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Comparative Study of Standard Urine Culture and Enhanced Urinary Culture Technique for Isolation of Pathogenic Bacteria from Urinary Tract Infection among Women at a Tertiary Care Centre, Rajasthan

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Abstract: ***Purpose: Nearly 50% of the women population experience at least 1 episode of urinary tract Infection (UTI) in their lifetime, with recurrent episodes of UTI seen in 20 - 40% of them. The clinical practice relies on standard urine culture (SUC) as the gold standard in diagnosing urinary tract infections. However, in clinically symptomatic patients, the majority do not show any growth on culture by SUC protocol. This study aimed to Comparison of SUC protocol and Enhanced Urinary Culture (EUC) technique in determining the bacteriological profile of urinary tract infection among women and to detect etiological agents which might be missed by SUC protocol to avoid therapeutic failures in symptomatic UTI patients. Material and methods: This hospital - based descriptive type of observational study included 100 women with symptoms of Urinary Tract Infection. The midstream clean catch urine sample obtained was cultured using SUC and EUC. Results for both methods were compared. Results: Among 100 women with clinical symptoms of UTI, SUC showed culture positivity in only 38% while EUC detected culture positivity in 69 % Conclusion: In clinically symptomatic patients with UTI, EUC is a better method to identify possible uropathogen.***

1. Introduction

Urinary tract infection (UTI) is a common and significant health issue worldwide, leading to considerable morbidity, mortality, and healthcare expenditure. [1] It is common among women, with the incidence rate increasing with age. Nearly half of women experience at least one UTI episode in their lifetime, and recurrent UTIs affect 20 - 40% of them. [2]

Diagnosis is based on clinical signs and symptoms along with the demonstration of bacteria in urine culture. [3] Standard Urine Culture (SUC) is the gold standard diagnostic test but has limitations in identifying specific pathogens, susceptibility to contamination, and inconsistent threshold definitions. [4] Enhanced Urine Culture (EUC), utilizing larger urine volumes, multiple growth media, longer incubation times, and varied atmospheric conditions, can overcome these limitations and detect microorganisms missed by SUC. [5]

Implementing EUC can aid in identifying causative organisms that may be overlooked by SUC, guiding clinicians in selecting appropriate antibiotics, ensuring complete treatment, reducing recurrence rates, and minimizing antimicrobial resistance. This approach can enhance understanding of UTIs and alleviate the associated economic burden.

2. Materials and Methods

A Hospital - based descriptive type of observational studywas carried out in the Department of Microbiology, SMS Medical College, Jaipur between July 2021 to December 2021. A total of 100 women with clinical suspicion of UTI were included.

Inclusion criteria for the study were women over 18 years of age with clinical suspicion of urinary tract infection. Patients who had been on antimicrobial therapy for a minimum of 48 hours prior to sample collection were excluded.

The urine samples were collected using clean catch, mid - stream technique and were processed within 2 hours of collection.

For SUC, urine specimens were inoculated on Blood agar and MacConkey agar plate with a standardized loop (semi - quantitative culture). A sterilized nichrome inoculating loop (HiMediaPvt. Ltd. Mumbai) calibrated to 0.001 mL was immersed into the well - mixed uncentrifuged urine sample. The loop was carefully removed and the entire volume within the loop was delivered on the surface of the agar plate by making a single streak across the center. The inoculum was then spread evenly at right angles to the primary streak to cover the entire surface. All plates were incubated at 37ºC for the isolation of organisms. After 18 - 24 hours of incubation, the number of bacteria in urine samples (CFU/mL) was estimated by counting the number of colonies on the surface of the agar.

For EUC three different volumes of urine samples were inoculated i. e.1µl, 10µl, and 100µl. Each of the three volumes of well - mixed urine sample was placed using a pipette and inoculated using a sterilized nichrome inoculating loop on Blood agar, MacConkey agar, and Chocolate agar. The chocolate agar plate was incubated in 5% CO2 at 37 °C for 18 - 24 hours. The Blood agar and MacConkey agar plate were incubated aerobically at 37ºCfor

18 - 24hrs. After incubation, the plates were examined for any growth. If growth was present it was identified by standard biochemical reactions and antimicrobial susceptibility was performed. If no growth was seen, the plates were reincubated and examined after 48 hrs.

Organisms grown were then identified on the basis of their colony morphology, Gram's staining characteristics, motility, and biochemical reactions as per the standard protocol using standard laboratory procedures. All gram - negative bacilli were identified to species level by their characteristic appearances on the media, Gram's stain, Oxidase test, motility, and the biochemical reactions as per standard laboratory protocol. All gram - positive organisms were identified to species level by their characteristic appearances on the media, Gram's stain, catalase test followed by coagulase test. Enterococcus was identified by Bile Esculin disc test and it was also confirmed by Salt tolerance test (6.5% NaCl).

