UGANDA MARTYRS UNIVERSITY

MOTHER KEVIN POSTGRADUATE MEDICAL SCHOOL NSAMBYA

PREDICTIVE VALUE OF DIPSTICK AND MICROSCOPY IN DIAGNOSIS OF URINARY TRACT INFECTIONS AMONG UNDER-FIVES PRESENTING WITH FEVER TO NSAMBYA HOSPITAL.

BY

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DEDICATION

I dedicate this work to my family: Dr. Tabule Frank, my dear husband for his support and encouragement, our children Alejo Anne Nicole and Drileba Isaac Pendo Phillip, for being very understanding, loving and patient. To my beloved Mum, Marie Luija Azikuru Ezati (Mrs.), for all the love, prayers and encouragement, my siblings; Juliet, Michael,Stella,Emmanuel and Benedict and my dearest cousins; Susan and Esther for all the support and encouragement.

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LIST OF ABBREVIATIONS:

UTI…….Urinary Tract Infection

POPD…….Paediatric out Patients' Department

SPA……..Supra pubic aspiration

CFU……..Colony forming unit

E. Coli……Escherichia coli

LE……..Leucocyte Esterase

PPV…….Positive predictive value

NPV…….Negative predictive value

WBC……White Blood Cell

RBC……..Red Blood Cell

CLED……Cystein- Lactose Electrolyte Deficient

HPF…….High Power Field

ml…….milliliter

µl…….microlitre

°C…….Degrees celcius

WHO……World Health Organization

OPERATIONAL DEFINITIONS

Urinary tract infection: Presence of bacterial pathogens anywhere in the urinary tract (kidney, ureters, bladder and/or urethra)(Steven L. Chang, 2006)

Fever: Axillary temperature ≥ 37.5ºC

Positive dipstick Was defined by positive leucocyte esterase or positive nitrite or both.

Significant pyuria: Presence of more than 5WBCs/HPF in a deposit of centrifuged urine sample.

Positive microscopy Was defined as positive leucocytes, ≥5WBCs/HPF or presence of bacteria on gram-stain or both.

Sensitivity: Ability of a test to correctly identify people who have a disease

Specificity: Ability of a test to correctly identify people who do not have a disease.

True positives: People with disease who test positive.

False positives: People without disease who test positive.

True negatives: People without disease who test negative

False negatives: People with disease who test negative.

Positive predictive value: Probability that the disease is present when the test is positive.

Negative predictive value: Probability of having no disease when the test is negative

ABSTRACT

Background: Urinary tract infection (UTI) is a common paediatric problem with the potential to cause long-term complications, globally. Children < 5 years often present with non-specific symptoms and signs making the diagnosis of urinary infection challenging. Fever may be the only sign of UTI in under-fives. Effective treatment depends on clinicians' high index of suspicion and laboratory urine test results. Early diagnosis of UTI is essential to institute prompt treatment and reduce lifelong morbidity. Urine dipstick for nitrites and leucocyte esterase, and microscopy for leucocytes and bacteriuria are good screening tests to select urine specimens for culture which is the gold standard for UTI diagnosis. The screening tests provide more rapid information than urine culture, which could be utilized as decision-making tool for initiating treatment of UTI.

Objective: To determine the predictive value of a combination of urine dipstick and microscopy in diagnosing UTI in children.

Methodology: A cross-sectional study was conducted at the paediatric out patients' department of Nsambya Hospital from December 2013 to April 2014.Children <5 years presenting with fever were approached and inquiries made about their prior antibiotic use. All children with temperatures ≥37.5^{o}C and aged ≤59 months who had not been on antibiotic therapy 48 hours prior to hospital visit were enrolled into the study upon obtaining consent from their parents/caretakers. Basic information was filled in a data collection form. Two urine samples were collected from every participant-one for urine dipstick and microscopy and the other for urine culture. Data was entered in a computer using Epidata version 3.1 and analyzed using SPSS version 19 software.

Findings: The sensitivity, specificity, positive predictive value and negative predictive value of combined urine dipstick and microscopy were 98.8%, 95%, 87.9% and 99.5% respectively. Urine dipstick alone had sensitivity of 46.9%, specificity 95.5%, positive predictive value of 79.2% and negative predictive value of 83.1%.Urine microscopy used alone had sensitivity of 95.1%, specificity 98.2%, positive predictive value 95.1% and negative predictive value of 98.2%.

Conclusions: A combination of urine dipstick and microscopy is reliable in the diagnosis of UTI

Recommendation: The MoH of Uganda should continue prioritizing the use of microscopy for

the diagnosis of UTI among children under-five years of age.

CHAPTER ONE

INTRODUCTION

1.0 Introduction

Urinary tract infection(UTI) refers to presence of pathogens, mostly bacteria, anywhere in the urinary tract, from the kidneys to the urethra(Steven L. Chang, 2006). There are generally two types-upper and lower UTI. Lower urinary infection involves the urethra (urethritis) and bladder (cystitis) and is less severe .Upper urinary tract infection, on the other hand refers to pyelonephritis which is more severe and can lead to renal injury with eventual scarring (pyelonephritic scarring) and renal function impairment. The perineal flora constitute the normal microbial flora of the distal urethra and voiding with a steady flow helps to washout contaminating bacteria. Normal urine is sterile. Pathogens, gain entry to the urethra and ascend upward to the bladder and may reach the ureters (causing ureteritis) and most significantly the kidneys (pyelonephritis).

Worldwide, UTI occurs among 2.7% of boys in their first year of life, and this prevalence decreases to 0.03% - 1.2% during school years. In the case of girls, 0.7% experience urinary infection during the first 12 months of life and this increases to 1% -3% during school years(Shortliffe and McCue, 2002a). In developing countries, UTI among under fives with fever has been estimated to be 8.08%(Wei-Chuan Chen, 2010.) In Uganda, a study done in the acute care unit of Mulago hospital in 2008, on children aged 2months to 12years with fever, showed a prevalence of 14.6% in this age group(Ojambo, 2008). An earlier study in Mulago hospital by Jolly Nankunda in 1995 on children aged 1 month to 12months presenting with fever had shown a prevalence of 13%(Nankunda, 1995).

Early diagnosis of infection in the urinary tract is very important in order to institute appropriate treatment and prevent renal damage and long term complications. The gold standard for diagnosis of urinary infection is urine culture which requires a high level of technical competence, is costly, and takes long to produce results. Urine dipstick for nitrites and leucocyte esterase, and microscopy for leucocytes plus presence of bacteria on gram stain are quicker and cheaper screening tests used to select urine specimens for culture and sensitivity(Kathy et al., 1998). Studies have shown that dipstick negative for leucocyte esterase and nitrite or microscopy negative for pyuria and bacteriuria may be used to rule out urinary tract infection and combinations of the positive tests could be used to rule in urinary infection(Whiting et al., 2005).

This study was designed to determine the predictive values of combined urine dipstick and microscopy in diagnosing UTI in children under- five years presenting with fever, against the gold standard.

1.1 Background to the study.

Urine infection is common in children and may present with fever, irritability, vomiting and failure to feed(WHO, 2013). These are non-specific and common presentations. Diagnosis is based on carrying out laboratory urine tests. A high index of suspicion is required in order to identify when to carry out the tests. Accurate and timely diagnosis of UTI is crucial to enable early treatment and avoid unnecessary treatment and costs. The recognized diagnostic test (the urine culture) is however difficult to perform and requires a high level laboratory set up, found only in a limited number of health institutions in developing countries-certainly in Uganda. The gold standard method of urine collection is suprapubic aspiration as it is associated with minimal contamination(Long and Vince, 2007).However, owing to its invasive nature, suprapubic urine aspiration is resisted by many people. The preferred methods of urine collection in children include bag collection, clean catch urine or midstream urine(SHETTIGAR et al., 2010). Often high index of suspicion plus urine microscopy and dipstick provide the diagnostic tools for managing UTI.

Urine dipsticks to detect presence of nitrites and /or leucocyte esterase are often used in the emergency department to screen for UTI due to their ease of use, rapidity and low cost. Using urine dipstick alone for screening patients may not reliably rule out UTI. Zorc et al in a clinical microbiology review of diagnosis and management of Paediatric UTI recommended back-up urine culture to detect 12% of UTIs that may be missed by the urine dipstick(Zorc et al., 2005). Urine microscopy to detect pus cells and bacteriuria is commonly used for the evaluation of patients with suspected UTI. It has been shown that presence of pyuria (>5WBCs/hpf) on microscopic examination is less sensitive and less specific than bacteriuria while presence of both pyuria and bacteriuria make the likelihood of UTI greater(VanDeVoorde, 2012).

