Original Research Paper

Microbiology

Evaluation of triphenyl tetrazolium chloride (TTC) test for quick diagnosis of Urinary tract infection (UTI) and for knowing its utility for follow up of patients on treatment by using its Optical density (OD).

Ingole Kishor V.	Professor and Head, Department of Microbiology, Dr. V M Government medical college, Solapur Maharashtra.
Mundhada Sapana G.	Assistant Professor, Department of Microbiology, Dr. V M Government medical college, Solapur Maharashtra.
Bhise Mayuri P.	Postgraduate student, Department of Microbiology, Dr. V M Government medical college, Solapur Maharashtra.
Powar Rajaram M.	Dean, Department of Microbiology, Dr. V M Government medical college, Solapur Maharashtra.

ABSTRACT

Aim: To evaluate reliability and simplicity of TTC test for diagnosis of UTI and its use for follow up of patient to check their response to prescribe antibiotic.
Methodology: Total 360 urine sample were collected with aseptic precaution and each of them was subjected for semi quantitative culture and for TTC test. Formation of red precipitate means positive test indicating significant bacteriuria. Bacterial growths were identified by standard biochemical procedure. TTC test was also use for follow up of patient to check response to antibiotics. **Results:** Out of 360 samples tested 189 (52.50%) had significant bacteriuria by semi quantitative culture. TTC test had showed 89.94% correlation with culture. All TTC test positive patients were properly followed on 3rd and if required 6th day of treatment to check response to prescribe antibiotic. **Conclusion:** TTC test is simple, reliable and economical for diagnosis and follow up of patients in resource limited area.

KEYWORDS

Optical density, semiquantitative culture, screening tests, TTC test.

Introduction: Urinary tract infections (UTIs) are the most common bacterial infections during pregnancy. Asymptomatic bacteriuria (ASB) is a major risk factor for the development of UTI during pregnancy. ASB is defined as persistently & actively multiplying bacteria in significant numbers i.e., $\geq 10^5$ bacteria per millilitre(ml) within the urinary tract without any obvious symptoms.[1,2] This is due to urinary stasis due to progesterone effect in pregnancy in addition to different morphological & physiological changes occurring during pregnancy.

Detection of ASB in antenatal women is important, as undetected and untreated ASB may lead to symptomatic infection during that pregnancy in 25% of culture positive patients, acute pyelonephritis, hypertension, postpartum UTI, anaemia, preterm labour in pregnant women, low birth weight & perinatal death of the foetus. [3,4] Due to this consequences it is important to screen pregnant women for ASB and treat them.

Urine culture by semi quantitative method is the gold standard screening technique for ASB during pregnancy but it is time consuming and increases workload of laboratory.[5,6] Hence a simple and reliable screening test for the diagnosis of significant bacteriuria would be of considerable value to the clinician and clinical microbiologist. It has been shown that urinary pathogens were able to reduce colourless soluble 2-3- 5 triphenyl tetrazolium chloride (TTC) to red coloured precipitate of triphenyl formazan by the metabolic activity of bacteria.[1] The formazan compound giving the coloured precipitate indicate significant bacteriuria i.e. colony count 10^5/ml or more.[7] The present study was aimed i) To evaluate reliability and simplicity of TTC test for diagnosis of UTI and ii) To evaluate its use for follow up of patient to check their response to prescribe antibiotic therapy.

Material and Methods: The study was conducted in at tertiary care hospital in the Department of microbiology from September 2015 to December 2015 in women coming for their antenatal check-up in obstetric OPD. They were asked to collect midstream urine sample in a sterile wide mouth container after proper explanation. Total 360 urine sample were collected and each of

them was divided into 2 parts, one for culture and other for TTC test.

The urine samples were processed within 1-2 hours of collection. The semi-quantitative culture was done on blood agar and MacConkey agar by standard loop method and incubated at 37^0C for 18-24 hours. The identification of organisms was done by standard microbiological procedures. [8] Growth was interpreted as Significant if colony count 10^5/ml or more. The antibiotic susceptibility testing (AST) was done by Kirby-Bauer disc diffusion method on Muller Hinton agar for following antibiotics - Norfloxacin (10μg), Nitrofurantoin (300 μg), Cephalexin (30 μg), Amoxicillin (25 μg), Gentamicin (10μg) and Cotrimoxazole (1.25/23.75 μg) (Hi-media Mumbai). [9] The TTC test was performed by adding 2 ml. of urine to a test tube containing the 0.5ml of TTC reagent. The tube was incubated at 37^0C for 4 hours and was examined against a white background under a fixed magnifying glass for the characteristic red triphenyl formazan precipitate, indicative of a positive test. [10] Optical density (OD) of positive test was calculated with the help of ELISA reader. (TransAsia Bio-medicals Mumbai) OD value depends on intensity of red colour which directly depends on bacterial count/ml of sample.

