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Etiology and Antibiogram of Asymptomatic Urinary Tract Pathogens in Selected Primary School Children in Uburu, South East Nigeria

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Abstract

The aim of this investigation is to assess the antibiotic resistance of urinary tract pathogens in primary school children in Uburu, Ohaozara local government area of Ebonyi State. A total of 50 clean mid-stream urine samples were collected from healthy children with the help of their parents and samples were processed by using standard microbiological techniques. Antimicrobial susceptibility of the isolated pathogen was determined using the Kirby and Bauer disc diffusion method. The result revealed that 38% of the samples were with significant bacteria growth. Six bacteria pathogens were isolated among which are *Proteus mirabilis* which is the most prevalent isolate 24% followed by *E. coli* (16%), *Proteus vulgaris* (12%), *Enterococcus faecalis* (8%), *Klebsiella pneumonia* (4%) while *Staphylococcus aureus* was the least prevalent (2%). Antibiotic sensitivity studies showed an alarming level of resistance to nearly all the antibiotics tested showing multi-drug resistant strains. Routine diagnostic exercise should be encouraged in primary schools for early detection and prompt treatment of bacteria pathogens.

1. Introduction

Urinary tract infection (UTI) is defined by the presence of organism in the urinary tract which is usually sterile. UTI is a term generally applied to a variety of clinical infections ranging from asymptomatic bacteriuria to several kidney damage and sepsis (Tanagho and Mcaninch, 2004). According to Reddy (2006) urinary tract infection is described as significant growth of organism of a single species in the urine accompanied with symptoms. Significant bacteriuria is growth with colony count of ≥ 10000 CFU/ml of clean catch mid-stream urine (Brian and Sarah, 2005), while asymptomatic bacteriuria (ABU) indicates a significant bacterial count present in the urine (usually 10^5 or 10^4 colony forming units (CFUs) per ml) in an individual without symptoms of a urinary tract infection.

Urinary tract infection is a serious bacterial infection causing illness in infants and children (Raya, 2009; Eltigani and Amira, 2009). The prevalence of UTI in children is difficult to estimate, particularly because the infections may be subclinical or the clinical signs vague and non-specific, secondly, collection techniques are prone to errors especially in younger patients, thirdly antibiotics are often given to the febrile children for other purposes eradicating the silent infection. As a result of these problems the relationship of symptomatic UTI and ABU is unknown. However, the disease is

commonly identified by fever, vomiting, pyuria, haematouria, pain sensation and neonatal jaundice. Different signs and symptoms may be observed among different age groups (Reddy, 2006). UTI could be classified into lower urinary tract infection localized to the bladder and urethra (cystitis and urethritis) and the upper urinary tract infection involving the ureter, pelvis and kidney (pyelonephritis). UTI infection is acquired mainly through bacteria infecting urinary tract, although hematogenous infection may occur in rare instances among young infants (Raya, 2009). The ascending infection is as a result of colonization of bacteria present in the gastrointestinal tract. The colonization is attributed to disturbances of normal flora by prolonged administration of antibiotics emerging from the environment or medical instrument such as invasive catheter.

The etiology of UTI is made up of several organisms, bacteria, viruses, protozoa and fungi are known to cause UTI. Most infections are caused by a large family of Gram-negative (aerobic bacilli known as Enterobacteriaceae) such as *Escherichia coli*, *Enterobacter*, *Pseudomonas*, *Salmonella*, and *Serratia* spp, of these *E. coli* is by far the most frequently isolated organism being responsible for approximately 80% of UTIs (Eltigani & Amira, 2009). The most common Gram-positive organisms include group B Streptococci, *Enterococcus* spp and *Staphylococcus aureus*.