1.2 Problem statement

Urinary tract infection is common in children, particularly under the age of five years presenting with fever. Yet, the diagnosis of UTI is often missed in children because the symptoms and signs are non specific. This leads to under diagnosis with eventual complications. On the other hand treatment of UTI is often based on suspicion and unclear diagnostic criteria.

In Uganda, there is scarcity of resources, both materials and skilled personnel especially in the lower health units to diagnose UTI using the gold standard (Urine culture). However, urine dipstick and microscopy can be made available in many centers and are easy to perform by the available laboratory personnel in these centers.

Dipstick and microscopy each used in isolation have been shown to lead to missing diagnosis of UTI in some cases as evidenced by studies quoted. Combination of dipstick and microscopy has been shown to produce better results. No studies have been done in Uganda to assess the accuracy of combined dipstick and microscopy.

1.3 Justification

Dipstick and microscopy may be available in lower health units and laboratory assistants, nursing staff and clinical officers can be trained to use them to screen out children with suspected UTI.

A Combination of urine dipstick and microscopy, if found to co-relate to urine culture could improve diagnosis of UTI and reduce incidences of complications.

The results from the study would support development of a protocol for the diagnosis of UTI at low level facilities.

This study sought to explore the reliability of combination of urine dipstick and urine microscopy as a basis for diagnosing UTI among children less than five years.

1.4 Research question

To what extent can combination of dipstick and microscopy accurately predict UTI among children less than five years of age presenting with fever?

OBJECTIVES

1.5 General objective

To determine the predictive values of combination of urine dipstick and microscopy in diagnosing UTI in children.

1.6 Specific objectives

1. To determine the sensitivity and specificity of combination of urine dipstick and microscopy in diagnosis of UTI in children.

2. To determine the sensitivity and specificity of urine dipstick in diagnosis of UTI in children.

3. To determine the sensitivity and specificity of urine microscopy in diagnosis of UTI in children.

CHAPTER TWO

LITERATURE REVIEW

Epidemiological studies have shown that occurrence of UTI varies by age and gender(Magliano et al., 2012). During infancy urinary infection is more common in boys, especially the uncircumcised ones, due to tendency of bacteria to accumulate under the prepuce and gain entry to the urethra(Ashkan, 2008). After infancy, girls are more likely to develop infection in the urinary tract because the short female urethra makes it easier for bacteria to move up the tract(Geoffrey A.Weinberg, 2006). Children are particularly prone to infection in the urinary tract due to several risk factors: congenital anomalies such as vesico-ureteric reflux, posterior urethral valves, and ureteric duplex have been associated with UTI in infants(50%) and in school-aged children (20%-30%) (Geoffrey A.Weinberg, 2006).) Other risk factors for urinary infection in children under age five years include young age (< 1year) and previous UTI(Zorc et al., 2005).

The American Family Physician journal has documented that by the age of 6 years, 7% of girls and 2 % of boys will have had symptomatic UTI. The prevalence of UTI in infants is greater with younger age(Brian S. Alper, 2005). A study done in a tertiary hospital in India revealed that prevalence of UTI in febrile preschool children aged 3 to 6 years was 4%; with female preponderance and a male to female ration of 1:1.5 (Ashok C, 2013). A cross-sectional analytical study conducted in Mwanza city, Tanzania, showed that prevalence of UTI in febrile under-fives was 20.3% (Bahati P Msaki, 2012).

2.2 Pathogenesis of UTI.

Infection of the urinary tract takes place when either the defence mechanisms are impaired or a virulent strain of bacteria has penetrated the urinary system.

Turbulent urine flow during normal voiding can facilitate entry of bacteria, from the distal urethra into the bladder; especially, following instrumentation (Stanley Hellerstein, 2002).

Disturbance of the normal peri urethral flora, following treatment of another disease, with broad spectrum antibiotics, can predispose to colonization of the distal urethra by pathogenic bacteria(Stanley Hellerstein, 2002).Stasis from obstruction also predisposes to UTI.

In the pathogenesis of UTI, the uropathogen first attaches to the epithelial lining of the urethra. Next, the pathogens spread throughout the mucosa with resultant tissue damage. Following the initial colonization, the pathogens then ascend into the urinary bladder, causing symptomatic or asymptomatic bacteriuria.

If the infection is unchecked, it can progress higher to cause pyelonephritis and renal scarring with resultant functional impairment.

2.3 Clinical features of UTI in children

In the under fives, UTI has been noted to have 2 peaks; the first peak occurs in infancy and the second occurs in 2 to 4 years of age. Frequency of UTI decreases after 6 years of life (Zorc, 2005.)

Clinical presentation of UTI in children is, generally, non specific(Mishra et al., 2013). Symptoms and signs depend on age. Thus, newborns may present with fever, vomiting, jaundice, sepsis and failure to thrive.

Infants and pre-school children commonly present with fever, hematuria, strong-smelling urine, abdominal or flank pain and new-onset urinary incontinence or nocturia(White, 2011). Infection in the urinary system is among the most common causes of fever in children under the age of five years and fever may be the only sign of UTI in this age group (Zorc et al., 2005). Shettigar *et al* studied urine cultures of 334 febrile children less than 5 years of age and found UTI prevalence of 8.08%. Twenty-four percent (24%) of these cases had fever as the only clinical presentation(SHETTIGAR et al., 2010).

Older (School-aged) children tend to have symptoms similar to adults-frequency, urgency or dysuria (White, 2011).

2.4 urine collection

Urine sample collection methods that are routinely employed include: suprapubic aspiration (SPA), catheter-specimen urine (CSU), bag-specimen urine (BSU) and clean catch or mid-stream urine (MSU)(Shortliffe and McCue, 2002b). Early morning urine specimen is preferred to allow for growth of optimal numbers of organisms per milliliter of urine. Following urine collection, urine processing should take place as soon as possible because microorganisms multiply rapidly in urine(PEZZLE, 1988).

SPA is taken as 'the "gold standard" of urine collection', as it greatly minimizes the risks of contamination (Long and Vince, 2007).

Elliot Long and John Vince having done Cochrane studies of systematic reviews, concluded that SPA and CSU are accurate methods of obtaining urine samples from infants who cannot void on command; and clean catch urine or MSU are methods of choice for urine sample collection(SHETTIGAR et al., 2010).

2.5 Diagnosis of UTI

2.5.1 Urine culture:

The gold standard for diagnosing UTI is urine culture of a suprapubic aspirate; or a sample obtained by catheterization or bag-specimen urine or clean catch, mid-stream urine sample. The significant bacterial growth is $\geq 10^5$CFU/ml of urine.

The urine specimen for culture should be processed within 2 hours of collection. Allowing urine to stand for too long increases false positive rates resulting from rapid multiplication of bacteria(Nader Snaikh, 2011)

2.5.2 Urine dipstick

The urine dipstick detects the presence of leucocyte esterase, nitrite, urobilinogen, glucose, bilirubin, ketones, blood, protein and the urine ph and specific gravity. The two important parameters for urine infection include urine nitrite and leucocyte esterase(Levart and Kenda, 2011).

The urinary nitrite is converted from dietary nitrates by the action of nitrate reductase enzyme produced by most gram-negative bacteria in urine(Roberts, 2011). The reaction requires urine to remain in the bladder for at least 4 hours. The nitrite test has a low sensitivity and high specificity. Hence, negative nitrite test results cannot reliably rule out UTI but positive tests are helpful with few false positive results(Roberts, 2011).

Leucocyte esterase detects leucocytes in urine by dipstick. The esterase released from neutrophils, following lysis of leucocytes, reacts with the ester on the reagent strip to release 3-OH-5-phenyl pyrrole which in turn reacts with a diazonium salt to produce pink to purple colour. The test is positive with leucocytes of ≥5/hpf (K Abirami, 2001, Abirami and Tiwari, 2001).

2.5.3 Urine microscopy

The most important parameters examined for in urine microscopy are leucocytes and bacteriuria. When a centrifuged sample of unstained urine is examined for WBCs (leucocytes), pyuria is defined as ≥5WBC/hpf. Bacteriuria is the presence of bacteria per HPF. The Leucocyte test has been noted to have high sensitivity but low specificity (Nader Snaikh, 2011).

