ETIOLOGICAL AGENTS AND ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF BACTERIAL AGENTS CAUSING URINARY TRACT INFECTION IN UNDER-FIVE CHILDREN, DAR ES SALAAM, 2010.

By

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CERTIFICATION

The undersigned certify that they have read and hereby recommend for examination of thesis/dissertation entitled, ***Etiological agents and antimicrobial susceptibility patterns of bacterial agents causing urinary tract infection in under-five children, Dar es Salaam, 2010*** in fulfillment of the requirements for the degree of Master of Science (Epidemiology & Lab Management) of Muhimbili University of Health and Allied Sciences.

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DEDICATION

This dissertation is dedicated to my beloved wife Beatha, our sons John and Shigongo whose believe in the importance of education has contributed much in making the way we are.

ABSTRACT

Background: The empirical therapy of urinary tract infections (UTI) relies on the predictability of the agents causing UTI and knowledge of their antimicrobial susceptibility testing patterns. This information need to be updated regularly. This study was conducted to assess the spectrum and patterns of antimicrobial resistance of UTI agents isolated from under-five children presenting with clinical symptoms suggestive of UTI at district hospitals in Dar es Salaam.

Methods: This was a cross-sectional study with epidemiological and laboratory components carried out at Amana and Mwananyamala Municipal hospitals. Epidemiological data was gathered from children's parents /guardian using a structured questionnaire for socio-demographic information and antimicrobial drug usage. Laboratory data included results of urine for culture in Cystine-Lactose-Electrolyte Deficient (CLED) agar media and susceptibility test done in Mueller Hinton agar using Kirby Bauer technique.

Results: Of the 270 children, 166 (61.5%) were male, mostly aged less than twelve months, 72.6% of mothers were housewives. Among cultured mid-stream urine samples, 78 (28.9%) revealed a significant single isolate growth of $\geq 10^5$ colon-forming units per milliliter of urine, 32 (11.9%) had mixed growth, 26 (9.6%) non-significant growth, and the remainder 134 (49.6%) revealed no bacterial growth. Among the isolated bacteria, *Escherichia coli* was the most common isolate 33 (42.3%), followed by *Klebsiella spp* 31 (39.7%), *Streptococcus spp* 4 (5.1%) while 3 (3.8%) were isolated each for *Staphylococcus aureus*, *Proteus mirabilis* and unidentified coliforms. *Pseudomonas spp* was isolated only once. The isolated bacteria had high resistance to amoxicillin 79 (98.7%), trimethoprim-sulfamethoxazole 77 (96.2) and ampicillin 76 (94.9%). They had low resistance against amikacin 6 (7.7%) and nitrofurantoin 16 (20.5%). Self medication showed no significant association with the development of multiple drug resistance.

Conclusions and Recommendations: In view of the high drug resistance amongst bacteria (95 – 98%), therapy should only be advocated, as far as possible, after culture and susceptibility test has been performed. Based on the results of this study, the empirical treatment of UTI should be done mostly with nitrofurantoin which revealed

lower resistance; also ciprofloxacin can be secondly considered as amikacin has limited usage to pediatrics due to side effects. Meanwhile more studies are required to get the baseline susceptibilities pattern of antibiotics to be incorporated in the National guideline for management of UTI for under-fives.

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LIST OF ACRONYMS/ABBREVIATIONS

AAP	American Academy of Pediatrics
ATCC	American Type Culture Collection
CLED	Cystine-Lactose-Electrolyte Deficient
DMSA	Dimercaptosuccinic acid
HIV	Human Immunodeficiency Virus
MNH	Muhimbili National Hospital
MOHSW	Ministry of Health and Social Welfare,
MUHAS	Muhimbili University of Health and Allied Sciences
NCTC	National Collection of Type Cultures
PABA	*p*-aminobenzoic acid
TFELTP	Tanzania Field Epidemiology and Laboratory Training Program
TPDF	Tanzania People Defense Forces
UK	United Kingdom
UTI	Urinary Tract Infections

CHAPTER ONE

1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 Background

Urinary tract infection (UTI) results from the presence and multiplication of microorganisms in one or more structures of the urinary tract with associated tissues invasion. It is the presence of multiplying micro-organisms in significant number of more than 10^5 bacteria per milliliter of urine. This can give rise to a wide variety of clinical syndromes. These include acute and chronic pyelonephritis (kidney and renal pelvis), urethritis (urethra), epididymitis (epididymis) and prostatitis (prostate gland). Infection may spread to surrounding tissues (e.g. perinephric abscess) or to the blood stream, (Sobel, 1997). Urinary tract infection may involve just the lower urinary tract or both the lower and upper urinary tracts. In children they are particularly important because their occurrence may be associated with some congenital abnormality of the urinary tract or an error in management. If not corrected, these may lead to recurrent infections causing damage to the urinary tract (Winberg, 1992).

In healthy children urine is sterile. The most important factor in maintaining the sterility of the urinary tract is emptying the bladder completely and frequently. The most common causative agent of UTIs is bacteria that initially settle (colonize) around the urethra, and then ascend into the rest of the urinary tract. Several factors can make this process more likely to occur (Winberg, 1992). UTI is a problem that is frequently encountered by pediatric healthcare providers. Over recent decades, the importance of UTI has been increasingly recognized, in particular the role of UTI as an occult cause of febrile illness in young children. The evolving state of knowledge about pediatric UTI leaves many questions and controversies.

1.1.1 Commonest causes of UTI

Clinically important infections usually occur due to bacteria, although viruses, fungi, and parasites can also cause infection. Common bacterial pathogens include Gram-negative species such as *Escherichia coli*, *Klebsiella*, *Proteus*, *Enterobacter*, *Pseudomonas*, and *Serratia* spp and Gram-positive organisms, including group B streptococci, *Enterococcus*

sp, and *Staphylococcus aureus*. In general, bacteria invade the urinary tract by ascending from the urethra, although hematogenous infection may occur in rare instances among young infants. Common non-bacterial causes of UTI include hemorrhagic cystitis from adenovirus and *Candida* infection in immunocompromised individuals. (Zorc *et al,* 2005).

