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Isolation and Characterisation of UTI Pathogens and Their Antibiotic Susceptibility

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Abstract: *The present study aimed to identify the causative organisms present in urine specimen and their antibiotic sensitivity profile among patients suspected with urinary tract infection (UTI). Ten mid-stream urine samples were collected from patients who suspected for UTI. Total 50 isolates were isolated by culturing on MacConkeys, Cetrimide, and Mannitol salt agar plates. Gram staining resulted that, 74% isolates were Gram negative and 34% were Gram positive. The isolates were processed further for identification, and the result reveals that, Escherichia coli aspredominant isolate which constituting 70%, followed by Enterobacter spp. (20%) and Staphylococcus aureus(10%) of total isolates. Antibiotic susceptibility and resistance result had shown that, E. coli is susceptible to Ampicillin as well as Amikacin and resistance to Ceftriaxone as well as Amoxicillin. S. aureus is susceptible Ampicillin, Amikacin as well as Ceftriaxone, and resistance toAmoxicillin. While, Enterobacter spp.showed resistance to all used antibiotics (Amikacin, Ampicillin, Ceftriaxone and Amoxicillin). Present work will be helpful for clinicians in order to improve the UTI treatment and prevent antibiotic resistance.*
Keywords*:Antibiotics, E. coli, Enterobacter, Isolates, UTI*

I. Introduction

Urinary tract infections are the second most serious health infections worldwide[1]. The most common cause of UTI is gram-negative bacteria from Enterobacteriaceae family. However, gram positive bacteria of same family were also reported to cause UTI infection in rare cases. Many researcher groups reported that the members of Enterobacteriaceae family are the most frequently detected in UTIs, causing 84.3% of the UTIs in both community, and healthcare settings[2,3,4,5]. *E. coli, E. faecalis, K. pneumoniae, P. aeruginosa, P. mirabilis, Proteus vulgaris, S. aureus, S. marcescens, and S. saprophyticus* are the most common bacteria causing UTI in humans[3,6]. Among these, *E. coli* accounts more than 80% of community acquired and ~50% of hospital acquired UTIs[7,8,9]. Different factors; age, gender, immune-suppression and urological instruments may affect prevalence of UTIs. Incidence of UTI is reported to be higher in women than men of whom 40% to 50% will suffer at least one clinical episode during their lifetime[10]. Women suffered more and risk factor may increase because of short urethra, absence of prostatic secretions, pregnancy and easy contamination of urinary tract with faecal flora[11]. ~ 90% of pregnant women develop ureteral dilation, which will persist until delivery[12]. It may contribute to increase urinary stasis and ureterovesical reflux. With this, during pregnancy period, increase in plasma volume decreases urine concentration and up to 70% of pregnant women developing glycosuria, which is responsible to boost the growth of the bacteria in urine[2]. Catheter-associated UTIs are one of the most dangerous health risks contributing 34% of all health care associated infections[13].

The emergence of extended-spectrum beta-lactamases has threatened the empirical use of cephalosporins and ciprofloxacin[14,15]. Hence, microorganism mediated drug resistance strategy is very helpful. Microorganisms use

various mechanisms to develop drug resistance, such as recombination of foreign DNA in bacterial chromosome, horizontal gene transfer and alteration in genetic material[16]. Resistance pattern of microorganisms may vary from country to country, state to state, large hospital to small hospital and hospital to community. There is no systematic national surveillance of antibiotic resistance and less data is available to quantify the problem[17]. Also, it is essential to detect UTI causing pathogens and resistance of these pathogens to commonly prescribe antibiotics in clinical set up for improvement and effectiveness in treatment[18]. To consider all above major issues, the objective of the present study was to highlight the bacterial etiology of UTIs and to determine the antibiotic sensitivity which will helpful to clinicians for improving the UTI treatment and prevent antibiotic resistance.

II. Material And Methods

Study Design and Collection of Urine Sample

This study was carried out in Department of Microbiology, H. V. Desai College, Savitribai Phule Pune University, Pune (MS), India. A total of 10 outpatients (5 males and 5 females in mean age of 42.1 years) were included in this study that was infected with UTI. Ten ml of clean and mid-stream of urine samples were collected in sterile containers (Himedia, India) from outpatients who visited private pathology laboratory from Pune city (Godbole Pathology Laboratory, Pune). All containers were labelled according to gender of each patient. The urine samples were processed further for bacterial cultivation and identification[19.]

