PREVALENCE AND SUSCEPTIBILITY PROFILES OF BACTERIAL PATHOGENS ASSOCIATED WITH URINARY TRACT INFECTIONS IN CHILDREN PRESENTING AT KISII LEVEL 5 HOSPITAL, KISII COUNTY, KENYA

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DECLARATION

I hereby declare that this thesis is my original work and has not been presented for the award of a degree in any other university.

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DEDICATION

I dedicate this work to my parents Mr. Ben Nyambane and Mrs. Mary Nyambane, my brother Albert, sisters Vane, Mercy and Daisy. My loving wife Judith and Son Williams.

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ABBREVIATIONS AND ACRONYMS

CFU   Colony forming units

CLED   Cysteine lactose electrolyte deficient

GI   Gastrointestinal tract

IMVIC   Indole test, Methyl red test, Voges-proskaur test and Citrate test

IVs   Intravascular devices

MDR   Multi Drug Resistance

MR   Methyl red

MRSA   Multi-resistant *Staphylococcus aureus*

NCCLS   National committee for clinical laboratory standards

NICE   The National Institute for Health and Clinical excellence

UTA   Urinary Tract Anomaly

UTI   Urinary Tract Infections

VP   Vogesproskaur

VUR   Vesicoureteral reflux

WHO   World health organisation

$\chi2$ Chi- square

ABSTRACT

Urinary Tract infection (UTI) is a serious infection causing illness in infants and children. It represents one of the most common diseases encountered in medical practice today. Despite the widespread availability of antibiotics, it remains the most common bacterial infection. Antimicrobial susceptibility testing therefore provides information that allows physicians to select the most appropriate antimicrobial agents for treating these infections and give the most effective antibiotic. This cross-sectional study was conducted to determine the prevalence of UTI among children presenting at Kisii Level 5 hospital and to evaluate the sensitivity patterns of the identified isolates to commonly used antibiotics. A total of 186 urine samples were collected from in and out-patients attending Kisii level 5 Hospital, Kisii County, Kenya between December 2012 and March 2013. Urine samples accompanied by microbiology request forms were delivered directly to the laboratory. All sample processing and patient's biodata were carried out centrally in Kisii level 5 hospital microbiology laboratory. The samples were cultured on Cystein lactose electrolytedeficient (CLED) media and incubated for 18 hours at $37^{o}C$. Criteria for defining significant bacteriuria was the presence of 10^{5}colony forming units per millimetre of urine. The bacterial isolates recovered were tested against Ampicilin, Tetracycline, Nitrofurantion, Nalidixic acid, Streptomycin, Co-Trimoxazole and Gentamicin using Kirby Bauer disc diffusion technique.Data was presented as frequencies. Chi square analysis ($\chi2$)was used in comparing of positive UTI cases according to individual characteristics. Evaluations were carried out at 95 % confidence level and $P< 0.05$ was considered statistically significant. Among the 186 samples examined 63.4 % of them were from female patients and 36.6 % from male patients; 26 (14 %) samples had positive bacteriuria with *Escherichia coli* isolates being the highest with 13 (50 %,),*Klebsiella* 8 (30.8 %), *Staphylococcus aureus* 4 (15.4 %) and *Pseudomonas aureginosa*1 (3.8 %). The isolates were sensitive to Nitrofurantion, Nalidixic acid, and Streptomycin while resistant to Ampicilin, Tetracycline, Gentamicin and Cotrimoxazole. There is therefore need to monitor the profile of etiological bacterial agents of UTI and the antimicrobial resistance regularly to keep track of effectiveness of serving therapeutic agents.

CHAPTER ONE

INTRODUCTION

1.1Background of the study

Urinary tract infection (UTI) is defined as an infection in the urinary tract. These infections are caused by microbes(organisms too small to be seen without the aid of a microscope) and they include fungi, viruses (adenoviruses), and bacteria (Watson, 2004). Urinary tract infections are almost exclusively due to bacteria. Diagnosis therefore cannot be made without bacteriological analysis of urine. Infections of bacteria are caused by members of the Enterobacteriaciae which include *Escherichia coli*, *Proteus mirabilis*, *Klebsiella*spp. and *Pseudomonas aureginosa*(Sharifinia*et al*., 2006). Urinary tract includes the organs that collect and store urine and release it from the body. These organs include the kidneys, ureter, bladder and urethra. An infection in the urethra is called urethritis. A bladder infection is called cystitis. Bacteria may travel up the ureter to multiply and infect the kidneys and this will cause an infection in the kidney called pyelonephritis (Farajnia, 2009).

The diagnosis of UTI in children is normally complicated; this is because the clinical presentation of UTI is often with non-specific clinical signs such as fever and abdominal pain which are also seen in many acute self-limiting childhood viral illnesses (Riccabona, 2003). Seeking laboratory confirmation of diagnosis requires the initial stage of collecting an uncontaminated urine sample and this is a challenge in infants and children who are not toilet trained (Colganand Williams, 2011). Failure to consider a diagnosis of UTI or delaying the antibiotic treatment can have the effect of reducing an acute clinical deterioration and in addition it may result in long term renal damage (Younis*et al.*, 2009).

Majority of children who have UTI recover promptly and do not have any long term complication. There is a small subgroup at risk of significant morbidity (Colgan and Williams, 2011). This group of children fall broadly into two categories; the first group during infection it may be a signal of a serious underlying congenital anomaly andin the other category, an infection may be associated with progressive loss of kidney function either in association with renal dysplasia or with recurrent episodes of acute pyelonephritis/upper urinary tract infection (Palikhe,2004). It is necessary, therefore to develop clinical pathways to identify these small but important sub-groups of children from the very many children presenting with urine infections who will recover with no residue ill health (Palikhe, 2004).

Urinary tract infections are among the most frequent bacterial infections worldwide (Gallati*et al*., 2006). In England and Wales, consulting rates in general practice for cystitis and other urinary infections were found to be approximately 3.5 % per 10,000 persons, whereas in Italy, in 2002, 2.4 % of a cohort of more than 450,000 people received a diagnosis of acute cystitis in preliminary care (Gallati*et al*., 2006). In a study in Nigeria, a prevalence of 14.2 % was obtained by Aiyegoro*et al*. (2007) among children in Ile-Ife. In studies that have been done regionallythe prevalence of UTI was found to be 13.3 % in Uganda (Andabati and Byomugisa, 2010).In Kenya Hannah*et al.* (2011) reported a prevalence of 18 % in a nosocomial study in children hospitalized in the Kenyatta National hospital.

In early childhood, Enterobacteriaciae and Enterococci are part of the normal periurethral flora. *E. coli* are the dominant Gram negative species in young girls whereas *Proteus* species predominate in boys (Jakobson*et al.,* 1999). Girls generally

tend to have UTI than boys (Ashkenazi*et al*., 2001). Boys are more susceptible to UTI than girls before the age of 6 months; thereafter, the incidence is substantially higher in girls than boys. Girls tend to have UTI more often than boys because bacteria can reach the bladder more easily in girls. This is partially due to the short and wider female urethra and its proximity to anus (Farajnia, 2009).

1.2 Problem statement and Justification

Urinary tract infections (UTI) are some of the most common infections of childhood.(Jakobson*et al.,* 1999). The prevalence and diagnosis of UTI in children in Kisii level 5 hospital on the basis of sensitivity profiles and sex has not been donedespite extensive published literature concerning UTI coming from other hospitals such as, Kebira*et al*. (2009), and Hannah *et al*. (2011). Since most of the uropathogens are of faecal origin, there is fear that the high levels of antibiotic resistance demonstrated for the enteric bacteria may also exist among the uropathogens (Farajnia*et al*., 2009).

In Kisii level 5 hospital children with UTI are mostly treated with antibiotics. This often leads to overuse of antibiotics thus causing antibiotic resistance. Therefore there was need to establish the prevalence of UTI among children who present with symptoms inorder to justify this practice. Therefore it is important to establish a very specific way of diagnosingUTI and determine the involved bacteria and their sensitivity patterns in this institution.

1.3 Research questions

i) What is the identity of pathogens causing UTI in children visiting Kisii level 5 hospital?

ii) Which is the most dominant pathogen causing UTI in children visiting Kisii level 5 hospital?

iii) Are these isolates from children visiting Kisii level 5 hospital resistant to antibiotics?

1.4 Hypotheses

i) Children visiting Kisii level 5 Hospital are not suffering from UTI.

ii) Bacterial isolates from children visiting Kisii level 5 hospital are not susceptible to antibiotics.

1.5 Objectives

1.5.1 General objective

To determine the prevalence of UTI among children visiting Kisii level 5 hospital, the common bacterial pathogen and their sensitivity profiles.

1.5.2 Specific objectives

i) To isolate and identify bacteria causing urinary tract infections in children visiting Kisii level 5 hospital using biochemical tests.

ii) To determine the common microbes causing UTI among children visiting Kisii level 5 hospital.

iii) Evaluate the antibiotic susceptibility patterns of the isolates causing UTI among children visiting Kisii level 5 hospital.

1.6 Significance of the study

The results of this study will assist various stake holders to individualize the antibiotics to be administered and monitor resistance patterns developing in Kisii level 5 hospital. In addition the study will make valuable contribution to the currently available literature in the field of medical microbiology.