2.6 Sensitivity and Specificity of microscopy and dipstick in diagnosis of UTI

2.6.1 Sensitivity and Specificity of urine dipstick

Whiting et al (2005) carried out a systematic review of rapid tests and urine sampling techniques in children under- five years of age. They reviewed the diagnostic accuracy of urine dipstick and found that the sensitivity of leucocyte esterase alone was 84% and the specificity was 77%.The sensitivity of nitrite alone was 58% with specificity of 99%.The sensitivity of leucocyte esterase in combination with nitrite was 92% with specificity of 70%(Penny Whiting, 2005).

A systematic review by NICE group in the United Kingdom found nine studies with twelve data sets examining use of urine dipstick in children where positive results from both leucocyte esterase and nitrite meant positive UTI. The sensitivity ranged from 30% to 89.2% and specificity ranged from 89.2% to 100%(National Collaborating Centre, 2007).

A meta-analysis by Gorelick MH and Shaw revealed that presence of either nitrite or leucocyte esterase had sensitivity of 88% and specificity of 96%(Gorelick and Shaw, 1999).

Shaikh and Hoberman studied urinary tract infection in childhood and reported urine dipstick sensitivity for nitrite and leucocyte esterase of 72% with specificity of 96%(Shaikh and Hoberman, 2010).

Epaphura et al conducted a cross sectional study in Tanzania to determine the predictors of urinary tract infection among febrile children of two months to five years of age. The total number of children recruited was 370 and the study revealed that sensitivity of leucocyte esterase was 8.8% and the specificity was 99.1% and that sensitivity of nitrite was 21.7% with specificity of 97%. They concluded that urine culture should be done in all children due to the low sensitivity of dipstick analysis(Epaphura Festo, 2011).

2.6.2 Sensitivity and Specificity of urine microscopy

Penny Whiting et al , in University of York in England, United Kingdom, did a systematic review of diagnosis of UTI in children under five years of age reported sensitivity of pyuria (>5WBCs) on microscopy as 78% with specificity of 87%.The sensitivity of bacteriuria was 88% with specificity of 93%(Penny Whiting, 2005).

Gatea, studying the sensitivity of microscopy for detecting UTI in children, in Babylon University, Iraq, analyzed urine samples of 81children aged three and half months to ten years. Sensitivity of urinary microscopy was high at 77% and the specificity was low at 36%. The positive predictive value was high while the negative predictive value was low. Gatea recommended that to get better results microscopy should be combined with dipstick test(Gatea, 2009).

Scott Moses of United States Navy, wrote in the Family Practice Notebook and put the sensitivity of leucocytes (≥5) on microscopy at 86% and specificity at 79%.According to Scott, sensitivity of urine bacteriuria present on microscopy is 93% with specificity of 40%(Moses, 2008).

In Uganda, Ojambo carried out a study on the prevalence, bacterial causes and antibiotic sensitivity of UTIs in children presenting with fever in Mulago Hospital acute care unit. He dealt with 486 children aged 2months to 12 years. He did urine dipstick and microscopy for all urine specimens collected. The findings of his study revealed presence of UTI in: 37 cases using presence of nitrites on dipstick, 16 cases basing on presence of leucocyte esterase on dipstick, 26 cases, basing on presence of bacteria on microscopy and 69 cases based on presence of WBC ≥5(Ojambo, 2008).

2.6.3 Sensitivity and Specificity of urine dipstick and microscopy combined

A systematic literature review on 402 articles about children between two months and two years of age with unexplained fever who were later found to have UTI was done. The review found sensitivity and specificity of components of urinalysis alone and in combination as shown below: Sensitivity of dipstick leucocyte esterase was 83% and the specificity was 78%; sensitivity of dipstick nitrite was 53% and specificity was 98%; sensitivity of leucocyte esterase or nitrite was 93% with specificity of 72%. Sensitivity of leucocytes on microscopy was 73% with specificity of 81%; the sensitivity of bacteria on microscopy was 81% and specificity was 83%.

The combination of urine dipstick and microscopy had a sensitivity of 99.8% and specificity of 70%(Alzalnia, 1999).

Robinson et al (2014) made a statement in the Canadian Paediatric association journal, focusing on diagnosis and management of acute UTI in infants and children> 2 months of age: They argued that using the different components of urine dipstick (nitrites and leucocyte esterase) and microscopy (pyuria and bacteriuria) could be associated with false positive results. The team stated that the combination of pyuria and bacteriuria on urinalysis should raise suspicion for a UTI. They further noted that a child with negative urine dipstick for nitrites and leucocyte esterase and no pyuria or bacteriuria on microscopic examination had < 1% chance of having a urinary infection.

Kathy et al in finding out what screening test is best for UTI in infants in the emergency department conducted a cross-sectional study on 3873 children aged <2 years. The urine specimens were collected by catheterization and subjected to dipstick, microscopy and culture. Their results showed that using microscopy to screen for when to send the urine for culture would eliminate 82% of cultures but 4% to 6% of the children with UTI would be missed(Kathy et al., 1998) .

Therefore a combination of urine dipstick and microscopy was thought likely to predict UTI better than when either screening test is used in isolation. Indeed, Wu and Wong working in a regional hospital in Hong Kong, China, conducted a retrospective review of two cohorts of children aged below 2 years. Data was collected to evaluate adequacy of screening for UTI by both dipstick and bedside microscopy in 87.6% cases on one hand and dipstick alone (63.9%) on the other hand. Wu and Wong reported sensitivity and specificity of urine dipstick alone for leucocyte esterase and nitrites as 88% to 93% and 72% to 93% respectively. They stated that by combining urine microscopy and dipstick tests one could increase the sensitivity to 99.8% with specificity of 70%, and pointed that 7% to 12% of genuine UTI cases would have been missed if only dipsticks were done(Wu and Wong, 2005) . Penny Whiting et al in their systematic review arrived at the conclusion that dipstick negative for leucocyte esterase and nitrite or microscopy negative for pyuria and bacteriuria may be used to rule out UTI and combinations of the positive tests could be used to rule in UTI(Penny Whiting, 2005).

2.6.4 Kappa statistics

Kappa statistics is a measure of agreement (Petrie A., 2000), which is often used to look at how accurately a test can be repeated (Taylor., 2008). Kappa is a way of assessing agreement between raters (Armitage P, 2002). In this study, it meant agreement between urine dipstick and the gold standard (culture) or between microscopy and culture or the agreement between the combination of dipstick and microscopy; and urine culture. It is only used with categorical data (ibid). Kappa statistic varies between 0 and 1 – with 1 showing perfect agreement and 0 showing agreement not different from chance. According to Di Eugenio (Di Eugenio B., 2000), having a Kappa of greater than zero is not sufficient to assess the quality of agreement, as such, various scales have been proposed to assess significance of Kappa. Accordingly, Krippendorff established the strictest scale. In this scale, Krippendorff discounts any variable with Kappa < 0.67, allows tentative conclusions when 0.67 < Kappa < 0.80 and definite conclusion when kappa >= 0.80(Krippendorff, 1980). Rietveld and Van Hout (1993) consider: 0.41 < Kappa < 0.60 as moderate agreement; 0.61 < Kappa < 0.80 as substantial agreement; and Kappa >= 0.80 as close to perfect agreement(Rietveld, 1993).

CHAPTER THREE

3.0 METHODOLOGY

3.1 STUDY SETTING:

The study was conducted at ST. Francis Hospital Nsambya, commonly known as Nsambya Hospital, at the Paediatric out patients' department.

3.1.1 Background to the study area

Nsambya hospital is a 361-bed hospital. It is a private not for profit, tertiary referral hospital. Nsambya hospital is, also, the teaching hospital for Mother Kevin postgraduate medical school of Uganda Martyrs' University. The hospital is located in the southern part of Kampala, about 3 kilometers from the city centre. Apart from offering services to the catchment area of Makindye West which has a population of 250,000 the hospital receives referrals from other facilities within Kampala, and beyond.

Nsambya hospital offers in patient care, research and teaching. It offers specialized services in Paediatrics, internal medicine, obstetrics and gynaecology, surgery and sub-specialties(Chief executive Officer Nsambya Hospital, 2011).

The paediatric department comprises the Paediatric Out Patients' Department (POPD), the paediatric specialists' clinic, St. Theresa ward and baby (neonatal care) unit.

All paediatric patients coming to the hospital are first attended to at the POPD, where: triaging is done to identify very sick patients. At the POPD, history is taken, physical examination done, and initial investigations are performed; as well as resuscitation of the very sick patients. On average, POPD receives 770 children less than five years of age in a month, with 300 presenting with fever(Nsambya hospital staff, 2013).

3.2 STUDY DESIGN:

It was a cross sectional study.

3.3 POPULATION

Children presenting at the POPD of Nsambya hospital

3.3.1 Target population

Children aged 59 months and below.