The study followed ethical guidelines and received approval from the ethical committee of SMS Hospital.

Data were recorded on the proforma and entered into Microsoft Excel Workbook 2019 and exported into SPSS software version 21.0 (IBM, USA). Data were presented as frequency, percentage and mean wherever applicable. Categorical variables between two groups were compared using Chi square test. P value < 0.05 was considered significant.

3. Results

A total of 100 urine samples from women with suspected UTI were processed from July 2021 to December 2021 at the Bacteriology Laboratory, Department of Microbiology, SMS Medical College to compare the two urine culture methodologies – Standard Urine Culture (SUC) and Enhanced Urine Culture (EUC). The majority of the study population belonged to the age group of 18 - 45 years. The mean age of the study population was 35.4 years. The most common presenting symptom was increased frequency of micturition and urgency seen in 55% of the study population followed by pain during micturition (47%) and abdominal pain (33%). Thirty - eight percent of samples showed growth on SUC while 69% of samples showed growth of uropathogens on EUC. Among 69 culture - positive samples, monomicrobial growth was seen in 94.2% of sample while polymicrobial growth was seen in 6.8% of samples. The highest rate of uro - pathogen growth was seen on 100 µl EUC (69%), followed by 10 µl (58%), and 1 µl (48%). The difference in the growth rate among the three different volumes of EUC was statistically significant (p - value = 0.011). By increasing the duration of incubation upto 48 hours for plates that showed no growth at 24 hours of incubation, 21% of organisms were detected when 1µl sample was inoculated as compared to 25.9% for 10 µl and 34.8% for 100 µl.

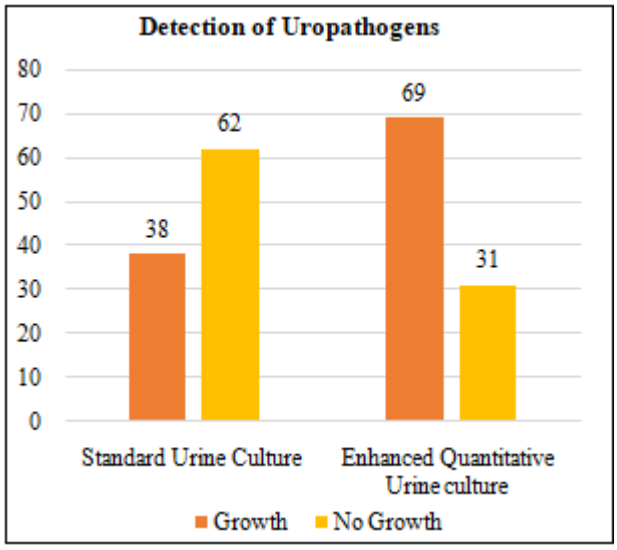

Figure 1: Detection of Uropathogens (n=100, % of total)

Table 1: Frequency distribution of isolates (n=73)

Isolate	SUC		EQUC	
	n	%	n	%
Staphylococcus aureus	2	5	6	8.2
Coagulase - negative Staphylococcus spp	0	0	1	1.7
Enterococcus	2	5	5	6.8
Micrococci	0	0	3	4.1
Diphtheroid	0	0	3	4.1
Escherichia coli	23	57.5	30	41.1
Klebsiella spp	6	15	9	12.3
Enterobacter cloacae	2	5	2	2.7
Acinetobacter spp	0	0	3	4.1
Pseudomonas aeruginosa	4	10	5	6.8
Candida	1	2.5	6	8.2
Total	40	100.0	73	100.0

4. Discussion

The present study compared the results of SUC and EUC techniques in detecting bacterial growth in urine samples. The study found that only 38% of samples showed bacterial growth on SUC, while EUC showed growth of pathogens in 69% of the samples. The difference in bacterial growth between the two techniques was highly significant (p - value <0.0001), indicating that the higher growth rate on EUC was not by chance but statistically significant.

Several other studies have also reported similar findings. For example, Hilt et al. [5] examined urine samples using both SUC and Enhanced Quantitative Urinary Culture (EQUC) techniques and found that 80% grew bacterial species using EQUC, while only 8% of samples tested using SUC reported growth. Coorevitset al. [6] reported that 73% of samples examined by SUC showed no growth, while 91% of samples examined by EUC grew bacterial species in large numbers. Thapaliya et al. [7] enrolled pediatric urine samples and found higher growth positivity in EQUC (16.15%) compared to SUC (12.80%). They also reported that about 20.6% of the significant isolates detected with EUC were missed on the SUC technique.

