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Original Research Paper

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# A BACTERIOLOGICAL STUDY OF URINARY TRACT INFECTION INVOLVING CULTURAL ANALYSIS

**Estibeiro AS*** Associate Professor, Department of Microbiology, Goa Medical College, Bambolim Goa, 403202 *Corresponding Author

**Dr. Fernandes AL** Junior Resident, Department of Microbiology, Goa Medical College, Bambolim Goa, 403202

## ABSTRACT

**Background:** Urinary Tract Infections are a common occurrence in the community and hospital and are associated with profound morbidity and decreased productivity at work. Timely diagnosis of UTI by cultural analysis is imperative in order to identify the offending agent and to initiate appropriate antimicrobial therapy. **Objectives:** The present study aimed at evaluating the bacteriological profile of UTI employing semiquantitative culture and to determine the antibiogram pattern of the bacteria isolated. **Methods:** The study was conducted on 200 urine samples, collected from clinically suspected cases of UTI. Semiquantitative analysis was done and antimicrobial sensitivity testing undertaken, as per CLSI guidelines. **Results:** Significant bacteriuria was observed in 66.5% cases. Gram negative bacilli were predominant pathogens (71.3%), amongst which, Escherichia coli and Klebsiella pneumoniae were the main agents (37.2% and 24.5 % respectively). Gram positive organisms accounted for 28.7% of the total; Enterococcus species being the predominant isolate type (78.04%; 32 out of 41 gram positive pathogens). Overall, Escherichia coli predominated (26.6%), followed by Enterococcus species (22.4%). Gram negative bacilli showed good sensitivity to Imipenem and Aminoglycosides. Gram positive cocci were all sensitive to Vancomycin and Linezolid. A large number of cases were 50 years and above (51%), followed by age groups 21-30 years (17.5%) and 31 to 40 years (16.5%). The Male: Female ratio was 1.2:1, males accounting for 53.5% of the total. **Conclusion:** Early cultural analysis of UTI cases with identification of the etiological agent and its antibiogram pattern will help in early treatment and use of appropriate antibiotics. This will reduce the use of empirical treatment, morbidity and chronicity.

**KEYWORDS :** Urinary Tract Infection, semiquantitative culture

## INTRODUCTION

Urinary tract infections (UTI) are common bacterial infections seen in the community and as well as in hospitals, the latter, usually being catheter associated. Acute infections involving the lower tract include urethritis and cystitis and upper tract infections present as pyelonephritis and prostatitis.

Uncomplicated UTIs affect individuals who are healthy with no identifiable risk factors. However, complicated UTIs are associated with various factors that compromise the host's defense, obstruction, indwelling catheters or malignancies.[1]

The most common bacterial etiologoical agents of UTI include members of Enterobacteriaceae, viz. Escherichia coli, Klebsiella pneumoniae, Proteus species, as well as Pseudomonas aeruginosa, Enterococcus faecalis and occasionally, Group B Beta hemolytic Streptococcus and Staphylococcus aureus.

Cultural analysis of UTI cases is essential, not only to identify the etiological agent, but most importantly, to obtain the antibiogram pattern so as to select appropriate antimicrobial therapy for quick and accurate management. Knowledge of the antibiotic sensitivity of the offending bacterial agents gains importance due to the overwhelming increase in antimicrobial resistance.

From a microbiological perspective, UTI exists when pathogenic microorganisms are detected in the urine. Semiquantitative cultural analysis of urine is the mainstay in diagnosis and is considered significant when a colony count equal to or more than $10^5$CFU/ml is obtained from a properly collected midstream clean catch urine sample. However, in symptomatic patients or in patients with an indwelling catheter, fewer organisms may signify infection.[2]

The present study was undertaken to evaluate the bacteriological profile of UTI using cultural analysis and to obtain the antibiogram pattern of the isolated etiological agents.

## MATERIALS AND METHODS

The study was undertaken in the Department of microbiology, Goa Medical College over a period of 6 months from July to December, 2022. Two hundred samples received from patients; attending Out Patient Departments of this institution, with a provisional diagnosis of Urinary Tract Infection formed the study material. Patients who received antimicrobial treatment were excluded from the study. Information was obtained with regard to the age and gender.

Every subject was asked to collect early mid-stream clean catch urine in a sterile container. The males were instructed to collect urine after retracting prepuce and the glans penis cleaned with soap and water. The women were instructed to collect urine after careful anogenital toilet with soap and water, while separating the labia with the fingers. The samples were refrigerated in case of delay of more than 1-2 hours for transportation of the samples.

