

Original Research Article

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Isolation of Urinary Tract Pathogens and Study of their Drug Susceptibility Patterns

Richa Mishra*, Deepti Saksena and Vinoy K. Shrivastava

Department of Biosciences, Barkatullah University, Bhopal, 462026 (M.P.), India

*Corresponding author

ABSTRACT

Keywords

Urinary Tract Infections, Drug Resistance, Bacterial Pathogens, *E. coli*, Antimicrobial Susceptibility Pattern.

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The objective of present study was to analyze urine specimen of patients suffering from UTI for the identification of pathogenic organisms and their drug susceptibility patterns. A total of 1255 (897 females and 358 males) patients with clinical symptoms of UTI were included in the study. Automated bioMérieux VITEK 2 compact bacterial identification and antibiotic susceptibility analyzer is used in the present study. A total of 1255 urine samples were collected and out of these 400 were growth positive isolates. Out of these isolates *Escherichia coli* (42.5%) was found to be the most frequent causative agent of UTIs followed by *Klebsiella spp* (23.25%), *Pseudomonas spp.* (12.5%) and *Enterobacter spp.* (9.75%), *Candida spp.* (5%), *Staphylococcus aureus* (3%), *Acinetobacter spp.* (2.5%) and *Proteus* (1.5%). All urinary isolates of *E.coli* including multidrug resistant strains were found to be highly sensitive for nitrofurantoin and pazufloxacin. Women are more prone towards UTI and *E. coli* is the main causative organism. The change in drug susceptibility pattern is notable since older drugs no longer show effectiveness, this is due to resistance developed by overuse or misuse of drugs. Comparative study should be repeated after regular intervals of time so that the changing patterns can be noted and choice of drug can be selected accordingly.

Introduction

In the clinical laboratory bacteria are cultured and precisely identified so that the appropriate medication can be selected. Gram negative bacteria are the most prevalent isolates from the UTI patients and urinary tract infection being commonest infection caused (Bashir *et al.*, 2008; Mehta *et al.*, 2012).

Microorganisms are not always harmful but when they are present in excess they become

pathogenic and cause infectious diseases. *Escherichia coli* which is a natural inhabitant of gastrointestinal tract and urinary tract but when their number increases it becomes pathogen and causes Urinary Tract Infection (UTI).

In fact, Gram negative bacteremia is caused mainly due to UTI in patients of all ages and in elderly patients there is a high risk of morbidity and mortality. In recent years there has been an increase in bacterial UTI

although several drugs are available and prescribed. This can be associated to the resistance developed by pathogens for antibiotics.

E. coli is the prominent causative uropathogen of UTI and among other pathogens of infection *Pseudomonas spp.*, *Candida spp.*, *Klebsiella spp.* and *Enterobacter spp.* are also found (Khoshbakht *et al.*, 2013). Resistance developed by microbes for antibiotics is a major problem which leads to improper treatment. An increase in the resistance for antibiotics is also because of haphazard use which is unchecked. Hence, antibiotics should be prescribed after urine culture & sensitivity test (Hryniewicz *et al.*, 2001).

In the present study emphasis has been given to the prevalence and susceptibility patterns of uropathogens. The data analyzed gives basic knowledge for future study and can be useful for controlling UTI.

Materials and Methods

A total of 1255 (897 females and 358 males) patients with clinical symptoms of UTI were included in the study. Sterile disposable containers were used for collecting mid-stream urine samples (4-5 ml) and transported immediately to laboratory. Urine routine examination was done using direct microscopy to count white blood cell (WBC). Cysteine lactose electrolyte deficient agar (CLED agar) plates were used for inoculation of urine samples by adopting Calibrated Loop Technique and kept for 24 hours incubation at 37°C aerobically.

Cultures without any colony at the end of 24 hours incubation were further incubated for 48 hours. Urine culture plates having significant growth were observed on the basis of colonial morphology and colony

counts. A growth of $\geq 10^5$ colony forming units/ml was considered as significant bacteriuria. Gram staining was performed to differentiate the Gram positive and Gram negative organisms. Bacterial identification and antibiotic susceptibility was done by automated bioMérieux VITEK 2 compact analyzer as per Clinical and Laboratory Standards Institute (CLSI) guidelines.

