

PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY PROFILE
OF BACTERIAL ISOLATES FROM CASES OF BACTERIURIA IN
ABUJA AND ENVIRON.

BY
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DECLARATION

I hereby declare that the work reported in this thesis was carried out by me under the supervision of Dr. Y.K.E. Ibrahim and Prof. J.A Onaolapo of Department of Pharmaceutical Microbiology. The work is original and has not been presented in anywhere for a higher degree. The works of other investigators are properly acknowledged by means of references.

CERTIFICATION

This Thesis entitled “PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF BACTERIAL ISOLATES FROM CASES OF BACTERIURIA IN ABUJA AND ENVIRON” submitted by SHUAIBU TAIRU ALHASSAN, meets the regulations governing the award of the degree of Master of Science (Pharmaceutical Microbiology) of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to my caring wife Mrs. H.U Shuaibu and our four children; Mukhtar Eyiene, Ahmad Onuche, Maryam Iye and Farouk Omaye for their love, patience and support in life.

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ABSTRACTS

Urinary tract infections (UTI) are systemic infections that pose great problem to the health care delivery system worldwide. More importantly, the uropathogenic isolates are becoming more resistant to commonly used antimicrobial agents.

In order to evaluate the extent of significant bacteriuria in Abuja and environs, pre-study survey by way of questionnaire was carried out. The outcome showed a high prevalence of significant bacteriuria in Abuja and surrounding settlements. Furthermore the research work was designed to undertake the isolation, characterization and assessment of the susceptibility profile of the pathogenic isolates involved in urinary tract infections. The degree of resistance and prevalence of multiple antibiotic resistant (MAR) of the isolates was examined using multiple antibiotic resistance index. The minimum inhibitory concentrations (MIC) of ampicillin and streptomycin against the seventeen (17) multiple antibiotic resistant (MAR) isolates was determined using the macrobroth dilution method. The involvement of either plasmid or chromosome in the drug resistant of the isolates was investigated using acridine-curing experiment. The biochemical mechanism of bacterial resistance was investigated by screening for the production of β -lactamase enzymes.

Out of a total of 305 suspected bacteriuria cases from five different healthcare Institutions in Abuja and environ, 96 (31.5%) of them were adjudged of having urinary tract infections. From the positive samples, 100

uropathogenic organisms were isolated. They comprised of *E. coli* (33%), *Proteus* spp (32%), *Klebsiella* spp (14%), *Staph. aureus* (11%), *Streptococcus* spp (6%) and *Ps aeruginosa* (4%). The increase in incidence of UTI pathogens in term of number of isolates was in the order of Asokoro > Gwagwalada > National Hospital (central area) > Maitama > Durumi.

The susceptibility test showed that a high percentage of the isolates was sensitive to gentimicin, ceftriaxone, ciprofloxacin, ofloxacin, pefloxacin, lincomycin, co-trimoxazole, rifampicin, chloramphenicol, nitrofurantoin, norfloxacin, streptomycin, nalidixic acid, ampicillin/cloxacillin (ampiclox^R) and cephalixin, while lower percentage of the isolates was susceptible to ampicillin and tetracycline.

The incidence of resistance to several antibiotics as judged by the multiple antibiotic resistance index (MARI) was in the order of *Pseudomonas aeruginosa* > *Streptococcus* spp > *Staphylococcus aureus* > *Klebsiella* spp > *Proteus* spp > *Escherichia coli*. The MICs of ampicillin and streptomycin to some of the resistant isolates were of the values between 73-1250 µg/ml and 156-2500 µg/ml respectively. From acridine orange curing experiment 71% and 83% of the resistant isolates showed the reduction in MIC values of ampicillin and streptomycin respectively suggesting that plasmids and in some cases chromosome were responsible for the resistance to ampicillin and streptomycin. Majority, (56%) of multiple antibiotic resistance (MAR) isolates were positive to β-lactamase enzyme production indicating that enzymatic detoxification is one of the major mechanisms of antibiotic resistance.

ABBREVIATIONS

Abbreviations	Meaning
M	Male
F	Female
A	Adult
C	Child
PN	Ampicillin
COT	Co-trimoxazole
NA	Naldixic acid
CP	Ciprofloxacin
CN	Gentimicin
PEF	Pefloxacin
AC	Amoxicillin/Clavulanic acid
OF	Ofloxacin
S	Streptomycin
CEP	Cephalexin
CNC	Chloramphenicol
PXN	Ampicillin/Cloxacillin
E	Erythromycin
NF	Norfloxacin
LC	Lincomycin
FLO	Flucloxacillin
RF	Rifampicin
N	Nitrofurantoin
TCN	Tetracycline
CEF	Ceftriaxone
s	Sensitive
R	Resistance

CHAPTER ONE

1.0 INTRODUCTION

1.1 General Introduction

The urinary system is one of the four excretory systems of the body. It consists of two kidneys, which produce urine and two ureters, which convey urine to the bladder. The bladder serves as a reservoir for urine and the urethra discharges urine from the bladder.