CHAPTER TWO

LITERATURE REVIEW

2.1 Urinary tract infections

Urine infection is the most common serious bacterial infection causing illness in infants and children (Schlager, 2001). It is one of the most common bacterial infections encountered by clinicians in developing countries including Kenya (Dulczac and Kirk, 2005). A complicated UTI describes infections in urinary tracts with structural or functional abnormalities. In neonates and infants, they are presumed to be complicated because of the high association between urinary tract malformation and concurrent bacteraemia (Schlager, 2001).

A urinary tract infection may involve only the lower urinary tract, in which case it is known as a bladder infection, which means the infection is confined to the bladder and urethra, this is much the same as cystitis (Yildiz*et al*., 2007). Alternatively, it may involve the upper urinary tract, in which case it is known as pyelonephritis, an infection that affects a kidney and/or tube called a ureter (Riccabona, 2003). It causes flank pain which is a pain in the side of the tummy (abdomen), often coming from a kidney. If the urine contains significant bacteria but there are no symptoms, the condition is known as asymptomatic bacteriuria. If a urinary tract infection involves the upper tract, and the person has diabetes mellitus, is pregnant, is male, or immunocompromised, it is considered complicated (Salvatore *et al*., 2011).

Throughout childhood, the risk of having a UTI is 2 % for boys and 8 % for girls (Gallati*et al*., 2006). Having an anomaly of the urinary tract, such as urine reflux from the bladder back into the ureter increases the risk of a UTI. Boys who are younger

than 6 months old who are not circumcised are at greater risk for a UTI than circumcised boys the same age (Riccabona, 2003). Surgically correctable causes of recurrent infection include; infection stones, infected non-functional renal segments, infected urethral stumps after nephrectomy, vesicointestinal or urethrorectal fistulae, vesicovaginal fistulae, infected necrotic papillae, unilateral medullary sponge kidney, infected urachal cyst, infected urethral diverticulum or periurethral glands(Tanagho and Mcaninch, 2004).

2.2 Risk factors of UTI

Risk factors for UTI can be categorized as anatomic and physiological, genetic and behavioural (Shaikh*et al*., 2008). Both anatomical and physiological anomalies restrain the flow of urine, delay bladder emptying or cause an increased post void residual volume. Such anomalies can be cystocoeles, rectoceles and bladder diverticula (Salvatore, 2011). Urinary incontinence is also a suggested risk factor for UTI but how it predisposes children to UTIis not entirely clear. Another contributory physiological factor is the effect of loss of oestrogen on the genitourinary mucosa which can lead to fragile mucous membranes (Dulczac and Kirk, 2005).

Genetic risk factor means that some children seem to have a genetic predisposition to UTI with a history of recurrent UTI (Hooton and Stamm, 1997). Interleukin 8, an inflammatory cytokine is another factor with genetic variability, that may influence the development of UTI and both urinary immune reactive interleukin 1 and interleukin 6 have been measured more frequently in urine in bacteriuria than in non bacteriuria children (Dulczac and Kirk, 2005). The most important associated factors for UTI vary between children living in the community and those living in

institutions. Studies have shown that the most common characteristics predisposing older children to UTI are urologic abnormalities, debilitating co morbid conditions, and functional impairment (Stauffer *et al*., 2004).

Co-morbid conditions seem to have a greater impact on contracting a UTI among old children living in institutions than those living in the community (Shaikh*et al*., 2008). One explanation could be that in these institutions diseases such as Alzheimer's, Parkinson's and cerebrovascular disease are common and that these diseases may be associated with impaired bladder control (Shaikh*et al*., 2008). This leads to impaired voiding, increased residual urine volumes, and sometimes ureteric reflux (Dolgan*et al*., 2012). However, studies regarding the association between residual urine and UTI have produced conflicting results, although it is generally assumed that residual urine is a risk factor for UTI because it creates a favourable environment for bacteria (Bakker *et al*., 2002).

A previous study has found that the use of diapers is a risk factor for UTI in young children (Shaikh*et al*., 2008). There is also a relationship between urinary stones and occurrence of UTI and these types of infections are often caused by urease producing Gram negative organisms (Wan *et al*., 1995). Urinary tract infection is also associated with hip fracture surgery and with other general fractures(Stauffer *et al*., 2004). The relationship between UTI and hip fractures may also depend on or be associated with malnutrition, dementia and polypharmacy (Gallati*et al*., 2006). Those who suffer from a hip fracture often have dementia, are malnourished and suffer from a UTI preoperatively but also postoperatively because they have been catheterized. Urinary

tract infection might also increase the risk of falls and fractures (Dulczac and Kirk, 2005).

Urinary tract infections can also be caused by a variety of predisposing factors either working alone or in combination with others leading to inoculation of the urinary tract. Urine and fecal elimination habits are considered an important possible cause for UTI (Dolgan*et al*., 2012). Infrequent micturation and incomplete emptying of the bladder in children represent important factors in the causation of incontinence during the day, and of urinary tract infections (Bakker *et al*., 2002). Children with daytime wetting with/without night wetting have very often bladder sphincter dysfunctions which are in turn correlated with recurrent urinary tract infections, 8 % of the school children (10-12 years old) report day time wetting with/without night wetting with some frequency. One percent of healthy children over the age of 5 years have troublesome day wetting (Shaikh*et al*., 2008).

Poor genital hygiene or toilet habits are sufficient to cause infections if they were combined with other functional abnormalities such as infrequent voiding, inadequate fluid intake, functional stool retention or voiding dysfunction (Mazzola*et al*., 2003). Another study in Switzerland stated that toilet habits affect strongly the development of urinary tract infections (Wan *et al*., 1995).

2.3 Epidemiology of UTI

The use of urinary catheters in young children, and in those experiencing nervous system disorders and children who are convalescing or unconscious for long periods may result in an increased risk of UTI (Molander*et al*., 2002). The urinary bladder

wall is coated with various mannosylated proteins, such as Tamm-Horsfall proteins, which block the binding of bacteria to the uroepithelium (Toth*et al*., 2003). As binding is an important factor in establishing pathogenicity for these organisms, its disruption results in reduced capacity for invasion of the tissues. Due to poor adhesion, the unbound bacteria are more easily removed from the bladder by voiding urine hence reducing the chance of infection(Toth*et al*., 2003).

The use of urinary catheters may physically disturb this protective lining, thereby allowing bacteria to invade the exposed epithelium (Esposito*et al.,* 2008; Alexander *et al.,* 2009). Elderly individuals are more likely to harbour bacteria in their genitourinary system at any time (Alexander*et al.,* 2009). This colonization may be accompanied by symptoms of infection that may necessitate treatment. The presence of bacteria in the urinary tract of older adults, without symptoms or associated consequences, is also a well recognized phenomenon which may not require antibiotics (Nurullaev, 2004). This is usually referred to as asymptomatic bacteriuria. The overuse of antibiotics in the treatment of bacteriuria is of concern and a controversial issue(Rodhe*et al*., 2009).

Escherichiacoli are facultative anaerobes, normal flora; found in gastrointestinal (GTI) of humans and animals. Transmission of pathogenic *E.coli* often occurs via fecal contamination,dirty hands, and through sexual contact (Brown and Foxman, 2000; Gupta *et al*., 2001).Since bacteria can enter the urinary tract through the urethra (an ascending infection), poor toilet habits can predispose to infection, but other factors such as pregnancy in women and prostate enlargement in men are also

important (Lane andMobley, 2007). However, in most cases the initiating cause is unclear. Among the elderly, UTI frequency occurs equally in women and men *(*Alraekand Baerheim, 2003).

2.4 Causative organisms

2.4.1 Bacterial UTI

Most of urinary tract infections are caused by Gram negative bacteria like *E. coli, Klebsiella*spp.*, Proteus* spp.*, P. aureginosa, Acinetobacter,* and*Serratia*(Shaikh*et al*., 2008)*.* A few of UTI cases are caused by Gram positive bacteria which include Enterococci*,* Staphylococcusand *Streptococcus agalactiae*(Foxman, 2003). *E. coli* causes 60 % to 80 % of UTI cases, *Proteus* (more common in boys and in children with renal stones), *Klebsiella*spp. 30-40 %,Enterococci and coagulase negative *Staphylococci* 10-20 % (Watson., 2004). *Escherichia coli* are the most common Gram negative bacteria responsible for UTI (Shaikh *et al*., 2008). *Proteus* spp. and *Klebsiella*infections account for 10 % and 6 %respectively (Ghedira*et al*., 2004; Yuksel*et al*., 2006).

Adherence properties of some organisms prevent their normal washout by bladder emptying and by mucosal host defence mechanisms. *Escherichia coli* are virulent due to the presence of P - fimbriae, organelles that may attach or adhere on specific receptors of uroepithelium cells and interfere with the washout of bacteria (Tanagho and Mcaninch, 2004). *Escherichia coli* are predominant in girls, whereas *Proteus* spp. and *Klebsiella*spp.are likely encountered in boys (Ghedira*et al*., 2004). High incidences of UTI are due to *Proteus* spp., *Klebsiella*spp. and *Enterobacter*spp. Infections are more common among children with recurrent UTI and in those treated with antibiotic prophylaxis (Tanagho and Mcaninch, 2004). Other uropathogens like

Pseudomonas, *Serratia,*and *Candida* are more common among children with urogenital abnormalities like urethritis and bladder cystis (Mangiarotti*et al*., 2000. In hospital acquired UTI 65 % are caused by *E. coli* and other pathogens including*Pseudomonas* spp.(Ashkenazi *et al*., 2001).