Isolation and identification of bacterial isolates

Considering the common causative pathogens of UTI, three types of agar media (Mannitol salt agar, Cetrimide agar, and Mac-Conkey's agar) were selected for isolation. Urine samples were inoculated by sterile loop (Himedia-India) on plates prepared with different agar media. The plates were incubated aerobically at 37 °C for 24-48 h. All single and pure bacterial colonies were identified on the basis of morphological and biochemical characteristics. Colony morphology, motility, gram stain, sugar fermentation and utilisation, oxidase, catalyse, nitrate reduction and IMViC tests were used for characterisation[19,20,21].

Antibiotics sensitivity testing and MDR

Antibiotic susceptibility testing was done by Kirby Bauer (1996) disc diffusion method following the guidelines of the National Committee of Clinical Laboratory Standards (NCCLS 1999). Bacterial inoculums were prepared by suspending the freshly-grown bacteria in 25 mL sterile nutrient broth.A sterile cotton swab was used to streak the surface of Mueller Hinton agar plates. By sterile forceps, all antibiotics disc were placed onto the surface of Mueller Hinton agar (Himedia, India) and incubated aerobically at 37 °C for 24 h. These antibiotics disks were used for antibiotic sensitivity as well as resistance pattern against isolated UTI causing pathogens.Four antibiotics used in the current study were obtained from Himedia Labs, Mumbai, India;Amikacin (AK 30µg), Ampicillin (AMP 10µg), Amoxicillin (AM 10µg), Ceftriaxone (CRO 30µg). *E. coli* ATCC-25922 was used as a control isolate.Inhibition zone diameter (mm) of each antimicrobial disc was measured, and the isolates were classified as resistant, and susceptible.

Data analysis

The experiment of antimicrobial analysis were replicated three times and the results are presented as mean±SD. Data were calculated by using 't-test' and analysed using in SPSS software.

III. Results

Isolation and Identification of isolates

A total 50 isolates of bacteria were collected from the 10 urine samples. In this study, 54% of isolates were recovered from females, and the remaining 46% were from males. The morphology of UTI causing isolates showed different characteristics including; size (1-4 mm), Shape (either circular or irregular), Colors (colorless, off-white, light, and dark pink, yellowish, greenish, and violet), margin (entire, curved or undulate), Opacity (opaque or transluscent), elevation (flat, raised, convex or undulate) and consistency (moist, rough, smooth, and dry). Few isolates showing maximum number of similar colonies MSA and MacConkey agar plates that are selected for identification. Whereas no growth was obtained on cetrimide agar (Table 1).

Table 1: Isolation of UTI pathogens on selective agar medium

Urine Samples	MacConkey agar	Mannitol salt agar	Cetrimide agar
I	+	-	-
II	+	-	-
III	+	-	-
IV	+	-	-
V	+	-	-
VI	+	+	-
VII	+	-	-
VIII	+	-	-
IX	+	-	-
X	+	-	-

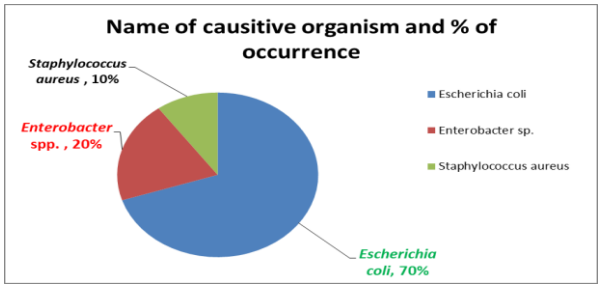
(+ sign indicates the presence of growth; - sign indicates No growth**)**

Gram staining resulted that, 90% isolates were Gram negative and 10% were Gram positive. Collected isolates were identified. Here, the isolates were checked with different biochemical test such as Indole, MR, VP, Citrate, Catalase and Urease. Result reveals that, 70% of the isolates from MacConkey agar plate showed pink colonies with dark centers and positive test for indole which indicates the presence of *E. coli*. Colorless colonies, negative test for indol (-), and, positive test for urease (+) confirmed the presence of *Enterobacter* spp. Whereas 10% of the total isolates from MSA plate showed positive test for catalase indicating the presence of *S. aureus* (Table 2; Graph 1).

