

Full Length Research Paper

An evaluation of leukocyte esterase activity as a rapid screening test for significant bacteriuria in children

Eyong K. I.^{1*}, Anah M. U.¹, Udo J. J.¹, Jombo G. T. A.², Ewa A. U.¹, Etuk I. S.¹ and Asindi A. A.¹

¹Department of Paediatrics, University of Calabar Teaching Hospital Calabar, Nigeria.

²Department of Medical Microbiology and Parasitology, College of Health Sciences, Benue State University Makurdi, Nigeria.

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The diagnosis of UTI is usually confirmed by microscopy and culture of properly collected urine specimens; however, due to scarce resources and other limitations, this is often not practicable in many resource poor nations. Since UTI if not identified early and treated could lead to serious complications, this study was therefore carried out to ascertain the clinical importance of Leukocyte esterase (LE) enzyme as a diagnostic tool for screening of Urinary Tract Infections (UTIs) in resource poor countries. The study was cross-sectional in nature comprising 250 asymptomatic pupils (120 males and 130 females) drawn from five nursery schools in Ikot Ansa in Calabar municipal area council in Nigeria. Subjects were selected using computer assisted random sampling methods. Urine specimens were collected, stored, transported, cultured and processed using standard laboratory procedures, while leukocyte esterase (LE) dipsticks were used as a screening tool for UTI and results compared with culture positive results. Significant bacteriuria through culture was recorded in 14 (5.6%) pupils and the commonest bacteria recovered were *Escherichia coli* 42.9% (6), *Proteus mirabilis* 21.5% (3) and *Klebsiella pneumoniae* 14.5% (2). Leukocyte esterase dipstick test correctly identified positive urine culture in 10 of 14 proven UTI (71.4%). The positive and negative predictive values were 25 and 98.1%, respectively. Leukocyte esterase test, though it has limitations in diagnosing UTI when compared to the culture methods, still proves useful in communities without facilities and requisite personnel for urine culture. The test is recommended for use in result limited communities, but where feasible, urine culture should be done.

Key words: Children, leukocyte esterase, screening, urinary tract infection.

INTRODUCTION

Urinary tract infection (UTI) is among the most common diseases in childhood (Bircan, 2002). The prevalence of UTI varies between 0.4 and 7.5% in different childhood populations and there is no age limit in this condition; the newborns are even susceptible (Bircan, 2002; Eyong et al., 2009). The symptoms of UTI vary with age; these include fever, frequency of urination, dysuria, foul-smelling urine and enuresis among others (Bircan, 2002; Elders, 2004).

Prompt diagnosis of all cases of UTI in children through a simple, sensitive test is desirable to initiate timely treatment to relief symptoms and minimize risk of renal scarring and other complications of UTI (Mori et al., 2007).

The standard test for diagnosis of UTI is urine culture yielding a colony count of greater than 10^5 cfu/ml of a pure growth of bacteria (Kass, 1962). However; this method requires an incubation period of 24 h or more and this could cause delay in the treatment of acutely ill children. Urine microscopy, looking for leucocytes and pus cells can provide immediate diagnostic information to enable the initiation of treatment; however, this requires

*Corresponding author. E-mail: komomoeyong@yahoo.com.

examination by trained staff and use of specialized equipment, which may not be available in resource poor nations.

Dipstick testing of freshly voided urine for leucocytes indicating the presence of white cells as in microscopy is particularly convenient, suitable for use at home or bedside and requires less skill (Whiting et al., 2006). Leukocyte esterase dipstick test proposed by Perry et al. (1982) demonstrates the presence of pyuria by histochemical method. Leukocyte esterase enzyme is a marker of pyuria from neutrophils found in infected urine. It is based on the enzymatic cleavage of an indoxyl ester contained in the test pad by esterase from leukocytes. The indoxyl released reacts with a diazonium salt to form a violet dye and gives a colour intensity proportional to the amount of leukocyte in the urine (Perry et al., 1982).

