

Bacteriological profile and its antibiotic susceptibility in patients with urinary tract infection in tertiary care hospital

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Introduction: Urinary tract infections (UTIs) are counted among the most common infections in humans. In spite of the availability and use of antimicrobial drugs, UTIs caused by bacteria have been showing increasing trends. The extensive and inappropriate use of antimicrobial agents has invariably resulted in the development of antibiotic resistance which, in recent years, has become a major problem worldwide. **Materials and Methods:** The study was conducted among the different ages of groups of both males and females attending KPC Medical College, Jadavpur, Kolkata during six months of the period included in this study. A total of 350 urine samples received from patients of the ICU and other wards of the medical college and hospital. All urine samples were processed within 1 hour after collection for aerobic bacterial culture. **Results:** Out of a total of 380 patients, isolates were detected in 350 (76.29%) samples. Out of these, 280 (80%) were female. The most common organism found positive was *Escherichia Coli*. *E. coli* was highly sensitive to Amikacin and Nitrofurantoin. Whereas, *E.coli* was highly resistant to Ampicillin and Nalidixic acid. The antibiotic sensitivity pattern of Klebsiella and Acinetobacter shows that they were also highly sensitive to Amikacin. Klebsiella and Acinetobacter were highly resistant to Ampicillin and Gentamicin. **Conclusion:** The pattern of resistance to commonly used antibiotics for treating UTI alerts us against indiscriminate usage of antibiotics.

Keywords: Urinary tract infection, Gram-negative, Antibiotic resistance

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Introduction

Urinary tract infections (UTIs) are counted among the most common infections in humans, exceeded in frequency among ambulatory patients only by respiratory and gastrointestinal infections. [1,2] Urinary tract

Infection (UTI) is a common bacterial infection known to affect the different parts of the urinary tract and the occurrence is found in both males and females. Despite the fact, that both the genders are susceptible to the infection, women are most vulnerable due to anatomy and reproductive physiology.

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It is estimated that 20% or more of the female population suffers some form of UTI in their lifetime. This study aimed at isolation, identification of bacterial contamination and also determining the antibiotic susceptibility pattern of the isolates to some commonly used antibiotic and identifying the multi-drug resistance bacteria isolated from urinary tract infected patients.

Infection in the male population remains uncommon through the fifth decade of life when enlargement of the prostate begins to interfere with the emptying of the bladder. The most common pathogenic organisms of UTI are *Escherichia coli*, *Staphylococcus saprophyticus* and less common organisms are *Proteus species*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Enterococci* and *Candida albicans* [3].

Treatment of UTI cases is often started empirically and therapy is based on information determined from the antimicrobial resistance pattern of the urinary pathogens. In spite of the availability and use of antimicrobial drugs, UTI is caused by bacteria that have been showing increasing trends. The extensive and inappropriate use of antimicrobial agents has invariably resulted in the development of antibiotic resistance which, in recent years, has become a major problem worldwide [4].

In patients with suspected UTI, antibiotic treatment is usually started empirically, before urine culture results are available. To ensure appropriate treatment, knowledge of the organisms that cause UTI and their antibiotic susceptibility is mandatory [5].

This study was planned to explore the common pathogens responsible for UTI and to determine the antibiotic susceptibility pattern of them. Pathogenesis of UTIs is not clear. A relationship exists between the human host, infectious and the environment. In the human host, the urethra connects the bladder to potentially infectious agents on the perineum.

A high-pressure zone exists within the urethra at a point where the urethra passes through the urogenital diaphragm. This zone creates a natural barrier to the ascent of organisms colonized in the distal urethra and the bladder itself has a natural defense against invading organisms. The interactions of host defense with bacteria determines whether or not the bacteria persist.

A small number of bacteria and some types of

Bacteria are controlled more effectively by natural bladder defense mechanisms and frequent bladder emptying than a large number of bacteria. *E.coli*, *Coliforms*, and *Enterococci* are considered common bacterial causes of UTIs and are found in high numbers on the perineum.

Intermittent catheterization is an effective way of bladder emptying but as an invasive procedure, it remains a risk factor in the development of UTI [6].

Virulence factors of recognized importance in the pathogenesis of UTI include adhesins (P fimbriae, certain other mannose resistant adhesins and type 1 fimbriae), the aerobactin system, hemolysin, K capsule and resistance to serum killing. Certain virulence factors especially favor the development of pyelonephritis, other favor cystitis and others favor asymptomatic bacteriuria [7].