Clinically, significant bacteriuria or urinary tract infection is said to exist when a significant number of bacteria, usually above 10^5 cfu/ml of urine, is detected from a properly collected mid stream “clean catch” urine or from a catheter specimen (Stamm, 1994). Infection of urinary tract is a global problem; the extent varies from country to country. For instance while the available information from Advicare (2005) shows that the extrapolated prevalence of UTI among Nigerian children was 532,510 and that of South Africa was 1,333,454. Others were; Ethiopia (2,140,097), Senegal (325,564), Sudan (1,174,444), Zambia (330,770), USA (8,809,662), United Kingdom (1,808,121) and China (38,965,428). The works of early researchers showed that UTI is one of the clinical conditions for which many people visit healthcare institutions. For instance, Ronald *et al* (2001) reported that UTIs are common with an estimated global incidence of at least 250 million, which is very costly to the patient, health care funding agencies and management strategies. The untreated urinary tract infection leads to pyelonephritis in 30% of women and the incidence

was similar in London and Birmingham Research Groups (William *et al*, 1972). In United States of America UTIs are commonly encountered clinical problems, affecting nearly half of women by their 20s and accounted for over five million visit to physician in the year 1983. Admission due to acute pyelonephritis was estimated at two hundred thousand and the yearly health care cost implication due to complicated lower UTI exceeded one billion American Dollar (Johnson and Stamm, 1998a). Johnson *et al* (1995) also reported that 94% of 162 urine samples evaluated in Washington Student health services met the culture criteria for UTI. In Nigeria, Ngwai (1999) and Ehimidu (2004) reported high incidence of UTIs in Zaria and environ. Akerele *et al* (2000) also reported a high prevalence of bacteriuria in Benin city, Nigeria while Ojiegbe and Nwaile (2000) recorded incidence of 1.2 % bacteriuria in children aged 6-15 years in Enugu. Oduyebo *et al* (2001) reported the prevalence rate of 20.3% of bacteriuria among patients undergoing pelvic radiotherapy at Lagos University teaching hospital, Nigeria.

Although no documented research work on UTI or bacteriuria in Abuja was cited during the period under review, there is circumstantial evidence that, Abuja which is a new growing metropolitan capital city, is witnessing influx of people and new residents of different background with different history of infections including that of urinary tracts. Available, though scanty and unpublished report of the Department of Health and Social Services of Federal Capital Development Authority in the year 2003 indicated that 20% of the annual visits to physicians in Abuja and its environ

are bacteriuria-related cases. Most of the population of Abuja and environ are living within indecent accommodation often with poor environmental sanitary condition and without pipe borne water. The above are positive signs of high prevalence of infections including bacteriuria in the area. It is on the basis of the above positive signs of high incidence of urinary tract infections in Abuja and environ that necessitated the choice of this research work and the study area.

1.2. The urinary system of human

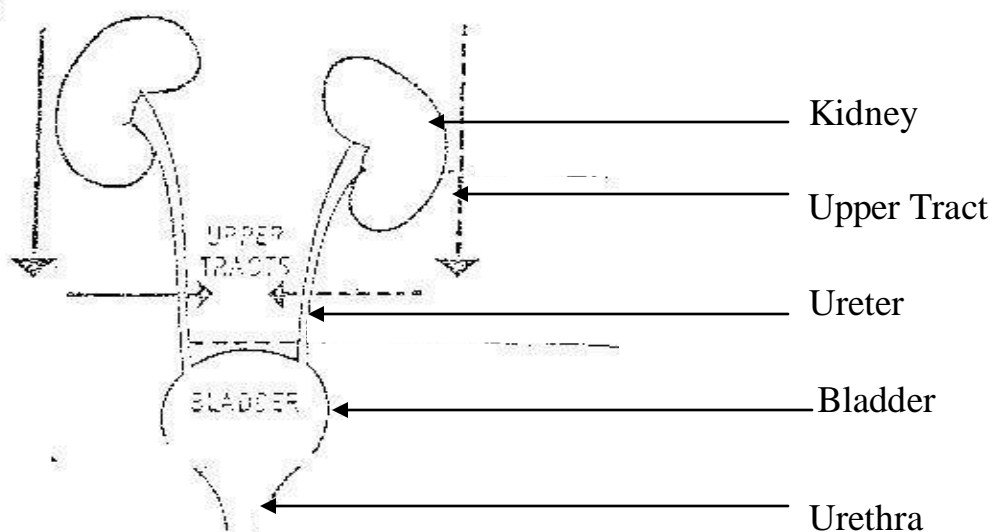


Fig. 1; Structure of urinary system of human

The urinary system is composed of two kidneys, ureter, bladder and urethra; all play a role in removing wastes from the body. The kidney is a pair of bean-shaped organ in the upper posterior abdomen that filter wastes from the blood. The ureters carry urine from the kidney

to the bladder where it is stored until exit from the body through the urethra (Mayo clinic.com, 2004).

Urinary tract infection is often presented or referred to as prostatitis, cystitis, urethritis or pyelonephritis depending on the primary focus of the infection (Johnson, 1999). However, once any component is infected, the entire system is susceptible to bacteria invasion. The term “urinary tract infection” (UTI) is therefore, descriptive of spectrum of urologic conditions of which the common link is the presence of bacteriuria (Oduola, 1986). Urinary tract infection is an umbrella term that embraces a wide range of clinical syndromes each with its own pathogenic mechanism, prognostic significance and unique requirement for diagnosis and treatment (Jill *et al*, 1998). It can be categorized as symptomatic or asymptomatic, complicated or uncomplicated; initial or recurrent; and acute or chronic. While symptomatic or non-symptomatic, chronic or acute are self-explanatory, complicated and uncomplicated describe whether or not structural or neurological abnormalities are present. A recurrent UTI can either be a relapse or a re-infection (Oduola, 1986). A relapse is a recurrent infection caused by the same bacteria that were present in the preceding infection while re-infection is used in describing a recurrent infection involving bacteria that differ in species or serotypes from those that caused the initial infection. Infections of the urinary tract are among the most common infectious diseases in human, possibly because the urinary tract is in direct communication with the exterior (EL-Bashir, 1991; Stamm, 1994).