2.4.2 Fungal and viral UTIs

Urinary tract infection may be caused by viruses and fungi. Fungi, such as *Candida*, is the second most cause of nosocomial UTI in children, it can be spread systemically and can be life threatening (Yildiz*et al*., 2007). Fungi infections are seen in infants and children who are on long term antibiotics, patients who are immunocompromised, or patients using invasive devices like IVs, and catheters (Watson, 2004). *Candida* and fungal infections are more prevalent in children with Urinary tract Anomaly (UTA); it is associated with infections after instrumentation of the urinary tract (Yildiz*et al*., 2007). The prevalence of UTI due to *Candida* increases gradually with the duration of hospitalization. Treatment of Candiduria includes stopping antibiotics, removing or changing indwelling catheters, and starting antifungal therapy with anti fungal agents like oral fluconazole, parental or intravesicalamphotercin B. Viral UTI can be caused by adenoviruses types 11 and 21, polyomavirus BK, and herpes simplex viruses (Watson, 2004).

2.5 Modes of bacterial entry

There are two major modes of bacterial entry into the genitourinary tract. Most UTI in children result from ascending infections, although hematogenous spread may be more common in the first 12 weeks of life (Yildiz*et al*., 2007).

2.5.1 The ascending route

Most cases of pyelonephritis are caused by the ascent of bacteria from the bladder through the ureter and into the renal parenchyma (Tonagho and Mcaninch, 2004). Most cases of UTI are caused by bacteria ascending from the perineum (Foxman, 2003).

2.5.2 Hematogenous route

This type usually occurs in neonates and immunocompromised patients (Dulczac and Kirk, 2005). In the first 8 to 12 weeks of life, urinary tract infection may be secondary to hematogenous source. Because of that, the diagnosis of UTI in young children is very important as it is considered a marker for urinary tract abnormalities in the newborns. UTI from hematogenous source may be associated with bacteraemia (Schlager, 2001). *S. aureus*, *Candida* species, and *M. tuberculosis* are common pathogens that travel through the blood to infect the urinary tract (Tonagho and Mcaninch, 2004).

2.6 Pathogenesis of UTI

Almost all UTI are ascending in origin and are caused by bacteria in the GI tract that have colonized the periurethral area (Handley *et al*., 2002). After birth, the periurethral area, including the distal urethra, becomes colonized with aerobic and anaerobic microorganisms. These organisms appear to function as a defense barrier against colonization by potential pathogens. Disturbance of the normal periurethral flora, such as it may occur when an upper respiratory tract infection is treated with a broad spectrum antibiotic, predisposes to colonization of the periurethral area by potential uropathogens (Lidefelt*et al.,* 2001).

Periurethral colonization with auropathogen plays an important role in the pathogenesis of recurrent infections in adults (Gallati*et al*., 2006). The perineal flora is a normal inhabitant of the distal urethra (Quigley, 2009). Urine in the proximal urethra, the urinary bladder, and more proximal sites within the urinary tract is normally sterile. Uropathogens must gain access to the urinary bladder and proliferate if an infection is to occur (Mysorekar*et al*., 2012). Bacteria in the distal urethra may gain access to the bladder because of turbulent urine flow during normal voiding, as a consequence of voiding dysfunction, or as a result of the use of instrumentation. In any case, normal voiding results in essentially complete washout of contaminating bacteria. Therefore, urinary bladder colonization does not usually occur unless bladder defense mechanisms are impaired or a virulent strain of bacteria has gained access to the bladder (Barnett and Stephens, 1997).

2.6.1 Bladder emptying

In the absence of normal bladder emptying, there is proliferation of bacteria in bladder urine and the risk of a UTI (Mulvey*et al*., 2000). Even with normal bladder emptying, adherence to uroepithelium cells by virulent organisms such as P-fimbriated *E. coli* may result in a UTI. P- Fimbriae (or pili) are organelles on *E. coli* that mediate attachment to specific receptors on uroepithelium cells and impair washout of the bacteria. The majority of UTI in neurologically and anatomically intact children are caused by *E. coli*. Children with intestinal carriage of P-fimbriae*E. coli* are at increased risk for UTI because of colonization of the periurethral area by these pathogens (Lidefelt*et al*., 2001).

The urinary tract (kidney, ureter, bladder, and urethra) is a closed, normally sterile space lined with mucosa composed of epithelium known as transitional cells (Mysorekar*et al*., 2012). The main defence mechanism against UTI is constant antegrade flow of urine from the kidneys to the bladder with intermittent complete emptying of the bladder via the urethra. This washout effect of the urinary flow usually clears the urinary tract of pathogens (Dromigyny*et al*., 2002). The urine itself also has specific antimicrobial characteristics, including low urine pH, polymorph nuclear cells, and Tamm-Horsfall glycoprotein, which inhibits bacterial adherence to the bladder mucosal wall (Toth*et al*., 2003). The UTI occurs when the introduction of pathogens into this space is associated with adherence to the mucosa of the urinary tract. If uropathogens are cleared inadequately by the washout effect of voiding, then microbial colonization potentially develops (Toth*et al*., 2003). Colonization may be followed by microbial multiplication and an associated inflammatory response.

2.6.2 Virulence factors for UTI

Bacteria that cause UTI in otherwise healthy hosts often exhibit distinctive properties known as virulence factors to overcome the normal defenses of the urinary system (Tena*et al*., 2008). In serotypes of *E. coli* frequently isolated in UTI, bacterial adherence to the uroepithelium is enhanced by adhesions, often fimbriae (pili), which bind to specific receptors of the uroepithelium. The interaction of fimbriae with the mucosal receptor triggers internalization of the bacterium into the epithelial cell, which leads to apoptosis, hyper infection, and invasion into surrounding epithelial cells or establishment of a bacterial focus for recurrent UTI (Mulvey*et al*., 2000).Uropathogenic strains of *E. coli* have been recognized to release toxins, including cytolethal distending toxin, alpha haemolysin, cytotoxic necrotizing factor-

1, secreted auto transporter toxin that causes cellular lyses, cause cell cycle arrest, and promote changes in cellular morphology and function (Uhlen*et al*., 2000). To promote survival, various uropathogens possess side phore systems capable of acquiring iron, an essential bacterial micronutrient (Uhlen*et al*., 2000). Uropathogenic strains of *E. coli* have a defensive mechanism that consists of a glycosylated polysaccharide capsule that interferes with phagocytosis and complement mediated destruction (Toth*et al*., 2003).

2.7 Diagnosis of UTI

Urinary tract infection is defined by a combination of clinical features and the presence of bacteria in urine (Mcloughlin and Joseph, 2003). It is also defined by the presence of more than 100,000 colony forming units (CFUs/ml) of single bacteria in cultured urine. The clinical features of UTI may include both specific and nonspecific signs and symptoms. Accurate diagnosis and treatment of UTI is essential to limit its associated morbidity, mortality and to avoid prolonged or unnecessary use of antibiotics (Tonagho and Mcanich, 2004). Diagnosis of UTI is difficult particularly in young children and infants. This is because in this age group, the clinical presentation of urine infection is often with non-specific clinical signs such as fever, irritability, and vomiting that are also commonly seen in other childhood viral illnesses (NICE, 2012). Evaluation of UTI relies on both lab investigations and clinical signs and symptoms. Lab investigations include both urinalysis and urine culture. Diagnosis of UTI is not always straight forward because one cannot look at its appearance and conclude an infection (Kolawole*et al.,* 2009). Criteria for the diagnosis of UTI vary greatly depending on the patients and context. A sample of urine is needed to confirm the diagnosis. Urine normally has no germs (bacteria) present, or only very few. A

urine infection can be confirmed by urine tests that detect bacteria and/or the effects of infection in the urine (Tonagho and Mcanich, 2004).

Ideally, the sample of urine should not come into contact with skin or other materials that may contaminate it with other bacteria (Hooton and Stamm, 1997). Adults and older children can do this by a midstream collection of urine. In young children, the usual way is to catch some urine in the specimen bottle whilst they are passing urine. In babies one method is to place a specially designed absorbent pad in a nappy (supplied by a doctor). Urine is sucked into a syringe from the wet pad. Another method is to use a plastic bag that sticks on to the skin and collects urine (Cox and Hinman, 2001).

According to Tena*et al.* (2008), there is no one best way of performing urine cultures. Guidelines for the diagnosis of UTI includes the use of sheep blood agar and either MacConkey agar or a similar selective medium for routine urine culture. To make the diagnosis of a urinary tract infection in children, a positive urinary culture is required (Manges, 2006). Contamination   poses frequent challenge depending on the method of collection used, thus a cut off of 10^5 CFU/ml is used for "clean catch" mid-stream sample. The use of "urine bags" to collect samples is discouraged by WHO due to the high risk rate of contamination. Catheterization is therefore preferred in those children who are not toilet trained (Mcloughlin and Joseph, 2003). There is considerable evidence of practice variation in use of diagnostic tests, interpretation of signs or symptoms and initiation of antibiotic treatment such as drug selection, dose, duration and route of administration (Gallati*et al*., 2006). Urine culture should be obtained for diagnosis of UTI in children if there is high clinical suspicion, cloudy urine, or

positive urine dipstick (Manges*et al*., 2006). Oral antibiotics should be used (when tolerated) instead of parenteral antibiotics to manage UTI in children. One day course of antibiotics should not be used to manage UTI in children instead; short courses of antibiotics (two to five days) may be used. Prophylactic antibiotics may be used to reduce the risk of recurrent UTI (Jamieson *et al*., 2006).