Laboratory facilities in most of the tropical and subtropical regions of the world are often confronted with varying degrees of challenges ranging from absent or inadequate quality equipment and reagents to unqualified personnel, as well as operational logistics (Jombo et al., 2006a, b, c). These pose serious challenges towards effective healthcare delivery. Furthermore, these could lead to morbidities and mortalities from diseases that could have been prevented through effective laboratory surveillance and screening mechanisms (Okwara, 2004; Hageman, 2003; Cassone, 2004). Methods to solve these problems should be encouraged.

The use of leukocyte esterase as a screening test for UTI has not been recently evaluated in our environment. This study was therefore undertaken to evaluate dipstick leukocyte esterase method in the diagnosis of UTI in this community with a view to recommend its use in both screening, as well as diagnosis of UTIs in resource poor communities so as to avert the long term complications of UTI in children (Effective Health Care, 2004; Scott, 1989).

MATERIALS AND METHODS

Setting

The study was carried out at Ikot Ansa, a community in Calabar Municipal council area. Based on the 2006 population census, the community has an estimated population of 30, 000 inhabitants. The major (over 95%) ethnic group is Efik who are over 99% Christians, however, the community is inhabited by people from other tribes in Cross River state and Nigeria notably, Ejaghama, Ibibio, Annang and Ibo. The community hosts five nursery schools, four primary schools, and two secondary schools. A primary healthcare centre and several patent medicine shops serve the community.

Sampling procedure

The study was carried out between May and July 2006. Subjects were selected using computer assisted random sampling methods involving the various classes and gender. All the five nursery schools in the area were used for the study.

Prior to commencement of the study, visits were made to individual schools to discuss the purpose of the study with the

school authorities. They liaised with parents/guardians of pupils to obtain written consents on behalf of their wards. With the aid of the serial numbers of children in the class registers, subjects were recruited with the aid of statistical table of random numbers in to the study. Structured questionnaires were administered to the parents/guardians of the recruited subjects to obtain information on: Age of pupils, history of urinary frequency, abdominal pain, bed wetting, educational status and qualifications of parents.

Measurement of biophysical parameters

Anthropometric and blood pressure measurements were carried out on the subjects using standards methods. Pupils were weighed using calibrated Beam balance and readings adjusted to the nearest 0.5 Kg; height was measured using an erect meter rule placed against a perpendicular wall; and blood pressure was measured using a standard mercury sphygmomanometer (Accoson).

Urine collection, transport and culture

Clean catch mid stream urine specimens were obtained from the subjects through normal voiding into sterile universal specimen containers (4 to 8 mls). Urine collection was carried out with the assistance of laboratory assistants at the respective schools. Specimens were processed using standard laboratory procedures shortly after collection or were stored in refrigerator at 4 to 8°C in an event of delay. Significant bacteriuria was determined using a graduated wire loop of internal diameter of 4 mm and uncentrifuged, uniformly mixed urine specimens were inoculated on CLED (Cysteine Lactose Electrolyte Deficient), blood agar, chocolate and Mc-Conkey agar media and incubated at 36.6°C overnight (Scott, 1989). Other sets of culture plates were incubated in Carbon dioxide Extinction Jar at the same temperature for isolation of anaerobes. Biochemical tests, such as catalase, oxidase, sugar fermentation, motility, citrate, urease, indole, hydrogen sulfide and gas production were subsequently carried out on significant growths based on the bacterial gram reactions, while antimicrobial susceptibility tests were carried out using modified Kirby-Bauer's diffusion methods where zones of inhibition were measured (Baron, 1994; Chessbrough, 2004). Those with positive urine culture results were treated appropriately.

Inclusion criteria

All children aged less than 60 months were selected through the sampling procedure, and consented for by parents or care givers.

Exclusion criteria

This involves children whose parents or care givers declined their involvement in the study and children 60 months and above.