Risk factors associated with UTI are anatomical and physiological, others may include age, gender, race, circumcision status, the method of detection and sexual and genetic activities in teenage girls, nocturnal enuresis and some unhealthy conditions have been indicated (Heffner & Gorelick, 2008). Other factors that could predispose children to UTI are delay urination; such children that have delayed urination are more likely to develop UTIs because regular urination helps keep the urinary tract sterile by flushing away bacteria, so holding in urine allows bacteria to grow. Producing too little urine because of inadequate fluid intake can also increase the risk developing UTI both in children and adults. Chronic constipation, a condition in which a child has fewer than two bowel movement a week can add to the risk of developing UTI, when the bowel is full of hard stool, it presses against the bladder and bladder neck, blocking the flow of urine and allowing bacteria to grow (Onuoha & Kayode, 2014)

Children are predisposed to these urinary tract infections as a result of the school children been exposed to the source of these bacteria pathogens through inadequate health care delivery, home treatment, poor feeding habits, lack of health education and poor local hygiene (Larcombe, 2009). Uncircumcised male infants are at increased risk of UTI, but thereafter UTI predominate in female partially because of the short female urethra and its proximity to the anus.

In many occasions asymptomatic UTI normally resolves without treatment but there is need to monitor it to avoid adverse effect. But symptomatic infections of UTI require treatment with regimen of antibiotics. However, antimicrobial resistance among urinary tract isolates has been known to increase worldwide, especially to commonly used

antimicrobials (Alemu *et al.*, 2012., Schmiemann *et al.*, 2012). The increasing antibiotic resistance trends are likely to have important clinical implication for antibiotic use. Hence, for this reasons knowledge of the etiology pathogen of UTI and their antimicrobial resistance pattern in a particular environment should be ascertained.

This study aims to investigate the prevalence of asymptomatic UTIs among children and also to ascertain the sensitivity pattern of the isolated bacteria to some antibiotics

2. Materials and methods

2.1. Study Area

The study was conducted in Uburu. Uburu is in Ohaozara local Government area of Ebonyi State. Obiozara is in uburu and is the headquarters of Ohaozara Local government area, is a fast growing city in Ebonyi State. It lies on latitude 6.20°N and longitude 7.46°E. There are many government and some private Primary Schools in the Town.

2.2. Samples Collection and Preparation

A total of fifty (150) samples comprising both male and female children were collected for the study and each of the selected children from different primary school within the town were adequately educated on how to take mid-stream urine sample of their first morning urine with the help of their parents into sterile capped, dry, wide necked and well labeled sample container, which were given to each of them to take home. The next day the urine samples for analysis were collected and immediately taken to the Microbiology Laboratory of Akanu Ibiam Federal Polytechnic Unwana within 2 hours of collection, were urine samples were subjected to a semi-qualitative test using combi-9 urine test Strip (SGL) to characterize the presence and levels of chemical entities such as Protein, Glucose, Blood, Urobilinogen, Bilirubin, Ketones, Nitrite and pH. Pus cells were examined microscopically. The microorganisms were isolated by pour plate method. The bacteria were identified on the basis of their morphological, biochemical and fermentation tests as mentioned by Cheesbrough (2006). The protozoa were identified according to the microscopic appearance as mentioned by(Cheesbrough, 2006).

2.3 Antibiotic Susceptibility Test

The susceptibility of the isolates to selected antibiotics agent was determined by the disc diffusion method (Bauer *et al.*, 1966) using antibiotic impregnated paper disc(s).

The following Gram- negative antibiotics disc were used: Ciprofloxacin (CIP) (5µg), Tetracycline (TET) (50µg), Norfloxacin (NOR) (10µg), Amoxycillin (AMX) (30µg), Ofloxacin (OFL) (5µg), Chloramphenicol (CHL) (10µg), Cefuroxime (CEF) (30µg), Ampicillin (AMP) (30µg), Gentamycin (GEN) (10 µg). The gram- positive antibiotic disc(s) include: Ciprofloxacin (CPX) (10µg), Norfloxacin (NOR) (10µg), Gentamicin (GEN) (10µg), Amoxil (AMX)