2.8 Urine collection

When collecting urine samples, two things must be taken in account, the way of collecting urine and the time until the sample is tested (Esposito *et al*., 2008). There are four ways in which urinary specimens are obtained in children, the bagged specimen which is a plastic bag attached to the perineum, the midstream clean catch voids, catheterization, or suprapubic aspiration (Toth*et al*., 2003). According to NICE a midstream clean catch urine sample is the recommended method for urine collection (NICE, 2012).Urine samples must be tested immediately after collection, but if urine cannot be tested and cultured within 4 hours of collection, the sample should be refrigerated or preserved with boric acid (NICE, 2012). Urine can be stored in the refrigerator for up to 24 hours (Tonagho and Mcaninch, 2004).

2.9 Treatment of UTI

It is very important to recognize and treat UTI rapidly (Palikhe, 2004). Treatment of UTI with the appropriate antibiotic can minimize mortality, morbidity and any renal damage from acute UTI. Choosing the appropriate antimicrobial agents sounds difficult, but advances in the understanding of the pathogenesis of UTI, the development of new diagnostic tests, and the introduction of new antimicrobial agents have allowed physicians to appropriately tailor specific treatment for each patient

(Tonaghoand Mcaninch,2004). Treatment of UTI depends on the age of the child, location of infection, etiology of the disease, degree of illness in the child, efficacy of antibiotic and resistance profile within the community (Romolo and Gaspari, 2005). Sick children and infants less than 3 months should be treated as inpatients, whereas healthy children and older infants may be treated as outpatients (Santen and Altieri, 2011). Predominant pathogens in the patient's age group, antibacterial sensitivity patterns in the practice area, the clinical status of the patient, and the opportunity for close follow up are important factors which must be taken in consideration when choosing the appropriate antimicrobial (Schlager, 2001).

The main treatment of UTI is to initiate appropriate antibiotic therapy promptly (Palikhe, 2004). Most organisms causing UTI originate from the gastrointestinal tract; the most common of them is *E. coli*. Antibiotics prescribed must be active against these organisms. In cases where children are seriously ill, broader spectrum antibiotics must be used. Majority of patients respond to oral antibiotics, but in some cases intravenous antibiotics must be used, these are cases of seriously ill or septic patients, children less than 1 month of age, and in the case of vomiting. The duration of traditional treatment with antibiotics is 7-10 days in acute pyelonephritis, whereas in lower tract UTI short course treatment from 3-4 days is effective in clearing the infection (Riccabona, 2003).

Antibiotics used in the treatment of UTI must be active against urinary pathogens with low rate of resistance, to be free of side effects, palatable, sugar-free preparations, available, and having no effect on normal gut flora (Riccabona, 2003). Antibiotics used in the treatment of UTI include: Sulphamethoxazole/ Trimethoprim,

Fluroquinolones such as Ciprofloxacin, Nitrofurantion, Amino glycosides such as, Gentamicin, and Amikacin, cephalosporin and Aminopenicillins such as Ampicilin and Amoxicillin (Tonagho and Mcaninch, 2004). Trimethoprim/Sulphamethoxazole, Cephalosporin's and Amoxicillin-clavulanate are considered to be the most acceptable antibiotics for the treatment of UTI in paediatrics in comparison to quoinolone, which have an effect on joint development, and first line therapy of amoxicillin which has a high prevalence of resistance to *Escherichia coli* in many communities (Colgan and Williams, 2011).

Parenteral antibiotics should be considered in children who are vomiting or dehydrated, or who have an abnormal urinary tract (Riccabona, 2003). A recent article has reviewed the evidence for treatment of acute pyelonephritis. The authors state that oral antibiotics, chosen to cover local uropathogens are as safe and effective as intravenous antibiotics in children with a clinical diagnosis of acute pyelonephritis and intravenous antibiotics should be reserved for those who are seriously ill or have persistent vomiting (Craig and Hodson, 2004). In complicated cases, longer course or intravenous antibiotics may be needed, and if symptoms have not improved in two or three days, further diagnostic testing is needed. It is therefore generally recommended to treat uncomplicated UTI in children for seven days with oral antibiotics, though short course (3-4 days) treatment has been shown in some studies to be as effective as single dose (Keren and Chan, 2002).

A urinary tract infection is a one off event (Riccabona, 2003). However, some children have more than one urine infection and some develop several throughout their childhood (recurring UTI). In some cases, an infection can be severe,

particularly if a kidney becomes badly infected. This can sometimes be a serious, even life-threatening in a minority of cases if treatment is delayed. A bad infection, or repeated infections, of a kidney may also do some permanent damage to the kidney. This could lead to kidney problems or high blood pressure later in life (Riccabona, 2003).

2.10 Prevention of UTI

Prevention of recurrent UTI focuses both on detection and correction if possible, of urinary tract abnormalities. Interventions that have been associated with a decrease in symptomatic UTI in children with a history of recurrent UTI include relief of constipation and voiding dysfunction (Schlager, 2001). According to the National Institute for Health and Clinical Excellence (NICE) guidelines, prevention of UTI recurrence includes: Relieving constipation and dysfunctional elimination syndromes in children who have had a UTI, encouraging them to drink an adequate amount, and ensuring that these children have ready access to clean toilets when required (NICE, 2012).

2.11 Resistance of uropathogens to antimicrobials

Infections of the urinary tract are among the most common infectious diseases in humans. Intestine is usually the source of organisms producing UTI (Manges*et al*., 2006). Antimicrobial resistance occurs in intestinal bacteria due to antibiotic therapy for treating infections outside the urinary tract. The use of antibiotics has an influence in the spread of antimicrobial resistance among bacteria. The etiology of UTI and the antibiotic resistance of uropathogens have been changing over the past years, both in community and nosocomial infection (Kahan*et al*., 2006). Indiscriminate use of

antibiotics has also increased resistance to β -lactam agents and Trimethoprim; in some regions rates of resistance to these drugs are above 20 % when empiric use is no longer recommended. Quoinolone resistance is also increasing; however resistance to Nitrofurantion is rare (Katherine*et al*., 2009).

In Africa, broad spectrum antimicrobial drugs such as Fluroquinolones, β-lactam inhibitors, and cephalosporin have lost their effectiveness over the years (Kariuki*et al.,* 2007). A study conducted in Tunisia revealed that the susceptibility of bacteria to the principal antibiotics used for the treatment of UTI was characterized by low sensitivity (Boukadida *et al.,* 2002). However, a similar study carried out in Kenya revealed a similar trend in antimicrobial resistance among uropathogenic*E. coli* (Kariuki*et al.,* 2007). Fluroquinolones have become popular treatment for patients with uncomplicated UTI, but a study conducted by Kariuki*et al.* (2007), showed that uropathogenic*E. coli* are becoming resistant to Fluroquinolonesamong other antibiotics. This high upsurge of resistance in Kenya is due to frequent misuse and prolonged use of certain drugs that expose these strains to adapt resistance (Mitema and Kikuvi, 2004)

The source of resistance may either be endogenous from contamination of the patient's urethra or perineum by the bacteria from the colonic flora or exogenous due to cross infection with bacteria from the infected urinary tract of another patient (Katherine*et al.,* 2009). Transmission is by instruments like cytoscopes and catheters or by the hands of doctors and nurses as in the case of MRSA. However, little information on etiology and resistance pattern of community acquired UTIs in Kisii is available. Antibiotic resistance is a major clinical problem in treating infections

caused by these microorganisms. The resistance to the antimicrobials has increased over the years. Resistance rates vary from one community to another and country to country depending on the commonly used antibiotics (Gales *et al.,* 2001).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

This study was conducted in Kisii level five hospital in Kisii central district, Kisii County, Kenya. It is located in Western Kenya, on Latitude: 0° 41' 0 S and Longitude: 34° 46' 0 E (Figure 3.1). This is a regional referral hospital covering south Nyanza, south Rift and the entire Gusii region. It has a catchment of 3 million people and a staff establishment of about 500 workers and 13 specialists. They offer all the four classes of services; curative, preventive, promotive and rehabilitative. It has a bed capacity of 379 and 20 cots (www.kisiihospital.org).

Source: www.wikipedia.com. Accessed on 1st February 2013 at 1300 Hrs GMT+3

Figure 3.1: Map of Kenya showing area of study.

3. 2 Study design and sampling method

This was a cross-sectional descriptive study. Purposive sampling was used to select children with UTI symptoms and then simple random sampling was used to choose children to include in the study with strict application of the inclusion criteria. Eligible participants were approached and requested to give a voluntary consent to participate in the study. Inclusion into the study was done consecutively until the required sample size was achieved. On average 12 patients were sampled every week.

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3.3 Study population and sample size

The study population consisted of children patients of the age between 0-12 years presenting with symptoms of urinary tract infection seeking treatment at Kisii level 5hospital. The symptoms included burning sensation during urination, loss of bladder control, micturation, lower back pain, and lower abdominal pain and cloudy or foul smelling urine. The urine was collected voluntarily into sterile universal bottles or by use of catheters for the young children. The sample size was determined using the formula by Lwanga and Lemeshaws (1991) using prevalence rate of 18% (Hannah*et al.*, 2011).