All patients with the significant bacteriuria were advised to take treatment based on their AST report. Patient with significant bacteriuria who had showed positive TTC test on their 1st day sample were again followed by using TTC test on 3rd and if required 6th day of their antibiotic course. If patient had responded well to antibiotic therapy bacterial count in urine will fall down compare to their initial count. Depending upon bacterial count TTC test either become negative or positive. If positive then optical density is calculated and compare with optical density of 1st day sample. To check result of TTC test during follow up semi-quantitative culture was performed as control for positive test.

Results: Among the 360 urine samples tested 189 (52.50%) showed significant bacteriuria (colony count 10^5/ml or above by semi-quantitative culture). Out of 189 sample showing significant

bacteriuria, 170 (89.94%) were positive by TTC test. Optical density (OD) was calculated for TTC test positive sample for follow up purpose. The test was considered false negative in 19 (10.05%) samples showing significant bacteriuria. 171(47.50) urine samples had not showed significant bacterial growth on culture but among them 2 showed positive TTC test giving 1.17% false positivity.(Table I)

Distribution of microorganism in samples (189) showing significant bacteriuria is as shown in Table II. Gram negative organisms were isolated from 157 samples, showing 96.17% correlation with positive TTC test. While gram positive organism were grown in 32 samples showing 59.37% correlation. Among 189 urine samples showing significant bacteriuria, E.coli (50.79%) was most common microorganism isolated followed by Proteus species (15.34%), Klebsiella pneumonia (13.22%), Enterococcus faecalis (9.53%), Staphylococcus aureus (4.23%), Pseudomonas aeruginosa (3.71%) and Staphylococcus saprophyticus (3.18%). For all the microorganisms isolated (Gram positive and Gram negative), nitrofurantoin (88.36%) was the most sensitive drug followed by norfloxacin (80.42%), Cotrimoxazole (69.31%), cephalexin (62.43%), gentamicin (57.14%) and amoxicillin (50.79%).

Antibiotic treatment was started to all patient having significant bacteriuria depending on AST report. They were followed on 3rd and 6th day of antibiotic course by using TTC test to check patient response. (Table III) Total 170 patients sample showing significant bacteriuria and positive TTC test on visit were followed on 3rd day. Among them 112 sample were negative on TTC test while 58 were still positive. OD value for each positive sample was calculated and compared with OD value of respective sample taken on day of visit. Out of 58 samples, 56 had showed decrease in OD value which indicate fall in bacterial count. These patients were advised to continue their treatment and come again for follow up on 6th day. While 2 patients sample were shown no change in their OD values i.e. no response to prescribe antibiotic in vivo. Another antibiotic was prescribe to them and they were also called for follow up again. During follow up on 6th day, TTC test was performed on 58 samples. 57 samples were negative and single sample was positive but there was fall in its OD value. It was one of those 2 sample for which antibiotic was change on 3rd day follow up. This patient was advised to complete antibiotic course. Semi quantitative culture was perform for samples who had showed positive TTC test during follow up of patient as a control.

Discussion: According to Purres et al [11], screening test for detection of significant bacteriuria must be reliable, simple and inexpensive. To be reliable it must correlate well with the bacterial count in the urine; that is, it must yield a minimum of false-positive and false-negative results. In terms of simplicity, the test should involve a minimum of manipulations and require no special laboratory or personnel to perform it, and the positive and negative results should be clear-cut and easily read. In our study we had evaluated TTC test for above mention points.

