66.66 %)	1 (33.33%)

Table 4: In Vitro Antibiotic Sensitivity Pattern Of Gram Positive Bacteria

	MRSA (4)		Enterococcus species (17)	
	S	R	S	R
Ampicillin	**2 (50 %)**	**2 (50 %)**	4 (**23.52%**)	13**(76.47%)**
Amoxyclavulanate	**1 (25 %)**	**3 (75 %)**	ND	ND
Amikacin	**4 (100%)**	**0**	ND	ND
Azithromicin	2 **(50 %)**	2 **(50 %)**	13 (**76.47%**)	4 (**23.52%**)
Gentamicin	**4 (100%)**	**0**	ND	ND
Ciprofloxacin	**2 (50 %)**	**2 (50 %)**	6 **(35.29%)**	11 **(64.70%)**
Cotrimoxazole	2 **(50 %)**	2**(50 %)**	5 **(29.41%)**	12 (70.**58%)**
Tetracycline	2 **(50 %)**	2 **(50 %)**	8 **(47.05%)**	9 **(52.94%)**
Clindamycin	2 **(50 %)**	2 **(50 %)**	ND	ND
Cefoxitin	0	4 **(100%)**	ND	ND
Linezolid	4 **(100%)**	0	17 **(100%)**	0
Vancomycin	4 **(100%)**	0	17 **(100%)**	0
Penicillin G	0	4	ND	ND
Norfloxacin	**2 (50 %)**	**2 (50 %)**	11 **(64.70%)**	6 (35.**29%)**
Nitrofurantoin	**3 (75 %)**	**0**	15 **(88.23%)**	2 **(11.76%)**
Erythromycin	2 **(50 %)**	2 **(50 %)**	12 **(70.58%)**	5**(29.41%)**
High Level Gentmicin	ND	ND	13**(76.47%)**	4 (**23.52%**)
High Level Streptomicin	ND	ND	12**(70.58%)**	5 (**29.41%**)
Teicoplanin	ND	ND	17 **(100%)**	0

Results

A total of 375 urine Samples were analyzed in the present study. Out of 375 samples, 149(39.73 %), samples were positive. Most common organism isolated were gram negative organisms. E.coli (51.67%), Klebsiella (14.76%),Enterococcus (11.40),Candida (6.04%). followed by Pseudomonas (5.36%), Citrobacter(4.02%),MRSA (2.68%), Proteus and enterobacter (2.01%) . E.coli showed low level resistance for Nitrofurantoin and Amikacin (7.79%), Norfloxacin (9.09%), Ceftazidime (12.98%.) with high resistance pattern for Cotrimoxazole (68.83 %), Fluoroquinolones (50.64%), Piperacilin &Tazobactam (50.54%),Gentamicin(31.1%) and Meropenem (28.57%). Klebsiella was less resistant for Nitrofurantoin(13.63%), Amikacin, Ceftazidime, Norfloxacin, (18.18%),Gentamicin (22.7%),whereas highly resistant for Amoxycillin (72.72%), Cotrimoxazole (68.18%), Piperacilin & Tazobactam (45.45%)and Meropenem (36.36%).

Pseudomonas showed low resistance for Nitrofurantoin, and Amikacin (12.51%) and Meropenem and Gentamicin (37.5%) Enterococcus faecalis showed low level resistance for Nitrofurantoin (11.76%), followed by High level Gentamicin (23.52%), Erythromicin and High levelStreptomicin (29.41%), Ciprofloxacin(52.94%),Cotrimoxazole(70.58%),Ampicillin (76.47%).MRSA was highly resistant to Amoxycillin (75%). Candida species isolated from various clinical specimens were identified up to species level by standard mycological techniques (77.77 %) non albicans Candida species and(22.22 %) was Candida albicans .

Statistical analysis

The collected data was summarized by using frequency, percentage, mean & S.D. To compare the qualitative outcome measures Chi-square test or Fisher's exact test was used. To compare the quantitative outcome measures Independent t test was used. If data was not following normal distribution, Mann Whitney U test was used. SPSS version 22 software was used to analyse the collected data. p value of <0.05 was considered to be statistically significant.

Discussion

High recurrence rates and the increasing antibiotic resistance among uropathogens constitute a large social and economic problem in current public health. Waske S et al studied antibiotic resistance pattern of Uropathogens in a tertiary care hospital of Central India. We assumed that combination of treatment that includes the administration ceragenins (CSAs), will reinforce the effect of antimicrobial LL-37 peptide continuously produced by urinary tract epithelial cells. It was concluded that the employment of combination of natural peptide LL-37 with synthetic analogs might be a potential solution to treat urinary tract infections caused by drug-resistant bacteria[7-12].