Bacteriuria is more prevalent in females than males (Akinkugbe *et al*, 1973). The frequency of occurrence in humans increases with age (Kass, 1960; Sweat, 1977). They often being associated with premature delivery and could lead to increased foetal mortality, loss of production, low birth weight and size (Williams *et al*, 1975; Naeye, 1979; Mc Grady *et al*, 1985; Sever *et al*, 1985). Infection of urinary tract is in fact, important causes of morbidity and mortality in both adult and children (Gerald *et al*, 1990). Infections of the urinary tract are probably second only to infections of the respiratory tract in terms of frequency and are more important because they often result in progressive and irreversible impairment of kidney functions (Cohen and Irew 1972).

Etiologically, gram-negatives organisms such as *Escherichia coli*, *Proteus mirabilis*, *Klebsiella aerogenes*, *Pseudomonas aeruginosa* and Gram positive organisms like *Staphylococcus aureus* and *Streptococcus faecalis* are often implicated in UTI (Gruenberg, 1984; Odutola, 1986 Gerald *et al*, 1990; Cheesbrogh, 1991; Stamm, 1994). In Nigeria approximately 90% of UTI were due to *Escherichia coli*, *Proteus spp* and *Klebsiella spp* (Olanipekun and montefiore, 1978).

1.3. Incidence of bacteriuria

Bacterial colonization of urinary tracts are more frequent in women (Odutola, 1986; Johnson,1991), probably because of the shorter urethra in females and consequently a shorter distance microorganisms need to travel to the bladder. During sexual intercourse, microbes can be pushed more easily into the urethra in

women. The urethra is located near the rectum in women hence bacteria from the rectum can easily travel up the urethra and cause ascending infections (Mayo clinic.com, 2005). There is evidence of antibacterial prostatic secretion that protects the urinary tract of males from bacterial colonization (Odotola, 1986), which may account for the lower incidence in males. It is estimated that at some time during lifetime, 12% of men and 10-20% of women experience acute symptomatic UTIs (Lipsky, 1989; Johnson and Stamm, 1998b) and greater number develop asymptomatic bacteriuria. The incidence of UTI increases at a rate of 20% per decade. Bacteriuria is a common feature of old age (Stamm and Hooton, 1993). In males, congenital urinary tract abnormalities cause the majority of UTIs especially in the advance-aged men (Lipsky, 1989)

1.4. **Habitat and uropathogenicity**

The ability of microorganisms to cause disease in the host is referred to as pathogenicity and this term is often used interchangeably with virulence (Johnson, 2003). However, the later term strictly refers to the degree of pathogenicity, which is different for different strains of pathogens. For a pathogen to establish an infection in its host, the invading organism must fulfill certain requirements (Onaolapo and Klemperer, 1986). These are: -

- The pathogen must gain entry into the host by penetration following attachment (Svanborg eden, 1986).

- The pathogen must be able to metabolize and multiply in the environment of the host.
- The organism must resist, void or interfere with the various defense mechanism of the host.
- They must cause damage to the tissues (Hammond *et al*, 1984; Onaolapo Klemperer, 1986).

It is generally accepted that most urinary tract infections are caused by organisms originally coming from the bowels (Gruenberg Bellelheim, 1969; Robertson, 1972). Blood, lymph or urethra are the three major routes by which bacteria can gain access to the urinary tract. Among them, the primary portal by which bacteria gain access to the urinary tract is the intraluminal ascent of the urethra (Odutola, 1986). The distal one-third of the urethra, and in woman, the vaginal vestibule, is contaminated with enteric bacteria. Poor micturition habits such as incomplete voiding or delayed urination can result in reflux or migration of bacteria into the bladder. Mechanical trauma such as the insertion of the catheters or urethra milking, which occurs during sexual intercourse can also force bacteria into the bladder (Kass, 1959; Odutola, 1986).

The species of bacteria that colonize, invade and occasionally cause symptomatic infections in part or all part of the urinary tracts are referred to as uropathogenic bacteria (Ngwai, 1999). Such species have been shown to originate from the gastrointestinal tract and

presumably colonize the vaginal introitus and periurethral area (Low *et al.*, 1984) and ascend the urethra and cause at least transient colonization of the bladder (Ngwai, 1999). The uropathogenic or extra intestinal pathogenic strain of bacteria especially *Escherichia coli* possess distinctive traits that confer enhanced extra intestinal virulent potentials (Johnson, 2003). The uropathogenic strains of *Escherichia coli* are quite distinct from ordinary intestinal *Escherichia coli* and belong to limited number of genetic lineages characterized by specific antigens and other properties that promote invasion, colonization and inflammation of urinary tract (Johnson, 2003). These properties include the presence of;

- ❖ Particular somatic (O) and capsular (K) antigens
- ❖ Toxins (α -hemolytic, cytoxin, necrotizing factor-1, cytolethal distending toxin, and secreted antitransporter toxin)
- ❖ Aerobatin
- ❖ Iron sequestration system
- ❖ Protectin lipopolysaccharide
- ❖ Metabolic genes
- ❖ Resistance to the killing action of normal serum.
- ❖ Ability to ferment salicin
- ❖ Production of colicin V
- ❖ P-fimbriae, which recognizes Gal containing receptor on host epithelial surface and with which binding to uroepithelial cells is mediated.