Table 2: Biochemical test and Identification of bacteria

Isolate	Biochemical test						Identified Organism
	Indole	MR	VP	Citrate	Catalase	Urease	
1	+	+	-	-	NA	NA	*E. coli*
2	+	+	-	-	NA	NA	*E. coli*
3	-	-	+	+	NA	+	*Enterobacter spp.*
4	+	+	-	-	NA	NA	*E. coli*
5	+	+	-	-	NA	NA	*E. coli*
6	-	+	+	+	+	NA	*S. aureus*
7	+	+	-	-	NA	NA	*E. coli*
8	+	+	-	-	NA	NA	*E. coli*
9	-	-	+	+	NA	+	*Enterobacter spp.*
10	+	+	-	-	NA	NA	*E. coli*

(Voges Proskauer (VP); Methyl Red (MR); +: sign indicates the positive test; - : sign indicates negative test; NA: Not Applicable**)**



Graph 1: Percentage of Causative organism found in samples of UTIs

Antimicrobial susceptibility testing and MDR

In-vitro sensitivity test for antimicrobial agents on Muller Hinton agar using the Kirby-Bauer disk diffusion method was carried out for the representative isolates, which include gram-positive organism, gram-negative organisms. The susceptibility was measured as a zone of inhibition (mm). Absence of zone of inhibition indicates the resistance. We have observed that, *E. coli* is susceptible to Ampicillin (12.3±0.7) as well as Amikacin (22.5±0.8) and resistance to Ceftriaxone as well as Amoxicillin. *S. aureus* is susceptible Ampicillin (10.3±0.9), Amikacin (29.2±1.0) as well as Ceftriaxone (9.4±0.4), and resistance toAmoxicillin (Table 1; Fig 1a and b). It was also recorded that, two species of *Enterobacter* genusi.e. *E. cloacae* and *E. hormaechei*, both showed the resistance to all used antibiotics (Amikacin, Ampicillin, Ceftriaxone and Amoxicillin)(Table 3; Fig 1b).

Table 3: Antibiotic susceptibility and resistant pattern of UTI causing bacteria

Sr. No.	**Name of the Bacteria**	**Used Antibiotics and zone of Inhibition (mm)**			
		Ampicillin	Amikacin	Ceftriaxone	Amoxycillin
01	*Enterobacter* spp.	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
02	*S. aureus*	10.3±0.9	29.2±1.0	9.4±0.4	0.0±0.0
03	*E. coli*	12.3±0.7	22.5±0.8	0.0±0.0	0.0±0.0

(The experiment was replicated three times and the results are presented as mean±SD)

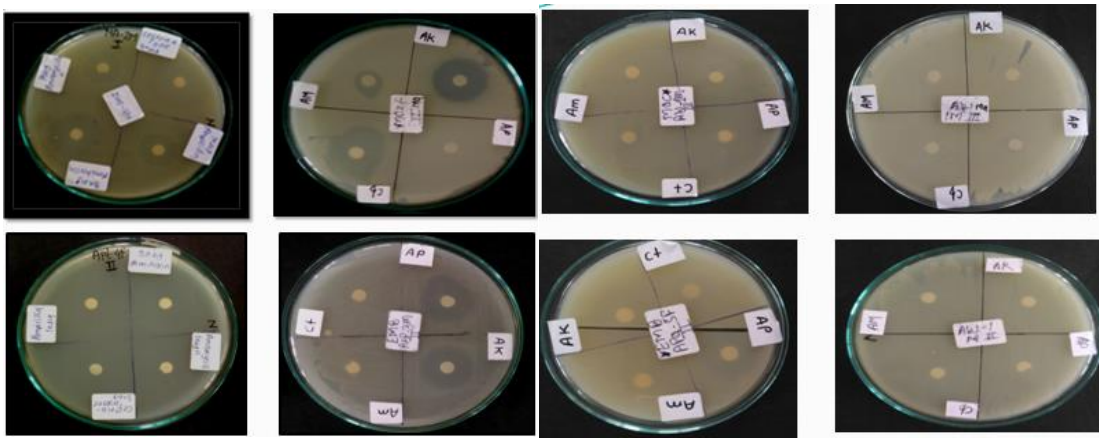


Figure 1 (a): Antibiotic sensitivity testing; isolates showing antibiotic susceptibility

Figure 1 (b): Antibiotic sensitivity testing; isolates showing antibiotic resistance

