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Original Research Article

Isolation, identification and frequency of isolated uropathogens with their antibiotic susceptibility pattern causing urinary tract infections in patients of Ujjain M.P. (India)

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ABSTRACT

Objectives: At present, resistance of uropathogenic bacteria towards different antibiotics is increasing worldwide due to improper, nonspecific and uncontrolled use of antibiotics; hence the treatment of UTI becomes difficult. These resistant uropathogens are major cause of increased rate of morbidity and mortality. This study was conducted to investigate frequency of causal bacterial agents of UTI and their antibiotics susceptibility pattern.

Materials and Methods: The present study was conducted on total 770 urine samples from suspected patients of urinary tract infections, these samples were collected over the period of one year November 2018- October 2019 from different hospitals of Ujjain. Uropathogens were isolated and identified from collected urine sample by biochemical tests and their susceptibility pattern was studied against different antibiotics by Kirby Bauer Disc Diffusion method.

Results A total of 770 urine samples were collected among which 486 (63.1%) samples were tested positive for urinary tract infection in their analysis and prevalence was found higher in female patients (71.6%) as compare to male patients (28.4%). The most common isolated uropathogens were *Escherichia coli* (45.6%), *Klebsiella pneumoniae* (27.1%), *Pseudomonas aeruginosa* (15.6%), *Enterococcus faecalis* (7.4%) and *Staphylococcus aureus* (4.1%).These isolated uropathogens were highly susceptible to Doripenem, Meropenem, Imepenem, Gentamicin, Piperacillin/Tazobactam, Vancomycin, Linezolid and Rifampin.

Conclusion: Due to high prevalence of uropathogens and increased rate of resistance among uropathogens, continued surveillance on uropathogens and their resistance is needed for its proper treatment. So the choice of drug for the treatment of urinary tract infections becomes narrow and its treatment is based on local antimicrobial sensitivity of uropathogens to prevent treatment failure and misuse of antibiotics.

1. Introduction

Urinary tract infection (UTI), is a major health related problem affecting large number of people all over the world and causes clinical and financial burden on human society. It is an inflammatory disorder which occurs in one or more part of urinary tract and causes cystitis, pyelonephrities and urethrietis.[1] The major symptoms of UTI are fever, burning and pain in urination, increased frequency of urination, pain in kidney and tissue damage.[2] UTI's are the second most common infection and several types of microorganisms such as bacteria, viruses, fungi and parasites causes UTI but bacteria are most common cause of UTI. Most of the UTI cases are mainly caused by Gram negative bacteria such

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as *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Enterobacter* spp. and some Gram positive bacteria like *Enterocoocus faecalis* and *Staphylococcus aureus*. Among these bacteria, *E. coli* is most predominant agent of UTI.[3–6]

Urinary tract infections are found in both male and female patients and in all age groups but females are more prone to UTI due to their anatomical and physiological factors of female urinary tract system. UTI may be Asymptomatic or Symptomatic and can occur in uncomplicated or complicated types. The uncomplicated UTI occur in a normal urinary tract while complicated UTI occur in abnormal urinary tract. If complicated UTI is not diagnosed and treated properly then it may spread and causes serious problems such as bacterimia, renal failure and premature delivery due to acute pyelonephritis.[7,8]

The proper treatment of UTI is done by timely identification of causative agent of UTI and detecting its susceptibility to various antibiotics but now a days resistance of uropathogens towards antibiotics is increases worldwide due to non-judicious, random and uncontrolled use of antibiotics, self-medication and incorrect investigations.[9] [9] The increasing drug resistance among uropathogens has made treatment of UTI difficult and it has became a serious problem so research is needed to identify causative agent of UTI and their antibiotic susceptibility patterns among patients of UTI in Ujjain , which may help in proper antibiotic therapy and prevent development of resistant microbes.[9,10]

2. Materials and Methods

The present study was conducted for one-year period starting from November 2018 to October 2019 to identify common uropathogenic bacteria causing UTI and antibiotic susceptibility of isolated uropathogens were determined against different antibiotics. The urine samples of suspected patients were collected in sterile container from different hospitals of Ujjain and processed in SRL laboratory Ujjain centre. Urine samples were examined physically for observing color and turbidity of urine sample and microscopically for detecting pus cells, epithelial cells, RBCs and bacteria.