1.1.2 Pathogenesis of UTI

Uropathogens can enter the urinary tract and cause UTI via the ascending route or the hematogenous route. The vast majority of UTI are thought to occur via the ascending route, and bladder infections are much more common than kidney infections. Sometimes, these ascending uropathogens reach the kidney and can cause pyelonephritis (in 5% of all UTI). The mechanism of ascension is uncertain, but motility mediated by flagella and pili appears to be important.

1.1.3 Symptoms of UTI

Symptoms of UTI include dysuria, urinary frequency, urgency, burning, flank pain, suprapubic pain, fever and haematuria. It is generally accepted microbiologically, that UTI exists when pathogenic bacteria are detected in the urinary tract, and the infection is considered significant and requires treatment when $\geq 10^5$ bacteria per milliliter of urine are present in a properly collected urine sample (Gleeson *et al* 1991). The condition ranges from cystitis (a mild but distressing inflammation that is limited to the bladder) to severe infections of the kidney such as pyelonephritis (when the infection has reached the kidney tissue itself (Gleeson *et al,* 1991).

1.1.4 Epidemiology and Public Health Importance of UTI

The epidemiology of UTI during childhood varies by age, and gender. The incidence of UTI is highest in the first year of life for all children (1%) but decreases substantially among boys after infancy (Jakobsson *et al,* 1999). Estimates of UTI incidence among infant boys have varied in different populations, likely due to factors such as circumcision, which has been associated with a reduction in risk of UTI (Jakobsson *et al,* 1999). Infants and young children presenting with UTI have traditionally been investigated extensively due to a concern that developing kidneys are more susceptible to damage from pyelonephritis and that the risk of identifying associated urinary tract abnormalities is statistically higher in this group (Berg *et al,* 1983). Originally there

appeared to be a difference in outcomes with infection occurring under the ages of 3 to 5 years based on studies which found a higher incidence of renal scarring in this age group (Berg *et al,* 1983).

1.1.5 Clinical Diagnosis

The question of when to screen for UTI has been evaluated in a number of studies and addressed by a subcommittee of the American Academy of Pediatrics (AAP, 1999). The presence of specific symptoms for UTI, including dysuria, frequency, urgency, suprapubic discomfort, and flank pain, should lead to screening. However, young children with UTI may present with nonspecific symptoms, such as poor feeding, vomiting, irritability, jaundice (in newborns), or fever alone, and a broader approach to screening may be appropriate. A patient with dysuria (painful voiding) and urinary frequency generally has to get a spot mid-stream urine sample sent for urinalysis, specifically for the presence of nitrites, leukocytes or leukocyte esterase (AAP, 1999). If there is a high bacterial load without the presence of leukocytes, it is most likely due to contamination. The diagnosis of UTI is confirmed by a urine culture to assess for significant growth of single uropathogen. In severe infection, characterized by fever, rigors or flank pain, urea and creatinine measurements may be performed to assess whether renal function has been affected. However, UTI in young infants must receive some imaging study, typically a retrograde urethrogram, to ascertain the presence/absence of congenital urinary tract anomalies (AAP, 1999).

1.1.6 Treatment

The appropriate treatment for UTI has been a subject of recent research. Traditionally, young children with a clinical diagnosis of pyelonephritis are admitted to the hospital for intravenous antibiotics, a clinical trial by Hoberman et al in 1999 compared intravenous cefotaxime with oral cefotaxime in a group of children with febrile UTI (Hoberman *et al*, 1999). Clinical outcomes, including detection of scarring by dimercaptosuccinic acid (DMSA) scan at 6 months, were similar between the two groups. The power to detect small differences in outcomes was limited, particularly among subgroups that may be at increased risk, such as younger children. However, the study suggested that outpatient therapy of pyelonephritis may be appropriate in selected children. (Hoberman *et al*, 1999).

Early treatment of asymptomatic bacteriuria and urinary tract infections in children can prevent adverse outcome. However, antimicrobial resistance can impede effective chemotherapy. A number of factors, which may promote antimicrobial resistance such as availability of antimicrobials without prescription, use of counterfeit or substandard antimicrobial drugs, suboptimal hygiene, immunosuppression due to malnutrition or HIV, may be more frequent in resource-limited countries. There is a need for treatment to be advocated as most of these children receive antibiotics without knowledge regarding the causative organism or its sensitivity to antibiotics (Baraff, 2000).

1.1.7 Antimicrobial resistance of UTI pathogens and Risk factors

Resistance to antimicrobial agents is an emerging problem worldwide among uropathogens that cause uncomplicated UTI. Resistance of uropathogens to antibiotics is increasing. Previous studies in adults and children have identified a variety of risk factors for the presence of resistant organisms, including prior antibiotic exposure,, and the use of prophylactic antibiotics (Jacobson *et al,* 1989)

Several investigations have examined predictors of drug resistance among uropathogens. Easily identifiable clinical characteristics associated with trimethoprim-sulfamethoxazole resistance have included recent antibiotic exposures, recent hospitalization, diabetes mellitus, three or more UTIs in the past year, and possibly use of oral contraceptives or estrogen-replacement drugs (Brown *et al*, 2002).