3.3.2 Study population
Children aged 59 months and below, presenting with fever.

3.3.3 Study unit
A child 59 months of age or younger with fever.

3.4 SELECTION CRITERIA

3.4.1 Inclusion criteria:
- Children, aged 59 months and younger coming to Nsambya hospital with fever whose parents/caretakers consented for the study.
- Temperature ≥ 37.5°C

3.4.2 Exclusion criteria:
- Children aged 59 months and younger with prior antibiotic use.

3.5 Sample size estimation
The sample size was estimated at 291, based on Buderer's formula:

Sample size (n) based on sensitivity= $\frac{Z^2_{1-\alpha/2} \times S_N \times (1-S_N)}{L^2 \times \text{Prevalence}}$

Where:

n= required sample size

S_N= anticipated sensitivity; 96% (0.96)

($Z_{1-\alpha/2}$)= standard normal deviate corresponding to the specified size of critical region estimated to be 1.96 at 95% level of confidence

L= absolute precision and in this study it was +/- 5%

Prevalence of UTI in children under the age of sixty months was 20.30% (0.203), based on a cross-sectional analytical study conducted on febrile under fives in Mwanza city, Tanzania(Msaki et al., 2012).This study was chosen because it was conducted in East Africa and it also looked at children under five years with fever, similar to the current study done in Nsambya. Earlier studies done in Uganda (Ojambo, 2008 and Nankunda, 1995) covered a wider age range (1month-12 years) though majority of cases of UTI were found among children below five years of age.

3.6 Sampling method

Between 8:30a.m and 1:30p.m daily, children presenting at Nsambya hospital POPD were screened to identify those between birth and sixty months, who came with a complaint of fever and had temperature of ≥ 37.5°C. The study subjects were consecutively enrolled over a period of 4 months.

3.7 Study variables

These included:

- Confirmed UTI by urine culture
- True positives using dipstick
- True negatives using dipstick
- False positives using dipstick
- False negatives using dipstick
- True positives using microscopy
- True negatives using microscopy
- False positives using microscopy
- False negatives using microscopy

3.8 STUDY PROCEDURE

3.8.1 Consenting procedure

Patients were approached at triage and those found to have axillary temperatures ≥37.5°C were considered possible candidates for the study, regardless of their presenting complaints. The ages of the children were inquired from their parents/caretakers to ascertain if the children were below 60months of age. Once found with fever (≥37.5°C) and below 60months of age, explanation about the study was given to the parents/caretakers of the children in the language best understood by the parent/caretaker. Any concerns/questions, arising about the study, were addressed. Parents/caretakers willing to have their children participate in the study were asked to sign a consent form. Those unable to write were asked to put their thumbprint on the consent form, which was translated in the local language (Luganda). Caretakers/parents were availed

with a copy of the consent form and a second copy of the consent form was filed in the patient's study records.

3.8.2 Recruitment and enrolment of participants

Any child presenting with fever of ≥ 37.5°C and below 60months of age was approached; a quick assessment of the child carried out to verify if he/she had taken prior medication. Children who had not been on treatment in the 48 hours preceding POPD visit or were only on paracetamol, were approached to be included in the study. Following explanation to the parent/caretaker by the principal investigator and/or a trained research assistant, consent was obtained. The duration of fever and body weight were recorded on a data collection guide form. Urine sample was then collected and immediately taken to Nsambya hospital laboratory for analysis.

3.8.3 Study instruments

- **Consent form**
- **Data collection guide**-this bore the date of visit, study number, outpatient number, child's age in months, child's gender, address and telephone number of the caretaker, duration of fever, body weight, results of urine dipstick (nitrite and leucocyte esterase), results of urine microscopy (leucocytes ≥ 5WBCs/HPF and presence of bacteria on gram stain), result of urine culture (10^5 or more bacteria per ml of fresh urine sample) and information on antibiotic sensitivity or resistance.

3.8.4 Collection of urine

This was done by the principal investigator or the 2 trained research assistants (nurses).Two urine specimens were collected from each patient, one for dipstick and microscopy and the other for culture. For children aged 2 years and below, the gold standard method of urine collection, suprapubic aspiration was not feasible due to its invasive nature; majority of the parents/caretakers refused to consent to the procedure. Therefore, patients' urine was collected using a standard adhesive sterile urine collection bag. In females, the bag was attached to the perineum following cleaning of the area with normal saline and drying with sterile gauze for the adhesion of the bag. In boys, the prepuce was retracted off and the area similarly cleaned and dried with gauze before the bag was attached to the perineum. The bags were left visible and one of the investigators kept observing for the urine in the bag and promptly removed the bag

immediately the urine was seen. The urine was transferred into 2 sterile laboratory urine bottles. Each bottle contained 5mls of urine.

For older children (above 2 years of age) midstream urine specimen (5mls) was collected into 2 sterile laboratory bottles: the perineum of the girl child was swabbed with normal saline and dried with sterile gauze before the child was led to squat over a clean pan and persuaded to pass urine. With the investigator's hands in sterile gloves, the girl's labia were spread apart as she passed urine. When the urine was in full flow, specimens were collected in the 2 sterile bottles. In the case of the male child, the penis was similarly cleaned with normal saline and the foreskin of the uncircumcised boy was retracted and the area underneath cleaned and dried with sterile gauze. The boy was left to stand naked; as he passed urine and mid-way, it was collected in 2 sterile laboratory urine bottles.

The 2 urine samples were properly labeled and taken to Nsambya hospital microbiology laboratory, located on the same block (2-storeyed building) as POPD, within 30 minutes of specimen collection and handed to the designated experienced laboratory technician in charge of urine examination. All the tests of urine dipstick, microscopy and culture were done by the same technician throughout the study period. A maximum of 15 urine samples were delivered per day to the laboratory between 8:30a.m and 1:30p.m

3.8.5 PROCESSING OF URINE

3.8.5.1 Materials and reagents:

These included: urine strips (10 parameters), microscope, centrifuge, cystein-Lactose electrolyte deficient (CLED) medium and culture incubator (35^{o}C-37°C).

3.8.5.2 Procedure:

Urine dipstick, microscopy and culture were done simultaneously. The urine dipstick and microscopy were performed on one of the urine samples and the second urine sample was cultured to identify organisms. Dipstick and microscopy were performed within 30 minutes to 1 hour of urine collection and culture was done within 1-2 hours from the time the urine specimen was collected.

3.8.5.3 Dipstick

The urine was examined for presence of nitrites and leucocyte esterase using a 10-parameter multi-reagent dipstick (multistix) strip supplied by cypress diagnostics (Langdorp city, Belgium). Two batches of dipsticks were used. The batch number of the first lot was 130715 and the expiry date was 2015.05; and the batch number of the second lot was 140312 and the expiry date was 2016.01.

A fresh test strip, from the multi-reagent strip container, was dipped in the urine sample, ensuring that all the test pads were immersed in the urine; and taken out immediately.

The excess urine on the strip was removed by drying the edge of the strip on an absorbent paper.

The manufacturer's instructions were adhered to by leaving the strip to stand for two minutes and the colours appearing on it were compared to the chromatin scale.

Urine was classified dipstick positive when either nitrite or leucocyte esterase or both, matched the colour on the chromatin scale of the manufacturer.

3.8.5.4 Microscopy

Microscopic urinalysis was performed on a centrifuged urine sample using Olympus microscope as described below:

Five mls of urine was transferred into a conical centrifuge tube and centrifuged at 3000 RPM for 5 minutes. The supernatant was decanted off into a jar containing a disinfectant, leaving the sediment in the tip of the tube. The sediment was shaken to make the mixture homogeneous.

3.8.5.4.1 Microscopic examination of the wet preparation

One drop of the homogenized urine sediment was placed on a clean dry glass slide which was then covered by a cover slip. The wet preparation was examined under a high power (X 40) microscope for presence of leucocytes. Presence of ≥5 leucocytes per high-powered field indicated pyuria.

3.8.5.4.2 Gram staining of the urine sediment

A drop of the homogenized urine sediment was placed on a new clean dry glass slide and evenly spread on the slide using the edge of a second glass slide in a sliding manner to make a thin smear. Gram staining was done according to the procedure described by Monica Cheesbrough(2006) as shown in appendix 111 on page 50(MonicaCheesbrough, 2006) . The dry smear was examined under a (X 100) microscope for bacteria. The gram positive bacteria appeared purple and the gram negative bacteria appeared pink.

Microscopy was considered positive with leucocyte count of ≥ 5WBCs/hpf or presence of bacteria on gram stain or both.