(20 μ g), Streptomycin (STX) (30 μ g), Rifampicin (RIF) (20 μ g), Ampiclox (AMP) (20 μ g) and Levofloxacin (LEV) (20 μ g), Erythromycin (ERY) (30 μ g), Chloramphenicol (CHL) (30 μ g),

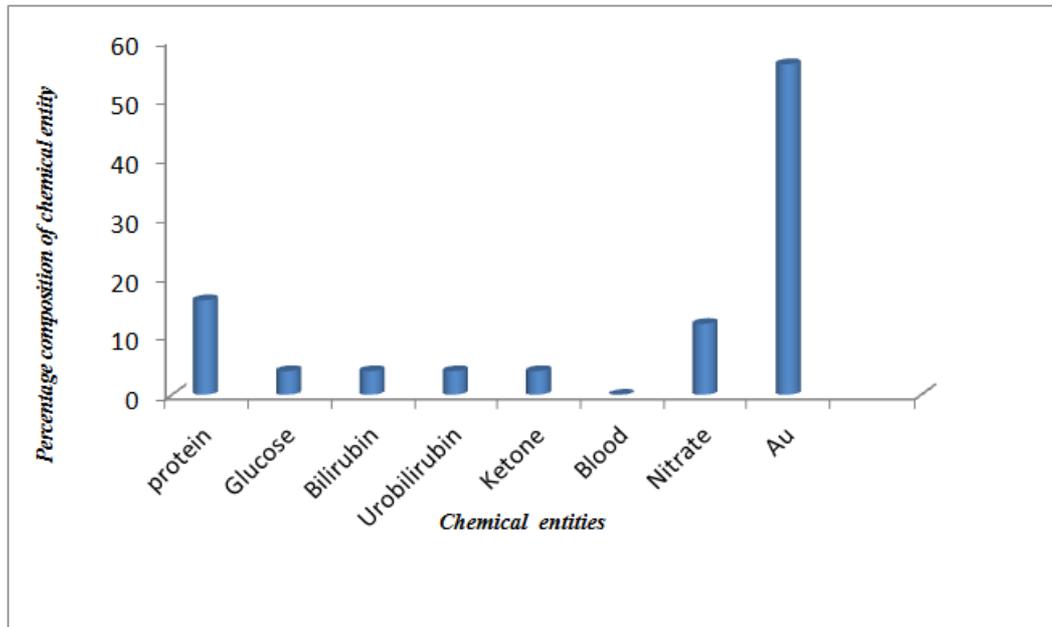


Figure 1. Urine analysis of Samples from school children.