Materials and Methods

The study was conducted among the different age groups of both males and females attending KPC Medical College, Jadavpur, Kolkata for six months (January to June 2019) of the period included in this study. A total of 350 urine samples received from patients of the ICU and other wards of the medical college and hospital. All urine samples were processed within 1 hour after the collection for aerobic bacterial culture.

Inclusion criteria

01. All indoor patients. Adult > 12 years with an indication of urodynamics assessment.
02. A patient who consented to participate.
03. Male and Female patient.

Exclusion criteria

01. Patients who refuse to sign the informed consent form.
02. Patients below 12 years of age.
03. Pregnant/Breastfeeding women.

Materials required

Glassware and other apparatus

- Spirit lamp
- Inoculating loop
- Laminar airflow
- Forceps
- Millimeter ruler

- Alcohol
- Cartridges of antibiotic discs
- Sterile Petri plates
- 37°C Incubator

Media used for culture

01. **UTI Agar-** Hi-chrome UTI Agar is a differential medium recommended for presumptive identification of microorganisms mainly causing urinary tract infections.
02. **MacConkey agar-** MacConkey Agar is recommended for selective isolation of *Escherichia coli* from pharmaceutical products. It is also recommended for selective isolation and differentiation of lactose fermenting and lactose non fermenting enteric bacteria.
03. **Mueller Hinton Agar for antibiotic sensitivity-** Recommended for determination of susceptibility of microorganisms to antimicrobial agents isolated from clinical samples.

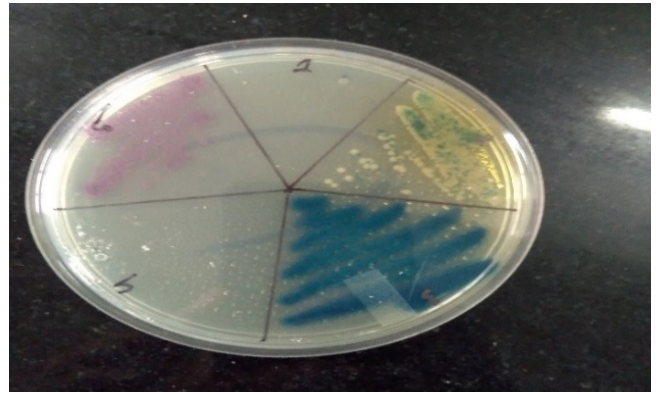
Specimen collection- Specimens of urine are collected in plastic universal containers, but midstream specimens from females are more conveniently collected in a wide-mouthed container such as 12 oz. (350 ml) glass jar or a sterile waxed cardboard container. From male patients, a *mid-stream specimen of urine* (MSU, the middle of the urine flow) was collected.

Transport of specimen- Once collected, a specimen of urine was transported to the laboratory without delay, for urine is an excellent culture medium and contaminating bacteria can readily multiply to reach apparently significant numbers.

Methodology- A prospective study was performed in 380 adults with suspected UTI attending OPD's respective department and admitted to the ward.

Microscopy of urine- Microscopical examination of urine was done principally to detect the presence of increased numbers of polymorphs (pyuria) as an indication of infection in the urinary tract when culture may fail to show significant bacteriuria [8].

Wet film examination- A leucocyte count sufficiently accurate for general purposes may be obtained from examination of a wet film of uncentrifuged urine, provided that the area of the microscope field is known and the depth of the film is standardized.

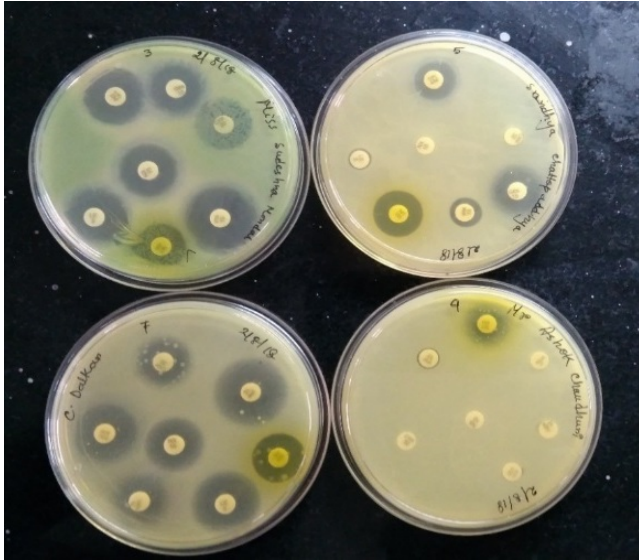


Standard loop method- An inoculating loop of standard dimensions was used to take up a small, approximately fixed and known volume of mixed uncentrifuged urine and it was spread over a plate of agar culture medium. The plate was incubated, the number of colonies counted or estimated, and this number used to calculate the number of viable bacteria per ml of urine. Thus, if a 0.004 ml loopful of urine yields 400 colonies the count per ml will be 10⁵, or just indicative of significant bacteriuria. [9,10]