In our study, prevalence of uropahtogens was 39.73%, which was similar to study done by (39.16%) Vicky P. Gandhi et al. and (39.16%) Nilofar S et al at Anand district, Gujarat. The most common isolate found in our study was E.coli (51.67%), which was followed by Klebseilla spp. (14.76%), Enterococcus spp. (11.40%), Candida (6.04%) , Pseudomonas 5.36% . Enterococcus spp and MRSA were most sensitive to Vancomycin and Linezolid, similar findings were reported by Choudhary et al. 100% Enterococcus sp were sensitive to Teicoplanin.Various other study groups like Patel J et al in Gujarat,Moghimbeigi A et al in isolates of Enterococci in Iran etc had similar findings like us and derived the same result[13-20].

Antimicrobial susceptibility pattern of the isolates obtained in the present study showed that most of the Gram negative bacilli were multidrug resistant. Enterobacteriaceae isolated showed a resistance to Carbapenems (28.57-66.66%). The study by Dash M, Padhi S was conducted to determine the prevalence of CA-UTI in rural Odisha, India, and the effect of gender and age on its prevalence as well as etiologic agents and the resistance profile of the bacterial isolates. The prevalence of UTI was significantly higher in females compared with males (females 45.2%, males 18.4%, OR = 2.041, 95% CI = 1.64-2.52, $P \leq 0.0001$). Young females within the age group of 18-37 years and elderly males (≥68 years) showed high prevalence of UTI. Escherichia coli (68.8%) was the most prevalent isolate followed by Enterococcus spp. (9.7%). Amikacin and nitrofurantoin were the most active antimicrobial agents which showed low resistance rate of 5.8% and 9.8%, respectively. Study revealed E. coli as the predominant bacterial pathogen. Nitrofurantoin should be used as empirical therapy for uncomplicated CA-UTIs. In the Indian setting, routine urine cultures may be advisable, since treatment failure is likely to occur with commonly used antimicrobials. Therefore, development of regional surveillance programs is necessary for implementation of national CA-UTI guidelines[21].

70.58%Enterococcus species, 68.83%E.coli,68.18% of Klebsiella pneumoniae isolates,66.66% Citrobacterand enterobacter were resistant to Cotrimoxazole. Shiva Verma at al have also found resistance rates as high as 86% in uropathogens to Cotrimoxazole. Resistance to COT in other studies is higher then our study. It could be because of demographic variation in number of samples.High resistance rates to Ciprofloxacin- a drug considered highly effective in the treatment of UTIs was another finding of our study. In our study, 63.63% of E.coli and Klebsiella were found tobe resistant to Ciprofloxacin. 64.70% Enterococcusand62.5% Pseudomonas aeruginosa, isolates were found to be resistant to Ciprofloxacin. Similar results are described by Shiva et al et al who found that 77.8% of Enterobacteriaceae were resistant to Ciprofloxacin and all

Pseudomonas aeruginosa isolates showed absolute resistance to Ciprofloxacin. The very high rate of Ciprofloxacin resistance observed among the isolates in our study warrants special attention and possibly is explained by the fact that in our setting, Ciprofloxacin constitutes one of the commonly prescribed drugs. Overuse of Fluoroquinolones in the last few years has contributed to this rise in resistance[22].Keten D et al studied catheter-associated urinary tract infections in intensive care units at a university hospital in Turkey.In this study, urinary catheter utilization rates, the causative agents for catheter-associated urinary tract infection (CAUTI) and their antimicrobial susceptibilities in intensive care units (ICUs) in 2009 were investigated at Gazi university hospital. The most common etiological agents of CAUTIs were Candida spp. (34.7%). The most frequently isolated Candida spp. was C.albicans (52.4%). All C. albicans spp. were sensitive to fluconazole. E.coli and Klebsiella spp. were found to be causative agents for CAUTI in 20.6% and 9.9% of cases respectively.

Third and fourth generation cephalosporins should not be used for empirical treatment because of the high prevalence of extended spectrum beta-lactamase production among E.coli and Klebsiella isolates[23].

Amongst the Candida isolates, 7 were Candida tropicalis and 2 Candida albicans was isolated. All the Candida isolates were sensitive to Amphotericin B while 50% were sensitive to Fluconazole. The results of anti-fungal susceptibility were found to be consistent with similar studies. In our study, Nitrofurantoin has showed better sensitivity against most organisms causing UTI. These results correlate with other studies also. Thus, Nitrofurantoin can be used as initial agent for empirical treatment till culture reports are available.

Conclusion

Our study concludes E. coli as most common organism causing urinary tract infection with female predominance. There is increasing resistance to commonly used antibiotics due to indiscriminate use of antibiotics without culture and sensitivity report. Amongst the Candida isolates, 7 were Candida tropicalis and 1 Candida albicans was isolated. All the Candida isolates were sensitive to Amphotericin B while 50% were sensitive to Fluconazole. In our study, Nitrofurantoin has showed better sensitivity against most organisms causing UTI.Thus, Nitrofurantoin can be used as initial agent for empirical treatment till culture reports are available