1.2 Literature review

In many literatures UTI is defined as colonization of a pathogen occurring anywhere along the urinary tract: kidney, ureter, bladder, and urethra (Smellie *et al,* 1998) Traditionally, UTI have been classified by the site of infection (i.e., pyelonephritis [kidney], cystitis [bladder], urethra [urethritis]) and by severity (i.e., complicated versus uncomplicated). A complicated UTI describes infections in urinary tracts with structural or functional abnormalities or the presence of foreign objects, such as an indwelling urethral catheter. This model does not necessarily reflect clinical management, however. In children, a simpler and more practical approach is to categorize UTI as a first infection versus recurrent infection. Recurrent infections can be further subdivided into (1) unresolved bacteriuria, (2) bacterial persistence, and (3) reinfection (Smellie *et al,* 1998).

Infections of the urinary tract generally resolve with adequate treatment in most children. In neonates and infants, however, they are presumed to be complicated because of the high association between urinary tract malformation and concurrent bacteremia, which predispose children to acute morbidity and long-term renal insufficiency (Benador *et al*, 1997).

The recurrence of UTI may be caused by several reasons. Unresolved bacteriuria is most commonly caused by inadequate antimicrobial therapy. Sub therapeutic levels of the antimicrobial agents may be a result of noncompliance, malabsorption, suboptimal drug metabolism, and resistant uropathogens unresponsive to attempted therapy (Pewitt *et al,* 1997). In these cases, infection typically resolves after altering the therapy according to antimicrobial susceptibilities determined by a proper urine culture. Young children with UTIs may only have a fever, poor appetite, vomiting, or no symptoms at all (Pewitt *et al,* 1997).The treatment for pediatric UTI varies considerably with several factors. The choice of antibiotic may be affected by local resistance patterns and other considerations. Amoxicillin was traditionally the first-line therapy for outpatient treatment of UTI in children (AAP, 1999). However, increased rates of *Escherichia coli* resistance have made amoxicillin a less acceptable choice, and studies have found higher cure rates for trimethoprim-sulfamethoxazole (AAP, 1999). Other choices include amoxicillin-clavulanate or cephalosporins such as cefoxime, cephalexin, cefprozil, or cefpodixime. Fluoroquinolones are widely used in adult patients, although concerns about potential effects on musculoskeletal joint development based on animal data have restricted their use in young children (AAP, 1999). A recent review of the use of fluoroquinolones for pediatric UTI noted a high rate of efficacy among patients with complex medical conditions or multidrug resistance, although data on the safety of these agents are limited (Koyle *et al,* 2003*)*. Inpatient treatment regimens may include the combination of ampicillin and gentamicin or third-generation cephalosporins. Urine should always be obtained for culture in children so that susceptibilities can be determined to guide antibiotic therapy.

Previous five - year retrospective study was performed to identify organisms isolated from the urinary tract of 2815 children in the community and 1314 children with underlying renal problems and their antimicrobial susceptibilities. Isolates from children

in the latter group were generally more resistant to commonly used antibiotics. In particular, up to a third of *E coli* isolates from children in the community and almost two thirds of *E coli* isolates from children with underlying renal damage was resistant to trimethoprim (Brown, *et al*, 2002).

First line drugs for Pediatric UTI treatment in Tanzania includes trimethoprim-sulphamethaxazole, amoxicillin, ampicillin, gentamicin and Nitrofurantoin, mostly they are empirically administered to suspect cases (MoHSW-NEMLIT, 2007). Results of a previous study done in Tanzania revealed that, among *E. coli* in urinary tract infections, more than 80% were resistant to ampicillin, cotrimoxazole and tetracycline while more than 80% of the isolates were still susceptible to nitrofurantoin, gentamicin and third generation cephalosporins (Urassa *et al,* 1997).

1.2.1 Antimicrobial agents and mechanisms of action for resistance

An antimicrobial agent is any chemical substance that is capable of inhibiting growth or causing the death of micro-organisms. They can either be bactericidal or bacteriostatic. They exert their activity through selective toxicity, by destroying the micro-organisms without causing harm to the body. Antibiotics first came to the forefront with the discovery of penicillin by Fleming in 1929. He observed that bacteria failed to grow in the vicinity of a colony of a fungus called *Penicillin rotatum* (Fleming, 1929). In 1940, Chain and Florey discovered that the above effect was due to a substance called Penicillin produced by these organisms (Chain *et al*, 1940). During the next 25 years, research on chemotherapeutic agents largely centered on substances of microbial origin called antibiotics. The isolation, concentration, purification and mass production of penicillin were followed by the development of streptomycin, tetracycline, chloramphenicol and many other agents (Brooks *et al*, 1998). These substances were originally isolated from filtrates of media in which their respective molds had grown. Subsequently, others have been synthesized and in recent year's biosynthetic modification of molecules have been a prominent technique for development of new antimicrobial agents (Brooks *et al*, 1998).
Mechanism of action of antimicrobial agents is based on selective toxicity. The selective toxicity may be a function of a specific receptor required for drug attachment, or it may depend on the inhibition of biochemical events essential to the organisms but not the host

cells (Brooks *et al*, 1998). Antimicrobial agents can act by inhibiting cell wall synthesis, which are found on bacterial cell and not human cells. Penicillins, vancomycin, cephalosporins, bacitracin and cycloserine inhibit various stages in the synthesis of the cell wall, making the bacterial cells fragile and easy to rupture. Amphotericin B, colistin, imidazoles, triazoles and polyene act by inhibiting bacterial cell membrane function, utilizing the existing structural and animal cells (Brooks *et al*, 1998). Protein synthesis inhibitors, such as erythromycin, tetracycline, chloramphenicol and aminoglycosides utilize the differences in the ribosomal make up between human and bacterial cells to achieve selective toxicity. Nucleic acid synthesis inhibitors, such as quinolones, trimethoprim, sulphonamides and rifampin act by inhibit various stages which are important in the synthesis of bacterial Deoxyribonucleic acid (DNA) (Brooks *et al*, 1998).