- ❖ Iron extraction mechanism which occurs mainly in three known variants including Class II and III, which are the main, causes of upper and lower UTIs respectively (Ngwai, 1999; Johnson, 2003).

1.5. **Clinical symptoms of bacteriuria**

Symptomatic bacteriuria can have either upper tract or lower tract involvement. Lower tract infection includes urethritis, prostatitis and cystitis while upper tract includes pyelonephritis (Odotola, 1986). Significant symptoms of lower tract infections are frequency and urgency of micturition, burning sensation, dysuria, pyuria, haematuria and vaginal discharge. On examination, the urine is dark and turbid with foul odor (Odotola, 1986; Johnson, 1991). Clinical presentation of pyelonephritis generally develops rapidly over a short period of time. The characteristics are aching pains in the lumbar region, fever, nausea, vomiting, headache, malaise, tenderness over the costovertebral angle and laboratory finding shows WBC cast, proteinuria, leukocytosis, positive blood and urine culture (Odotola, 1986; Stamm, 1994).

1.6. **Laboratory detection of significant bacteriuria**

Significant bacteriuria is said to exist when the significant number of organisms usually above 10^5 cfu/ml of urine is detected in properly collected midstream “clean catch” urine or from a catheter specimen (Stamm, 1994). The need for simple yet accurate test to detect bacteriuria has been widely recognized and some of these tests are: -

1.6.1 Microscopic method

- Microscopic examination of Gram-stained film of uncentrifuged Urine (Lenette *et al*, 1990)

1.6.2 Culture plate method

- Spreading of standard bacteriological loopful of urine over the surface of a whole culture plate. The plate is then incubated at specific temperature for a specific period of time and the resulting colonies counted (McGeachile and Kennedy, 1963).
- Use of a measured area of a blotting paper strip to transfer a constant volume of urine onto a surface of a culture medium, incubate and resulting colonies is counted (Leigh and William, 1964)
- Dip-spoon and dip- slide method as described by Guttmann and Naylor (1967).

1.6.3 Chemical method

- Nitrite test which depends on the reduction of nitrate to nitrite by bacteria
- Catalase test as described by Kincaid *et al* (1964)
- Glucose test according to the description of Schersten *et al* (1967).

1.7. Causative Organism

It is generally accepted that the most common cause of bacteriuria in human is by ascending of bacteria derived from faeces (Cohen, 1972). Gram-negative organisms such as *Escherichia coli*, *Klebsiella aerogens*, *Proteus mirabilis* and Gram-positive organism like *Streptococcus faecalis* are implicated in urinary tract infections (Gruenberg, 1984; Gerald *et al*, 1990; Hooton and Stamm, 1997). The primary cause of acute uncomplicated urinary tract infections are *Escherichia coli* and *Staphylococcus saprophyticus* and in Nigeria approximately 90% of urinary tract infection are due to *Escherichia coli*, *Proteus* spp and *klebsiella* species (Ngwai, 1999). *Escherichia coli*, which is a wide spread intestinal parasite of mammals and birds are the most frequent primary agents of urinary tract infection (Onaolapo *et al*, 1997). It has also been observed that in man, the most common and important septic infections including those of urinary tract are caused by *Escherichia coli* (Holmes and Gross, 1995).

1.8 Characteristics of common causative organisms

1.8.1 *Escherichia coli*

It is a member of the bacterial order *Eubacteriale* and belongs to the Family *Enterobacteriaceae*. It was first isolated from faeces and named *bacterium commune* in 1885 by Theodor Escherich but renamed *Escherichia coli* in the year 1920 by Castedani and Chalmers (Holmes and Gross, 1995). Following the descriptions of Ewing (1986), *E. coli* is aerobic, Gram negative, asporogenous rod and usually motile by peritrichous flagella. Some strains of *Escherichia coli* possess polysaccharide capsules, grow very well at temperature of

15-45°C within 18 hours to form a large circular, low convex, smooth and colorless colonies on nutrient agar, rose-pink colonies on MacConkey agar and rose-pink or red with metallic sheen colonies on Eosin–Methylene blue or Endo agar media. In most cases, blood agar is discolored around the growth accompanied by haemolysis. It produces both acid and gas from fermentable carbohydrate like lactose and glucose. At the temperature of 37°C and 44°C, it produces indole but unable to utilize urea and citrate (Ewing, 1986). It gives positive methyl red (MR) and negative Voges Proskauer (VP) reactions. Most strain of *E. coli* do not produce hydrogen sulfide (H₂S) gas detectable in Kligler Iron (KI) agar but decarboxylate lysine and ornithine while gelatin is not liquefied (Ewing, 1986; Ngwai, 1999). *Escherichia coli* do not deaminate and oxidize phenylalanine and gluconate respectively.