IV. Discussion

In developing countries and among all age group, UTI infection considered most common infection of bacterial etiology[22,23]. In elderly people, UTI infection found to be the most common cause of hospitalisation for infection. Also in primary treatment, it is the most common cause of antibiotics prescriptions. It also constitutes over 30% of all infectious complications in patients after kidney transplantation[24,25]. Still, it is challenging to clinicians to

treat and diagnose the upper and lower urinary tract infections because of their frequent appearance, recurrence, and a worldwide increase of antibiotic resistance. This indicates that urine culturing and antimicrobial susceptibility testing is necessary for a definitive diagnosis and treatment of UTI [22]. Hence, the present work aimed todetermine the antibiotic susceptibility isolates of bacteria from the urine of patients suffering with UTI. In this study, total fifty isolates were isolated from ten different samples (five male and five female urine samples) using selective media. In present work, we have observed the prevalence of *E. coli* in UTI, as well as their antibiotic sensitivity pattern. Similar to our study, Raeispour and Ranjbar in 2018 reported that *E. coli* is considered as the cause of 80–90% of UTIs and it is one of the most common bacterial infections in today's life[26]. Gupta and co-researcher reported, among 65 patients (including children, adults, and women) of UTI, *E. coli* found to be causative agents for UTI in 53 patients[27]. In the present study, identified the presence of *S. aureus* in samples collected from UTI infected patients. Akortha and Ibadin reported the gram-positive bacteria, particularly *S. aureus* as the most commonly implicated pathogen in patients with UTIs because of virulent nature of the organism, which gives it the ability to overcome body defence mechanisms and resistance to antibiotics[28]. After isolation of different bacteria and their confirmation through different biochemical tests, we performed antibiotic sensitivity test. The knowledge of antibiotic sensitivity of pathogen is very necessary and helpful for guidance and treatment of pathogens[29]. We have observed that, *E. coli* are susceptible to Ampicillin as well as Amikacin and resistance to Ceftriaxone and Amoxicillin. Similar types of results were reported by Bano et al. 2012, *E. coli* showed the highest sensitivity (56%) to Amikacin and low susceptibility (5%) to Ciprofloxacin[1]. Some of the researcher also reported that highest effectiveness of Amikacin against *E. coli* while Ciprofloxacin showed the lowest sensitivity against this pathogen[30]. Sabir et al. (2014), reported that the *E. coli* was highly resistant to Amoxicillin[31]. *S. aureus* is resistant to only Amoxicillin andsusceptible to other used antibiotics (Ampicillin, Ceftriaxone and Amikacin). Shittu and Mandere in 1999, reported the Sensitivity patterns of *S. aureus* to antibiotics, results reveals the 100% sensitivity to gentamicin and cephalosporin, but resistant to Amoxicillin/Clavulanate and Nitrofurantoin[32]. Abuse or misuse of antibiotics among general population favored the emergence of resistance strains which may show the differences in sensitivity pattern of *S. aureus*[33]. We have observed that, two species of *Enterobacter* spp. are resistance to all used antibiotics (Amikacin, Ampicillin, Ceftriaxone and Amoxicillin). Davin-Regli and Pagès reported that the *E. aerogenes* and *E. cloacae* is naturally resistant to Ampicillin, Amoxicillin–Clavulanic acid, Cephalothin, and Cefoxitin by low production of the natural inducible cephalosporinase of Bush group 1[34].

V. Conclusions

Maximum Gram negative and few gram positive isolates were recorded in present studywhich indicates the gram-negative bacteria were responsible for urinary tract infections. Present study concluded that, *E. coli*. recorded aspredominant isolate followed by *Enterobacter sp.* and then *S. aureus*out of total isolates collected from the samples of patient suffering from UTI's. Antibiotic sensitivity results showed that, *E. coli* are (susceptible; Ampicillin, Amikacin and resistance; to Ceftriaxone and Amoxicillin) and *S. aureus* is resistant to only Amoxicillin andsusceptible to other used antibiotics (Ampicillin, Ceftriaxone and Amikacin). While, *Enterobacter* spp. showed resistance to all used antibiotics (Amikacin, Ampicillin, Ceftriaxone and Amoxicillin). This study will be helpful to guide physicians in making right choice of drugs while treating patients thus ensuring effective and treatment of the infection and preventing antibiotic resistance.

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