Semi quantitative culture was performed by using a calibrated bacteriological loop streak plate method. A platinum loop with a diameter of 1.3 mm calibrated to deliver 0.001 ml of urine was used. One loopful of well mixed uncentrifuged urine was inoculated on blood agar and MacConkey agar. Streaking method employed on macConkey agar plate was inverted cone technique.[3] The streaking on blood agar plate was done using a primary, secondary and tertiary streak. After overnight incubation at $37^0$C, colony count was done and significant bacteriuria was reported at a count of >/=$10^5$CFU/ml. Growth on the plates were processed according to standard microbiological techniques.[4] Antimicrobial susceptibility testing was performed using Kirby Bauer Disc Diffusion Method, as per CLSI Guidelines.[5]

## RESULTS

This prospective study included a bacteriological analysis of 200 randomly selected urine samples from patients with clinical suspicion of UTI. The study extended over a period of 6 months, from July to December 2022.

Table no. 1 depicts the age and sex distribution of subjects included in the study

**Table No 1: Age And Sex Distribution Of Subjects Under Study**

| Age group in | Female | | Male | | No. of cases | |
|---|---|---|---|---|---|---|
| years | No. | % | No. | % | No. | % |
| 0-10 | - | - | - | - | - | - |
| 11-20 | 3 | 50 | 3 | 50 | 6 | 3 |
| 21-30 | 15 | 42. 9 | 20 | 57.1 | 35 | 17.5 |
| 31-40 | 13 | 39.4 | 20 | 60.6 | 33 | 16.5 |
| 41-50 | 14 | 58.3 | 10 | 41.7 | 24 | 12 |
| >50 | 48 | 47.1 | 54 | 52.9 | 102 | 51 |
| Total | 93 | 46.5 | 107 | 53.5 | 200 | 100 |

From the above Table no. 1, it can be seen that a large number of cases belonged to the older age group, above 50 years (51%), followed by the age groups 21-30 years (17.5%) and 31 to 40 years (16.5%). Children and adolescents contributed to a very low number i.e. 6 out of 200 (3%). The Male: Female ratio was 1.2:1, males accounting for 53.5% of the total.

Culture result of urine samples by the semiquantitative loop method revealed significant bacteriuria in 66.5 % cases, while no growth was obtained in 18% cases. Doubtful bacteriuria and growth of contaminants was seen in 0.5% and 15% cases respectively. (Table no. 2)

**Table No.2: Culture Result Of Urine Samples By Semiquantitative Loop Method**

| Culture result | No. of samples | Percentage |
|---|---|---|
| Significant bacteriuria | 133 | 66.5 |
| Doubtful bacteriuria | 01 | 0.5 |
| Growth of contaminants | 30 | 15 |
| No growth | 36 | 18 |
| Total | 200 | 100 |

Among significant bacteriuria cases, a single pathogen was obtained in 92.4% cases (123 out of 133 culture positive) while two bacteria were isolated concomitantly in 7.6% cases. (20 out of 133 culture positive). All polymicrobial cases included two Gram negative bacilli.

Bacteriological profile of significant bacteriuria cases can be observed in Table no 3.

**Table No.3: Organisms Isolated On Culture**

| Organisms | Number | % |
|---|---|---|
| Escherichia coli | 38 | 26.6 |
| Klebsiella pneumoniae | 25 | 17.4 |
| Enterobacter species | 10 | 7 |
| Proteus mirabilis | 02 | 1.4 |
| Citrobacter diversus | 11 | 7.7 |
| Pseudomonas aeruginosa | 11 | 7.7 |
| Acinetobacter baumanii | 05 | 3.5 |
| Staphylococcus aureus | 09 | 6.3 |
| Enterococcus species | 32 | 22.4 |
| Total | 143 | 100 |

From the Table no. 3, it can be observed that isolation of gram negative bacilli were to the tune of 71.3%, while gram positive cocci were 28.7% of the total (41/143) The commonest etiological agents of UTI were Escherichia coli (26.6%), followed by Enterococcus species (22.4%) and Klebsiellapneumoniae(17.4%).

Table No. 4 A Depicts The Antimicrobial Sensitivity Pattern Of Gram Negative Bacilli Isolated In The Study (Depicted At The End).

Escherichia coli showed 76.3% sensitivity to Imipenem, 60.5% sensitivity to Amikacin, 68.4% to Gentamicin and 52.6% to Trimethoprim+ Sulfamethoxazole. Klebsiella pneumoniae sensitivity to Cefepime, Amikacin and Imipenem was to the tune of 52% each. Pseudomonas aeruginosa showed 81.8% sensitivity to Aminoglycosides i.e. Amikacin and Gentamicin while it was 72.7% each to Ciprofloxacin, Imipenem and Piperacillin- Tazobactam combination.

All Staphylococcus aureus isolates were Methicillin sensitive strains and were sensitive to Vancomycin. Linezolid sensitivity was 100%, while Teichoplanin sensitivity was 66.6% (Table no. 4B, depicted at the end).

Enterococcus species were all sensitive to Vancomycin and Linezolid (100%), while to Ciprofloxacin and Tetracyclines, the strains showed 81.2% and 68.8% sensitivity respectively.