$N=Z^2 \times PQ/D^2$

N= Desired minimal sample size

Z=Standard normal deviation (1.96 from tailed normal table)

P=Prevalence of condition under study

Q=1-P

D=Precision required for the study at 95 % confidence level (0.05)

$N= (1.96)^2 \times (0.24).0.874/0.05^2$

N=242

However during the entire period of study from December 2012 to March 2013, only 186 patients presented with such symptoms.

3.4 Inclusion criteria

The study included all those children of the age below 12 years and whose parents consented to participate in this study. It also included children who presented with symptoms of UTI, and those who had not taken antibiotics in the last 14 days as these would prevent the growth of bacteria.

3.5 Exclusion criteria

The study did not include children whose parents refused to consent to participate in this study and also those who were under antibiotics. In addition, children without UTI symptoms were also excluded.

3.6 Data collection instrument.

Data was collected using structured questionnaire. The questionnaire used had information about; age, sex, hospital status, schooling status, previous diagnosis of UTI and if the children had taken antibiotics in the last 14 days.It was availed to the study participants by principle investigator or the researchassistants (Appendix II).

3.7 Laboratory procedures

3.7.1 Collection of urine samples

Midstream urine samples were collected using sterile universal bottles. Patients were instructed to collect mid stream urine into a sterile bottle up to at least half the

capacity and delivered the specimen in the laboratory within an hour. In very young children not able to pass urine voluntary; urine bags were used.

3.7.2 Culturing urine samples

The cysteine lactose electrolyte deficient (CLED) (Oxoid limited) was used as selective media for isolation. The media were prepared according to the manufacturer's instructions and 0.001 ml of sample inoculated onto media using a platinum wire loop. Plates were then incubated for 24 hours at 37°C. The number of pure colony forming units was then multiplied by 1000 to determine the number of micro-organisms per milliliter in the original specimen of urine. Those with more than 10^5 colonies were selected for further tests. Plates with no growthor tiny colonies were returned to the incubator for another 24 hours before discarding the plates since antimicrobial treatment or other factors may inhibit initial growth (WHO, 2003).

3.7.3 Identification of bacteria

A total of 186 samples were collected and tested bacteriologically using standard procedures. Isolation of uropathogens was performed by a surface streak on CLED agar (Oxoid Ltd) and incubated aerobically at 37°C for 24 hours and those cultures that became negative at the end of 24 hours of incubation were further incubated for 48 hours (Tena*et al*., 2008). A significant bacterial growth was considered in cases where the bacteria were above a concentration of 10^5 CFU/ml (Tonagho and Mcanich, 2004).*S.aureus* was isolated by direct plating and enumerated by direct countingof colonies. Colonies greater than 100 was considered significant. The bacteria were confirmed by use of biochemical tests; Indole, methyl, Vogesproskaur and Citrate

test, oxidase, Hydrogen sulphide production, lactose fermentation, gas production, catalase and coagulase tests.

3.7.3.1 Triple sugar iron test

This test was used in Gram negative colonies. TSI agar has glucose with a 0.1 % concentration and lactose and sucrose with a concentration of 1 %. Sterile TSI slants with agar were taken from the refrigerator and wiped using a dry cotton towel. The cap was removed and then the neck was flamed (WHO, 2003). An inoculating straight loop was sterilized in the blue flame of the Bunsen burner and then allowed to cool. A colony of the suspected organism from CLED agar was picked, stabbed into the medium up to the butt of the TSI tube and then it was streaked back and forth along the surface of the slant. Again the neck of the TSI was flamed, capped and placed in the incubator for 18 hours at a temperature of 37° C. Triple sugar iron agar tube was used to test for the fermentation of only glucose (yellow butt), fermentation of lactose and sucrose (all over yellow), CO_2 formation (crack in agar), or ferrous ammonium sulphate produced (black precipitate) (WHO, 2003).

3.7.3.2 Catalase test

This test was used to differentiate suspected Staphylococci spp. colonies which appeared with a uniform yellow colour. Two drops of 3 % hydrogen peroxide were put onto a clean glass slide using a dropper; a pure colony of the organism was picked from CLED agar using a wooden applicator stick(WHO, 2003). Placing the colony on the hydrogen peroxide on the glass slide; emulsification was done. Observation for bubble formation was done within 30 seconds(WHO, 2003).

3.7.3.3 Free coagulase test

This test was used to differentiate suspected*S. aureus* (pathogenic) from *S. albus* which is non pathogenic (WHO, 2003). Dilute plasma from human blood was used with peptone water. A loopful of the test organism was put into the diluted plasma which made a complete suspension. Incubation of the suspension was done at a temperature of 37°C then examination for clot formation was made.

3.7.3.4 Indole, methyl red, vogesproskaur and citrate test (IMVIC) tests

This test was used in organisms suspected to be *E. coli* and *Klebsiella*. Indole test determines the presence or absence of the tryptophanase, an enzyme which breaks down tryptophan (WHO, 2003). A 1 % Tryptone broth was used during the test (WHO, 2003). Kovac'sreagent was added to theTryptone broth and if indole is present then a red coloration forms at the top (WHO, 2003).

A MR-VP broth was used to look for mixed acid and butanediol fermenters in the test organisms. One tube was used for each test. Half of the broth, once incubated, was removed and placed into a different tube. Methyl red was added to one tube to see if the pH is neutral (yellow) (WHO, 2003). Barritt's solution (alphahapthol and potassium hydroxide) was added to the other tube to test the Butanediol fermenters and if the bacteria are butanediol fermenters then the broth turns red.

Citrate test was used to test for the presence of citrate which is the sole source of carbon for bacteria (WHO, 2003). An agar slant with synthetic medium containing small amounts of mineral salts (citrate and ammonium) was used to perform the test (WHO, 2003). Bromothymol blue (pH indicator) was added to the agar slant and if

there is growth (presence of citrate) the agar is blue and if there is no growth the agar is green.

3.7.7.5 Oxidase test

One colony of the suspect organism was transferred to a filter paper soaked with oxidase reagent (tetramethyl-p-phenylenediaminedihydrochloride). Appearance of a blue colour within 10 seconds indicates a positive result (WHO, 2003).

3.7.4 Bacterial antibiotic susceptibility assays

The antimicrobial susceptibility tests were performed using the Kirby Bauer disk diffusion technique (Bauer *et al*., 1966) with commercially available disks(Hi media laboratories) on Mueller Hinton agar plates (Plate 4.1). Antibiotics disks viability was quality controlled using *E. coli* ATCC 25922. This was performed weekly.The agar was poured to a uniform depth of 4 mm and allowed to cool and solidify according to Clinical and laboratory standards institute (CLSI, 2007) and international guidelines.

A 0.5 McFarland turbidity standard was prepared according to the method described by Dulczak and Kirk (2005). A solution with 9.95 ml of 1 % chemically pure sulphuric acid was mixed with 0.05 ml of 1.175 % barium chloride to form a barium sulfate precipitate which causes turbidity. This standard was used to adjust the turbidity of the inoculums for the antimicrobial susceptibility test. Well isolated single colonies were transferred to the tube with sterile saline and suspensions compared to 0.5McFarland turbidity. After the turbidity of the inocula was adjusted, a sterile cotton swab was dipped into the suspension, pressed firmly against the inside wall of the tube; the swab was streaked over the surface of the medium 3 times rotating the

plate after each application to ensure an even distribution and allowed to stand at room temperature for 10 minutes (Tena*et al*., 2008).

Antimicrobial disks containing specified concentrations in micrograms were placed on the agar plates after 10 minutes (to allow the agar to dry) using a pair of sterile forceps and then gently pressed down on the agar to ensure contact. The plates were inverted, and then incubated at a temperature of 37°C for 24 hours.*E. coli* ATCC 25922 was used as reference. After incubation the zone diameters with complete inhibition, including the diameter of the disk were measured using a ruler and recorded in millimeter on the under surface of the plate without opening the lid. The diameter of the zone of inhibition for each antibiotic was measured and interpreted as resistant, intermediate and sensitive according to Clinical Laboratory Standards Institute criteria (2007) (Table 3.1).

Table 3.1: Standard antimicrobial inhibition zones according to Clinical Laboratory Standards Institute

Antibiotics	Resistant	Intermediate	Sensitive
Gentamicin (10µg)	≤12	13-14	≥15
Ampicilin (10µg)	≤13	14-16	≥17
Nitrofurantion (10µg)	≤14	15-16	≥17
Tetracycline (30ug)	≤11	12-14	≥15
Nalidixic Acid (30µg)	≤13	14-18	≥19
Cotrimoxazole (30µg)	≤10	11-15	≥16
Streptomycin (10µg)	≤10	12-14	≥15
Sulphamethoxazole (30µg)	≤13	14-18	≥15

3.7.5 Data analysis

Data collected was labeled appropriately. Patients names were not used, numbers and letters were used to label the samples. The raw data was entered into excel spreadsheets and later imported to Statistical package for social sciences (SPSS version 15) for analysis. Data were presented as frequencies. Chi square analysis ($\chi2$) and students T-test was used in findings on comparison of positive UTI cases according to individual characteristics. Evaluations were carried out at 95 % confidence level and P< 0.05 was considered statistically significant.

3.7.6 Ethical considerations

The study obtained approval from the medical officer in charge of Kisii level 5 hospital. This study also applied and was granted ethical clearance from the Kenyatta University ethical review committee. Informed patient's consent was sought and obtained through writing from the participants parents/guardians. All patients' data and bacterial isolates gathered in this study were handled confidentially by the researcher. Further, acceptable protocols of handling patient data were strictly adhered to. In addition, laboratory coding was used to identify patients from whom the data was obtained.