The antimicrobial resistance may be genetic or non-genetic. Non-genetic resistance includes failure to act on metabolically insert bacteria, intracellular bacteria and those bacteria that have lost the specific target structure for a drug (Brooks *et al*, 1998). Genetic origin of antimicrobial resistance may be due to spontaneous mutation or due to acquisition of plasmids, which code for enzymes that are capable of destroying drugs. Bacteria have also evolved mechanisms of evading the action of antimicrobial agents, these include production of enzymes that destroy drugs, for example staphylococci produced β-lactamase which destroys the β-lactam ring in penicillin and hence rendering the drug ineffective (Brooks *et al*, 1998, O'Brien, 1997). Bacteria can also alter their outer membranes and hence impairing the permeability of the drug, for example, tetracyclines accumulate in susceptible bacteria but not in resistant bacteria. Other mechanisms include altered structural targets for the drug, for example, penicillin resistance in *S. pneumoniae* and enterococci is due to alteration in penicillin binding proteins (Brooks *et al*, 1998). Bacteria can also develop an altered metabolic pathway that bypasses the reactions inhibited by antibiotics, for example, sulfonamide resistant bacteria can utilize preformed *p*-aminobenzoic acid (PABA) instead of extracellular PABA. Bacteria can alter their enzymes which are still functional but which are not affected many drugs, for example, in trimethoprim resistant bacteria, the dihydrofolic

acid reductase is inhibited less efficiently than in trimethoprim sensitive bacteria (Brooks *et al*, 1998).

1.2.2 Antimicrobial susceptibility patterns

The increasing prevalence of bacterial resistance to commonly prescribed antimicrobial agents, especially in the developing countries poses a major challenge in the management of bacterial infections (Kariuki *et al*, 1997, Kunin, 1993). The global magnitude of antimicrobial resistance is unknown and more so in the developing countries where the literature is very scanty and in most cases deficient (WHO, 1997). The frequency of resistance to antimicrobial agents for individual pathogens and the patterns of multiple resistances vary between countries and often in different parts of the same country. However, the situation has become worse in the last few years (Shears, 1993, Turnidge, 1998, Belihu *et al,* 1999). Antibiotic resistance is rapidly evolving, hence a need for a constant surveillance. In Tanzania, Blomberg in 1998 conducted a surveillance of antimicrobial resistance at a tertiary hospital. Certain trends in antimicrobial susceptibility were identified by comparison with data from other studies. While resistance to ampicillin, tetracycline and sulfonamides in Gram-negative bacteria was frequent already in the seventies, it was worrying that resistance to trimethoprim-sulfamethoxazole, chloramphenicol, nitrofurantoin, nalidixic acid and amoxicillin-clavulanate appeared to have increased compared to previous studies (Blomberg *et al*, 1998).

1.2.3 Factors influencing antimicrobial resistance

Development of antimicrobial resistance occurs as a result of complex interactions, which encourage the emergence, persistence and enhances transmission of resistant organisms (Cohen, 1994). In a study done in a Finnish hospital between 1971 and 1984, the trimethoprim resistance pattern of *E. coli* isolates from urine specimen was related to the antimicrobial use (Huovinen *et al*, 1986). In a study done on patients with cancer and neutropenia reported that between 1983 and 1990, there was no resistance to fluoroquinolones amongst *E. coli* isolates, however, 28% were found to be resistant in 1991 through 1993. This change corresponded to an increase in the rate of fluoroquinolone use from 1.4% of patients between 1983 and 1985 to 45% between 1991 and 1993 (Cometta *et al*, 1994).

In another study done in Gonder, Ethiopia, it was found that, a high proportion of the common pathogens isolated were resistant to the frequently used antibiotics including tetracycline, chloramphenicol, sulphamethaxazole/trimethoprim and ampicillin (Asseffa, 1996). A similar relationship has been reported in a study that was done in Sidamo, Ethiopia between 1985 and 1997, which showed that resistance pattern correlated with the commonly prescribed antibiotics (Belihu *et al,* 1999). In a study done in Tanzania between 1978 and 1979, reported that 50%-90% of all coliforms were resistant to ampicillin, tetracycline and sulphonamides which were the commonly used antimicrobials (Maselle *et al,* 1980). Mhalu showed that excessive use of tetracycline during the cholera epidemic in the late 1970's in Tanzania contributed to a significant increase in the widespread resistance to the antimicrobial agent (Mhalu *et al,* 1979).

Self- medication is very common in many developing countries (Okeke *et al,* 1999). In a study done in Kenya, it was found that about 64% of all antibiotics sold by the chemists were without prescriptions from a doctor (Indalo, 1997). Due to lack of control, broad-spectrum antibiotics can be found being sold freely over the counter and even at open market places (Kunin *et al,* 1987, Okeke *et al* 1999). Some antibiotics being sold are often of substandard qualities; these are therefore cheap and thus preferred by many people (Okeke *et al,* 1999). Other factors that favor development of antimicrobial resistance include a heavy burden of infectious diseases, large populations with rudimentary primary health care. Others are poor diagnostic services prompting health care workers to prescribe indiscriminately, poor sanitation and overcrowding, all of which tend to favour the rapid spread of resistant organisms (Okeke *et al*, 1999, WHO, 1998). The consequences of antimicrobial resistance may include limitation in therapeutic options, high costs of alternative drugs and an increase in morbidity and mortality (Cohen, 1992).

1.3 Problem statement and rationale of the study

In Tanzania, treatment of UTI is done empirically with little information regarding the spectrum, magnitude and patterns of antimicrobial resistance to urinary bacterial isolates from children. Also the existence of irrational drug use that contributes to development of antimicrobial resistance which includes taking medication at home without hospital prescription, incomplete dosage taking, use of counterfeit drugs from unregistered medical stores, and side effects following prolonged drug usage.