Detection of antibacterial activity in urine- Antibiotic sensitivity describes the susceptibility of bacteria to various antibiotics. It is often done by Kirby-Bauer methods [11].

Kirby Bauer Disc Diffusion Method- Three to five identical colonies were picked from an overnight grown primary agar plate with a sterile loop and was suspended in 0.5ml of Peptone water. The turbidity was matched with 0.5 McFarland turbidity standards. A fresh, sterile cotton-tipped swab was dipped into this suspension and the excess of inoculum was removed by pressing it against the sides of the tube.



Antibiotic susceptibility testing disc diffusion

01. *Pseudomonas aeruginosa* sensitivity testing showing in sample no. 3, sensitive to NX, LE, GEN, IPM, PIT.
02. *Escherichia coli* sensitivity testing showing in sample no. 5, resistant to CXM, NX, LE.
03. *Escherichia coli* sensitivity testing showing in sample no. 7, sensitive to NX, LE, GEN, IPM, PIT, NIT.
04. Multi-drug resistant *Escherichia coli* showing in sample no. 9, resistant to NX, LE, GEN, IPM, PIT, NIT, and CXM.

Biochemical reactions for identification of bacterial isolates- Presence of lactose fermenting large, dry, colonies with irregular margins and lactose fermenting large mucoid colonies on MacConkey agar medium confirmed by gram staining. These were then subjected to preliminary tests and biochemical tests:-

Organisms	Indole test	Citrate utilization test	Ureas e test	Mannitol fermentation test	Triple sugar iron test	Coagulase test
E. coli	Positive (+ve)	Negative(-ve)	Negative(-ve)	Fermented	A/A+/- Gas	-
K. pneumonia	Negative(-ve)	Positive (+ve)	Positive (+ve)	Fermented	A/A+ Gas	-
Pseudomonas aeruginosa	Negative(-ve)	Positive (+ve)	Negative(-ve)	Not Fermented	K/ NC	Negative(-ve)
Enterococcus faecilis	Negative(-ve)	Negative(-ve)	Negative(-ve)	Fermented	K/ NC	-

S. aureus	Negative(-ve)	Positive (+ve)	Positive (+ve)	Fermented	K/ NC	Positive (+ve)
CONS	Negative(-ve)	Positive (+ve)	Positive (+ve)	Fermented	K/ NC	Negative(-ve)

"K"?= Alkaline, "A"?= Asaccharolytic, "NC"?= No change.

Results

In this study, a urine sample of a total of 380 patients clinically diagnosed with urinary tract infection was collected and tested for microorganisms. Out of a total of 380 patients, isolates were detected in 350 samples. Out of these, 280 were female and 70 were male, found positive for *Proteus*, *Staphylococcus* species, etc. It was seen that *E.coli* was highly sensitive to Amikacin and Nitrofurantoin. Whereas, *E.coli* was highly resistant to Ampicillin and Nalidixic acid. The antibiotic sensitivity pattern of *Klebsiella* and *Acinetobacter* shows that they were also highly sensitive to Amikacin. *Klebsiella* and *Acinetobacter* were resistant to Ampicillin and Gentamicin.

Table-1: Gender wise distribution of various urinary pathogens (n-350).

Isolates	Infected male (%)	Infected female (%)	Total
<i>Escherichia coli</i>	35 (50)	168 (60)	203
<i>Klebsiella</i>	17.5 (25)	70 (25)	88
<i>Acinetobacter</i>	8.75 (12.5)	14 (5)	24
<i>Pseudomonas</i>	3 (5.75)	11 (4)	15
Others	4 (7.25)	16 (6)	20
Total	70 (20)	280 (80)	350 (100)

Table-2: Percentage of in vitro antibiotic sensitivity pattern of most frequently isolated microorganisms.