1.8.2 *Pseudomonas aeruginosa*

It belongs to the bacterial order *Pseudomonadale* and Family *Pseudomonadaceae* and is referred to as blue pus organism that is frequently isolated from the pus of wounds and is one of the major causes of various human lesions (Winslows, 1957). Most strains of *Pseudomonas aeruginosa* are aerobic or facultative anaerobic, Gram negative rod of 0.5-0.6 by 1.5µ occurring simply in pairs or short chains, motile and possessing one or three polar flagella (Adedare, 1998). The colonies appear large, spreading and grayish with dark centre and translucent edge on nutrient agar. *Pseudomonas aeruginosa* produces heavy sediment with marked turbidity and thick

pellicle in nutrient broth. Most strains of *Pseudomonas aeruginosa* reduce nitrates to nitrites and nitrogen. Kligler iron agar, Urea, Voges – Proskauer, methyl red (MR) and indole reactions are negative while catalase, citrate, oxidase and nitrate tests are positive (Cheesbrough, 1991). They do not ferment glucose, lactose, mannitol, galactose, sucrose, glycerol, inulin and dextrin but glucose can be oxidized to gluconic acid, 2-ketogluconic acid and other intermediates and has optimum growth temperature of 37°C-42°C and the growth has a remarked odor of trimethylamine (Winslows, 1957)

1.8.3 *Klebsiella* species

Klebsiella is a member of the bacterial order *Eubacteriale* and the Family *Enterobacteriaceae* and named after the early German bacteriologist; Edwin Kleb (Winslows, 1957). The bacteria occur as aerobic or facultative anaerobic, non-motile, non-encapsulated and short rod. They are Gram–negative bacteria, occurring mostly single and frequently encountered in respiratory, intestinal and urogenital tracts infections, appearing dirty white, smooth, opaque and entire on nutrient agar (Adedare, 1998). Species of *Klebsiella* do not liquefy gelatin but reduce nitrate to nitrite and nitrogen, Methyl red and Voges Proskauer (MR – VP) reactions are variable depending on the species (Winslows, 1957). Lactose, glucose, mannitol, sucrose, and rabinose are fermented by most species. The reactions to urea and citrate tests are positive while those of indole and Kligler iron tests are negative (Ewing, 1986; Adedare, 1998).

1.8.4 *Proteus mirabilis*

It is a member of the order *Eubacteriale* and belongs to the Family *Enterobacteriaceae*. *Proteus mirabilis* is aerobic or facultative anaerobic, motile at temperature of 25°C by means of peritrichous flagella, Gram negative short rod of 0.5-0.6µ by 1.0µ (Winslows, 1957). It is reported as one of the major cause of gastroenteritis and can be isolated from abscesses and putrefying materials. The colonies are irregular, swarming on gelatin and gray, irregular, swarming on nutrient agar (Adedare, 1998). They produce turbid, thin, grey pellicle sediment in nutrient broth and are colorless on MacConkey agar (Bashir, 1991). Most strains of *Proteus mirabilis* convert nitrate to nitrite and nitrogen and their reactions to indole, Voges-Proskauer and liquefaction of gelatin are variable depending on the species involved (Lapage *et al*, 1979; Adedare, 1997). Reaction tests of Citrate, urea, catalase, kligler and fermentation of carbohydrate like fructose and glucose are positive but not lactose (Lapage *et al*, 1979).

1.8.5 *Staphylococcus* species

Staphylococcus is a member of the bacterial order *Eubacteriale* and the Family *Micrococcaceae* and named after the early researcher on this organism *Staphylococcus* (Winslows, 1957). The genus consists of *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Staphylococcus saprophyticus* (Baird-Parker, 1979). They are facultative anaerobic, non- motile, spherical cells occurring singly or in pairs and irregular cluster (Adedare, 1998). They are Gram positive and produce orange or yellow pigment particularly on media containing high level of sodium chloride (Winslows, 1957). They are

neither sporing nor capsulated and growth in nutritionally adequate broth is abundant, usually with heavy uniform turbidity and slight ring pellicle (Olayinka, 1998). *Staphylococcus* species are circular, smooth and orange to white, abundant and entire on nutrient agar and grow on Mannitol salt agar to produce yellow and abundant colonies (Cheesbrough, 1991). *Staphylococcus* spp are some time found on the nasal mucosal membrane, skin and skin glands. They produce exotoxin like haematoxin, dermatotoxin, and enterotoxin, which is the main source of food poisoning in mammals (Winslows, 1957). Most species of *Staphylococcus* convert nitrate to nitrite and nitrogen. Reaction to MR, VP, citrate, catalase tests are positive while those of indole, urea, oxidase and kligler tests are negative (Baird- Parker, 1979). Species of *Staphylococcus* ferment glucose, sucrose, lactose, manitol and glycerol into acid and gas, while inulin, raffinose, and arabinose are not fermented. The optimal growth temperature is 37–45°C (Baird Parker, 1979; Olayinka, 1998).

1.8.6 *Streptococcus* species

The genus *Streptococcus* belongs to the bacterial order *Eubacteriale* and Family *Loctobacillaceae* and are motile or nor motile Gram-positive bacteria, appearing in chains each of not less than seven cells and produce tiny grey colonies on nutrient agar and tiny pink colonies on MacConkey agar (only after 48 hours of incubation) (Winslows, 1957). Strains of *Streptococcus* can be isolated from human faeces, urinary tract, blood streams and heart lesion in sub-acute endocarditis cases (Winslows, 1957). Optimal growth temperature of *Streptococcus* is 45°C. Reactions of *Streptococcus* species to urea,

citrate, Methyl red, Voge-Proskauer are positive and negative to catalase, oxidase and indole tests (Elisabeth, 1979). The fermentation of carbohydrate compounds is variable, depending on the species involved (Adedare, 1998).