The measure of a microorganism's susceptibility or resistance to an antibiotic was reported as the Minimum Inhibitory Concentration (MIC). *E. coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923) and *Pseudomonas aeruginosa* (ATCC 27853) were used as quality control strains. The antibiotics tested were Amikacin, Gentamycin, Amoxicillin, Imipenem, Meropenem, Ertapenem, Cefoperazone, Cotrimoxazole, Pazufloxacin, Levofloxacin, Norfloxacin, Ciprofloxacin, Nitrofurantoin, (Piperacillin + Tazobactam) and (Ceftazidime + Clavulanic).

Statistical Analysis

Clinical data were entered in the worksheet of Statistical Package for Social Science (SPSS) software of version 21.0. Percentage and frequency were analyzed as descriptive findings.

Results and Discussion

A total of 1255 urine samples, 358 (28.52%) were from males and 897 (71.47%) were from females. Out of these samples 400 were growth positive isolates. Frequency of growth positive samples in males were 23% and the frequency of growth positive samples in females were 77%. Frequency pattern of males and females were shown in Table 1

The present study findings showed that

Escherichia coli (42.5%) was found to be the most frequent causative agent of UTIs followed by *Klebsiella spp* (23.25%), *Pseudomonas spp.* (12.5%) and *Enterobacter spp.* (9.75%), *Candida spp.* (5%), *Staphylococcus aureus* (3%), *Acinetobacter spp.* (2.5%) and *Proteus* (1.5%). The percentage of isolated uropathogens were shown in Table 2 and distribution of isolated uropathogens were shown in Fig 1.

High degree of resistance was observed to cefoperazone, ciprofloxacin, amoxicillin, cotrimaxozole whereas combination drugs (piperacillin + tazobactam), (ceftazidime + clavulinic) along with amikacin, gentamycin, ertapenam, meropenem, imipenem, levofloxacin, norfloxacin exhibit low degree of resistance. Although all urinary isolates of *E.coli* including multidrug resistant strains were found to be highly sensitive for nitrofuratoin and pazufloxacin.

E.coli and *Klebsiella spp* were highly sensitive to pazufloxacin, nitrofuratoin and imipenem and they were highly resistant to amoxicillin. *Pseudomonas spp.* was highly sensitive to (Piperacillin + Tazobactam) and pazufloxacin and it was highly resistant to amoxicillin. Percentage of antibiotic sensitivity pattern of most frequently isolated microorganisms were shown in Table 3 and distribution of antibiotic sensitivity pattern were shown in Fig 2.

Many researchers have observed that the cause of UTI and the drug susceptibility patterns of pathogens were found to be changing since recent years (Saleh *et al.*, 2009; Shalini *et al.*, 2011). In our study UTI is the most common bacterial infection caused by *E.coli*. It was also seen that females get UTIs more often than males (Ronald *et al.*, 2009; Chaurasia *et al.*, 2015).

Table.1 Incidence of Urinary Tract Infection according to Sex

Sex	No. of Samples Studied	Frequency (%)	No. of Positive Samples	Frequency (%)
Male	358	28.52	92	23
Female	897	71.47	308	77
Total	1255	100	400	100

Table.2 Number of Isolated Uropathogens

Isolated Uropathogens	Number
<i>Escherichia coli</i>	170 (42.5%)
<i>Klebsiella spp.</i>	93 (23.25%)
<i>Pseudomonas spp</i>	50 (12.5%)
<i>Enterobacter spp.</i>	39 (9.75%)
<i>Candida spp.</i>	20 (5%)
<i>Staphylococcus aureus</i>	12 (3%)
<i>Acinetobacter spp.</i>	10 (2.5%)
<i>Proteus</i>	6 (1.5%)

Table.3 Percentage of Antibiotic Sensitivity Patterns of Most Frequently Isolated Microorganisms

Class of Antibiotics	Antibiotics	<i>E.coli</i> (n=170)	<i>Klebsiella</i> (n=93)	<i>Pseudomonas</i> (n=50)
Aminoglycosides	Amikacin	75.62	75.03	76.58
	Gentamycin	64.43	61.84	63.08
Aminopenicillin	Amoxicillin	14.24	19.05	10.13
Carbapenems	Imipenem	86.55	80.34	85.83
	Meropenem	83.66	78.48	81.53
	Ertapenem	81.96	79.98	79.01
Cephalosporins	Cefoperazone	38.83	40.83	34.13
Trimethoprim + Sulphamethoxazole	Cotrimaxozole	35.60	38.96	29.38
Quinolones	Pazufloxacin	94.62	81.28	90.05
	Levofloxacin	68.14	65.73	60.34
	Norfloxacin	71.27	68.23	62.89
	Ciprofloxacin	41.53	44.03	23.53
Nitrofurans	Nitrofuratoin	87.98	74.58	18.97
Combinations	(Piperacillin + Tazobactam)	78.27	72.09	92.07
	(Ceftazidime + Clavulinic)	77.61	71.53	75.93