In present study, TTC test positivity was 89.84% well correlated with significant bacterial count with less number of false positive (1.17%) and false negative (10.05%) results. Similar finding of higher correlation were noted in other studies by Agrawal et al [12] (81.3%), Simmons et al [13] (94%) and Purres et al [11] (92.5 %). Studies by Wagle et al [7] (0.23%) and Simmon et al [13] (2%) had reported low false positivity rate of TTC test which was comparable to finding of our study (1.17%). The frequency of positive TTC test was directly related to number of bacteria per ml of samples and not by other non-specific factors like glucose, ketones, urobilinogen , cast, crystals, number of pus cells, RBCs in sample.[7,10] Similar finding were noted in present study that TTC test positivity rate was 100% if sample contain bacteria >10^7 per ml. In study by Hinton et al [10] and Umar et al [14], significant difference was observed between TTC test positivity and type of pathogen isolated. In present study we had observed such significant difference which was in contrast to finding by Wagle et al. [7] Gram negative organisms had showed 96.17% correlation

with positive TTC test while gram positive had only 59.37% correlation. According to Hnatko et al [15], highest correlation of TTC test positivity was obtained when the urine sample showing significant bacteriuria contained E. coli or one of the enteric bacteria. Similarly in our study, E.coli and Klebsiella pneumonia had showed 100% correlation with TTC test positivity. Other studies by Wagle et al [7], Agrawal et al [12], and Hardas et al [16] were noted similar results.

In present study follow up of patient was done with the help of TTC test to check their response to prescribe antibiotic. Negative test indicated that patient had responded well to prescribe antibiotic. If test is positive, optical density (OD) was calculated. Fall in OD value compared to 1st day value of respective sample indicate decrease in bacterial count per ml of sample i.e. good response to antibiotic. No change or increase in optical density indicate that patient had not responded to antibiotic and need change in it. Out of 170 patients followed on 3rd day and 6th day, 112 and 57 had showed negative TTC test respectively. On 6th day follow up single sample was showing positive TTC test but with fall in its OD value indicating good prognosis. In present study, we were able to follow our all patients properly to check their response to prescribe antibiotic by using TTC test and its OD value. Till our best knowledge there were no similar studies for comparison purpose who had used TTC test and its OD value for follow up of patients showing significant bacteriuria.

According to our study, TTC test is a simple and reliable test for not only detecting significant bacteriuria but also for follow up of patients to check their response to prescribe antibiotic in resource limited area for microbiologist as well as physician. Its use also reduce workload and prevent unnecessary use of resources by reducing sample size requiring culture.

Conclusion- TTC test is an economical, simple and quick method which makes it suitable for screening large number of samples for the detection of significant bacteriuria in suspected cases of UTI specially in high risk group and resource limited areas. It does not appear to be in any respect a replacement for quantitative culture where adequate laboratory facilities are available. In present study, we had first time evaluated use of TTC test and its optical density (OD) for follow up of patients with significant bacteriuria to check their response to prescribe antibiotic and we got good response. For performing this test expertise and highly equipped laboratory not required. Depending on our result, we would like to recommend use of TTC test for follow up of patients in resource limited area with high patient pool.

Table I: Correlation between Viable Count in Urine and TTC Test

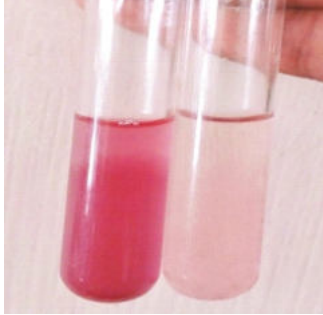| Colony count /ml	Semi-quantitative culture No.(n = 360)	%	TTC Test Positive	Negative
No Growth	124	34.44	0	124
< 10^5	47	13.06	2	45
10^5 to 10^7	113	31.38	94	19
>10^7	76	21.12	76	0

Table II: Distribution of microorganism in samples showing significant bacteriuria and their correlation with TTC test positivity.

Significant bacteriuria (n=189) (colony count 105/ml or above)	Number of isolate (189)	TTC test positive (170)	Correlation of Significant bacteriuria with positive TTC test (%)
Gram negative isolates	**157**	**151**	**96.17**
E.coli	96	96	100
Proteus species	29	26	89.65
Klebsiella pneumonia	25	25	100
P.aeruginosa	7	4	57.14
Gram positive isolates	**32**	**19**	**59.37**
Enterococcus faecalis	18	11	61.12
Staphylococcus aureus	8	5	62.5

| Staphylococcus saprophyticus	6	3	50

Table III: Follow up of patients showing significant bacteriuria and TTC test positive on 1st visit by using TTC test.