1.9. **Antimicrobial susceptibility of causative organisms**

Various bacteriuria causative Gram negative organisms like *E.coli*, *Proteus* sp and *Klebsiella* sp are generally sensitive to a wide variety of antimicrobial agents, notably, the amino penicillin (Ampicillin), fluoroquinolone (ciprofloxacin), cephalosporin (ceftriaxone), tetracycline, aminoglycoside, chloramphenicol, trimethoprim, sulphamide, nalidixic acid and nitrofurantoin. Gram positive organisms like *Staphylococcus* and *Streptococcus* species are exclusively sensitive to erythromycin, lincomycin, clindomycin and oleandomycin, (Child, 1991; Prasad, 1994; Holmes and Gross, 1995).

1.10. **The Chemotherapy of urinary tract infection**

Urinary tract infections are heterogeneous diseases and many factors must be taken into account when considering antimicrobial agents for the treatment of the infection (Brumfitt *et al*, 1972). For example, urinary tract infections encountered in domiciliary practice differ in many respects from those in the hospitals, therefore, the selection of therapeutic approach depends largely on the infecting organisms (sensitivity), the patient's past history of urinary tract infection, site of infection (upper tract or lower tract), presence or absence of predisposing condition which can influence the prognosis of the

current infection, pharmacology and pharmacokinetics of the drugs (Brumfitt *et al*,1972; Odutola,1986). Basically, antimicrobial agents that have been found useful in chemotherapeutic treatment of urinary tract infection are:

1.10.1 Ampicillin

Ampicillin is amino penicillin with an extended spectrum of activity and structurally composed of a β -lactam ring fused to a thiazolidine ring to form the 6-amino penicillanic acid (6-APA) nucleus (Russel, 1998). Ampicillin is a white crystalline powder, sparingly soluble in water and acid stable (Power, 1987). It is however, destroyed by the action of penicillinase enzymes (Ngwai, 1999). Although the exact mechanism of action is not well understood, the basic phenomenon has been described as a bactericidal by its ability to selectively inhibit bacterial cell wall synthesis through the inhibition of several enzymes like penicillin binding protein 1a, 1b, 2, 3, 4, 5 and 6 located in the cytoplasmic membrane which are involved in the final transpeptidation reaction of peptidoglycan synthesis (Waxman and Strominger, 1983). This inhibition triggers membrane associated autolytic enzymes that destroy cell wall, causing cell lyses (Waxman and Strominger, 1983; Reiner, 1984; Yao and Moellerng Jnr, 1995). Other mechanisms of action have also been reported and these include.

- Inhibition of bacterial endopeptidase and glycosidase enzymes involved in bacterial growth (Yao and Moellerng Jnr, 1995).

- Inhibition of bacterial RNA synthesis in some bacterial cells causing death without cell lyses (Mc Dowell and Reed, 1989). Due to the susceptibility of causative organisms' ampicillin is used in the treatment of bacteriuria at a dose of 2gm daily (Simon *et al*, 1993).

1.10.2 Cephalosporin

Cephalosporin is another group of antibiotics that is widely used in the treatment of urinary tract infections. It is mostly the second and third generation like cefuroxime (Zinat^R), ceftriaxone (Rocephin^R) and ceftazidime (Fortum^R) that are commonly employed in the chemotherapy of urinary tract infections (Yao and Moellerng Jnr, 1995). They are administered intramuscularly or intravenously, and are less effective orally (Prasad, 1994). They share almost the same mode of antimicrobial activity with penicillin, which is through the inhibition of the action of transpeptidase enzyme thereby preventing cell wall formation in all susceptible organisms (Waxman and Strominger, 1983). Cephalosporin is employed in the treatment of other infections like typhoid fever, salmonella meningitis, pneumonia gonorrhoea and syphilis. The most serious side effects are gastrointestinal disturbance, pains at the site of injection and blood disorder (Prasad, 1994).

1.10.3 Chloramphenicol

Although chloramphenicol is now synthetically produced for the treatment of most infection, it was originally produced by

fermentation using *Streptomyces venezuela* (Russel, 1987). The mechanism of action is by selectively inhibiting protein synthesis in bacterial cells by binding to the 50s subunit of the 70s ribosome. As a result of this attachment, it inhibits peptidyl transferase enzymes, which is found in the ribosome preventing it from forming a new peptide bond with the growing peptide chain (Lambert, 1998). Clinically it is used in the treatment of urinary tract infection involving prostate gland. It is first drug of choice in the treatment of *Salmonella typhi* and *paratyphi* infections, *Salmonella* septicemia and meningitis (Prasad, 1994). Chloramphenicol is rapidly absorbed from gastro-intestinal tract and enters the bile, cerebrospinal fluid, urine and passes through placenta in therapeutically effective concentration. The level of toxicity, which involved renal and gastrointestinal systems, blood and hearing disorder reduces the use of the drug. For effective treatment of urinary tract infection and prostaticitis, the daily dose of 2gms for 5-days is employed (Prasad, 1994)