3.7.7 Validity and Reliability of data

Reliability refers to the degree of consistency and precision of collected data. Cronbach's alpha was used to determine the coefficient of reliability using Statistical package for social science (SPSS). It was 0.705.

3.7.8 Scope and limitation of the study

The study encountered several limitations. First the study was restricted to children patients with UTI symptoms who sought medical attention at kisii level 5 hospital. Finally this was a cross sectional survey study.Consequently data on key variables was only collected once at a given period of time. Further the trends in the resistance of bacterial isolates to commonly used antimicrobials in the study community over time could not be established. The findings of this study that required such rich data to clarify were therefore only speculated upon based on previous studies from elsewhere.

Despite taking all due precautions, and instructing children and their guardians how to take a clean catch, urine specimen, the possibility of contamination cannot be completely eliminated.

CHAPTER FOUR

RESULTS

4.1 Characteristics of the samples collected at Kisii level 5 Hospital

A total of 186 fresh urine samples were collected from children patients by the standard mid-stream catch method in sterile universal bottles (Appendix VII).Among the sampled population, 14.0 % had UTI while 86.0 % had no UTI.

4.1.1 Demographic information of children presenting with UTI symptoms at Kisii level 5 hospital

In regard to sex of the children, the males were 68 while the females were 118. The age of children who presented with urinary tract symptoms and from whom urine was collected were in the age groups of 0-2 years, 3-4 years, 5-7 years, 8-9 years and 10-12 years. The mean age of the children was 5.0 ±1.8.Prevalence of UTI among the children in the different sex showed that there was no significant difference ($\chi 2$ = 2.369, P= 0.187).

In the age group 0-2 years, only one female had UTI, 3-4 years one male and one female presented with UTI, 5-7 years one male and 8 female had UTI, 8-9 years 4 male and 6 female had UTI, 10-12 years only 4 female had UTI (Table 4.1). In regard to sex and age distribution of children patients presenting with UTI it was as follows; 0-2 years only 7 male and 1 female presented with UTI, 3- 4 years 1 male and 1 female, 5-7 years 1 male and 8 female, 8-9 years 4 male and 6 female, and 10-12 years no male presented with UTI while 4 females presented with UTI. Therefore the age group that presented with most UTI cases was between 8-9 years (10 children) and the least age group with UTI cases was between 0-2 years (1 child). Most female

with UTI cases were between the age group 5-7 years (8 female) whereas most male were between 8-9 years (4 male) (Table 4.1). When using Paired sample T-test on the number of female and those of male having UTI, the result showed there was no significant difference (T = 1.670, P = 0.170) (Table 4.1).

Table 4.1: Urinary tract infection status among the age groups that presented at Kisii level 5 hospital

| Age group | Urinary Tract Infection status | Sex of the children | | Total |
		Male	Female	
0-2 years	Positive	0	1	1
	Negative	7	0	7
3-4 years	Positive	1	1	2
	Negative	13	19	32
5-7 years	Positive	1	8	9
	Negative	18	26	44
8-9 years	Positive	4	6	10
	Negative	20	40	60
10-12 years	Positive	0	4	4
	Negative	4	13	17
Mean no. having UTI		12.40 ± 3.08a	19.60 ± 6.65a	

4.1.2 Hospital status of the children presenting with UTI symptoms at Kisii level 5 hospital

The hospital had both inpatients and out patients attending for their UTI services offered. Among the population sampled, majority, 88.2 % were out patients while only 11.8 % were in patients. Patients who had UTIwere mainly outpatients in this hospital showing a significant difference in the hospital status of the children (inpatient or outpatient) ($\chi 2$= 0.961, P= 0.001).

A significant difference in the patients UTI status to the gender of the patients was also noted ($\chi 2$= 2.369, P = 0.001). More female patients (76.9 %) had UTIthan their male counterparts (23.1 %).

4.1.3 UTIs among school going children

The results showed that, more children sampled were going to school (78.5 %). Only 21.5 % of the children were not going to school. Among those going to school, 13.7 % had UTI while among those not going to school, (15.0 %)had UTI. There was no significant difference in the prevalence of UTIs among the school going and those children not going to school ($\chi 2$= 0.044, P = 0.801).

4.1.4 Recurrence of UTI among children patients presenting at Kisii level 5 hospital

Most of the UTI patients (96.2 %) who attended Kisii level 5 Hospital did not experience any recurrence. Only 3.8 % had experienced recurrence of UTI. Recurrence of UTI was significantly more among the female children (4.2 %) than the male children (2.9 %) ($\chi 2$= 0.655, P = 0.001).

4.2 Identification of uropathogens isolated from children presenting in Kisii level 5 hospital

Various biochemical tests were performed to identify the uropathogens. They were identified as follows (Table 4.2).

Table 4.2: Biochemical tests used to identify the organisms isolated from children presenting in Kisii level 5 hospital

Indole	MR	VP	Citrate	Oxidase	Catalase	Coagula	Motility	G.status		Conclusion
P	P	N	N	N	N	N	P	N	*E. coli*	
N	N	P	P	N	N	N	N	N	*Klebsiella*	
N	N	N	N	N	P	P	N	P	*S. aureus*	
N	N	N	N	P	N	N	N	N	*P. aureginosa*	

MR-Methyl red,   VP-VogesProskaur, P-Positive, N - Negative

Four genera of bacterial agents were isolated. Three Gram negative rods including *E. coli* andone Gram positive cocci (Table 4.3).

Table 4.3: Etiological bacterial agents isolated from children presenting with UTI at Kisii level 5 Hospital

Microorganism	No. of isolates	%
Gram negative rods		
P. aeruginosa	1	3.8
Klebsiella	8	30.8
E. coli	13	50.0
Gram positive cocci		
Staphylococcus aureus	4	15.4
Total	**26**	**100**

4.3 Prevalence of uropathogens among children presenting with UTI in Kisii level 5 Hospital

In reference to the culture results obtained, the overall prevalence of UTI was 26 out of 186 samples. UTI prevalence among themale children was 6 out of 68, (8.8 %) while among the female children was 20 out of 118,*E. coli* was isolated in 4 males out of 68 and 9 female children out of 118. Prevalence of *S. aureus*among male children was 2 out of 68. Among the female children, prevalence of *S. aureus* was 2 out of 118. *Klebsiella*was isolated in 4 male children out of 68 and 4 females out of 118. *P aeruginosa* was isolated in one female child.

4.4 Evaluation of antibiotic susceptibility patterns of isolates to antibiotics used in the treatment of UTI in children presentingat Kisii level 5 Hospital

Antimicrobial susceptibility test showed that *E. coli* was sensitive to Sulphamethicilin, Tetracycline, Streptomycin, Gentamicin,Nalidixic acid, Ampicilin andNitrofurantion. *P.aeruginosa*was sensitive to Ampicilin, Nalidixic acid, Streptomycin and Cotrimoxazole. However it was resistant to Nitrofurantion, Tetracycline, Sulphamethicilin and Gentamicin. *Klebsiella*was sensitive to Ampicilin, Nalidixic acid, Sulphamethicilin, and Cotrimoxazole. It was resistant to Ampicilin, Nalidixic acid, Sulphamethicilin and Cotrimoxazole. *S. aureuswas* sensitive to Nalidixic acid, Streptomycin and Nitrofurantion while it was resistant to Ampicilin, Sulphamethicilin, Cotrimoxazole, Tetracyclineand Gentamicin (Table 4.4).

Table 4.4: Susceptibility profiles of bacterial isolates from children presenting in Kisii level 5 hospital to antibiotics

Antibiotics	Bacteria isolated								
	E. coli		*P.aeruginosa*		*Klebsiella*			*S. aureus*	
	N = 13		N = 1		N = 8			N = 4	
	%S	%R	S	R	S	I	R	%S	%R
Nitrofurantion	53.8	46.2	0	1	0	8	0	3	1
Ampicilin	53.8	46.2	1	0	0	8	0	0	4
Nalidixic acid	61.5	38.5	1	0	3	0	5	4	0
Tetracycline	76.9	23.1	0	1	0	8	0	1	3
Streptomycin	76.9	23.1	0	1	0	8	0	4	0
Sulphamethoxazole	84.6	15.4	0	1	2	0	6	0	4
Gentamicin	69.2	30.8	0	1	0	8	0	1	3
Cotrimoxazole	38.5	61.5	1	0	3	1	4	0	4