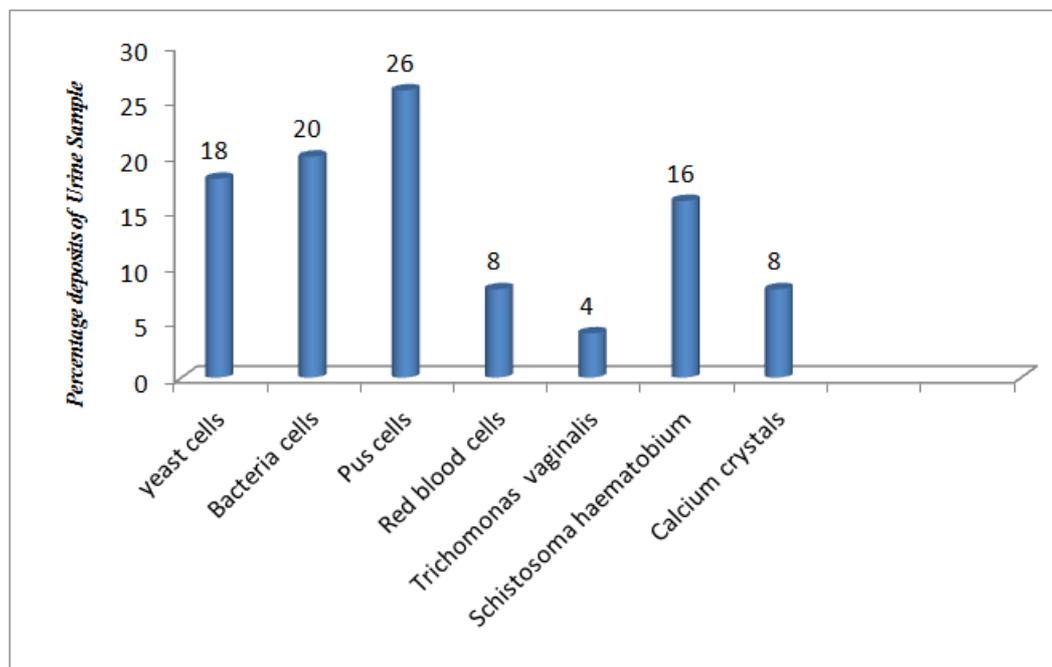


Figure 2. Microscopic examination samples of urine samples.

3. Results

A total of one hundred fifty (150) urine samples were collected from selected primary school children, out of which 57 (38%) of the school children showed significant bacteriuria, while 93 (62%) of the children were negative. After chemical and microscopic analysis, followed by urine culture using CLED agar as described by Cheesbrough (2006). The results showed that 6 samples (12%) tested positive for nitrate; traces

of protein were recorded in 8(16%) samples, whereas 2 (4%) samples tested positive for glucose, ketones, bilirubin and urobilirubin respectively. About 28 (56%) samples showed abnormal urine pH, while there was no trace of blood in the urine (Figure 1). Microscopic examination of the samples showed a high percentage of pus cells (26%), followed by bacteria (20%), yeast (18%) and a low percentage of red blood cells (4%) and Schistosoma haematobium ova (16%) (Figure 2). The distribution of UTI among the ages of the children

showed that age group within the range of 11-13 years recorded the highest incidence(36.8%) of UTI, whereas those of them above 13 years showed the least incidence (15.8%) as shown in (Figure3). Microbiological studies showed that a total of six (6) bacterial species were isolated and identified based on standard methods. Four (4) of the bacteria isolates were Gram-negative and two (2) were Gram-Positive. The

result also revealed that *Proteus mirabilis* is the most common pathogen which constitute 24% of the pathogens isolated, this was followed by *E. coli* (16%), *Proteus vulgaris* (12%), *Enterococcus faecalis* (8%), *Klebsiella pneumoniae* (4%) and *Staphylococcus aureus* was the least pathogen which had 2% prevalence rate (Figure 4).