Fig.1 Showing Distribution of Isolated Uropathogens

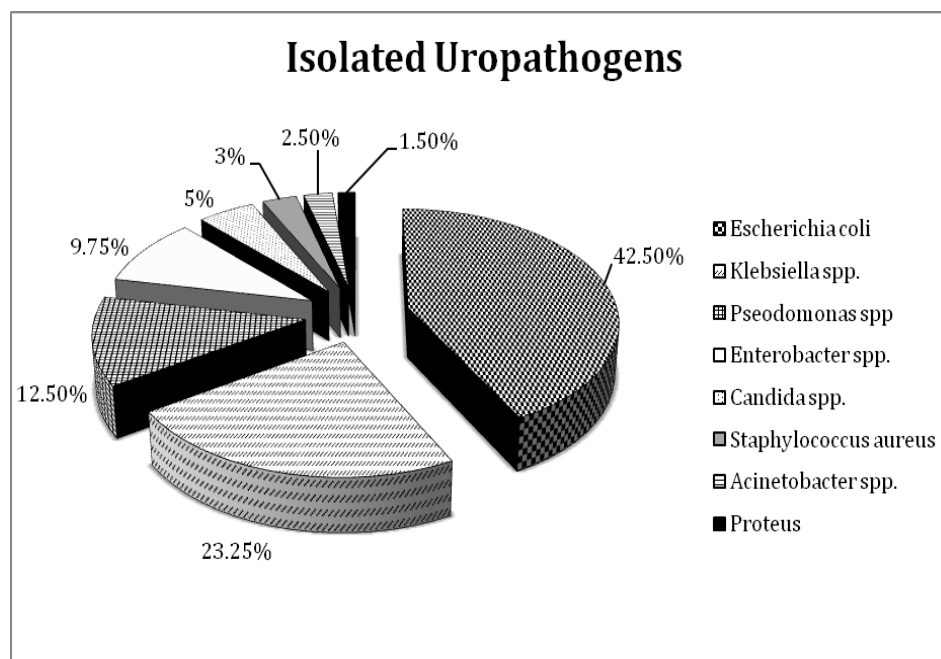
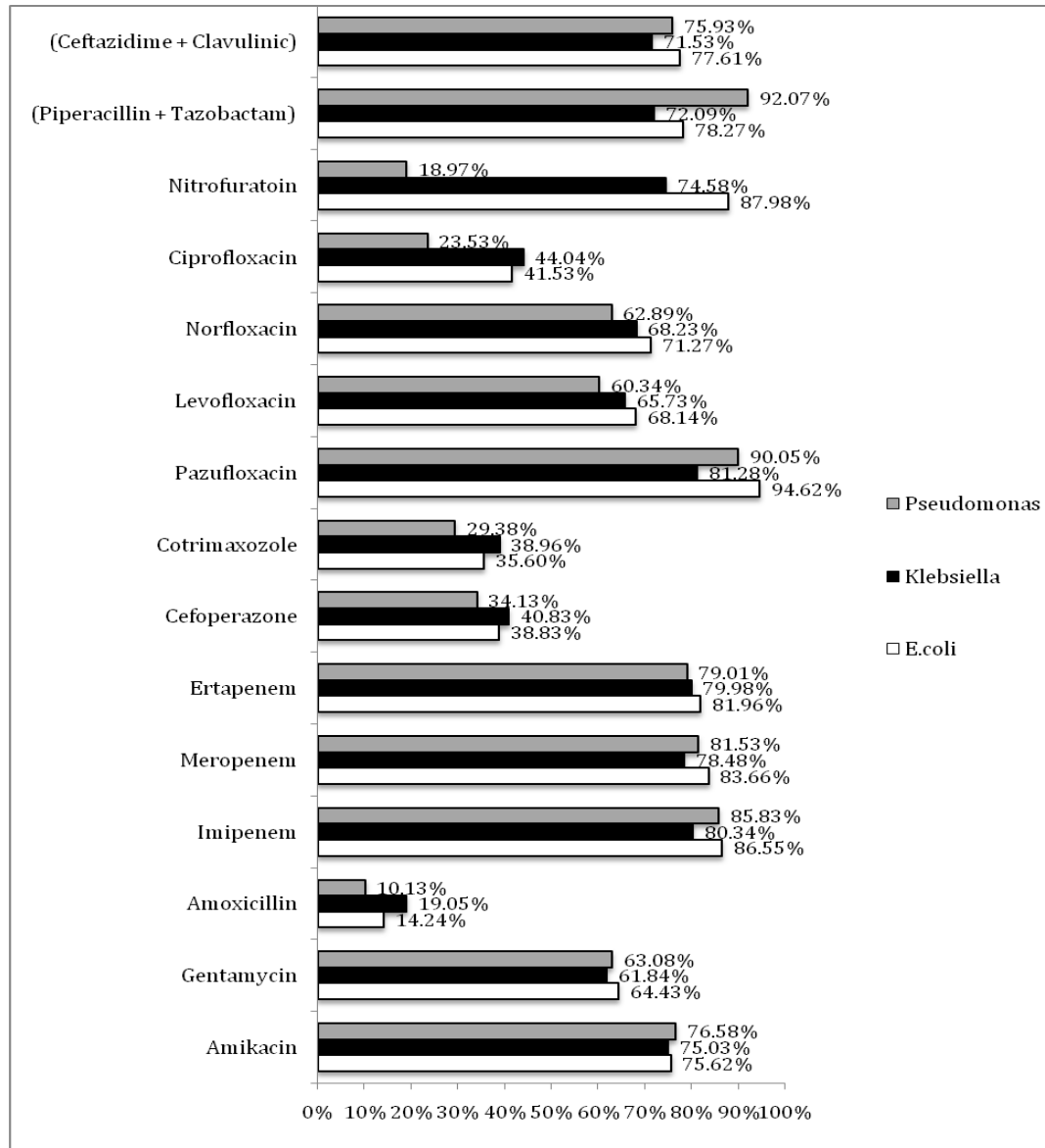