After examination urine samples were inoculated and cultured on different types of culture media blood agar, Chrome agar and Mac'Conkey agar plate and incubated at 37^oC for 24 hrs. After incubation if the growth of bacterial culture was> 10^5 (CFU)/ ml then it was considered as a positive sample. All the bacterial isolates were further identified and confirmed by their motility, morphological and biochemical characters as per the standard procedures.[11]

2.1. Antibiotic susceptibility test

Antibiotic susceptibility test was performed on Muller Hinton Agar by using Kirby Bauer Disc Diffusion method. This test and interpretation of results was done according to Clinical Laboratory Standards Institute (CLSI) guidelines for determination of susceptibility of uropathogens causing UTI against antimicrobial agents. Bacterial colony was selected then inoculated in peptone water broth and incubated at 37^0C for 2 hrs. After adjustingto 0.5 McFarlands standard this test organism was spread on Muller Hinton Agar by sterile swab using lawn method and commercially available antibiotic Himedia discs were placed on this lawn and plate was incubated at 37^oc for 24-48 hours. The incubated plates were observed for size of zone of clearance and the size was interpreted as sensitive, intermediate and resistant according to CLSI guidelines.[12]

The following antibiotic discs (drugs concentrations in mcg) were applied for observing susceptibility pattern of uropathogens: Amikacin (30mcg), Gentamicin (10mcg), Tobramycin (10mcg), Ampicillin (10mcg), Amoxicillin-Clavulanate (20/10mcg), Piperacillin (100mcg), Piperacillin/Tazobactam (100mcg), Ciprofloxacin (5mcg), Norfloxacin (10mcg), Tetracycline (30mcg), Doxycycline (30mcg), Nitrofurantoin (300mcg), Cotrimoxazole (1.25/23.75mcg), Ceftazidime (30mcg), Imipenem (10mcg), Meropenem (10mcg), Doripenem (10mcg) and Aztreonam (30mcg) were used for gram negative bacteria. In addition to this Teicoplanin (30mcg), Vancomycin (30mcg), Penicillin (10), Cefoxitin (30mcg), Rifampin (5mcg) and Lenizolid (30mcg) were used for gram positive bacteria.

Quality control strains used were:

1. *Staphylococcus aureus* ATCC 25923
2. *Escherichia coli* ATCC 25922
3. *Pseudomonas aeruginosa* ATCC 27853

3. Results

A total of 770 midstream clean catch urine samples were collected from patients during the studies and 486 urine samples were found positive and prevalence of UTI in patients was (63.1%). The percentage of 430(88.4%) gram negative bacteria and 56 (11.5%) gram positive bacteria was detected. Out of 321 positive urine samples, 349(71.8%) were from female and 137(28.1%) were from male patients found UTI (Table 1).

Table 1: Prevalence of UTI in different genders

Gender	Number of UTI patients	Percentage of UTI (%)
Male	137	28.1
Female	349	71.8
Total	486	63.1

Table 2: The frequency of isolated uropathogenic bacteria from urine culture

Isolated Bacteria	**Number of isolated bacteria**	**Percentage of occurence (%)**
Escherichia coli	222	45.6
Klebsiella pneumoniae	132	27.1
Pseudomonas aeruginosa	76	15.6
Enterococcus faecalis	36	7.4
Staphylococcus aureus	20	4.1
Total	486	

Table 3: Antibiotic susceptibility pattern of isolated gram negative bacteria. (N=430)