Drugs	E.coli(n=280)	<i>Klebsiella</i> (n=70)	<i>Acinetobacter</i> (n=14)
Amikacin	74.07	41.67	41.18
Ampicillin	3.70	16.67	11.76
Gentamycin	32.41	12.50	17.65
Ciprofloxacin	25.93	16.67	29.41
Nitrofurantoin	88.89	25.00	35.29
Nalidixic Acid	5.56	16.67	29.41
Norfloxacin	10.19	29.17	25.53

Discussion

In community and hospital settings, the etiology of UTIs and the antimicrobial susceptibility of UTI causing bacteria have been changing over the years [12,13]. Over the last decade, the treatment of choice for urinary tract infections (UTIs) has

Changed from co-trimoxazole to quinolones owing to the rate of resistance to Co-trimoxazole and its high level of therapeutic failure [14]. Antimicrobial resistance has been associated with an increased rate of clinical failure, and reports from Canada and the US indicate that the prevalence of Co-trimoxazole resistance exceeds 15% and can be as high as 25%. Use of fluoroquinolones is recommended for uncomplicated UTIs in areas where the incidence of cotrimoxazole resistance exceeds 10%, as well as for the treatment of complicated UTIs and acute pyelonephritis [15].

In the present study, the prevalence rate of isolation of urinary pathogens was 76.29%. In a similar study by Das RN et al isolation rate was 71.6% [16]. Another study done in Karnataka had reported 71.72%. The prevalence of UTIs was more in females when compared to males [17].

This was in agreement with other studies by Bashir MF et al [18]. Women are more prone to UTIs than men because, in females, the urethra is much shorter and closer to the anus [19]. The most commonly isolated organism in UTI among female outpatients in the present study was *E. coli*. But nowadays *Klebsiella*, *Pseudomonas*, *Proteus*, *Enterococcus*, *Acinetobacter* and *Candida* have been reported increasingly from UTI. The proportion of bacterial species isolated was similar to those described in several previous studies [20,21,22].

In the present study, *E-coli* was most resistant to Ampicillin, followed by Nalidixic acid and Norfloxacin. It was most sensitive to Nitrofurantoin followed by Amikacin. Similar findings were seen in a study by Bashir MF et al who concluded that the organisms showed resistance to older urinary antimicrobial agents such as Ampicillin which indicates that increased consumption of a particular antibiotic can be a pathway to its resistance. All the three most frequently isolated organisms showed resistant to commonly used antibiotics like Ampicillin, Norfloxacin, and Nalidixic acid.

Antimicrobial resistance is a natural biological response of microbes to antimicrobial drugs. Resistance may be inherent. Nowadays also found in non-hospitalized patients are seen. So, ESBL may throw more information on this resistance. This can monitor the therapy for resistant bacteria. *E-coli*, *K. pneumoniae* and *Enterococcus* species were the primary isolates found to cause UTI from the present study. These findings are in agreement with the results from previous studies from Cameroon,

Pakistan, Israel and Turkey [23,24] that found *E-coli* and *K. pneumoniae* were the most significant causes of UTIs. However, the development of Enterococcus species as a cause of UTI shows that other pathogens may be starting to cause UTI. ESBL pathogens are often known to be multi-drug resistant. Our results showed ESBLs were highly resistant to Ampicillin and Nalidixic acid [25].

Most *E-coli* from community infections investigated in this study were susceptible to oral drugs commonly used in general practice such as trimethoprim/sulphamethoxazole, norfloxacin, ciprofloxacin, nitrofurantoin, cephalexin, and fosfomycin.

Limitation: The sample size too small to generalize the findings.

Conclusion

Urinary Tract Infection was more common among females than males. *E. coli* was the most commonly isolated organism in UTI. Urinary pathogens showed resistance to commonly used antibiotics like Ampicillin, Norfloxacin, and Nalidixic acid. This pattern of resistance to commonly used antibiotics for treating UTI alerts us against indiscriminate usage of antibiotics. This study concludes that *E-coli* is the principal pathogen of UTI. It also indicates a high resistance to the most commonly used antibiotics due to the indiscriminate use of antibiotics. Thus, in order to prevent the development of resistance antibiotic susceptibility patterns must be continuously and periodically evaluated to select the appropriate regimen to treat UTI and to avoid complications.

What does the study add to the existing knowledge?

Regular screening is necessary for the presence of symptomatic or asymptomatic bacteriuria in community practice. Periodic surveys should be done for the prevalence and susceptibility pattern of the common pathogens causing UTI in the local region.

Author's contribution

Dr. Tamasi Mukherji: Concept, study design, manuscript preparation.

Dr. Mayur Bahan Mukherji: Data analysis.

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