1.10.4 Gentamicin

Gentamicin is one of the chemotherapeutic agents used systemically in the treatment of urinary tract infection (UTI). Gentamicin belongs to group of antibiotic collectively referred to as aminoglycoside (Russel, 1998). Other members include streptomycin, kanamycin, neomycin, amikacin, and netilmicin. Gentamicin is produced by bacterial species called *Micromonospora purpurea*. The mechanism of action of gentamicin as described by Lambert (1998) is by interfering with the protein synthesis of microorganisms. This is however due to the ability of gentamicin to bind to the protein component of 30s subunit

preventing the initiation and assemblage of the ribosome. The binding distorts the shape of A - site on the ribosome and interferes with the positioning and transferring of RNA molecules during peptide chains elongation. Gentamicin, therefore exert two-effect viz: inhibition of protein synthesis by freezing the initiation complex and misreading of the condone on messenger RNA through the distortion of 30s subunit leading to the formation of toxic non-functional protein in the bacterial cells. It is bactericidal against Gram-negative bacteria such as *E. coli*, *Klebsiella*, *Nesserisia*, *Proteus*, *Salmonella*, *Pseudomonas* and Gram-positive organisms like *Staph aureus*, *Haemophylus* and *Streptococcus* spp (Simon *et al*, 1993). Gentamicin is clinically used in the treatment of infection like UTI, meningitis, pneumonia, endocarditis, *Salmonella typhi* and *paratyphi* infections (Prasad, 1994). It is administered intravenously or intramuscularly in the treatment of infection at a dose of 40 – 280mg daily (Prasad, 1994).

1.10.5 Lincomycin and clindamycin

The agents are teffective in the treatment of urinary tract infections (Russel, 1998). They are structurally similar and belong to Lincosamide group of antibiotics and exert their antibiotic properties through the inhibition of biosynthesis of protein by selectively binding to the 50s ribosome subunit close to the binding site of chloramphenicol and erythromycin (Lambert, 1998). This leads to the inhibition of peptidyltransferase thereby preventing the elongation of the peptidyl chains (Lambert, 1998). The antibiotics are active against gram positive and negative bacteria including penicillin resistant *Staphylococcus*. Owing to their high concentration in the prostate

fluid, bones and joint, they are useful in the treatment of the infection involving the organs (Reeves *et al*, 1972).

1.10.6 Erythromycin

Erythromycin is a member of the Macrolide group of antibiotics. As reported by Reeve *et al* (1972), out of the drugs examined only the basic macrolides (erythromycin and oleandomycin) were secreted in prostate fluid in concentration high enough to suggest any therapeutic potential. For this reason, erythromycin is vital in the treatment of urinary tract infection in which prostate is involved (Reeves *et al*, 1972). The action of the agents is through the inhibition of the biosynthesis of protein in bacterial cells. It acts by interfering with the process of translocation by binding to 50s subunit of the 70s ribosome, and prevent the transfer-RNA from the donor site, consequently inhibiting the occupation of the p-site by the peptidyl transfer RNA (Lambert, 1998). It is used in the treatment of prostatitis, endocarditis and peptic ulcer caused by *Helicobacter pylori*. The main toxic effects are gastrointestinal disorder and hepatotoxicity (Prasad, 1994).

1.10.7 Nitrofurantoin

It belongs to the group of furan antibiotic (Russel, 1998). It is used in the therapy of urinary tract infection. The antibacterial activity is by the breakage of DNA strands (Lambert, 1998). The drug is very effective against Gram positive and negative bacteria (Simon *et al*, 1993). It is generally active against *Enterobacteriaceae* hence useful in the chemotherapy of urinary tract infections since most of the

causatives of infection of urinary tract are in the family of *Enterobacteriaceae* (Yao and Moellerng Jnr, 1995).

1.10.8 Tetracycline

Tetracycline group includes chlortetracycline, tetracycline, doxycycline and oxytetracycline (Reiner, 1984). Some of the agents were obtained as by-product of *Streptomyces aureofaciens* while some were semi synthetic (Russel, 1998). The action of the group is by the inhibition of the biosyntheses of proteins. It binds to the 30s subunit of the bacteria ribosome and prevents the attachment of the aminoacyl transfer RNA to the A-site, thereby preventing the introduction of new amino acid to the nascent peptide chain (Lambert, 1998). Clinically, tetracycline is orally administered at a dose of 1- 2gm daily in the treatment of urinary tract infection and other disease like bronchitis, cholera, trachoma and brucellosis (Prasad, 1994). Generally its spectrum of activity is against Gram- negative and positive like *Streptococcus and Bacillus*. The main toxicity are gastrointestinal disorder and dental staining.

1.10.9 Co trimoxazole

It is a combination of sulphamethoxazole and trimethoprim in the ratio of 5: 1. Although individual component is bacteriostatic, the combination is synergetic and bactericidal (Adedare, 1998). The components of cotrimixazole act by synergistically inhibiting the biosynthesis of folic acid at different stages thereby preventing the formation of functional nucleic acid in the bacteria (Lambert, 1998). The agent has wide spectrum of activity against a large number of

bacterial cells including *Streptococcus Staphylococcus*, *Escherichia coli*, gonococci, and pneumococci (Simon *et al*, 1993). It is clinically employed in the treatment of typhoid fever, respiratory and urinary tract infections at daily dose of about 0.96-1.92gms (Prasad, 1994).