## DISCUSSION

Urinary Tract Infections are common and need medical care. They account for the second most common infections after respiratory tract infections in the community. Approximately 150 million people are affected worldwide, each year.[1] If left untreated, UTI can lead to various complications, chronicity and permanent kidney damage.

UTI can affect individuals of all ages and gender. In the present study, a large number of cases belonged to age group 50 years and above (51%), 21-30 years (17.5%) and 31-40 years (16.5%). Similar observation was encountered in the study of Eshwarappa et al in their study, the occurrence being common in age group above 50 years.[6] In a study conducted by Naik et al, a large number of cases were seen in the age group 21-30 years (40.5%) followed by the age group of more than 50 years (20.5%).[7] Increased number of cases in the older age group is probably related to obstruction, urinary retention and immunosuppression. Younger age involvement can be attributed to increased sexual activity, which often predisposes to UTI.

Although UTI is predominantly a disease of females, a total of 53.5% cases were males in the present study. Similar male predominance was seen in the study of Eshwarappa et al[6] and Bhayani et al.[8] However Prakash et al recorded a higher occurrence in females (73.57%).[9] A higher involvement of males in the present study is probably related to its occurrence in the elderly, who have age related comorbidities, diabetes mellitus, kidney stones and urinary retention due to enlargement of prostate.

Culture analysis of UTI involves determination of CFU per ml of urine by semi quantitative method. This serves as a gold standard to diagnose UTI. In the present study, significant bacteriuria was observed in 66.5% cases. Similar finding was seen in the study of Naik et al (62%).[7] Shah et al obtained a culture positivity of 42% in their study in Ahmedabad.[10] A very low culture positivity rate was seen in the study of Eshwarappa et al, it being 9.17%, probably related to non-specific symptoms of fever and abdominal pain in their study.[6] A high UTI rate in the present study is probably related to selection of patients with careful history undertaken and exclusion of those who had received antimicrobial therapy.

In the present study, a total of 144 organisms were isolated from 133 culture positive cases, monobacterial etiology being 92.4%. Similar observation was seen in the study of Naik et al (91.9%).[7]

Escherichia coli was and continues to be the predominant causative agent of UTI, as seen in the present and other studies.[6,7,8] The second common pathogen in the present study was Enterococcus species (22.4%) and as well as in the study of Naik et al.[7] Enterococcus species are now recognized as important urinary pathogens, often associated with poor hygiene and among hospitalized patients.

Gram negative bacteria have several factors that assist in adherence to the uroepithelial cells, such as adhesins, pili and fimbria.[11] Escherichia coli carrying K antigens are commonly responsible for pyelonephritis. These bacteria can replicate and persist in intracellular and extracellular niches.[12]

In the present study, gram negative organisms showed overall maximum sensitivity to Imipenem and Aminoglycosides i.e. Amikacin and Gentamicin.

A high degree of resistance was seen to Cephalosporins and Quinolones. Similar findings were observed in the study of Naik et al,[7] Eshwarappa et al[6] and Dash et al.[13]

Enterococcus species were all sensitive to Vancomycin and Linezolid (100% isolates), while sensitivity to Ciprofloxacin was 81.2%.

Similar findings were encountered in the study of Naik et al.[7]

**Table No. 4: Antimicrobial Sensitivity Pattern Of Isolates Encountered In The Study**

**A) Gram Negative Bacilli**

| Organism | Amoxicillin-Clavulanic acid | Trimethoprim-Sulfamethoxazole | Cefuroxime | Ceftriaxone | Ceftazadime | Cefepime | Ciprofloxacin | Gentamicin | Amikacin | Tobramycin | Imipenem | Piperacillin-Tazobactam |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Escherichia coli (n=38) | 7 | 20 | 7 | 10 | 7 | 14 | 12 | 26 | 23 | - | 29 | 21 |
| % | 18.4 | 52.6 | 18.4 | 26.3 | 18.4 | 36.8 | 31.5 | 68.4 | 60.5 | - | 76.3 | 55.2 |
| Klebsiella pneumoniae (n=25) | 6 | 7 | 6 | 6 | 5 | 13 | 9 | 10 | 13 | - | 13 | 9 |
| % | 24 | 28 | 24 | 24 | 20 | 52 | 36 | 40 | 52 | - | 52 | 36 |
| Enterobacter species (n=10) | 2 | 3 | - | 2 | 9 | 2 | 8 | 6 | 5 | 4 | 6 | 4 |
| % | 20 | 30 | - | 20 | 90 | 20 | 80 | 60 | 50 | 40 | 60 | 40 |
| Proteae (n=2) | 2 | 1 | 1 | 2 | 2 | 2 | 1 | 2 | 2 | 1 | 2 | 1 |
| % | 100 | 50 | 50 | 100 | 100 | 100 | 50 | 100 | 100 | 50 | 100 | 50 |
| Citrobacter species (n=11) | 4 | 7 | - | 5 | 6 | 9 | 5 | 7 | 10 | 8 | 11 | 10 |
| % | 36.3 | 63.6 | - | 45.4 | 54.5 | 81.8 | 45.4 | 63.6 | 90.9 | 72.7 | 100 | 90.9 |
| Pseudomonas aeruginosa (n=11) | - | - | - | 4 | 5 | 6 | 8 | 9 | 9 | - | 8 | 8 |
| % | - | - | - | 36.3 | 45.4 | 54.5 | 72.7 | 81.8 | 81.8 | - | 72.7 | 72.7 |
| Acinetobacter baumanii (n=5) | 2 | 4 | - | 3 | 1 | 4 | 4 | 2 | 2 | 2 | 3 | 3 |
| % | 40 | 80 | - | 60 | 20 | 80 | 80 | 40 | 40 | 40 | 60 | 60 |