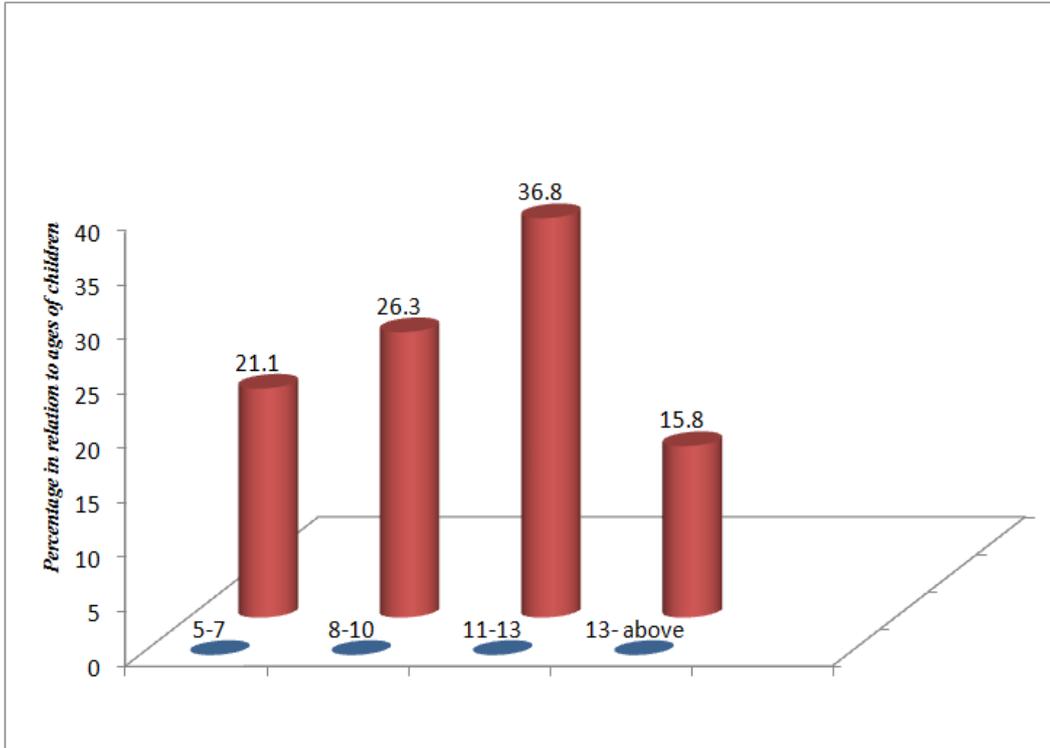


Figure 3. Percentage distribution of urinary tract pathogen in relation to age.

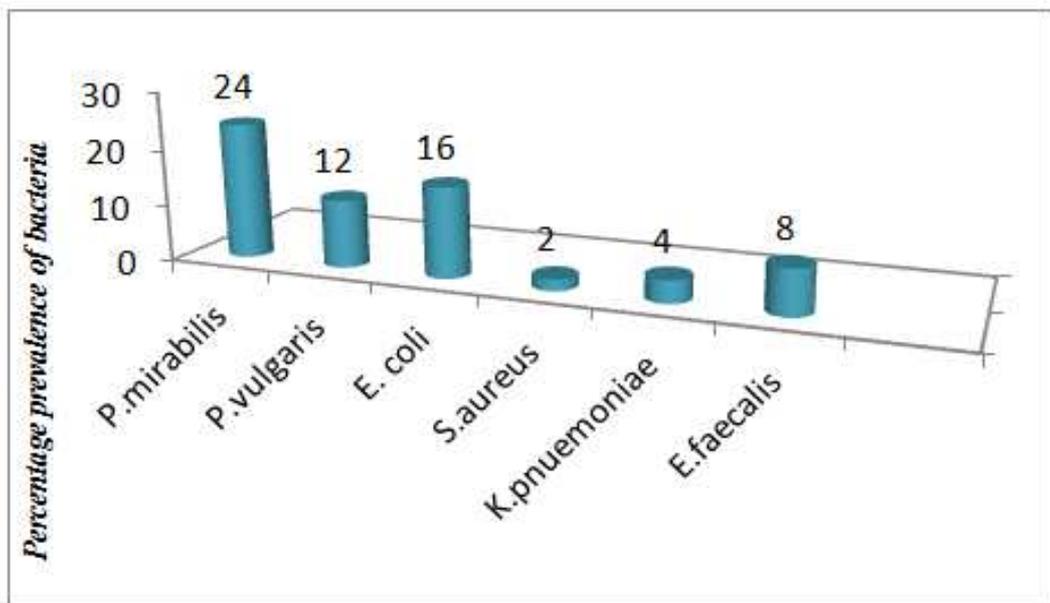


Figure 4. Percentage occurrence of bacteria in urine samples.

The result of the antibiotic susceptibility test showed that Gram-negative bacteria, *K. pneumoniae* showed the highest

susceptibility of 30% to the applied antibiotics, followed by *E. coli* with 20%, while the least susceptibility of 10% was

obtained from *P. mirabilis* and *P. vulgaris*. *P. mirabilis* showed the highest resistance of 80% to the tested antibiotics, while the least resistance of 40% was seen in *E. coli* (Table 1). Gram-positive bacterial isolates showed between 20 - 30% susceptibility on the antibiotics, while exhibiting between 30 - 67% resistance to all the antibiotics (Table 2).