The rationale for conducting this study based on the fact that, previous study that investigated UTI among children was conducted more than ten years ago. Thus, current knowledge on the prevailing urinary pathogens and their antimicrobial susceptibility patterns is important for the proper selection and use of antimicrobial drugs for management of patients and for the development of an appropriate prescribing guideline.

1.4 Hypothesis

There is no burden of antimicrobial resistance among UTI isolates from under-five children.

1.5 Broad objective

To determine the spectrum of bacterial aetiological agents and antimicrobial resistance patterns of UTI agents in under-five children in Dar es Salaam, Tanzania.

1.6 Specific objectives

1. To determine the spectrum of bacterial etiologic agents causing UTI in children by sex and age.
2. To determine the spectrum and pattern of anti-microbial resistance among bacteria isolated from children with UTI
3. To relate self medication and antimicrobial resistance of bacterial agents causing UTI in children.

CHAPTER TWO

2.0 METHODOLOGY

2.1 Epidemiological Methods

2.1.1 Study design

A cross-sectional health facility based study was conducted for two months from February to April 2010.

2.1.2 Study sites

Pediatric Wards of Amana and Mwananyamala Hospitals-Dar es Salaam, Tanzania

Amana and Mwananyamala are district hospitals serving as Municipal's health facility within Dar es Salaam Region. Amana hospital provides care to one million residents of Ilala Municipality. It sees approximate 1400 people per day. Mwananyamala attends over 1000 patients daily from Kinondoni Municipal.

2.1.3 Study population

Study subjects included under-five children presenting with clinical symptoms suggestive of UTI (i.e. Dysuria, frequency, burning, fever and haematuria).

2.1.4 Inclusion criteria: All new cases under-five years presenting with symptoms of UTI at pediatric wards after written informed consent of their parents or guardians.

2.1.5 Exclusion criteria: all cases aged five years or above with symptoms of UTI, all children with known co-existing renal diseases, all children with known co-existing diabetic mellitus, and all children with severe forms of malnutrition

2.1.6 Sample size

Sample size was calculated with the following formulae

$$n = \frac{z^2 p(1-p)}{\varepsilon^2} \quad \text{or} \quad n = \frac{z^2 p(100-p)}{\varepsilon^2}$$

$$n = \frac{1.96^2 \times 0.20(1-0.20)}{0.5^2}$$

$$\frac{3.84 \times 0.20 \times 0.80}{} =$$

$$\frac{3.84 \times 0.16}{0.0025} = 245.76$$

Sample size was 250.

By adding 10 percent for the non respondents the minimum sample size was 270 children under five years of age.

n = Minimum sample size

p = Proportion/Prevalence of antimicrobial resistance =20% (Urassa *et al,* 1997)

E = Tolerable error / level of significance = 5%.

z =Standard deviation of the normal distribution = 1.96

2.1.7 Sampling technique

All new cases during the period of February and April 2010 were reviewed by clinicians at Pediatric wards; those with clinical symptoms suggestive of UTI were selected to participate in the study after parental/guardian consent.

2.1.8 Data collection

A structured questionnaire was used to interview parents/guardians of children on socio-demographic and antimicrobial use such as source of prescription, previous dosage completion and other history of medication of the child. Urine samples were taken for culture and antibiotic susceptibility test that was done at MUHAS Microbiology Laboratory.

2.2 Laboratory Methods

2.2.1 Specimen Collection and Storage

Mid-stream urine specimens were collected in sterile bottles by standard method for urine collection (Collee, *et al,* 1996). (i.e. Parents were given a sterile, dry, wide-necked, leak-proof container, and explained the importance of collecting a specimen with no contamination [clean-catch specimen], they were instructed to cleanse the area around the urethral opening with clean water, dry the area and collect the urine, for female with the

labia held apart) collected urine was transported to the laboratory for examination within six hours of collection and if not; it was stored at 4 °C until the following day.

2.2.2 Urine Cultures

Urine samples were cultured in Cystine-Lactose-Electrolyte Deficient (CLED) agar media for isolation of uropathogens. Standard quantitative culture was performed in a laboratory. A loop calibrated to deliver approximately 0.001 mL was used to inoculate urine in CLED agar plates. All plates were incubated at 35°C and examined for growth after 24 hours. A significant bacteriuria result was defined as growth of a single urinary tract pathogen at $\geq 10^5$ CFU/mL of urine. Bacterial isolates were identified by standard microbiological methods as described in Mackie & McCartney Practical Medical Microbiology (Collee, *et al,* 1996).Thus, important biochemical feature of most *E.coli* strains is the production of indole from peptone water containing tryptophan, *Proteus* rapidly hydrolyzes urea (within 4 hours), *Klebsiella* gives a positive urease test after 18 – 24 hours, *Pseudomonas spp* are oxidase positive, catalase positive, indole negative and mostly citrate positive.

2.2.3 Antibiotic Susceptibility testing

Susceptibility testing was performed by Kirby-Bauer disk diffusion method on Mueller-Hinton agar plates as recommended by Clinical and Laboratory Standards Institute (CLSI 2009). Isolates were suspended in peptone water, inoculated onto the agar plate and followed by antibiotic-impregnated discs within 15 minutes and the control strain at the respective media. The antibiotic disk was placed precisely at the interface between the surface areas inoculated with the clinical isolate and the control strain. After overnight incubation, the relative size of the inhibition zones of the clinical isolate and the control strains were measured using a metric ruler and compared with respect to the antibiotic disc manufacturer's guidelines to determine if they were susceptible, intermediate and resistance. The test results were classified as susceptible (S), intermediate (I) or resistant (R) by evaluation of the difference between the inhibition zones of the clinical isolate and the control strain as per CLSI guideline. Resistance level of isolates to antibiotics were further classified as higher (80 – 100%), significant (50 – 80%) and moderate to lower (0

– 50%). The control strains were used are *S. aureus* ATCC 25923, *E. coli* ATCC 25922 or *Pseudomonas aeruginosa* ATCC 27853.