Dipstick test

Screening urine for leukocytes esterase was done using multistix urine test strip, batch number: (IVD) CE PBA 9309/UTSH/01.1. The strip was immersed into freshly voided urine briefly at room temperature for about two seconds. The colour reaction was read by comparing the colour of the immersed strip with the code provided on the container after 60 s (National Committee for Clinical Laboratory Standards, 2003).

Table 1. Age and gender distribution of the nursery school pupils at Ikot Ansa, Calabar.

Age(Months)	Males (%)	Females (%)	Total (%)
24	22 (8.8)	31 (12.4)	53 (21.2)
25-35	32 (12.8)	34 (13.6)	66 (26.4)
36-47	35 (14.0)	33 (13.2)	68 (27.2)
48-59	31 (12.4)	32 (12.8)	63 (25.2)
Total	120 (48.0)	130 (52.0)	250 (100)250

Table 2. Bacteria recovered from urine specimens of pupils with sub-clinical significant bacteriuria at Ikot Ansa, Calabar.

Organism	Number of isolate	Percentage
<i>Escherichia coli</i>	6	42.9
<i>Proteus mirabilis</i>	3	21.5
<i>Klebsiella pneumoniae</i>	2	14.3
<i>Pseudomonas aeruginosa</i>	1	7.1
<i>Enterobacter spp.</i>	1	7.1
<i>Staphylococcus aureus</i>	1	7.1
Total	14	100.0

Data management and analysis

Data obtained were analysed using simple descriptive methods of arithmetic sum, mean, and frequency (Sahm, 2001).

Ethical considerations

Ethical approval for the study was obtained from the ethical committee of the University of Calabar Teaching Hospital (UCTH) Calabar, Nigeria. A written informed consent from the parents and permission from the heads of the nursery schools were obtained.

RESULTS

Out of a total of 250 children recruited into the study, 120 (48%) were males and 130 (52%) females (Table 1).

Significant bacteriuria was recorded in 5.6% (14 of the 250) of the pupils. *Escherichia coli* was the commonest organism isolated 42.9% (6 out of 14), followed by *Proteus mirabilis* 21.5% (3 out of 14), and *Klebsiella pneumoniae* 14.3% (2 out of 14), while *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterobacter* were isolated from one child each representing 7.1% (Table 2).

A review of the activity profile of Leukocyte esterase dipstick method vis-à-vis the culture method showed that 71.4% (10 out of 14) of those positive for significant bacteriuria by culture method were also positive by

dipstick method, while 86.6% (206 out of 238) of those negative by culture method were also negative by dipstick method. Thus, the test has a low positive predictive value of 25% and a high negative predictive value of 98.1%.

DISCUSSION

In this study, the dipstick Leucocyte Esterase (LE) test was found to have a sensitivity of 71.4% and a specificity of 86.6% (Figure 1). The positive and negative predictive values were 25.0 and 98.1%, respectively. This implies that using the dipstick LE test alone to detect pyuria would result in a large number of false-positive and some false-negative results. Our study shows a higher sensitivity compared to the study by Hoberman and Wald (1994) who had a sensitivity of 52.9% and a positive predictive value of 82.1%, but the specificity was not reported in their study in febrile children younger than 2 years. The reason for this difference is not clear, but shows the variability of this test. However, the positive and negative predictive values were similar to that of Wammanda et al. (1999) in Nigeria and Wiggelinkhuizen et al. (1988) in South Africa. The need to look for a more reliable test is further stress by these differences as some patients with pyuria and possible UTI would be overlooked and this could be dangerous. It is of note that the risk of renal damage from UTI is greatest in children younger than 5 years; thus, early diagnosis and prompt