3.8.5.5 Urine culture

The bag or mid stream urine sample was cultured on Cystein-Lactose Electrolyte Deficient (CLED) culture medium. The culture medium was, first, dried at 37ºC for 15minutes-30minutes.

Urine specimen was innoculated on the culture medium using a nicrome wire loop which held 1/200µl of urine. The culture plate was incubated at 35°C-37ºC for 24hours.

The plate was examined after 24 hours and after 48 hours for colonies of isolates.

Discrete colonies growing within clear zone of inhibition were counted. Single colonies were picked out for sensitivity tests and identified directly from the CLED plate. Sensitivity testing was done by Kirby Bauer diffusion method.

The culture results were reported after 48 hours. Significant growth was indicated by colony counts of more than 10^5 organisms/ml (> 100,000 cfu per ml); which indicated infection.

3.8.5.6 Collection of laboratory results:

Each result, once ready, was placed in a special file in the laboratory. Results of urine dipstick and microscopy were collected on the same day that the tests were done and the urine culture results were collected on the 5^{th} day. The information on the laboratory result form was transferred onto a pre-prepared data collection form and later entered in Epidata 3.1.

3.9 QUALITY AND ACCURACY OF LABORATORY RESULTS

Precautions taken included:

- Having competent laboratory personnel who handled all urine samples and analysis himself.
- Immediate processing of the urine samples, aimed at the quality and reliability of the results.
- All the preparations and procedures done to cultivate or culture or grow organisms were done aseptically and sterility of the media and materials used was strictly observed.
- Exposing the organisms that had grown to varied biochemical tests to rule out contaminants, whereby the biochemical reactions for contaminants did not match the true reactions for an organism. The colonial appearance of one organism differed from another on the culture plates.

- For the urine microscopy and the biochemical strip test, they were controlled by a urinometer machine that measured up to 11 parameters for the biochemical tests including, leucocytes, nitrites, and leucocyte esterase among others.
- The laboratory used is a 4-star laboratory, due for accreditation to the International organization for standardization (ISO 15189) in October, 2014. There were two monthly external quality control checks of the laboratory.

3.10 DATA MANAGEMENT AND ANALYSIS

Data was entered and cleaned using Epidata 3.1 software.

Data was analyzed using SPSS version 19.

The urine culture was the gold standard to identify children with and without UTI against which the sensitivity and specificity of the urine dipstick and microscopy were measured.

The sensitivity and specificity of each dipstick and microscopy were established independently and later in combination. Sensitivity and specificity for combination of dipstick and microscopy were done simultaneously. Combination of any 2 or 3 positive tests increases the sensitivity and a negative result when combining 2 or 3 tests produces a higher negative predictive value (Othman et al., 2003).

Two –by-two table Conventions

	Disease (based on urine culture test**)**		
Test(dipstick or microscopy or combination)	**Present**	**Absent**	**Total**
Positive	True Positive a	False Positive b	a + b
Negative	False Negative c	True Negative d	c + d
Total	a+c	b+d	a+b+c+d

Where: a=True positives, b=False positives, c=False negatives, d=True negatives

Cross tabulations using 2x2 contingency tables comparing each of the urinalysis markers towards the urine culture were constructed, as illustrated in the above dummy table. The results were presented in the 2x2 table of the combination of urine dipstick and microscopy against urine culture. The urine culture was regarded as the gold standard test. The sensitivity, specificity, positive predictive value and negative predictive value where calculated as shown below. Individual tests of sensitivity, specificity, positive predictive value and negative predictive value were reported as percentages.

$$\text{Sensitivity} = \frac{a}{a + c} \times 100 = \text{Disease (UTI) patients correctly identified}$$

$$\text{Specificity} = \frac{d}{d + b} \times 100 = \text{Non-disease patients correctly identified as healthy.}$$

Positive predictive value = $\frac{a}{a+b}$ X 100

Negative predictive value = $\frac{d}{d+c}$ X 100

3.10 Quality control and quality assurance

Basic data was checked for validity.

Double data entry was employed to ensure the liability.

Special codes were assigned to all the children to prevent missing or multiple entries of data.

3.11 Ethical considerations

Ethical approval to conduct this study was sought from department of paediatrics and child health of Nsambya hospital, Institutional Review Board of Nsambya hospital and Uganda National Council of Science and Technology.

Participation in the study was voluntary.

Informed consent was obtained from parents/caretakers after offering to them explanations about the study.

Participants were told to feel free to withdraw from the study at any point, in case they felt the need to, without any consequences on their medical care in Nsambya hospital.

All study patients received routine care for their fever with antipyretics. Results of the urine tests were given to the patients as they came out; with explanations on the result offered by the principal investigator. In the event that the results delayed the participants were contacted on phone, by the principal investigator, through their caretakers to come for the results. Patients identified as having UTI by urine culture and sensitivity results were put on appropriate treatment by the principal investigator who called the caretakers of these patients by phone to bring back the patients to the hospital for their definitive treatment.

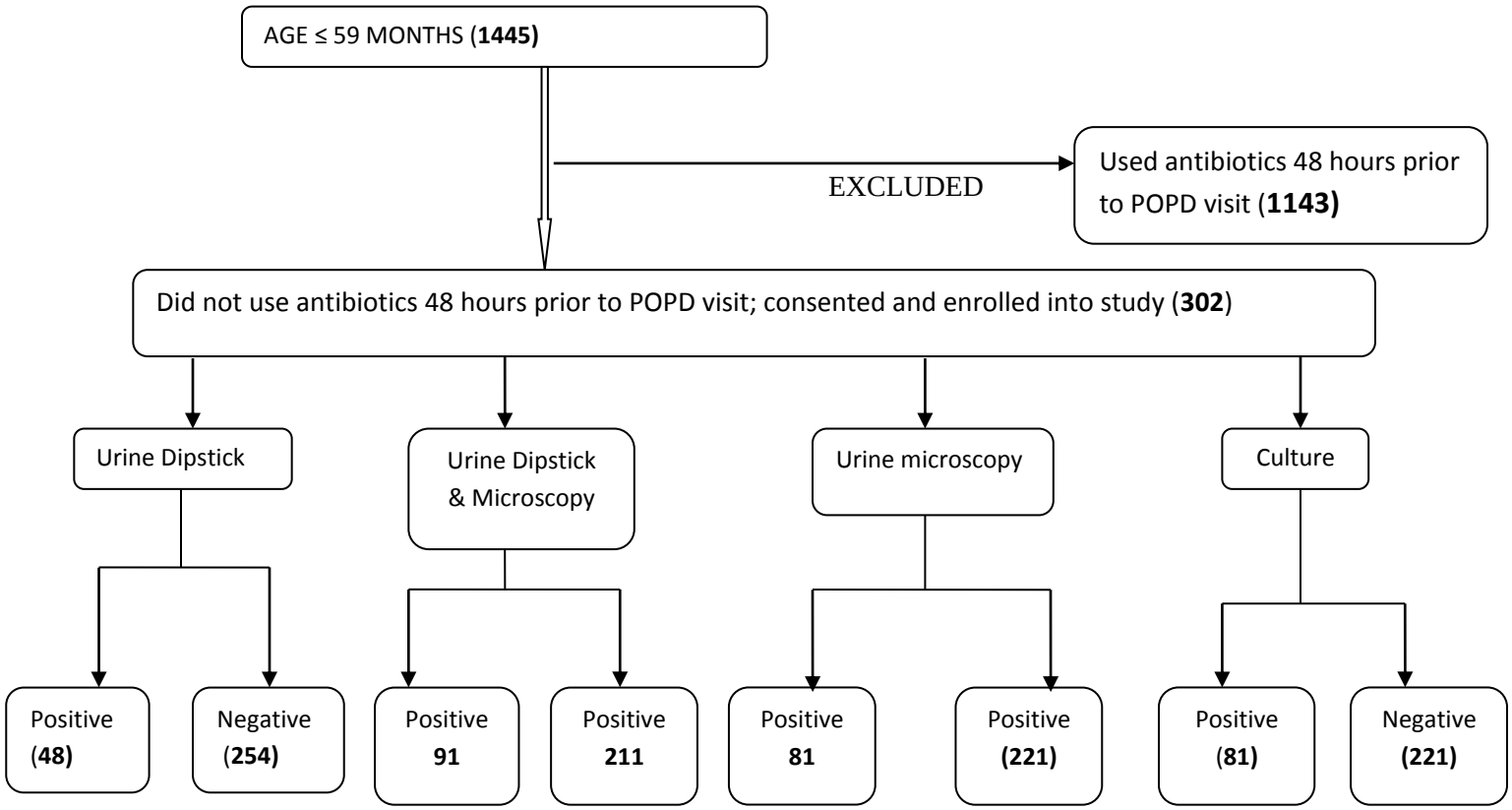
CHAPTER FOUR

RESULTS

4.1 Response rate

A total of three hundred and two (302) children aged 0 to 59 months presenting with fever to Nsambya hospital paediatric out patients' department were recruited into the study during the period between 17th December, 2013 and 18th April, 2014. This represents a response rate of 103.80%.