S – Sensitive, I – Intermediate, R – Resistant, N – Number of isolates

Susceptibility of the four bacteria strains showed that, *E. coli* was more susceptible to Sulphamethicilin; *P. aeruginosa* was more susceptible to Ampicilin, Cotrimoxazole and Nalidixic acid; *Klebsiella* was more susceptible to Nalidixic acid and Cotrimoxazole; *S. aureus* was more susceptible to Nalidixic acid and streptomycin (Figure 4.4). *P. aeruginosa*and *Klebsiella* were susceptible to only 3 out of the 8 tested antibiotics; *S. aureus* was susceptible to 5 while *E.coli* was susceptible to all the 8 tested antibiotics (Figure 4.1).
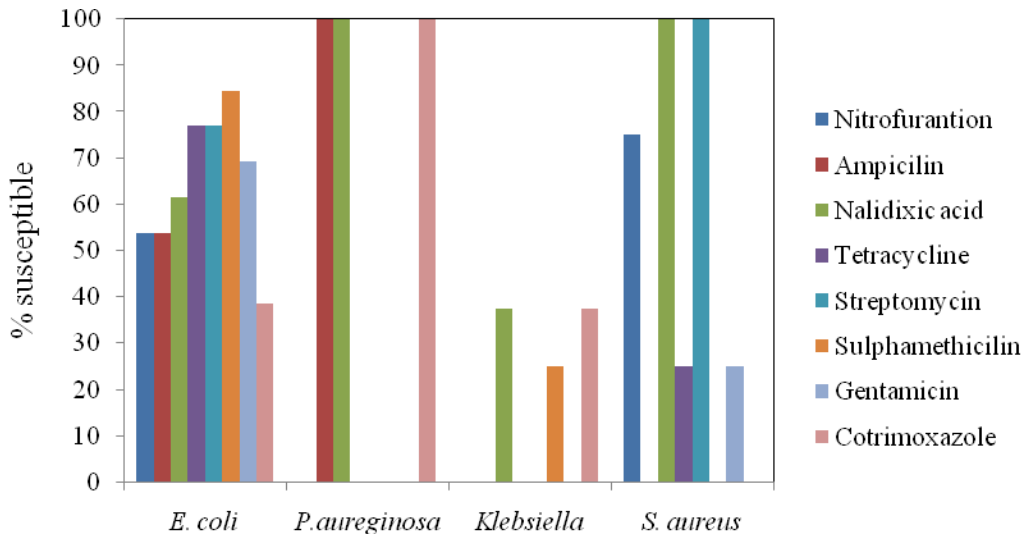
Figure 4.1: Susceptibility of the four bacteria strains to the antibiotics tested.

4.4.1 Quality control

Quality control of antimicrobials was done weekly using *E.coli* ATCC 25922 (Figure 4.2).
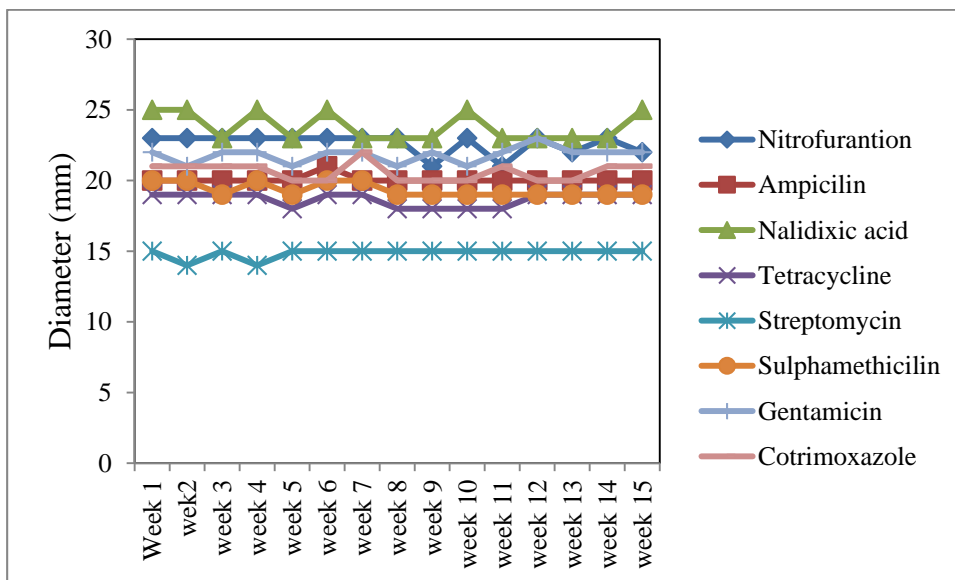
Figure 4.2: Quality control of antimicrobials using *E. coli* ATCC 25922

Plate 4.1: Antibiotic sensitivity plate of *S.aureus.*

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

A majority of patients recruited with UTI symptoms in this study were female. This could be due to the tendency of clinicians sending females with symptoms of UTI to the laboratory based merely on the observation that females are more pre-disposed to UTIs than males. It can also be attributed to the fact that more females presented with UTI symptoms than males. The high prevalence of infection in females (16.9 %) reported in this study is related to anatomical and pathogenic factors, for example, the short length of the urethra hence lesser distance of bacteria ascending up the urinary tract, and lack of antimicrobial properties of prostatic fluid as in males (Daikos and Sharifi, 2007). This high prevalence of UTI in females is comparable to a prevalence rate of 17 % reported in Nigeria byAsinobi*et al*.(2003). However, this study is in agreement with other reports which stress that UTI is more frequent in females than in males (Ibeawuchi and Mbata, 2002; Olaitan, 2005; Mbata, 2007). Misdiagnosis of other conditions as UTI and the subsequent unnecessary therapy represents an additional cost and also gives an opportunity for exposure of antimicrobials to normal flora that may lead to development of resistance (Foxman, 2003).

This study showed a significant difference in the hospital status. Outpatient children mainly presented with UTI;this could be attributed to predisposing factors like congestion, contaminated toilet facilities in schools and poor drainage (Hannah *et al*., 2011). *S. aureus* was isolated from the samples collected from the hospital. This is probably due to the fact that *S. aureus*is a member of the normal flora of both asymptomatic carriers and sick persons thus takes advantage of the weak immune

system. This organism can be spread by the hands, expelled from the respiratory tract or transmitted by animate or inanimate objects (Pelczar*et al.,* 2003). There are numerous reported cases of emerging UTI caused by Methicilin resistant *S. aureus*(MRSA), Vancomycin resistant *S. aureus*(VRSA) and other multi-drug (MDR) resistant strains in the world (Zhanel*et al*., 2000). These findings agree with others by Ashkenazi *et al*. (2001) that hospital acquired UTI are caused by *S. aureus.*

In this study, of 186 patients from whom urine samples were taken, only 14 % had UTI. This is possibly because UTI symptoms are not a reliable indicator of infection in children younger than 2 years of age. This is similar to figures reported in previous studies by Aiyegoro*et al*. (2007) among children in Ile-Ife, Nigeria. This finding is also comparable to the 14.2 % incidence reported by Farajnia*et al*. (2009) in a similar study among children in the academic hospital of Jordan University, Jordan. This incidence is however lower than the prevalence rate of 25.6 % by Nedolisa (1998) at the Jos University Teaching hospital, Nigeria, 18 % reported by Hannah *et al*. (2011) in children patients hospitalised at the Kenyatta National hospital and by Kebira*et al*. (2009) who reported a prevalence rate of 24 % in Thika level 5 hospital.

In antimicrobial susceptibility profiles, high resistance was recorded in Ampicilin. Out of the 4 isolates, 3 of them were resistant to Ampicilin. This is attributed to the use of this drug which is a first line treatment hence organisms have developed resistance to it (Kariuki*et al*., 2007). The other reason is that β lactams are relatively ineffective in clearing Gram negative rods from the vagina and colonic mucosa, thus possibly predisposing to recurrences when used to treat UTI (Vasquez and Atal,2004). According to previous studies high rate of resistance to Ampicilin was reported in

several countries such as Senegal (77 %), Spain (65 %), Taiwan (80 %), India (88 %) and Iran (88 %) (Lau*et al*., 2004, Minal*et al*. 2012, Daikos and Sharifi, 2007).

Cotrimoxazole also had a high level of resistance. Three isolates out of four were resistant to Cotrimoxazole. This is because it does not achieve systemic antibacterial levels used for treatment of UTIs (Lau*et al*., 2004). Amino glycosides such as Streptomycin has improved antibacterial activity compared to Cotrimoxazole and therefore should be incorporated in the treatment of UTIs (Rakaa*et al*., 2004). Streptomycin is the most potent antibiotic on the organisms tested. This amino glycoside (Streptomycin) was sensitive to all the 4 isolates. It is an irreversible inhibitor of protein synthesis and its mode of action is by binding to specific 30s subunit of the ribosomal proteins.

All isolates were susceptible toNitrofurantion; this finding agrees with a previous report in Kenya (Mitema and Kikuvi, 2004). It is one of the oldest urinary antibacterial agents still in use. This antibiotic is thought to have many modes of action that delay the emergence of resistance. *E. coli* isolates were highly susceptible to 7 antibiotics but less sensitive to Cotrimoxazole, a finding similar to that already reported by Mitema and Kikuvi (2004). This susceptibility rate contrast greatly with findings in the U.S.A where regional resistance rates to Cotrimoxazolerange from 11.8 to 21.8 percent (Daikos and Sharifi, 2007). This can be attributed to the fact that bacteria undergo mutation which makes their susceptibility vary from one geographical region to the other (Gupta *et al*., 2001). Cotrimoxazole can thereforebe used as empiric therapy for uncomplicated UTIs in some regions. This finding advises

the use of Streptomycin and Nitrofurantion as empirical therapy for UTIs in the study community.