Tested antibiotics	***E. coli* n=222**		***K. pneumoniae* n=132**		***P. aeruginosa* n=76**	
	No. of sensitive bacteria	(%)	No. of sensitive bacteria	(%)	No. of sensitive bacteria	(%)
Amikacin	186	(83.7)	111	(84.0)	56	(73.6)
Gentamicin	162	(72.9)	108	(81.8)	60	(78.9)
Tobramycin	170	(76.5)	96	(72.7)	52	(68.4)
Ampicillin	21	(9.4)	15	(11.3)	NA	NA
AMC	26	(11.7)	21	(15.9)	NA	NA
Piperacillin	69	(31.0)	109	(82.5)	50	(65.7)
PTZ	182	(81.9)	111	(84.0)	61	(80.2)
Ciprofloxacin	38	(17.1)	23	(17.4)	22	(28.9)
Norfloxacin	67	(30.1)	27	(20.4)	39	(51.3)
Tetracycline	138	(62.1)	72	(54.5)	NA	NA
Doxycycline	158	(71.7)	68	(51.5)	NA	NA
Nitrofurantoin	188	(84.6)	70	(53.0)	NA	NA
COT	88	(39.6)	54	(40.9)	NA	NA
Ceftazidime	58	(26.1)	82	(62.1)	44	(57.8)
Imipenem	202	(90.9)	109	(82.5)	64	(84.2)
Meropenem	205	(92.3)	119	(90.1)	68	(89.4)
Doripenem	209	(94.1)	124	(93.9)	71	(93.4)
Aztreonam	168	(75.6)	104	(78.7)	54	(71.0)

Value in columns indicates number of sensitive bacteria & values in parenthesis indicate percentage of sensitive bacteria.
N- Total no. of bacteria, n- Number of sensitive bacteria, NA-Not Applicable
AMC-Amoxicillin/clavulanate, PTZ- Piperacillin/Tazobactam, COT- Co-trimoxazole

Table 4: Antibiotics sensitivity pattern of isolated gram positive bacteria. N=(56)

Tested Antibiotics	***E. faecalis* n=36**		***S. aureus* n =20**	
	No. of sensitive bacteria	(%)	no. of sensitive bacteria	(%)
Amikacin	NA	NA	15	(75.0)
Gentamicin	NA	NA	14	(70.0)
Teicoplanin	32	(88.8)	18	(90.0)
Vancomycin	33	(91.6)	19	(95.0)
Tetracycline	03	(8.3)	15	(75.0)
Doxycycline	05	(13.8)	18	(90.0)
Penicillin	02	(5.5)	06	(30.0)
Ampicillin	19	(52.7)	08	(40.0)
AMC	NA	NA	10	(50.0)
Ciprofloxacin	04	(11.1)	06	(30.0)
Norfloxacin	03	(8.3)	10	(50.0)
Nitrofurantoin	29	(80.5)	16	(80.0)
Co-trimaxazole	NA	NA	08	(40.0)
Rifampin	30	(83.3)	18	(90)
Linezolid	34	(94.4)	19	(95)

Value in columns indicates number of sensitive bacteria & values in parenthesis indicate percentage of sensitive bacteria.
N- Total no. of bacteria, n- Number of sensitive bacteria, NA-Not Applicable
AMC-Amoxicillin/clavulanate
NA- Not applicable

The most commonly isolated gram negative uropathogens included *Escherichia coli* (45.6%) followed by *Klebsiella pneumoniae* (27.1%), *Pseudomonas aeruginosa* (15.6%) and gram positive uropathogens included *Enterococcus faecalis* (7.4%) and *Staphylococcus aureus* (4.1%) (Table 2).