**B) Gram Positive Cocci**

| Organism | Penicillin | Ampicillin | Augmentin | Azithromycin | Clindamycin | Tetracycline | Vancomycin | Linezolid | Teicoplanin | Ciprofloxacin |
|---|---|---|---|---|---|---|---|---|---|---|
| Staphylococcus aureus (n=9) | 5 | 5 | 6 | 6 | 4 | 6 | 9 | 9 | 6 | 6 |
| % | 55.5 | 66.6 | 66.6 | 66.6 | 44.4 | 66.6 | 100 | 100 | 66.6 | 66.6 |
| Enterococcus (n=32) | 19 | 19 | - | - | - | 22 | 32 | 32 | - | 26 |
| % | 59.3 | 59.3 | - | - | - | 68.8 | 100 | 100 | - | 81.2 |

## CONCLUSION

Management of UTI requires urine culture and antimicrobial sensitivity testing to be undertaken as important diagnostic modalities. Treatment of UTI has become complex due to the advent of growing resistance among the urinary pathogens, thus leaving very few options for oral therapeutic agents. Quinolones and Cephalosporins once used widely to treat UTI are now experiencing a setback due to increased resistance and clinicians have to resort to parenteral drugs.

## REFERENCES


1. Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. Urinary tract Infections: Epidemiology, mechanisms of infection and treatment options. Nat Rev Microbiol 2015; 13:269-84.
2. Kass EH. Asymptomatic infections of the urinary tract. Trans Assoc Am Physicians. 1956; 69: 56-64.
3. Schaus R. Griess' Nitrate Test in Diagnosis of Urinary Infection. JAMA. 1956; 161(6):528-529.
4. Collee JG, Duguid JP, Fraser AG, Marmion BP. Laboratory strategy in the diagnosis of infective syndromes in Mackie and McCartney Practical Medical Microbiology 1989, Thirteenth Edition, Vol 2, Longman Group UK. P.640-647.
5. Performance standards for Antimicrobial susceptibility Testing. Clinical and Laboratory Standards Institute, M100, 32th Edition, January 2022, Wayne, PA, 19087, USA.
6. Eshwarappa M, Dosegowda R, AprameyaIV, Khan MV, Shiva Kumar P, Kempegowda P. Clinico-microbiological profile of urinary tract infection in South India. Indian journal of Nephrology. January 2011; 21(1).
7. Naik P, Pinto MJ. A Bacteriological Study of Urinary Tract Infections (U.T.I.) For Diagnosis of Significant Bacteriuria. Ann Int Med Den Res. 2019; 5(5):MB05-MB12.
8. Bhayani P, Rawekar R, Bawankule S, Kumar S, Acharya S, Gaidhane A, et al. Profile of urinary tract infection in a rural tertiary care hospital: Two- year cross- sectional study. J Data Meghe Inst Med Sciences University. 2019; 14:22-6.
9. Prakash D, Ramchandra SS. Distribution and antimicrobial susceptibility pattern of bacterial pathogens causing urinary tract infection in urban community of Meerut city, India. ISRN Microbiol. 2013.
10. Shah A, Vinzuda M, Prajapati B, Rajput A, Kadam M. Clinico- Microbiological Profile of Urinary Tract Infection in Tertiary Care Hospital in Ahmedabad, Gujarat, India. Int J Curr Microbiol App Sci, 2015; 4(9):288-295.
11. Das RN, Chandra S., Joshi HS, Shreshta N. Frequency and susceptibility profile of pathogens causing urinary tract infections at a tertiary care hospital in Western Nepal. Singapore Med J. 2006; 47:281-5.
12. Gawel D, Seed PC. Virulence. 2011; 2(3):222-32.
13. Dash M, Sanghamitra P, Indrani M. J Family community Med. 2013; 20(1):20-26.