The present study also investigated the effect of increasing volumes of EUC on the detection of uropathogens. The results showed that the proportion of urine samples detecting

uropathogens increased with increasing volumes of EUC. The highest rate of uropathogen growth was seen with 100 µl EUC (69%), followed by 10 µl (58%), and 1 µl (48%). The difference in growth rate among the three different volumes of EQUC was statistically significant (p - value = 0.011). These findings were consistent with a study by Price et al. [8], which reported higher uropathogen detection with expanded - spectrum EQUC urine volumes.

Regarding the type of bacterial growth, the present study found that out of the total samples showing bacterial growth on EUC, 94.2% showed monomicrobial growth, and 6.8% showed polymicrobial growth. The low prevalence of polymicrobial growth in the study participants was likely because all the cases were community - acquired UTIs. Polymicrobial UTIs are more commonly seen in hospital - acquired UTIs and among immunocompromised patients.

The most frequently identified pathogen in both SUC and EUC was Escherichia coli, followed by different pathogens depending on the culture technique used. Klebsiella species were the second most common pathogen on SUC, while Candida was the second most common on EQUC. Similar findings were reported by other studies, where E. coli was predominantly the most common cause of UTIs. [9]

In summary, the present study demonstrated that EUC had a significantly higher bacterial growth rate compared to SUC. Increasing volumes of EQUC also increased the detection of uropathogens. Monomicrobial growth was more prevalent in community - acquired UTIs, while polymicrobial growth was associated with hospital - acquired UTIs. Escherichia coli was the most frequently identified pathogen in both culture techniques.

5. Conclusion

Standard Urine Culture is the gold standard in the diagnosis and treatment of UTI. But in symptomatic women, the majority of SUC fail to grow any uropathogens. Women are treated with empirical antibiotics, some recover while others manifest as recurrent UTIs or other complications. Enhanced Urine Culture detects more uropathogens and thus a more clinically relevant antibiotic susceptibility can be reported. It is recommended to re - incubate the SUC plates with no growth for another 24 hours so more organisms can be detected and this will lead to appropriate antibiotic therapy and prevent empirical treatment which may lead to many complications and the development of infection with multi - drug resistant bacteria. It is also suggested that in patients with UTI - like symptoms with a sterile report by standard urine culture method, Enhanced Urine Culture examination with extended volume may be performed.

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References

[1] Abou Heidar NF, Degheili JA, Yacoubian AA, Khauli RB. Management of urinary tract infection in women: A practical approach for everyday practice. Urol Ann 2019; 11: 339 - 46
[2] Medina M, Castillo - Pino E. An introduction to the epidemiology and burden of urinary tract infections. Ther Adv Urol.2019 May 2; 11: 17562872198 32172. doi: 10.1177/1756287219832172. PMID: 31105774; PMCID: PMC6502976.
[3] ProcopG. W, Church D. L, Hall G. S, Janda W. M, Koneman E. W, SchreckenbergerP. C, Woods G. L. Koneman'sColor Atlas &Textbook of Diagnostic Microbiology.7th edition. Wolters Kluver.2017.81 - 85.
[4] Bjerklund Johansen, T. E.; Bonkat, G.; Cai, T.; Tandogdu, Z.; Wagenlehner, F.; Grabe, M. Grey Zones in the Field of Urinary Tract Infections. Eur. Urol. Focus 2016, 2, 460–462.
[5] Hilt, E. E.; McKinley, K.; Pearce, M. M.; Rosenfeld, A. B.; Zilliox, M. J.; Mueller, E. R.; Brubaker, L.; Gai, X.; Wolfe, A. J.; Schreckenberger, P. C. Urine is not sterile: Use of enhanced urine culture techniques to detect resident bacterial flora in the adult female bladder. J. Clin. Microbiol.2014, 52, 871–876.
[6] Coorevits, L. et al. (2016) "The resident microflora of voided midstream urine of healthy controls: Standard versus expanded urine culture protocols, " European Journal of Clinical Microbiology & Infectious Diseases, 36 (4), pp.635–639. Available at: https: //doi. org/10.1007/s10096 - 016 - 2839 - x
[7] Thapaliya, J., Khadka, P., Thapa, S. et al. Enhanced quantitative urine culture technique, a slight modification, in detecting under - diagnosed pediatric urinary tract infection. BMC Res Notes 13, 5 (2020).
[8] Price, T. K. et al. (2016) "The clinical urine culture: Enhanced techniques improve detection of clinically relevant microorganisms, " Journal of Clinical Microbiology, 54 (5), pp.1216–1222. Available at: https: //doi. org/10.1128/jcm.00044 - 16.
[9] Patel HB, Soni ST, Bhagyalaxmi A, Patel NM. Causative agents of urinary tract infections and their antimicrobial susceptibility patterns at a referral center in Western India: An audit to help clinicians prevent antibiotic misuse. J Family Med Prim Care 2019; 8: 154 - 9.