The study of antibiotic susceptibility patterns of isolated uropathogens showed that *E. coli* showed highest sensitivity against carbapenems such as Doripenem (94.1%) and Meropenem (92.3%) closely followed by Imipenem(90.9%) beside these antibiotics more than 70% sensitivity was found against Amikacin, Gentamicin, Tobramycin, Doxycycline, Nitrofurantoin and Aztreonam antibioticsandleast sensitivity found against to Ampicillin (9.4%). Similarly, *K. pneumoniae* showed highest sensitivity to Doripenem (93.9%), Meropenem (90.1%) while susceptibility to Ampicillin was (12.9%). It was also found that *P. aeruginosa* was highly sensitive against Doripenem, and Meropenem and their susceptibility rates are (93.4%) and (89.4%) respectively and least sensitivity was seen for Ciprofloxacin (28.9%). In case of gram positive bacteria *E. faecalis* was predominant uropathogen and it was highly sensitive for Teicoplanin, Vancomycin and Linezolid, for these antibiotics its sensitivity rate was (88.8%), (91.6%) and (94.4%) respectively which was followed by Rifampin (83.3%) and Nitrofurantoin (80.5%) and it showed lower sensitivity for Penicillin (5.5%). Similarly *S. aureus* was highly susceptible for Tiecoplanin, Vancomycin, Rifampin and Linezolid and least susceptible to Ciprofloxacin and Penicillin (Tables 3 and 4).

4. Discussion

The present study represents prevalence and antibiotics susceptibility patterns of uropathogens which can differ in same country with time and place. In this study prevalence of UTI was found (63.1%). This findings similar with other studies done in India.[13–15] The frequency of occurrence of UTI was found higher (71.6%) in female than (28.4%) in male patients. The findings with this regard are in consonance with the studies done in India and Ethiopia.[4,15] In the present study among gram negative bacteria *E. coli* has been reported as the predominant uropathogen, responsible for causing UTI and its frequency of occurrence was (45.6%) which was followed by *K. pneumoniae*, *P. aeruginosa* and gram positive bacteria *E. faecalis* and *S. aureus* uropathogens. These results are in accordance with the studies done in Pakistan, Ethiopia and Saudi Arabia.[4,7,8] In this study *E. coli* showed highest sensitivity for Doripenem (94.1), Meropenem (92.3%) and Imipenem (90.9%) and these findingsare same as with the study done in India.[5,14,16] However study done in Pakistan showed opposite results to our study and they reported low susceptibility to Imipenem (39.5%).[17] *E. coli* also showed high susceptibility for Amikacin, Nitrofurantoinand Piperacillin/Tazobactam and their percentage of susceptibility was (83.7%), (84.6%), (81.9) respectively. These findings are similar to the study done in Southern Iran[18] while *E. coli* showed least susceptibility for Ampicillin (9.4), Ciprofloxacin (17.1%). These results of susceptibility are correlate with the study done in Gujarat, India.[5] In case of *K. pneumoniae* uropathogen it shows higher susceptibility to Carbapenems, Piperacillin /Tazobactam, Gentamicin and Amikacin and very low susceptibility for Ampicillin these results are same as the studies done in India and Southern Iran.[5,18,19] In this study *P. aeruginosa* shows higher sensitivity towards Carbepenems, Piperacillin / Tazobactam, Aztreonam, Amikacin and Gentamicin and their sensitivity was more than 70 percent while for Norfloxacin it showed 51.3% percent sensitivity and low sensitivity to Ciprofloxacin (27.2%). These results are same to the studies performed in India and Ethiopia.[5,20,21] In this study it was found that gram positive bacteria *E. faecalis* and *S. aureus* were highly susceptible to Vancomycin, Linezolid, Rifampin and Teicoplanin and their percentage of sensitivity was in between 80-95% while low sensitivity was demonstrated against Ciprofloxacin, Norfloxacin and Penicillin. These findings are in accordance with the previous studies done in India.[1,22]

5. Conclusion

The findings of this study showed that UTI's have become a major health related problem and affect large number of people through the World. In this study *E. coli* isolates were the predominant uropathogen and all isolates are sensitive for carbapenems, aminoglycoside, antibiotic groups while these isolates are resistant to commonly prescribed antibiotics so very few options of drugs are available for physicians for the proper treatment of UTIs. Therefore, to ensure appropriate therapy current knowledge related to the organisms that cause UTI and their antibiotic susceptibility is mandatory.

6. Source of Funding

None.

7. Conflicts of Interest

None.

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