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A COMPARATIVE STUDY OF TRIPHENYLTETRAZOLIUM CHLORIDE (TTC) TEST AND URINARY CULTURE IN DETECTION OF ASYMPTOMATIC BACTERIURIA IN SCHOOL CHILDREN.

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ABSTRACT

Objectives: The present study was undertaken to study the accuracy of TriphenylTetrazolium Chloride (TTC) Test as a screening procedure for detecting asymptomatic bacteriuria (ASB) in school children.

Materials and Methods: A total of 1000 healthy children of both sexes aged between 5 to 14 years were selected from schools of Mysore city. A midstream clean catch specimen of urine was collected and transported immediately to the laboratory. Urine specimens were screened for significant bacteriuria by culture and TTC Test. The results were recorded and calculated for sensitivity of TTC Test.

Results: TTC Test was positive in 39 (3.9%) cases while culture was positive in 11(1.1%) cases. There was a female preponderance among culture positive cases (90.9%). All culture positive cases were positive for TTC Test. The sensitivity of TTC Test was 100%.

Conclusion: TTC Test can be applied as a screening test for bacteriuria due to its high sensitivity in resource limited settings.

INTRODUCTION

Asymptomatic Bacteriuria (ASB) is defined as the presence of significant number of bacteriain the urine of a person without symptoms[1]. In school aged children, the reported prevalence of ASB range from 0.12% to 1.8% with female preponderance[1,2]. Detection of significant bacteriuria in children might lead to detection of treatable urinary tract abnormalities and prevention of renal scarring, hypertension and renal insufficiency[3]. Culture is the gold standard method to detect significant bacteriuria but is time consuming, expensive and requires laboratory facilities with trained personnel. Hence a simple and reliable screening test for the diagnosis of significant bacteriuria would be of considerable value to the clinician. Among the various chemical tests devised, TTC test introduced by Simmons and Williams in 1962 has shown the greatest promise.TTC test is based on the observation that respiring bacteria in the urine reduce the colourless 2-3-5- TTC to pink or red colored precipitate of triphenylformazan. The formazan compound giving the colored precipitate indicates significantbacteriuria (colony counts $\geqslant 10^5$/ml). Reagent can be easily prepared in the laboratory[4]. A high degree of correlation between TTC test and significant bacteriuria has also been reported[5-7]. The present study was therefore undertaken to know the sensitivity of TTC test in detecting significant bacteriuria.

MATERIALS AND METHODS

This cross sectional study included 1000 school children aged 5-14 years from different schools in Mysore city.Before each school was visited, the Principal/ Headmaster/ Headmistress was notified and informed consent was taken. Mid - stream clean catch method of urine collection was explained to the students and urine specimens were collected in sterile bottles. Urine samples were transported to the laboratory within half to one hour and tested immediately.

Urine culture:

Specimens were cultured in MacConkey's agar, Blood agar and Thioglycolate agar. A culture was said to be positive when the bacterial colony count was $\geqslant 10^5$/ml.

TTC test:

Preparation of working solution of TTC reagent:750 mg of TTC was dissolved in 100 ml of saturated solution of disodium hydrogen phosphate to make it alkaline as

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reduction of TTC takes place only at an alkaline pH. A working solution was prepared by taking 4 ml of the solution and diluting it to 100 ml with water. 2 ml of urine was poured into a test tube and 0.5 ml of the working solution of TTC reagent was added with a sterile pipette, shaken thoroughly and incubated at37°Cfor 4 hours. At the end of 4 hours the deposit was examined with the naked eye. A positive result was shown by a red precipitate and a negative test by the absence of red precipitate.

The results of TTC test were analyzed and correlated with the results of culture.

RESULTS

A total of 1000 children aged between 5-14 years were enrolled in the study.Table 1 depicts the age and sex distribution. Out of 1000 children investigated for ASB, TTC test was negative in 961 cases and positive in 3.9% (39/1000). Culture showed significant colony counts in 1.1% (11/1000)of cases.Out of the 11 culture positives,90.9% was present in girls. Results of TST test and cultureare summarised in Table 2. Sensitivity of TTC test observed was 100% as all the culture positive cases were also TTC test positive. True negative cases was also 100% as all the TTC Test negative cases were also negative by culture.

The common organisms isolated were E.coli followed by Klebsiella.

Table 2: Comparison of Urine Culture and TTC Test

Culture	TTC Test		Total
	Positive	Negative	
Positive	11	0	11
Negative	28	0	28
Total	39	0	39

DISCUSSION

Urinary tract infections (UTI) are the third most common bacterial infections in children in developing countries. They may remain unrecognised because of vague and non specific symptoms and in some instances they may be asymptomatic[8]. Symptomatic and ASB in infants is generally characterised by a benign outcome. However, in some children episodes of renal damage has been reported[9]. Moreover in children ASB may be a sign of underlying urinary tract abnormalities hence the need for a simple test capable of detecting significant bacteriuria.

The incidence of ASB in school children varies among different studies[1,2,10]. In the present study the incidence was 1.1%. Approximately 5-6% of girls have atleast 1 episode of bacteriuria between first grade and their graduation from high school and as many as 80% of cases experience recurrent infections with higher risk of developing bacteriuria during pregnancy[11].In the present study ASB was higher in girls (10/11) compared to that of boys. Kumar et al[10] have also an increased incidence of ASB in girls aged 11-15 years ref thereby stressing the need for screening tests in school children particularly girls.

The reliability of TTC Test in detecting ASB was evaluated in our study and the sensitivity was 100% as all the culture positive cases were also TTC Test positive which is consistent with a study conducted by Hnatko[6] in which the sensitivity of the test was 97.2%. The false positive results of TTC Test obtained in our study might be due to contamination of urine with bacteria from external genitalia.

The organisms most frequently isolated in ASB and UTI include Enterobacteriaeceae especially E.coli and Gram negative bacilli[2,10,12-13]. In our study the predominant organisms isolated were E. coli and Klebsiella which was similar to other studies[2,10,13].

TTC Test is very simple to apply to large number of urine samples and the results agree well with the results of culture. The test is not only reliable but is also very easy to perform. It can be carried out in any place provided with an electrical outlet for plugging in an office incubator. Performing of even a dozen tests requires only a few hours. The tubes are ready for examination in 4 hours and in the meantime one is free for other duties. Reading of results is rapid and in majority of instances the negative results can be easily recognised. Finally the cost is not prohibitive and is very cheap.

Although the TTC Test appears sufficiently reliable and practical to be adopted as a screening procedure for bacteriuria, it has no place in the diagnosis or

investigation of symptomatic UTI which should be investigated by accepted methods to determine the etiology of infection and the offending organism[14]. This type of investigation becomes mandatory once ASB is disclosed by any screening procedure.

CONCLUSION

Our study has shown that ASB was present in 1.1% of school children of which 90.9% was in girls. The TTC Test shows good correlation with bacterial counts, is inexpensive and simple to perform. It can be used as an alternative to culture for screening purposes in resource limited settings.

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