4.2 study profile



4.3 Socio-demographic and clinical characteristics of study participants

Majority, 171 (56.60%) of the children were male and 131(43.40%) were female. The mean age and standard deviation were 22.60 and 15.40 months respectively. More than one quarter, 97(32.10%) of the children were between 1-12 months of age. There were more males than females in the first month of life and between 13months and 48 months. All children enrolled into the study had fever with the temperature ranging from 37.5°C-40°C. The mean fever duration was 2.6 days (SD=2.10). Table 1, below, shows the age and sex distribution of the study participants.

Table 1:

Age and sex distribution of the under-fives at paediatric out patients' department, who participated in the study between December, 2013 and April, 2014.

Age category	Male	Female	Total
Less than 1 month	6(75%)	2(25%)	8
1-12 months	47(48.50%)	50(51.50%)	97
13-24 months	47(65.30%)	25(34.70%)	72
25-36 months	36(54.50%)	30(45.50%)	66
37-48 months	34(63.00%)	20(37.00%)	54
49-59months	1(20.00%)	4(80.00%)	5
Total	**171(56.60%)**	**131(43.40%)**	**302**

4.4 Prevalence of urinary tract infection

Eighty-one children had positive urine cultures; the prevalence of UTI was therefore 26.80%.

4.4.1: Prevalence of urinary tract infections by age

Eight (8) of the study participants were below 1 month of age and only 1 had positive urine culture result and hence UTI. The prevalence of UTI among children aged less than 1 month of age was therefore 12.50%, which was the lowest in all the age categories of the study participants. Table 2 shows the prevalence of urinary infections by age.

Table 2: Prevalence of urinary tract infections by age

Age category	Culture Results		
	Positive	**Negative**	**Total**
less than 1 month	**1(12.50%)**	7(87.50%)	8(100.00%)
1-12 months	28(28.90%)	69(71.10%)	97(100.00%)
13-24 months	15(20.80%)	57(79.20%)	72(100.00%)
25-36 months	15(22.70%)	51(77.30%)	66(100.00%)
37-48 months	19(35.20%)	35(64.80%)	54(100.00%)
49-59months	3(60.00%)	2(40.00%)	5(100.00%)
Total	**81(26.80%)**	**221(73.20%)**	**302(100.00%)**

4.5: Sensitivity, specificity, positive predictive value and negative predictive value of dipstick and microscopy in identifying urinary tract infections

A 2 x 2 table was constructed and used to calculate the sensitivities, specificities and predictive values of urine dipstick, microscopy and the combination. The sensitivity, specificity and predictive values were computed based on the culture results as gold standard.

4.5.1: Summary of results

Table 3 shows the summary of sensitivities, specificities, positive predictive values, negative predictive values and accuracies of dipstick, microscopy and the combination of dipstick and microscopy.

Table 3: Summary of the sensitivities, specificities and predictive values of urine dipstick, microscopy and combination of dipstick and microscopy for the under-fives at paediatric out patients' department who participated in the study.

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Observed level of agreement (%)
Dipstick	46.90	95.50	79.20	83.10	82.50
Microscopy	95.10	98.20	95.10	98.20	97.40
Combined dipstick/microscopy	98.80	95.00	87.90	99.50	96.00

From table 3, above, sensitivity of combined dipstick and microscopy is higher than sensitivities of either dipstick or microscopy alone.

The specificity of combined dipstick and microscopy is similar to that of dipstick alone, but lower than the specificity of microscopy alone. The positive predictive value of combined dipstick and microscopy is higher than the positive predictive value of dipstick alone but lower than the positive predictive value of microscopy alone. The negative predictive value of the combination of urine dipstick and microscopy is much higher than the negative predictive values of either dipstick or microscopy alone.

The observed level of agreement was highest with microscopy, followed by combined dipstick and microscopy with dipstick showing the least observed level of agreement.

4.5.2: Combined dipstick and microscopy

The research team combined dipstick and microscopy in order to ascertain predictive validity of the tests in combination. Table 4 summarises the findings.

Table 4: Results of combined dipstick and Microscopy against Culture for the paediatric out patients' department under-fives who participated in the study between December, 2013 and April, 2014.

		Culture(gold standard)			
	Results	Positive	Negative	Total	Kappa statistics
Combined dipstick and microscopy (test)	Positive	80	11	91	0.90
	Negative	1	210	211	
	Total	81	221	302	

From table 4, the sensitivity of urine dipstick and microscopy (combined) is very high. The combined tests showed close to gold standard sensitivity of 98.80%. They showed relatively lower specificity of 95.00% compared to that of the two tests when assessed independently. At UTI prevalence of 26.80%, the positive predictive value of microscopy was 87.90% and the negative predictive value was almost at gold standard (99.50%). The observed level of agreement was high at 96.00% with very strong level of agreement of 0.90 using kappa statistic and this observation is statistically significant (p-value < 0.01).

4.5.3: Dipstick

The sensitivity, specificity, positive predictive value, and negative predictive value of the dipstick test were 46.90%, 95.50%, 79.20%, and 83.10% respectively. The kappa statistics was used to assess the level of agreement between urine dipstick and culture.

The level of agreement using the kappa statistics between dipstick test and the gold standard (culture) was 0.49. This indicates that there was only moderate agreement between dipstick and culture.

4.5.4: Microscopy

Urine microscopy showed high levels of validity with sensitivity and specificity values greater than 90% each (sensitivity of 95%; and specificity of 98.20%). At UTI prevalence of 26.80%, the positive predictive value of microscopy was 95.10% while the negative predictive value was similarly high at 98.20%. The observed level of agreement between urine culture and microscopy was 97.40%. Kappa statistic showed a very strong level of agreement of 0.93 and this was statistically significant (p-value < 0.01).

CHAPTER FIVE

DISCUSSION, STUDY STRENGTH AND LIMITATIONS

5.1 Introduction

In this chapter the key findings of the study are discussed in relation to existing literature and implications of the findings herein.

5.2 Discussion

The prevalence of UTI in febrile children under the age of 5 years in Nsambya Hospital was 26.80% which was double the prevalence of UTI in children reported in previous studies conducted in Uganda. The earlier Ugandan studies had a different study population – having looked at children aged 1 month to 12 months of age (Nankunda et al. 1995; and Ojambo, 2008). The observed deviation in prevalence could have been attributable to age difference in the study populations. The determinants of urinary tract infection in children include pyuria (5 WBCs/hpf), presence of bacteria in urine, positive urinary leucocyte esterase and positive urinary nitrite test; however, the prevalence of UTI varies by age and sex. The prevalence of UTI in the present study is comparable to that of a study done in Mwanza city (Bahati and Msaki, 2012) that looked at children in a similar age group and found prevalence of 20.30% though the sample size was smaller (231).

5.2.1 Combined dipstick and microscopy-sensitivity, specificity, and predictive values:

For the combined diagnosis of UTI using dipstick together with microscopy, the sensitivity and specificity stood very high-98.80% and 95% respectively. The findings of our study confirm what Wu and Wong projected that the combination of urine microscopy and dipstick could increase sensitivity to 99.80% (Wu and Wong, 2005).However, the specificity of the combined test in our study was higher than that anticipated by Wu and Wong of 70% .

In terms of sensitivity, our study findings were similar to that of a study published by the American Academy of Paediatrics journal on combination of urine dipstick and microscopy as a screening test for UTI in febrile infants aged 1 day to 90 days(Glissmeyer et al., 2014).According to the study, sensitivity of combined urine dipstick and microscopy was higher (94.70%) than the sensitivity of dipstick alone(90.80%).Regarding the specificity, urine dipstick was reported to produce a better result than the combination urinalysis giving a specificity of 93.80% which was higher than the specificity of the combined urinalysis of 87.60%.In our study, the specificity of

the combination of urine dipstick and microscopy was similar to the specificity of either test alone.