The antibiotic discs and their concentrations per disc (mg) included: Trimethoprim-sulfamethoxazole (25), nitrofurantoin (300); representative antibiotics of aminoglycosides such as gentamicin (10), amikacin (10); quinolones such as nalidixic acid (30), ciprofloxacin (5); and cephalosporin such as, cefotaxime (30), as well as aminopenicillin, amoxicillin and clavulanic acid

2.3 Data management and Analysis

Results datasheet were checked for accuracy and entered into a computer and analyzed by using Epi info version 3.5.1 software package to calculate frequencies and cross tabulations of bacterial aetiological agents by sex and age, its antibiotic susceptibility patterns. Comparisons between proportions were tested using Chi-square test, Odds ratio, and 95% confidence interval. A p-value of < 0.05 was regarded as statistically significant.

2.4 Ethical Considerations

The study proposal received ethical approval from the Senate Research and Publication committee of Muhimbili University of health and Allied Sciences. Obligations of investigators were to adhere to ethical guidelines. All information attained during the study was kept confidential.

Informed consents were sought from the parents or guardians of all children included in this study and code numbers were used instead of names. Refusal to participate or withdrawal from the study did not attract any penalty or loss of any benefits to which non consenting participants were otherwise entitled. Results were shared with hospital authority for proper clinical management of patients

CHAPTER THREE

3.0 RESULTS

A total of 270 under-five children presenting with clinical symptoms suggestive of UTI were enrolled from Pediatrics Wards of Amana (140) and Mwananyamala (130) Municipal hospitals. Of 270 children, 166 (61.5%) were male (Table 1). Many 125 (46.3%) came from Kinondoni Municipality, while 97 (35.9%) and 48 (17.8%) were from Ilala and Temeke Municipalities respectively. A total of 259 (95.9%) children was under the care of their mothers, 10 (3.7%) taken care by guardians and only 1 (0.4%) was under care of the father when attended to hospital for treatments.

Many of the children 149 (55%) had parents with primary education, followed by those 49(18.1%) with secondary school completed, 39 (14.4%) primary not completed, 32 (11.9%) had no formal education and only one had tertiary education. Most of the mothers 196 (72.6%) were housewives, 54 (20.0%) business entrepreneurship and only 20 (7.4%) were government employees (Table 1).

Table 1. Socio-demographic characteristics and antibiotic usage/history of study population (n =270)

Characteristics	Number	%
Sex		
Male	166	61.5
Female	104	38.5
Education (Parents)		
No formal education	32	11.9
Primary completed	149	55.2
Primary not completed	39	14.4
Secondary	49	18.1
Tertiary	1	0.4
Occupation		
Housewives	196	72.6
Business	54	20.0
Government *	20	7.4
Location		
Ilala	97	35.9
Kinondoni	125	46.3
Temeke	48	17.8
Healthy facility		
Amana	140	51.9
Mwananyamala	130	48.1

** Government included Teachers, health workers and other institutions*

Of the 270 cultured mid-stream urine samples, 78 (28.9%) revealed a significant single isolate growth of $\geq 10^5$ colon-forming units per milliliter of urine, 32 (11.9%) mixed growth, 26 (9.6%) non-significant growth, and while the remainder 134 (49.6%) had no bacterial growth. The most common bacteria isolated was *Escherichia coli* which accounted for 33 (42.3%), followed by *Klebsiella spp* 31 (39.7%), *Streptococcus spp* 4 (5.1%) while *Staphylococcus aureus*, *Proteus mirabilis* and unidentified coliforms caused 3 (3.8%) each, *Pseudomonas spp* isolated only once (Table 2)

Table 2. Distribution of UTI pathogens in the study population (n=78)

Bacteria isolated	Number	%
i. *E. coli*	33	(42.3)
ii. *Klebsiella spp*	31	(39.7)
iii. *P. mirabilis*	3	(3.8)
iv. *Pseudomonas*	1	(1.3)
v. *S. aureus*	3	(3.8)
vi. *Streptococci spp*	4	(5.1)
vii. Unidentified coliforms	3	(3.8)
Total	**78**	**100**

Most isolates were from male children 49 (61.3%) while infants less or equal to twelve months of age accounted for 38 (47.5%) of all culture results with significant growth of uropathogens (Table 3).

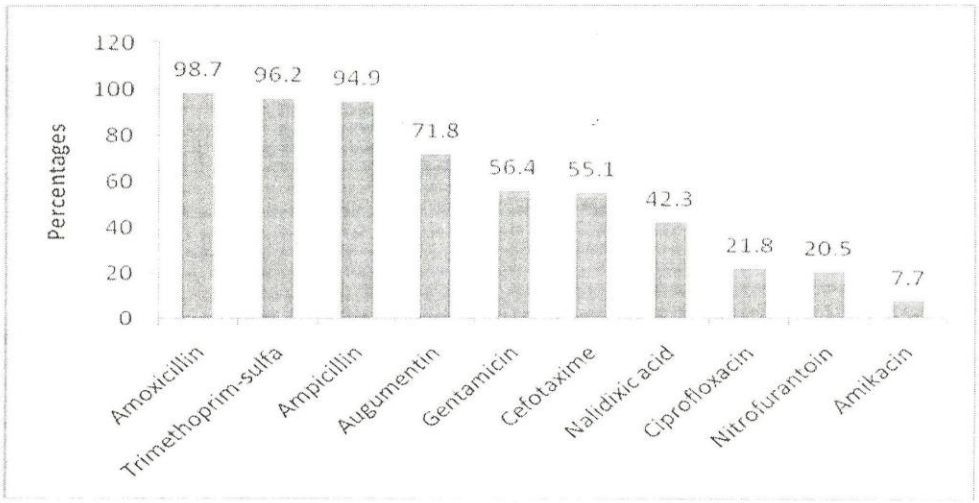
There is no significant difference (OR 1.077) on the risk of getting urinary tract infection on sex and age of children.