Table 1. Antibiotic susceptibility pattern of Gram-negative bacteria isolates.

Antibiotics	<i>P. mirabilis</i>	<i>P. vulgaris</i>	<i>E. coli</i>	<i>K. pneumonia</i>
Nitrofurantoin	R	R	S	R
Ciprofloxacin	R	R	R	R
Tetracycline	R	R	S	I
Norfloxacin	R	R	R	R
Amoxicillin	R	R	I	R
Ofloxacin	R	R	R	R
Cephalosporin	S	I	I	S
Penicillin	R	I	I	S
Ampicillin	R	R	R	R
Gentamicin	I	S	I	S
Susceptible	10%	10%	20%	30%
Intermediate	10%	20%	40%	10%
Resistant	80%	70%	40%	60%

KEY: NIT= Nitrofurantoin; CPX=Ciprofloxacin; TET=Tetracycline; NOR= Norfloxacin; AMX =Amoxicillin; OFL=Ofloxacin; CEP=Cephalosporin; PEN=Penicillin, AMP=Ampicillin; GEN=Gentamicin

Table 2. Antibiotic susceptibility pattern of Gram-positive bacteria isolates.

Antibiotics	<i>S. aureus</i>	<i>E. faecalis</i>
Ciprofloxacin	R	R
Vancomycin	I	S
Amoxicillin	R	R
Tetracycline	S	R
Cephalosporin	S	S
Erythromycin	S	I
Chloramphenicol	R	R
Ampicillin	I	R
Levofloxacin	R	R
Susceptible	33.3%	22.2%
Intermediate	22.2%	11.1%
Resistant	33.3%	66.6%

KEY: CPX= Ciproflox, VAN =Vancomycin, AMX= Amoxil, TET= Tetracycline, CEP=Cephalosporin, ERY =Erythromycin, CHL= Chloramphenicol, AMP=Ampiclox, LEV=Levofloxacin

4. Discussion

The present study on the etiology and antibiotic sensitivity of asymptomatic UTIs among primary school children revealed the presence of pathogenic bacteria that are associated with urinary tract infection. The high prevalence of 38% bacteriuria recorded from this study showed that majority of the children harbor urinary tract pathogen as carriers and could serve as source of transmission and spread of the pathogen. The high incidence of UTI among apparently healthy children could be as a result of the school children being exposed to the sources of these bacteria pathogens through inadequate healthcare delivery, home treatment, poor feeding habits, lack of health education, poor local hygiene,

circumcision, bladder and urethral colonization and improper catheterization (Larcombe, 1999). The above findings are in agreement with several works that have been carried out in Nigeria. The report is comparable to 31.7% incidence rate reported by Isa *et al.*, 2013, 21.4% by Adedoyin *et al* 2003, 24.1% by Musa-Asien *et al.*, 2003. The result represents high incidence when compared to earlier reports such as Ogomaka *et al.*, 2013 who obtained 8.1% , 3.0% (Muoneke *et al*, 2012), 9% (Ibadin & Abiodun, 2004), 11.3% (Rabassa & Shettima, 2002). However, similar studies conducted in other parts of the world have reported the different variations in UTI incidence from one geographical location to another (Moghadas and Irajian, 2009), 3.7% (Mobasheri *et al.*, 2002), 28% (Hamdan *et al.*, 2011), 22.7% (Mulugeta and Bayeh, 2014), the differences could be attributed to the differences in UTI perception, mode of screening and compounding risk factors, such as age, socio-economic status of the family, standard of personal hygiene and education.