The positive predictive value for combined dipstick and microscopy was higher than for dipstick alone, but lower than microscopy alone. The negative predictive value for the combination was close to 100% (99.50%), much higher than negative predictive value of either dipstick or microscopy alone. This means that the combined value for dipstick and microscopy has a lower probability of identifying sick children as a function of positive tests compared to microscopy alone but it is still superior to dipstick alone. The very high negative predictive value for the combination of dipstick and microscopy suggests its strength in identifying healthy children among negative test results. In the present study the combination of urine dipstick and microscopy had a higher positive predictive value than that of dipstick alone but much lower than for microscopy alone. The negative predictive value for the combination of urine dipstick and microscopy was higher than either dipstick or microscopy alone. According to the findings of the study on combination of urine dipstick and microscopy as a screening test for UTI in febrile infants aged 1 day to 90 days, there was no difference in the negative predictive values of urine dipstick, microscopy and the combination urinalysis. However, the positive predictive value of the combined urinalysis was lower than either dipstick or microscopy alone(Glissmeyer et al., 2014). The positive predictive value being dependent on prevalence implies that the findings of the predictive values herein, are only transferable to a context with similar prevalence but not generalizable. Our study yielded different results from the above study possibly because of variation in prevalence of UTIs since positive predictive value increases with prevalence and negative predictive value decreases with increase in prevalence.

5.2.2 Urine dipstick-sensitivity, specificity, and predictive values:

This study revealed very low sensitivity of dipstick at 46.90% and a much higher specificity of 95.50%. This finding is inconsistent with that of a study conducted on UTI in febrile under-fives in Muhimbili hospital in Dar es Salaam that revealed high sensitivity and specificity of dipstick (85.90% and 79.60% respectively) (Fredrick, 2010) though the specificity was lower than that for this study. The possible explanation for this difference could be because Fredrick dealt with children admitted on the ward. This means that there was a high likelihood that the children were very sick as compared to the children at Nsambya hospital POPD. Our findings for the sensitivity

and specificity were comparable to the values quoted in a systematic review by NICE group in the United Kingdom that reviewed 9 studies containing 12 data sets.

The sensitivity ranged from 30% to 89.20% while the specificity ranged from 89.20% to 100%(National Collaborating Centre, 2007).

There was moderate level of agreement between urine dipstick and culture (kappa=0.49)

The low capacity of the dipstick to correctly identify children with UTI explains the low value of kappa statistic due to high levels of false negatives.

The low sensitivity of urine dipstick implies very high level of false negative error and this can lead to missed or late diagnosis, inappropriate treatment, complications and poor treatment outcome with increased UTI case fatality. This casts doubt on the feasibility of dipstick alone in diagnosis of UTI among children in Uganda.

5.2.3 Urine microscopy-sensitivity, specificity, and predictive values:

Both sensitivity (95.10%) and specificity (98.20%) for microscopic diagnosis of UTI were very high. This level of sensitivity and specificity are still comparable to the ones for combined dipstick and microscopy. These findings suggest that microscopy can be used both as a screening tool as well as a diagnostic tool. This evidence is further supported by the high positive predictive value (95.10%) and negative predictive value (98.20%) for the given level of prevalence. The level of agreement between urine microscopy and culture was almost perfect (kappa=0.93). The accuracy of microscopy was also found to be very high; supporting the argument that microscopy, on its own can be used as a diagnostic tool. Our study is comparable to a study by Ali in Karbala University in Iraq, where the sensitivity of urine microscopy for pyuria and bacteria was 95.70% and the specificity was 99.20%(Ali, 2010).Ali's study was a comparative study of dipstick, urine microscopy and culture in diagnosis of UTI in children under the age of five years. The sensitivity and specificity of urine microscopy in the current study were in the range of sensitivity and specificity reported by NICE group in a systematic review. The review reported on 8 studies including 10 data sets on pyuria or bacteriuria on microscopy where a positive result from either test was indicative of UTI. According to the

systematic review, sensitivity of microscopy ranged from 75% to 100% and the specificity ranged from 32.20% to 92.90%.

5.3 Strengths and Limitations of the study

5.3.1 Strength of the study

Sterile urine specimens were collected from patients as young as babies, less than one month of age.

5.3.2 Limitations of the study

All urine samples were collected between 8:30a.m and 1:30p.m; as such patients reporting later in the afternoon were not included in the study. This could have introduced selection bias.

Although one microscopist was used, he had the experience and expertise needed to do the analyses.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATION

6.1 Conclusions

The sensitivity, specificity, PPV and NPV of combined dipstick and microscopy were 98.80%, 95%, 87.90% and 99.50% respectively. Urine dipstick alone had sensitivity of 46.90%, specificity of 95.50%, PPV of 79.20% and NPV of 83.10%.The sensitivity of urine microscopy alone was 95.10%, the specificity was 98.20%, the PPV was 95.10% and the NPV was 98.20%. The findings of our study reflect that urine microscopy; and combination of dipstick and microscopy had high levels of sensitivity, specificity and negative predictive values. The use of microscopy and the combined tests is highly reliable in diagnosis of UTI among children under five years of age.

This study confirms that urine dipstick alone has a low sensitivity and a high specificity and is inappropriate for use as a diagnostic tool for UTI.

6.3 Recommendation

The Ministry of health of Uganda should continue prioritizing the use of microscopy for the diagnosis of UTI among children under-five years of age.

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APPENDIX I

DATA COLLECTION GUIDE

TITLE: PREDICTIVE VALUE OF DIPSTICK AND MICROSCOPY IN DIAGNOSIS OF URINARY TRACT INFECTIONS AMONG UNDER FIVES PRESENTING TO NSAMBYA HOSPITAL.

Date of visit			|__|__|/|__|__|/|__|__|__|__| dd / mm / yyyy
Study number			|__|__|__|
OPD No.			|__|__|__|__|__|__|__|__|
A. Socio-demographics			
1	**Age in months**		|__|__| **AGE**
2	**Sex**		|__| 1=Male 2=Female
3	Address……..……. Telephone number (of caretaker)…….		
B. Clinical history			
4	**Fever, duration**(in days) |__|		
5	Weight (kg) |__|__|		

C. **Laboratory results**		
Dipstick	Nitrites	|__| 1=Positive 2= Negative
	Leucocyte esterase	|__| 1=Positive 2= Negative
Microscopy	Leucocytes ≥ 5WBCs/HPF	|__| 1=Yes 2=No
	Presence of Bacteria on gram stain	|__| 1=Yes 2=No
Culture (10^5 or more bacteria per ml of fresh urine sample)		|__| 1=Positive 2= Negative
Sensitive to:		
Resistant to:		

Appendix II

CONSENT FORM

PREDICTIVE VALUE OF DIPSTICK AND MICROSCOPY IN DIAGNOSIS OF URINARY TRACT INFECTIONS AMONG UNDER FIVES PRESENTING TO NSAMBYA HOSPITAL.

Introduction

I am Dr Ocokoru Christine from department of Paediatrics and child health, Uganda Martyrs' University, Nsambya hospital. I am conducting a study titled **Predictive value of dipstick and microscopy in diagnosis of urinary tract infections among under fives presenting to nsambya hospital.**

My telephone number is: ***0772-303436***

You are being requested to allow your child to take part in this study.

Purpose of the study

To assess the accuracy of combining dipstick with microscopy in the diagnosis of UTI among under fives presenting with fever in Nsambya hospital.

Study procedure

Your child has been identified to participate in this study and requires your consent. You will be asked questions about your child's illness and physical examination will be carried out on the child. Upon your permission, a urine sample will be obtained from the child for laboratory test to check for urinary tract infection.

Rights of the patient

Entry into the study is entirely voluntary and no penalty will be earned for non participation. Should you choose to withdraw your child from the study at any point, for any reason, you are free to do so and it will not affect the management of your child. For any questions related to the study, feel free to contact me anytime during or after the study.

Benefits and risks

Your child will benefit from the urinalysis results. If it turns out that there is infection, it will be treated.

In case of delay of results you will be contacted by telephone as soon as the results are available. The results will be used to institute the appropriate treatment as the germs causing the urinary tract infection will be known. No serious risks; only the pain of a prick if urine is aspirated from the bladder by pushing a needle through the lower part of the abdomen. There is a possibility of infection; however, this is minimal because sterility will be observed.

Confidentiality

The identity of your child as well as yours will not be revealed in any presentation or publications of this study. Confidential information will only be used for research purposes. None of the information obtained in the course of the study will be released to anyone without your permission.

Compensation

In case of any procedure-related injury incurred during the study, standard management will be instituted promptly.