The pattern and frequency of occurrence of thebacterial isolates found in this study is similar to what hasbeen previously reported. *E. coli*was the most predominant microorganism isolated and was prevalently more among the female patients. This could be due to the close proximity of the vagina to the anus and the short urethra. It also accounted forhalf of the reported cases; this is due to the fact that *E. coli* are the most Gram negative bacteria responsible for UTI. It is also attributed to the presence of P- fimbriae which enable them to attach to the uroepithelium cells and interfere with the normal wash out of bacteria (Tanagho and Mcaninch, 2004). It was followed by *Klebsiella* spp., *S. aureus*, and *P. aeruginosa*. This finding is similar to other reports which suggest that Gram negative bacteria particularly *E. coli*is the most common isolated pathogen in patients with UTI (Shaikh*et al*., 2008). Also reported in their study is that *E. coli* was the most commonly isolated pathogen in significant bacteriuria. In a similar study by Nwanze*et al*. (2007) the common isolates were *E. coli*, *S. aureus*, and *Klebsiella* spp. respectively. This same pattern was also reported by Kolawole*et al*. (2009). The 30.8 % incidence rate reported by *Klebsiella* in this study brings to light the fact that they are achieving more prominence as aetiological agents for UTI than previously reported (Obaseki, 1988; Abdulrahnaman*et al*., 1992; Nwanze*et al*., 2007; Kolawole*et al*., 2009). The pattern of isolates reported in this study is thus consistent with the usually reported pattern, with *E. coli* being the most common organism isolated in cases of UTI followed by *Klebsiella*, *S. aureus*, and *P. aeruginosa* was the least common isolate.

Only 3.8 % of the confirmed UTI cases in this study were recurrent. This study also showed that occurrence of UTI in children who were school going and those who are not school going was not significantly different, this could be due to the fact that most of the children attend baby care units with caretakers who ensure that children are kept clean after urinating and their diapers are changed regularly and lastly older children are toilet trained. This trend can also be attributed to the high levels of literacy among parents in the study region.

5.2 Conclusion

The findings of this study revealed that the important infecting organisms were the commensals of perianal and vaginal regions. This calls for increase in personal hygiene. Enterobacteriaciae particularly *E.coli* are important etiological agents causing UTIs in children presenting at Kisii level 5 hospital. Other etiological agents identified in this study include *Klebsiella, P. aureginosa,* and *S.aureus*. Urine culture assists in identifying bacteria causing UTIs therefore treatment based on positive culture may lead to resolution of acute symptoms and eradication of the pathogens.

E. coli was more susceptible to Sulphamethicilin; *P. aeruginosa* was more susceptible to Ampicilin, Cotrimoxazole and Nalidixic acid; *Klebsiella* was more susceptible to Nalidixic acid and Cotrimoxazole; *S. aureus* was more susceptible to Nalidixic acid and streptomycin. *P. aeruginosa*and *Klebsiella* were susceptible to only 3 out of the 8 tested antibiotics; *S. aureus* was susceptible to 5 of the antibiotics.

The overall prevalence of UTIs is 14.0 % in children presenting at kisii level 5 hospital with UTI symptoms.Females are more susceptible to UTI than males. This

prevalence should be of great concern, as not only do UTIs pose a threat to health, but they also impose an economic and social burden due to the stigma associated with these infections.

5.3 Recommendations

Based on the results of this study the following can be recommended:

i) There is need to monitor the profile of etiological bacteria of UTIs and the antimicrobial resistance regularly. This would show emergence of resistance to newer therapeutic agents as well as keep track of effectiveness of serving therapeutic agents.

ii) It is recommended that the treatment of UTIs at Kisii level 5 hospital should be based on available evident data in order to achieve effective therapy for patients, limit emergence of antimicrobial resistance and utilize resources optimally instead of regular prescription of outdated drugs.

iii) Health workers should mobilize patients to ensure that they have duly completed the prescribed antimicrobial therapy as not finishing it leads to resistance.

iv) Parents should not assume their children automatically need antibiotics and should not routinely ask for them when their children are sick this will greatly reduce antimicrobial resistance instead they should seek a physician's advice.

v) Several future research gaps worth filling are recommended. There is need to establish the influence of resistance on recurrent UTIs, and the risk factors

involved in infection with resistant bacteria strain. Elaborating of in vivo effectiveness of the commonly used antimicrobial agents particularly the ones showing high resistance prevalence rates such as Ampicilin and investigating the endogenous nature of the identified uropathogenic isolates are other fruitful future research areas.

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APPENDICES

APPENDIX I

CONSENT FORM

Dear parent/guardian,

I am a student at Kenyatta University undertaking a study on prevalence and susceptibility profiles of bacterial pathogens associated with urinary tract infections in children presenting at kisii level 5 hospital, kisii county, Kenya.

Purpose of the study: To identify bacteria that cause urinary tract infections in children and test if they are inhibited or resist commonly used antibiotics.

Procedure to be followed: Your child will give a urine sample in a clean sterile bottle which will be examined for presence of bacteria and other indicators of urinary tract infections. The sample will be cultured in the laboratory and any bacteria that grow will be identified and tested if they are eliminated or resist inhibition by antibiotics.

Risks involved: The procedure for collection of urine sample is not invasive and does not pose pain or harm to your child.

Benefits: Results obtained from this study will be used in your child's treatment.

Confidentiality of the records: Personal information gathered from your child will be encoded for purposes of confidentiality and the name of the child will not be disclosed. Only the code numbers will be used in reports and publications.

Participation: It is important for you to know that you can decline to allow your child to participate in this study.

Consent: I have carefully read the above information and was given the opportunity to seek clarification. I have fully understood that there are no risks associated with the collection of the urine sample.

Signature……………………………………Date…………………………………..

Age of the child…………………………………………………………………..

Sex of the child…………………………………………………………………

Study number (To be provided)…………………………………………………..

I the undersigned have fully explained the relevant details of this study to the parent/guardian of the patient.

Signature…………………………………Date…………………………………...

APPENDIX II

QUESTIONNAIRE

Dear parent/guardian,

I am a student at Kenyatta University undertaking a study on prevalence and susceptibility profiles of bacterial pathogens associated with urinary tract infections in children presenting at kisii level 5 hospital, kisii county, Kenya.

Please fill in accurately. The information obtained will be confidential and used for the above stated study.

Study number (to be provided)………………………………….

A) GENERAL INFORMATION

Tick where appropriate

1. Age of the child

0-2years ☐   3-4years ☐   5-7years ☐   8-9years ☐   10-12 years ☐

2. Sex of the child

Male ☐   Female ☐

3. Is the child admitted in hospital or outpatient?

Inpatient ☐   Outpatient ☐

4. Is your child school going? Yes ☐   No ☐

5. Has the child been diagnosed of a urinary tract before? ....If yes when did s/he last use drugs (Antibiotics)?

....

APPENDIX III

PREPARATION OF MUELLER HINTON AGAR

It is used for determination of susceptibility of microorganisms to antimicrobial agents.

Ingredients	Grams/Litre
Beef infusion	300.0
Casein acid hdrolysate	17.50
Starch	1.50
Agar	17.0

Directions

1. Suspend 38.0 Grams in 1000 ml distilled water.

2. Heat to boiling to dissolve the medium completely.

3. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

4. Mix well before pouring onto sterile petri plates.

APPENDIX IV

PREPARATION OF TRIPPLE SUGAR IRON AGAR

It is used for identification of gram negative enteric bacilli on the basis of dextrose,

lactose and sucrose fermentation and hydrogen sulphide production.

Ingredients	Grams/Litre
Peptic digest of animal tissue	10.0
Casein enzyme hdrolysate	10.0
Yeast extract	3.0
Beef extract	3.0
Lactose	10.0
Sucrose	10.0
Dextrose	1.0
Sodium chloride	5.0
Ferrous sulphate	0.20
Sodium Thiosulphate	0.30
Phenol red	0.024
Agar	12.0

Directions

1. Suspend 64.52 grams in 1000ml distilled water.

2. Heat to boiling to dissolve the medium completely.

3. Mix well and distribute into tubes.

4. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

5. Allow the medium to set in slopped form with a butt about 1 inch long.

APPENDIX V

PREPARATION OF MACCONKEY AGAR

It is used for selective isolation and differentitiation of coliform organisms and other enteric pathogens.

Ingredients	Grams/Litre
Peptic digest of animal tissue	1.50
Casein enzyme hdrolysate	1.50
Pancreatic digest of gelatine	17.0
Lactose	10.0
Bile salts	1.50
Sodium chloride	5.00
Crystal violet	0.001
Neutral red	0.03
Agar	15.0

Directions

1. Suspend 51.5 grams in 1000 ml distilled water.

2. Heat to boiling with gentle swirling to dissolve the agar completely.

3. Sterilize by autoclaving at 121ºC.

4. Avoid overheating.

5. Cool to 45ºC and pour into sterile petri plates.

6. The surface of the medium should be dry when inoculated.

APPENDIX VI

PREPARATION OF CLED (BEVIS) MEDIUM (CYSTEIN LACTOSE ELECTROLYTE DEFICIENT)

This is a differential medium for the enumeration of urinary tract pathogens.

Formulation	Gram/Litre
Balanced peptone No.1	4.0
Beef extract	3.0
Tryptone	4.0
Lactose	10.0
L-cysteine	0.128
Bromothymol blue indicator	0.02
Andrade's indicator	0.08
Agar No.1	15.0

Directions

1. Weigh 36 grams of powder to a conical flask; disperse in 1 litre of deionised water.

2. Allow soaking for 10 minutes; swirl to mix.

3. Sterilize by autoclaving for 15 minutes at 121ºC.

4. Cool to 47ºC and mix well before pouring to the sterile petri plates.

APPENDIX VII

**URINE SAMPLES COLLECTED FROM CHILDREN PATIENTS IN KISII
LEVEL 5 HOSPITAL**

APPENDIX VIII

EscherichiaColi **GROWING ON MACCONKEY AGAR**