Table 3. Distribution of UTI pathogens by Sex and Age (n=270)

Variable	Culture results		p - value
	Positive	Negative	
Sex			
Male	49	113	0.7856
Female	29	79	
Total	**78**	**192**	
Age			
< 12 Months	38	67	0.3221
12 – 24	19	59	
24 – 36	11	23	
36 – 48	8	25	
< 60 Months	4	16	
Total	**80**	**190**	
Occupation			
Housewives	60	137	0.8543
Business	15	38	
Government	5	15	
Total	**80**	**190**	

Higher proportions of antimicrobial resistance were seen with Amoxicillin 79 (98.7%), Trimethoprim-sulfamethoxazole 77 (96.2%) and Ampicillin 76 (94.9%). Significant resistance revealed with Augumentin 58 (71.8%), Gentamicin 46 (56.4%) and Cefotaxime 45 (55.1%). Moderate to lower resistance seen with, Ciprofloxacin (21.8%), Nitrofurantoin 16 (20.5%) and Amikacin 6 (7.7%) (Figure 1)

Figure 1. Antimicrobial resistance patterns in the study population



The resistance pattern of the isolates to some prescribed antibiotics is shown in Table 4. While *E. coli* showed high resistance to amoxicillin and ampicillin (100%), it showed low resistance to amikacin (8.8%) and nitrofurantoin (14.7%). All isolates, however, showed high resistance to amoxicillin, trimethoprim-sulfamethoxazole and ampicillin. Of all bacteria isolated 99% were resistant to amoxicillin, and 95% were resistant to trimethoprim-sulfamethoxazole.

Table 4. Antimicrobial resistance patterns in specific isolated bacteria

		Antimicrobial agent (resistance in percent)									
Pathogen	n	CIP	AMP	AK	CTX	NA	F	GEN	AUG	SXT	AML
E. coli	34	29.4	100	8.8	47.0	47.0	14.7	50	79.4	97	100
Klebsiella spp	32	12.5	93.7	0	62.5	25	15.6	68.7	71.8	96.8	96.8
Proteus	3	33.3	100	66.6	66.7	33.3	66.7	100	100	100	100
Pseudomonas	1	0	100	0	100	100	100	0	100	100	100
S. aureus	3	0	66.7	0	33.3	66.7	0	33.3	0	100	100
Streptococcus	4	25	75	25	75	100	50	25	50	75	100
Unidentified coli	3	33.3	100	0	66.7	33.3	33.3	66.7	66.7	100	100
General (%)		**19**	**90**	**14**	**64**	**58**	**40**	**49**	**66**	**95**	**99**

CIP; Ciprofloxacin, AMP; Ampicillin, AK; Amikacin, CTX; Cefotaxime, NA; Nalidixic Acid, F; Nitrofurantoin, GEN; Gentamicin, AUG; Augumentin, SXT; Trimethoprim-sulfamethoxazole, AML; Amoxicillin

Antimicrobial usage response revealed that 188 (69.6%) of all interviewed parents/guardians had given their children antibiotics at home after onset of symptoms before attending hospital, with 44 (24.4%) getting their prescribed drugs from private medical stores. Fifty-five percent of the kids were given antibiotics for three days during the previous episode of the disease. Most of children 262 (97%) presented at pediatric clinics with persistent fever and 75 (97%) of those with positive culture results presented at hospital with fever symptoms only. Meanwhile 166 (63.1%) was recurrent cases of UTI.

Table 5. Association between self medications to drug resistance

Variable	Multiple drug resistance		Crude OR
	Yes	No	(95% CI)
Self medication			
Yes	61	127	1.592 (0.8767, 2.8932)
No	19	63	
Self medication	**Antibiotic resistance (Amp)***		
Yes	58	3	1.074 (0.1051, 10.9752)
No	18	1	
		(Gen)*	
Yes	34	27	0.734 (0.2544, 2.1210
No	12	7	
		(Cip)*	
Yes	14	47	1.58 (0.369, 9.670)
No	3	16	

** Amp; ampicillin, Gen; Gentamicin, Cip; Ciprofloxacin*

Self medication has shown no significant association with the development of multiple drug resistance.

CHAPTER FOUR

4.0 DISCUSSION

The aim of the current study was to assess the spectrum and patterns of antimicrobial resistance of UTI agents isolated from under-five children presenting with clinical symptoms suggestive of UTI in major hospitals in Dar es Salaam.

The study findings showed that 28.9% of suspect cases had significant bacteriuria. As all study subjects were symptomatically identified, this low proportion of significant bacteriuria can be due to the fact that some of the children were given antibiotics at home prior to onset of symptoms before attending hospital and contamination due specimen collection procedure where 11.9% had mixed growth. There are limited data on pediatric UTI as a significant health care burden to Tanzanian public, unlike in America where data from the Urologic Disease revealed that infections of the urinary tract affect 2.4% to 2.8% of children every year and account for more than 1.1 million office visits annually (Freedman, 2005).

When comparison is made between males and females; male children has shown higher percentage of infections and those of age less or equal to one year, but the difference was not statistically significant (OR 1.077). Results of this study are similar to those described in other study showing that during the first year of life, boys have a higher incidence of UTI; but in all other age groups, girls are more prone to developing UTI (Wettergren *et al*, 1989). During the first year of life, the incidence of UTI in girls is 0.7% compared with 2.7% in boys (Wettergren *et al*, 1989).