References

1. Faraz MAA, Mendem S, Swamy MV and Patil S: Prevalence of urinary tract infections and related antimicrobial resistance in india: a systematic review and meta-analysis. Int J Pharm Sci & Res 2021; 12(8): 4314-21. doi: 10.13040/IJPSR.0975-8232.12(8).4314-21
2. Al-Khikani FHO, Abadi RM, and Ayit AS: Emerging carbapenemase Klebsiella oxytoca with multidrug resistance implicated in urinary tract infection. Biomedical and Biotechnology Research Journal 2020; 4: 148-51.
3. Kumar A, Banik A, Sanjeev H, Palit A, Mustaqim S, Joshi M. Prevalence of Uropathogen and their Antimicrobial Resistance Pattern in a Tertiary Care Teaching Hospital in Port Blair, India. Int J Med Public Health. 2018;8(1):38-41.
4. Liperky BA. Urinary tract infection in men: Epidemiology, pathophysiology, diagnosis and treatment. Ann Intern Med 1989;111:138.
5. Sanjaykumar More et al. Antibiotic resistance pattern of urinary isolates in a Rural Medical College. International Journal of Medical Microbiology and Tropical Diseases, April-June, 2017;3(2):79-82
6. Bailey & Scott's, Diagnostic Microbiology, 15th edition, Infection of urinary tract, Page No.842-855
7. Waske S et al. Antibiotic resistance pattern of Uropathogens in a tertiary care hospital of Central India International Journal of Medical Microbiology and Tropical Diseases, April-June, 2017;3(2):61-64
8. Wnorowska U, Piktel E, Durnaś B, Fiedoruk K, Savage PB and Bucki R: Use of Ceragenins as a potential treatment for urinary tract infections. BioMed Central Infectious Diseases 2019; 19: 369-82.
9. Patel J and Deshpande S: Antibiotic Prescribing Pattern in Uncomplicated Urinary Tract Infection Patients at a Tertiary Care Hospital of North Gujarat. International J of Pharmaceutical Science Invention 2020; 9: 10-14.
10. Moghimbeigi A, Moghimbeygi M, Dousti M, Kiani F, Sayehmiri F, Sadeghifard N and Nazari A: Prevalence of vancomycin resistance among isolates of Enterococci in Iran: a systematic review and meta-analysis. Adolescent Health, Medicine and Therapeutics 2018; 9: 177-88.
11. MortazaviTabatabaei SA, Ghaderkhani J, Nazari A, Sayehmiri K, Sayehmiri F and Pakzad I: Pattern of antibacterial resistance in urinary tract infections:A systematic review and metaanalysis. International Journal of Preventive Medicine 2019; 10: 169-85
12. Collee GJ, Marimon BP, Frazer AG, Simmons A Makie McCartney Practical Medical Microbiology. Collee JG, 14ed;20:53-94.
13. Koneman EW, Allen S, Janda W, Schreckenberger P, Winn WC. Color Atlas and Text book of Diagnostic Microbiology, 7thedn. New York: Lippincott; 2017
14. CLSI. Performance Standards for Antimicrobial Susceptibility Testing; M10-30th edition Informational Supplement. Wayne, PA: Clinical and Laboratory Standards Institute; 2021.
15. Chander J. A text book of Medical Mycology. 4thed. New Delhi Interprint, 2018; 212-230.
16. Gandhi1 V, Patel M, Nerurkar A et al. Bacteriological profile and its antibiotic susceptibility in patients with Urinary. Int J of Med Microbiol and Tropical Diseases, April-June, 2017;3(2):57-60
17. Nilofar S, Trivedi S et al. Microbiological profile and antibiotic susceptibility pattern of uropathogens in Anand district, Gujarat. Journal of Biomeidcal and Pharmaceutical research, 4:4, July-august 2015;49-56.
18. BL Choudhary, Charu Chandra and Snehanshu Shukla: "Bacteriology of urinary tract infection and susceptibility pattern among diabetic patients". International journal of Bioassays, 2014,3(08),3224-3227.
19. Mahesh E, Ramesh D, Indumathi V.A, Punith K, Kirthi Raj, Anupama H.A, "Complicated urinary tract infection in a tertiary care centre in south India", Al amen journal of medical sciences;2010;3(2):120-127.
20. Jubina Bency A. T., Priyanka R., Ponnu Jose. "A study on the bacteriological profile of urinary tract infection in adults and their antibiotic sensitivity pattern in a tertiary care hospital in central Kerala, India". International Journal of Research in Medical Sciences Bency JAT et al. Int J Res Med Sci. 2017 Feb;5(2):666-669
21. Muktikesh Dash, Sanghmitra Padhi, Indrani Mohanty, Pritilata Panda, Banojini Parida: "Antimicrobial resistance in pathogens causing urinary tract infections in rural community of Odisha, India". Journal of Family and community medicine. April2013;Vol20:20-26
22. Shiva Verma et al. Etiology and risk factors of catheter associated urinary tract infections in ICU Patients. International Journal of Medical Microbiology and Tropical Diseases, April-June, 2017;3(2):65-70
23. Keten D, Aktas F, Tunccan OG, Dizbay M, Kalkanci A, Biter G, et al. Catheter-associated urinary tract infections in intensive care units at a university hospital in Turkey. Bosn J Basic Med Sci. 2014 November;14(4):227-233.

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