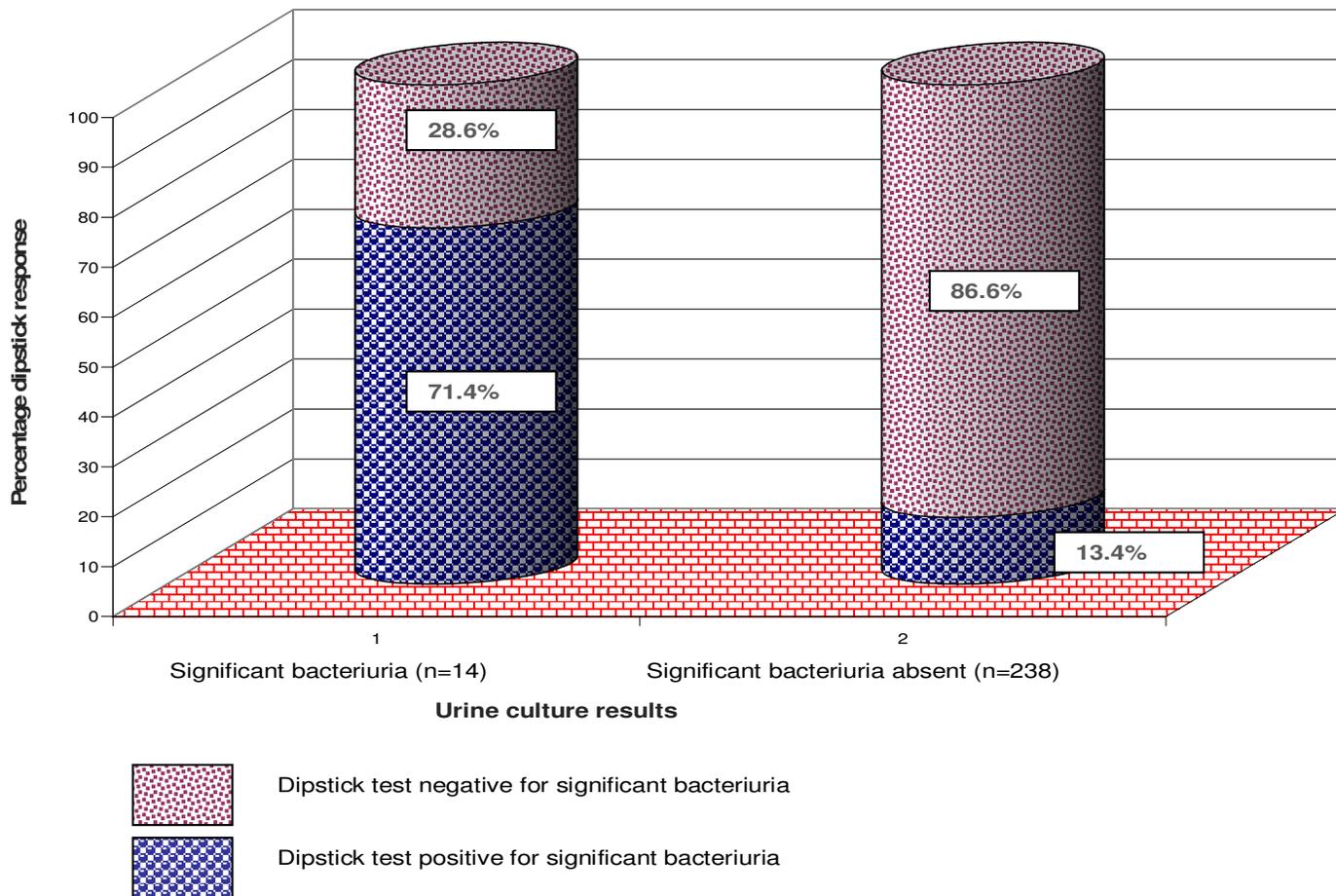


Figure 1. Comparison of Leukocyte esterase dipstick test with urine culture method among nursery school pupils in Ikot Ansa, Calabar.

treatment are important (Mohammed et al., 2008).

Interestingly, studies of the dipstick LE test in adults have shown that the test is both sensitive and specific in detecting pyuria (Hoberman and Wald, 1994; Mohammed et al., 2008). The differences between studies in adults and children might relate to either the degree of pyuria and the enzyme content of immature leukocytes, or both (Hoberman and Wald, 1994).