In this study *E. coli* was the most common bacterial agent isolated in the sample population. The predominance of *E. coli* as the commonest aetiological agent of UTI has also been reported in studies conducted previously in Tanzania (Bloomberg *et al,* 2005) and in Kuwait (Al Sweih *et al*, 2005). In most cases, *E. coli* which originates as a harmless microorganism in the intestines spreads to the vaginal passage where it invades and colonizes the urinary tract. Contamination with fecal matter during specimen collection can also reflect predominance of *E. coli* as the commonest aetiological agent of

UTI. The procedure used for urine collection was mid-stream urine clean-catch which involves higher degree careness especially for pediatrics to avoid contamination.

The results of antimicrobial susceptibility tests showed that the isolates were generally highly resistant to amoxicillin (99%) and trimethoprim-sulfamethoxazole (95%) and less resistance to amikacin (14%) and ciprofloxacin (19%). Also there are increased antimicrobial agent resistance to different bacteria, *Escherichia coli* shown 100% resistance to Ampicillin and Amoxicillin and 97% to Trimethoprim-sulfamethoxazole as shown by prior studies which have shown increasing rates of resistance to Ampicillin, trimethoprim-sulfamethoxazole, and first-generation cephalosporins (Gupta *et al* 1999). When compared with previous study done in Dar es Salaam, there is increase in resistance to specific antibiotics as shown in results of a study done in Tanzania which revealed that, among *E. coli* in urinary tract infections, more than 80% were resistant to ampicillin, cotrimoxazole and tetracycline while more than 80% of the isolates were still susceptible to nitrofurantoin, gentamicin and third generation cephalosporins (Urassa *et al* 1997).

This also is in consistent with the data previously reported from a study done in Muhimbili whereby more than 80% of *Escherichia coli* were resistant to ampicillin (Bloomberg *et al,* 2005), this could possibly be as a result of extensive use or misuse of the drugs by the correspondents since the drugs are commercially available, however this could not be proven in that study.

The study findings suggest that empirical treatment of UTI should be done mostly with nitrofurantoin which revealed lower resistance, also ciprofloxacin can be secondly considered as amikacin has limited usage to pediatrics due to side effects. Meanwhile more studies are required to get the baseline susceptibility pattern of antibiotics to be incorporated in the National guideline for management of UTI for under-five. The National guideline with the first line treatment for UTI in children is trimethoprim-sulphamethaxazole, amoxicillin, ampicillin, gentamicin and Nitrofurantoin, (MOHSW-NEMLIT, 2007). Most of these antibiotics revealed higher level of resistance to uropathogens, thus the guideline need to be reviewed.

Study findings showed purchasing drugs from private medical stores, 44 (24.4%) and the habit of parents to give their children antibiotics at home (Self medication) prior to onset

of fever symptoms 188 (69.6%) before attending hospital. Although self medication behavior shown no statistical significant association with the development of multiple drug resistance as revealed in this study, it may be due to the fact that those used antibiotics at home (self medication) revealed no significant growth of uropathogens, thus it was difficult to prove statistically the association with drug resistance. To manage this, health education to community is needed, also a need for reinforced guidelines prohibiting selling of antibiotics in irrelevant medical stores. Self- medication is very common in many developing countries (Okeke *et al,* 1999). In a study done in Kenya, it was found that about 64% of all antibiotics sold by the chemists were without prescriptions from a doctor (Indalo, 1997). Thus due to lack of control, broad-spectrum antibiotics can be found being sold freely over the counter and even at open market places (Kunin *et al,* 1987, Okeke *et al,* 1999). Some antibiotics being sold are often of substandard qualities; these are therefore cheap and thus preferred by many people (Okeke *et al,* 1999). Also this study show more than 149 (55%) of the parents complete primary education, while 32 (11.9%) had no formal education; Ignorance and poverty are other contributing factors such that some patients stop taking medication before completing recommended dose when they cannot afford the full dose (Okeke *et al,* 1999, Kunin *et al,* 1987).

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The study revealed that there is a significant increase in antimicrobial agent resistance to different bacteria, *Escherichia coli* shown 100% resistance to Ampicillin and Amoxicillin also 97% to Trimethoprim-sulfamethoxazole which are the first line antibiotic for treatment of UTI in Tanzania and commonly used antibiotics in the community. These drugs are mostly purchased from private unregistered medical stores and administered empirically, thus reflects to be the problem of public health importance, proper guideline is needed to ensure proper use of antibiotics to reduce the drug resistance burden.

In view of the high drug resistance amongst bacteria, (95 – 98%), therapy should only be advocated, as far as possible, after culture and sensitivity has been performed. This would not only help in the proper treatment of the patients but would also discourage the indiscriminate use of the antibiotics and prevent further development of bacterial drug resistance.

Based on the results of this study, the empirical treatment of UTI should be done mostly with nitrofurantoin which revealed lower resistance; also ciprofloxacin can be secondly considered as amikacin has limited usage to pediatrics due to side effects.

Health education using methods that can make those with no formal education understand the proper usage of antibiotics is needed and if delivered, will build their capacity on understanding the importance of acquiring appropriate drugs from appropriate suppliers and appropriate usage.

This should be associated with legal guidelines to control sales of antibiotics in unauthorized medical stores and without prescriptions of a doctor.

5.2 Recommendations

Based on the study findings, it is recommended that there should be deliberate efforts to ensure that information on antimicrobial resistance patterns is updated regularly for appropriate treatment of patients and avoiding the use of resistant antimicrobial agents.

There is a need for health education to the community on the proper use of antibiotics and adherence to the hospital prescribed dosage to reduce resistance and disease recurrence burden in the community.

As most of the bacteria that shown higher resistance (*E. coli and Klebsiella*) are Extended Spectrum Beta – Lactamase producers, there is a need for continuous ESBL surveillance in clinical and environmental isolates.

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