Contacts and questions

In case of any queries now or later, you are encouraged to contact the persons below:

Researcher conducting this study: Dr.Ocokoru Christine (0772-303436)

Supervisors: DR. Nantulya Florence (0776867149); DR. Nannyonga Maria Musoke (0772415010)

Chairman Institutional Review Board of St. Raphael of St. Francis Hospital Nsambya; Professor Kakande Ignatius (0772501745)

Consent statement

I have been informed about the study on predictive value of dipstick and microscopy in diagnosis of urinary tract infections among under fives presenting to nsambya hospital**.** The purpose of the study, the nature of the study, the benefits and risks to my child has been explained to me. I have been made aware that urine tests will be done on my child. I have also been informed that the information given will be kept confidential and that my child's participation in this study is voluntary and that no consequences will result if I refuse to participate or withdraw from the study.

I hereby give my informed consent to allow my child to participate in this study.

…….	…….	…….
Name of parent/caretaker	caretaker's signature or thumb print	Date
…….	……..	…….
Name of investigator	signature	Date
…….	…….	……..
Witness	signature	Date.

APPENDIX II CONSENT FORM IN LUGANDA

EKIWANDIIKO EKILAGA OKUKIRIZA OKWENYIGIRA MUKUNONYEREZA

Mukisa gw'okuzuula edwadde okusinziira kubiba bivudde mukukebeza okukoleddwa n'olupapula olutono olulaga endwadde n'okukebeza okukoleddwa n'ekyuma ekikebera ekimanyiddwa nga microscope mukuzuula endwadde z'ebitundu mumubiri ebilina akakwate n'okufulumya omusulo mubaana abaggwa wansi w'emyaka etaano abaletebwa muddwaliro ly'ensambya

Enyanjula

Nze Dr.Ocokoru Christine okuva mu kitundu ky'eddwaliro eky'obujanjabi bw'abaana n'embeera y'obulamu bw'abaana, mu yunivasite ya Uganda Martyrs', muddwaliro ly'eNsambya. Nkola okunonyereza okulina omutwe ogugamba nti **Omukisa gw'okuzuula edwadde okusinziira kubiba bivudde mukukebeza okukoleddwa n'olupapula olutono olulaga endwadde n'okukebeza okukoleddwa n'ekyuma ekikebera ekimanyiddwa nga microscope mukuzuula endwadde z'ebitundu mumubiri ebilina akakwate n'okufulumya omusulo mubaana abaggwa wansi w'emyaka etaano abaletebwa muddwaliro ly'ensambya**

Ennamba yange eyessimu eri: 0772-303436

Osabibwa okukiriza omwanawo okwenyigira mukunonyereza kunno.

Omugaso gw'okunonyereza

Okupima enkola y'okugatta okukebeza n'olupapula olutono olulaga endwadde n'okukebeza okukolebwa nekyuma ekikebera ekimanyiddwa nga microscope mukuzuula endwadde z'ebitundu mumubiri ebilina akakwate n'okufulumya omusulo mubaana abaggwa wansi w'emyaka etaano abajja n'omusujja mu ddwaliro ly'eNsambya.

Omutendera gw'okunonyereza

Omwanawo alondedwa okwenyigira mukunonyereza kunno era yetaaga okukiriza kwo. Ojja kubuzibwa ebibuuzo ebikwata kundwadde y'omwanawo n'okwekebejja kujja kukolebwa kumwana. Ng'owadde olukusa, sampolo y'omusulo ejja kufunibwa kumwana okusobola okukola okukebera mu laabu okw'endwadde y'ebitundu ebilina akakwate n'okufulumya omusulo

Eddembe ly'omulwadde

Okuyingira mukunonyereza kwa kyeyagalire mungeri yonna era tewali kibonerezo kijja kufunibwa olw'obutenyigiramu. Ssinga onoba osazeewo okujja omwanawo mukunonyereza obudde bwonna, olw'ensonga yonna, oli waddembe okukikola era tekijja kukosa bujanjabi bwamwanawo. Ku bibuuzo byonna ebikwata kukunonyereza, oli waddembe okuntukirira obudde bwonna ng'okunonyereza kugenda mumaaso oba nga kuwedde.

Eby'okuganyulwa n'obubi

Omwanawo ajja kuganyulwa mubinaba bivudde mukukebera omusulo. Ssinga binaba bilaze nti waliwo endwadde, ejja kujanjabibwa.

Ssinga ebinaba bivudde mukukebera binaba biludeyo tujja kukutukirira kussimu amangu ddala nga ebivudde mukukebeza bifulumye. Ebinaba bivudde mukukebeza bijja kukozesebwa okuwa obujanjabi obusanidde engeri obuwuka obuleeta edwadde y'ebitundu ebilina akakwate n'okufulumya omusulo gy'ebunaba bumanyiddwa. Tewali bubi bwamaanyi; okugyako obulumi bw'empiso ssinga omusulo gubera gusikiddwa okuva mukawago nga basindika empiso ng'eyisibwa mukitundu ky'olubuto ekyawansi. Endwadde esobola okufunibwa; wabula obusobozi bunno butono kubanga tujja kufuba okulaba nti obuyonjo bukumibwa.

Okukuuma obubaka nga bwakyama

Ebyawula omwanawo n'ebikwawula ng'omuntu tebijja kulagibwa mukwolesa kwonna oba mumpapula ezinafulumizibwa mukunonyereza kunno. Obubaka obw'ekyama bujja kukozesebwa kumigaso gy'okunonyereza gyokka. Tewali kyonna kububaka obunaba bukunganyiziddwa ng'okunonyereza kukyagenda mumaaso bujja kuwebwa muntu yenna nga towadde lukusa.

Okudizibwawo

Ssinga wanabawo obuvune bwonna obulina akakwate n'emitendera ng'okunonyereza kugenda mumaaso, obulabirizi obwabulijjo bujja kuwebwa amangu ddala.

Endagiliro n'e bibuuzo

Bwoba olina kyeweebuuza osobola okukuba esimu ya:

Omunonyereza omukulu (07723034360

Abalaburwa okunonnyereza: DR. Nantulya Florence (0776867149); DR. Nannyonga Maria Musoke (0772415010)

Ssentebe W'Akakiiko akanonnyereza mu ddwaliro e'Nsambya: Professor Kakande Ignatius (0772501745).

Sitatimenti y'okukiriza

Ntegezeddwa kukunonyereza okukwata kumukisa gw'okuzuula endwadde okusinziira kubiba bivudde mukukebeza nga bakozesa olupapula olutono olulaga endwadde n'okukebeza nga bakozesa ekyuma ekikebera ekimanyiddwa nga microscope mukuzuula endwadde ez'ebitundu ebilina akakwate n'okufulumya omusulo mu baana abaggwa wansi w'emyaka etaano abaletebwa muddwaliro ly'eNsambya. Omugaso gw'okunonyereza, ekikula ky'okunonyereza, eby'okuganyulwa n'obubi eri omwana wange binyonyoddwa. Ntegezeddwa nti okukebera omusulo kujja kukolebwa kumwana wange. Era ntegezeddwa nti obubaka obuwereddwa bujja kukumibwa nga bwakyama era nti okwenyigira kw'omwana wange mukunonyereza kunno kwa kyeyagalire era nti tewali kibonerezo kijja kuvaamu ssinga ngaana okwenyigiramu oba ssinga nva mukunonyereza.

Mpa okukiriza okusinziddwa kububaka obuwereddwa kukunonyereza okukiriza omwana wange okwenyigira mukunonyereza kunno.

……. ……. …….

Erinya ly'omuzadde/omulabirizi Omukono gw'omulabirizi oba ekinkumu Ennaku z'omwezi

……. …….. …….

Erinya ly’omuntu anonyereza Omukono Ennaku z’omwezi

……. ……. ……..

Omujulizi omukono Ennaku z’omwezi

APPENDIX III

Grams stain procedure:

One drop of the sediment was placed on a glass slide, evenly spread on the slide to form a thin smear and air-dried for 5minutes.

The dry smear was heat fixed by quickly passing it over a flame 3 times.

The smear was, next, flooded with 0.5% crystal violet solution and allowed to stand for 30 seconds.

The excess crystal violet was poured off and gently rinsed with tap water.

The smear was flooded with lugol's iodine and left to stand for 3 seconds.

The iodine was gently rinsed with tap water and decolourised by adding 50% acetone to the smear.

The slide was held at an angle to allow the decolouriser to drain over 3 seconds.

The excess decolouriser was rinsed off with tap water.

The smear was flooded with neutral red counter stain and left to stand for 30seconds.

The excess neutral red stain was rinsed off with tap water, drained and allowed to air dry.