A further question that arises is whether the dipstick test could act as a screening test for selecting urine samples for microscopy. The high negative predictive value (98.1%) appears favourable in this regard though four (28.6%) of the 14 confirmed cases of UTI tested negative according to the LE test. If microscopic examination had not been performed, these four patients would not have been confirmed to have UTI. Hence, the dipstick LE test cannot be used solely as a screening test in selecting urine samples for microscopic examination or bacterial culture.

Although Leukocyte esterase dipstick method appear limited in applicability in diagnosis of UTIs in children, we support the view that the test still proves a veritable fall back tool for laboratory diagnosis in several urban and

rural communities across Africa where both requisite laboratory personnel and facilities for appropriate diagnosis are still lacking (Akpede and Akenzua, 2001).

In remote areas, it is not practicable to do microscopy and so, practitioners can fall back on LE test.

The limitations of the LE dipstick test should be factored into the final outcome of the test. These include the period in the course of the infection when the urine specimen was collected; the conditions of transport and storage, as well as the degree of precision in the interpretation of the test (Olowu and Oyetunji, 2003). These variables adversely affect the results of LE test. This position was also corroborated by Whiting et al. (2006) in Bristol, UK and Lammers et al. (2001) in Kalamazoo, USA in which both emphasized that the dipstick test, though useful, should not be interpreted in isolation, but along with culture results where practicable.

Conclusion

The present study has shown that Leukocyte esterase dipstick method for diagnosis of UTI has a high sensitivity

and specificity, but low positive predictive value. The test therefore, should not be interpreted in isolation, but along with culture results where practicable, as such, recommended for screening purposes in communities without adequate laboratory facilities.