Number of sample showing significant bacteriuria and TTC test positive on 1st visit.	Follow up of patient during Antibiotic course by using TTC Test.			
	3rd day		5th day	
	Positive	Negative	Positive	Negative
170	58	112	1	57

Photograph no 1: TTC test
Left side–Positive test, Right side-Negative test
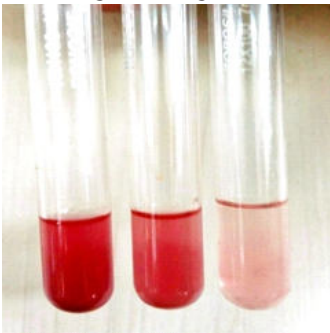
Photograph no 2: TTC test result on follow up (From left to right)
i) Positive on the day of Visit and OD- 0.124, on culture > 10^7 colony count/ml
ii) Positive on 3rd day and OD- 0.094, on culture >10^5 but <10^7 colony count/ml
iii) Negative on 6th day, on culture <10^5 colony count/ml)

References:

1. Hankins GD, Whalley PJ. Acute urinary tract infections in pregnancy. Clin Obstet Gynaecol 1985;28:266-78.
2. Jayalakshmi J, Jayaram VS. Evaluation of various screening tests to detect asymptomatic bacteriuria in pregnant women. Indian J Pathol Microbiol 2008;51:379-81.
3. Kerure SB, Surpur R, Sagarad SS, Hegadi S. Asymptomatic bacteriuria among pregnant women. Int J Reprod Contracept Obstet Gynecol 2013;2:213-16.
4. Dr.Kerure RD, Dr.Umashanker. Prevalence of asymptomatic bacteriuria among pregnant women in tertiary care hospital. International Journal of Scientific and Research Publications 2013;Vol3(11):1-3.
5. Patterson TF, Andriole VT. Bacteriuria in pregnancy. Infect Disclin North Am. 1987;1(4):807–22.
6. Gayathree I, Shetty S, Deshpande SR , Venkatesh DT. Screening for asymptomatic bacteriuria in pregnancy. An evaluation of various screening tests in Hassan District Hospital, India. JCDR 2010;4(4):2702-706.
7. Wagle N, Vaidya A, Joshi S, Merchant S. Triphenyl Tetrazolium Chloride (TTC) Dye Test for Quick Diagnosis of Urinary Tract Infection. Indian Journal of Pediatrics1989 sept;Vol56(5):635-38.
8. Forbes BA, Sahm DF, Weissfeld AS. Overview of bacterial identification methods and strategies. Bailey and Scott's Diagnostic Microbiology. 12th ed. Missouri: Mosby Elsevier;2007.p.218-47.
9. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Second Informational Supplement. CLSI document M100 S24. Wayne, PA: Clinical and Laboratory Standards Institute; 2014.
10. Hinton NA, Hoeve E. The Detection of Significant Bacteriuria: An Assessment of the Triphenyltetrazolium Chloride Reduction Test. Canad. Med. Ass. J.1965;Vol93:639-42.
11. Purres J, Jaworskiz F. A comparative study of the triphenyltetrazolium chloride (Uroscreen) test and conventional methods for detection of bacteriuria. Canad. Med. Ass. J.1965;Vol92:1161-65.
12. Agrawal SK, Das R, Goel M, Kumar M. Tetrazolium reduction test in diagnosis of urinary tract infections. Indian I Pathol Microbiol 1986;29:61-65.
13. Simmons NA, Williams JD. Use of a solid reagent in the triphenyl tetrazolium chloride test for bacteriuria. J Clin Pathol. 1967 Sept;20(5):767–69.
14. Dr. Umar N, Dr. Sirwar SB. Prevalance of urinary tract infection in pregnant women. Journal of evolution of medical and dental science 2012 oct;Vol1(4):315-20.
15. Hnatko SI. The Triphenyl Tetrazolium Chloride (Uroscreen) Test Alone and in Combination with the Gram Smear as a Screening Procedure for Significant Bacteriuria in Hospital Patients. Canad. Med. Ass. J. 1966 July;Vol95:10-13.
16. Hardas UD, Iurmarkar RV. Evaluation of TTC for detection of significant bacteriuria. Indian I Pathol Bacteriol 1969;12:25-27.