Fig.2 Showing Distribution of Antibiotic Sensitivity Patterns of Most Frequently Isolated Microorganisms



The most common organisms isolated in these patients were *Escherichia coli*(42.5%), *Klebsiella spp.*(23.25%), *Pseudomonas spp.* (12.5%) and *Enterobacter spp.* (9.75%), *Candida spp.* (5%), *Staphylococcus aureus* (3%), *Acinetobacter spp.* (2.5%) and *Proteus* (1.5%).All the micro-organisms obtained during this study were of clinical significance and were similar to the data recorded by (Hryniewicz *et al.*, 2001).

Our study indicate that *E.coli* and *Klebsiella spp* were highly sensitive to pazufloxacin, nitrofuratoin and imipenem. Same has been recommended by (Shamataj and Vishwanath, 2012). *Pseudomonas spp.* washighly sensitive to (Piperacillin + Tazobactam) and pazufloxacin. (Niranjanand Malini, 2014) study is very much closely related to our study.

The pathogen showed least sensitivity towards generally used antibiotics example amoxicillin etc. The present finding demonstrate that increased resistance in the amoxicillin against *E. coli* may reflect the overuse of aminopenicillin for treatment of UTI. Cefoperazone, ciprofloxacin, amoxicillin and cotrimaxozole are losing effectiveness which is alarming because these antibiotics have been one of the best options for treatment of UTI.

In conclusion, Women are more prone towards UTI and among uropathogens Gram negative bacteria were most commonly isolated. *E.coli* was prominent among these. Broad-spectrum drugs can change normal microbial content of body (normal flora) which leads to secondary infection by multi drug resistant organism. Hence susceptibility patterns should be studied for the pathogens before prescribing antibiotics so that development of resistance against antibiotics can be checked.

The change in drug susceptibility pattern is notable since older drugs no longer show effectiveness, this is due to resistance developed by overuse or misuse of drugs.

The present study proves to be beneficial as it helps to identify the changing trends in the pattern of drug susceptibility. Hence, comparative study should be repeated after regular intervals of time so that the changing patterns can be noted and choice of drug can be selected accordingly. Persons with previous other infections or compromised immune system are much prone to UTIs hence, the frequency of occurrence of UTI is also responsible for the resistance developed for antibiotics in pathogens.

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