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REFERENCES

- Akpede GO, Akenzua GI (2001). Management of children with prolonged fever of unknown origin and difficulties in the management of fever of unknown origin in children in developing countries. *Paediatr. Drugs*, 3(4): 247-262.
- Baron E, Peterson LR, Finegold SM (1994). Methods of testing antimicrobial effectiveness (In: Bailey and Scott's Diagnostic Microbiology). 9th edn.; U S A-Missouri, St Louis: Musby Publishers, pp. 168-188.
- Bircan Z (2002). Urinary tract infections and the Paediatrician. *Int. J. Paediatr.*, 17: 143-144.
- Cassone M, Campanile F, Pantosti A, Venditti M, Stefani F (2004). Identification of a variant "Rome clone" of methicillin-resistant *Staphylococcus aureus* with decreased susceptibility to vancomycin, responsible for an outbreak in an intensive care unit. *Microb. Drug Resist.*, 10(1): 43-49.
- Chessbrough M (2004). *Medical laboratory manual for Tropical Countries*. Cambridge; Cambridge University Press 2004, 4th edn., pp. 143-167.
- Effective Health Care (2004). Diagnosing urinary tract infection (UTI) in the under fives. *Centre Rev. Dissem.*, 8(6): e.
- Elders JS (2004). Urologic disorders in infants and children. In: Behrman RE, Kliegman RM, Jenson HB, eds. *Nelson textbook of Pediatrics*. 17th ed. Philadelphia: WB Saunders Company, pp. 1787-1789.
- Eyong KI, Asindi AA, Anah MU, Udo JJ, Jombo JT, Meremikwu MM (2009). Asymptomatic Bacteriuria Among Preschool Nigerian Children. *Nig. Med. Pract.*, 56: 40-42.
- Hageman JC, Fridkin SK, Mohammed JM, Steward CD, Gaynes RP, Tenover FC, NNISSH (2003). Antimicrobial proficiency testing of national nosocomial infections surveillance system hospital laboratories. *Infect. Control Hosp. Epidemiol.*, 34(5): 356-361.
- Hoberman A, Wald ER (1994). Pyuria and bacteriuria in urine specimens obtained by catheter from young children with fever. *J. Pediatr.*, 124: 513-519.
- Jombo GTA, Ayilara AO, Bello K, Dakum NK, Enenebeaku MNO (2006c). Antimicrobial Susceptibility profiles of Uropathogenic Bacterial Isolates from Community- and Hospital-Acquired Urinary Tract Infections in Yobe State, Nigeria. *J. Med. Lab. Sci.*, 14(2): 54-61.
- Jombo GTA, Egah DZ, Ayeni JA (2006b). Antibiotic susceptibility patterns of bacterial isolates from urine samples of acquired immunodeficiency syndrome (AIDS) patients in Jos, Nigeria. *Mary Slessor J. Med.*, 6(2): 40-49.
- Jombo GTA, Egah DZ, Banwat EB, Ayeni JA (2006a). Nosocomial and Community Acquired urinary tract infections at a teaching hospital in north central Nigeria: Findings from a study of 12,458 urine samples. *Nig. J. Med.*, 15(3): 230-236.
- Kass EH (1962). Pyelonephritis and bacteriuria. A major problem in preventive medicine. *Ann. Int. Med.*, 56: 46-53.
- Lammers RL, Gibson S, Kovacs D, Sears W, Strachan G (2001). Comparison of test characteristics of urine dipstick and urinalysis at various test cut off points. *Ann. Emerg. Med.*, 38(5): 505-512.
- Mohammed W, Abu G, Anahtar K (2008). Screening Test for Detection of Urinary Tract Infections: Evaluation of the Urinary Leukocyte Esterase Dipstick Test. *TAF Prev. Med. Bull.*, 7: 187-190.
- Mori R, Lakhanpaul M, Verrier-Jones K (2007). Diagnosis and management of urinary tract infection in children: Summary of NICE guidance. *BMJ*, 335: 395-397.
- National Committee for Clinical Laboratory Standards (NCCLS) (2003). Performance standards for antimicrobial disk susceptibility tests; Approved standard - Eighth edition. Wayne, PA: NCCLS, 2003. NCCLS document no. M02-A8.
- Okwara FN, Obimbo EM, Wafula EM, Murila FV (2004). Bacteremia, urinary tract infection and malaria in hospitalized febrile children in Nairobi: is there an association? *East Afr. Med. J.*, 81(1): 47-51.
- Olowu WA, Oyetunji TG (2003). Nosocomial significant bacteriuria: Prevalence and pattern of bacterial pathogens among children hospitalized for non-infective urinary tract disorders. *West Afr. J. Med.*, 22(1): 72-75.
- Perry JL, Mathews JS, Seesner DE (1982). Evaluation of leukocyte esterase activity as a rapid screening technique for bacteriuria. *J. Clin. Microbiol.*, 15: 852-854.
- Sahm DF (2001). Surveillance of antimicrobial resistance patterns and data for urinary tract pathogens in the USA. *Clin. Drug Invest.*, 21(Suppl. 1): 1-5.
- Scott AC (1989). Laboratory control of antimicrobial therapy. In: Mackie and McCartney Practical medical microbiology (Edited by Collee JG, Duguid JP, Fraser AG and Marmion B P) 13th Edn. Vol. 2, United Kingdom-Edinburgh: Churchill Livingstone, pp. 161-181.
- Wammamanda RD, Aikhionbare HA, Ogala WN (1999). Evaluation of leukocyte esterase Dipstick test in the diagnosis of UTI in children. *Nig. J. Paediatr.*, 26: 21-24.
- Whiting P, Westwood M, Bojke L, Palmer S, Richardson G, Cooper J (2006). Clinical and cost-effectiveness of tests for the diagnosis and evaluation of urinary tract infection (UTI) in children: A systematic review and economic model. *Health Technol. Assess.*, p. 10.
- Whiting P, Westwood M, Bojke L, Palmer S, Richardson G, Cooper J (2006). Clinical effectiveness and cost-effectiveness of tests for the diagnosis and investigation of urinary tract infection in children: A systematic review and economic survival. *Health Technol. Assess.*, 10(36): iii-iv, xi-xiii.
- Wiggelinkhuizen J, Matten D, Hanalo DH (1988). Dipstick screening for urinary tract Infection. *S. Afr. Med. J.*, 74: 22-24.