The result of the urinalysis showed that, six(6) urine samples, representing 12% of the samples contained nitrate, two (2) which represents 4% of the samples had glucose, ketones, bilirubin and urobilirubin in the urine, while high number of 8 children had protein in their urine. The presence of protein in the urine could be due to pathological disorders. Proteinuria in some of the samples could be a non-specific biomarker for UTI. Nitrites were always detected in urine samples for which Gram-negative bacteria were isolated. Gram-negative organism like *Escherichia coli*, *Klebsiella pneumonia* and *Proteus mirabilis* are able to reduce nitrate present in urine to nitrite, hence could account for the nitrate present. This agrees with Cheesbrough (2006) who had linked bacteria invasion to the presence of protein and nitrite in urine.

Microscopic examination of urine samples showed that 26% of the samples had pus cells, while 18% of the samples had yeast cells. The high percentage of pus cells in the urine samples is an indication of the presence of urinary tract infection. Also, the presence of high percentage of glucose in this study might be responsible for high number of yeast cells in the urine. Sugar is known to be a good culture medium for the growth of fungi and usually diabetic patients suffer more from fungi infection

The present study showed that the highest prevalence of UTI was recorded among age group between 11- 13 years. While, the least prevalence was among age group above 13 years. This finding was in agreement with the result of studies done in Nigeria and other countries(Onuoha & Kayode 2014; Ogoamaka *et al.*, 2013; Onuh *et al.*, 2006; Desai *et al.*, 2012; Adeleke *et al.*, 2013). The reason for high incidence of UTI among children with higher age group could be as a result of delayed voiding of urine. The consequences of retaining urine could lead to multiplication and growth of urinary tract pathogens which could cause UTIs. However, the low incidences of UTIs among the lower age group could be attributed to constant flushing of urine from the bladder which is a host defense mechanism against the development of UTIs as most of the children urinate as often as they have the urge(Ogoamaka *et al.*,2013; Chukwu *et al.*, 2010).

The most common uropathogen isolated from the urine of infected subjects in this study was *Proteus mirabilis* which constituted 24%. It was followed by *Escherichia coli* (16%), *Proteus vulgaris* (12%), *Enterococcus faecalis* (8%), *Klebsiella pneumonia* (4%) and *Staphylococcus aureus* (2%), *Proteus mirabilis* and other pathogens in this study were comparable to the rates documented previously (Onuoha and Kayode, 2014; Demile *et al.*, 2012, de Francesco *et al.*, 2007; Aiyegoro *et al.*, 2007 and Onifade *et al.*, 2005). However in my study, *E. coli* was not the most isolated organism as has been the case in majority of previous reports in Nigeria and elsewhere. The reason for this could not be known since *E. coli* is responsible for majority of pre-hospital antibiotic administration to the organism profile.

The result of the antibiotic susceptibility tests showed that majority of the Gram-negative bacteria exhibited high resistance between 40 - 80% to the tested antibiotics, while it showed minimal susceptibility of 10- 30% to the antibiotics. Gram-positive bacterial isolates showed between 20- 30% susceptibility on the antibiotics, while exhibiting between 30 - 67% resistances to all the antibiotics. The high level of resistance to these medications could probably be because they have been in the market for a long time, thus allowing microorganisms time to develop resistance mechanisms towards the antibiotics. In addition, this high level of resistance noticed could equally be attributed to easy access of antibiotics across the counter in developing countries like Nigeria especially in the absence of a prescription and the running of pharmacies by unlicensed personnel.

5. Conclusion

The result of the study revealed a high prevalence of asymptomatic urinary tract infection among apparently healthy school children which poses public health concern. The study also indicates a high incidence of microbial resistance to many of the common antibiotic used in the study. Based on this, routine urine screening exercise should be encouraged in primary schools in order to ensure early detection of those that carry the pathogens for adequate and prompt treatment of the infection. Regular monitoring is required also to establish reliable information about resistance pattern for optimal empirical therapy of patients with UTIs.

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