1.10.10 Fluoroquinolone

Quinolone derivatives of carboxylic acid are widely employed in the chemotherapy of bacteriuria (Adedare, 1998). Among the group, ciprofloxacin and norfloxacin (Norbactin) are commonly used in the treatment of the infections (Prasad, 1994). They exert their action through the specific inhibition of microbial enzymes- DNA gyrase (Lambert, 1998) which is bacterial topoisomerase II which catalyses ATP dependent negative super coiling of DNA. It acts together with DNA topoisomerase - I to promote super coiling and relaxation of the bacterial chromosome. The agents are active against Gram positive and negative organism like *Enterobacteriaceae*, *Shigella* and *Pseudomonas* but nalidixic acid is mainly active against Gram negative organism (Simon *et al*, 1993). They are clinically useful in the treatment of urinary tract infections at daily dose of 0.5 -1g and the main side effects of quinolones are nausea, vomiting, diarrhea, neurotoxicity and skin rashes (Prasad, 1994).

Suppressive therapy is generally instituted in patients who have persistent focus of infection such as prostatitis or urinary calculi.

The preferred drug of suppression is methenamine based on its mechanism of action (antiseptic). The drugs can be used with a urinary acidified agent so as to maintain acidic pH of the urine

(Odutola, 1986). At the acidified pH methanamine is converted to formaldehyde which then exerts the antiseptic activities.

It is critical to treat all forms of urinary tract infections. The required intensity and duration of therapy increases in proportion to the severity of the clinical syndrome (Odutola, 1986).

1.11. **Bacterial resistance to antimicrobial agents**

Antibiotic resistance is a clinical condition in which microorganism continue to proliferate at its therapeutically achievable in-vitro minimum inhibitory concentration. The bacterial cells survive the bacteriostatic or bactericidal concentration of the agent. Antibacterial resistance has been recognized since the first drug was introduced for clinical use. It was first noticed and reported by Paul Ehrlich at the beginning of the chemotherapeutic era in 1909 (Rainer, 1984) and since then, it has been on the increase. Antibacterial resistance has been a major problem in the chemotherapy of bacterial infections with bacteria being extremely adept at becoming resistant to many agents. In recent years, antimicrobial resistance has emerged explosively among diverse bacterial types largely as a consequence of decades of unrestricted antimicrobial use in agriculture, human and veterinary medicine (Johnson *et al*, 1999). Bacterial resistance is a world wide problem with the report of its occurrences in different parts of the world (Sang *et al*, 1985; Johnson, *et al*, 1999). The incidences of resistance to either a single chemotherapeutic agent or many agents at the same time (multiple antibiotic resistant) have been reported severally in *Escherichia coli* and *Shigella* spp (Franklin, 1987).

Antibiotic resistance has serious clinical and economic consequences. The increasing prevalence of multiple antibiotic resistance (MAR) Gram negative bacilli, methicillin resistant *Staphylococcus epidermidis* and vancomycin-resistant enterococci (VRE) has further increased the morbidity, mortality rate and prolonged hospitalization with its attendant high cost of health care.

1.11.1 Types of bacterial drug resistance

Considerable progress has been made in understanding more fully the responses of different types of bacteria to antimicrobial agents. As a result, antibiotic resistance is classified into two broad types; intrinsic (natural) or acquired resistance.

a. Intrinsic resistance, which is also termed as innate, suggests that inherent properties of the bacterium are responsible for preventing antibacterial action. Intrinsic resistance is associated with the permeability barrier of the complex outer membrane layer (absent in Gram-positive bacteria) to some antibiotics that prevent the attainment of their MIC within the cell. Gram negative cell envelope is therefore effectively impermeable, preventing certain antibiotic from reaching their intracellular targets (Lowbury and Ayliffe, 1974; Franklin, 1987; Power, 1998).

b. On the other hand, acquired resistance may occur through the spontaneous mutation in the chromosome that controls susceptibility to a given drug. For instance streptomycin resistance in which the drug structural receptor, Protein-S₁₂ on 30s subunit of the 70s ribosome is altered. Spontaneous mutations occur with a very low

frequency therefore it is an infrequent cause of the emergence of clinical drug resistance in a patient (Jawetz *et al*, 1974).

The acquired resistance can also be through the acquisition of genetic materials of either chromosomal or extra chromosomal (plasmid or transposon) originating from one bacterium to another between species or genera. The spread of antibiotic resistance gene is largely by extra chromosomal (plasmid) transfer through the process of conjugation, transduction and transformation (Franklin, 1987; Power, 1998). Though both types are clinically important and can result in treatment failure but acquired antibiotic resistance is more of a threat in the spread of antibiotic resistance (Franklin, 1987; Power, 1998)

1.11.2 Roles of genetic elements in acquired resistance

Antibiotic resistant organisms have emerged as a result of genetic changes, followed by the selective pressure (Jawetz *et al*, 1974; Franklin, 1987; Simon *et al*, 1993; Olayinka, 1998). In most cases the introduction of new drugs initially marks a “victory” on the causal organism such that very high rate of success is reported in therapy. However, reports of treatment failure are soon encountered after continuous therapy as a result of the emergence and spread of resistant strains of the causative organisms (Franklin, 1987). The acquired ability of the organism to thrive in the presence of the new drug is a reflection of the differences in the genetic composition of the resistant strains which gradually replace the sensitive strains as a result of the antibiotic selective pressure (Franklin, 1987; Simon *et al*, 1993). The mechanisms of bacteria acquiring drug-resistant are often classified on

the source of the genetic changes that are responsible for the acquired-resistant (Simon *et al*, 1993).

1.11.3 Chromosomal mediated antibiotic resistance

It is the acquired resistant brought about by the changes in the chromosomal DNA sequence called mutation, which can occur due to single base pair change (Power, 1998). The transition involves the substitution of one purine (A or G) for one another and one pyrimidine (C or T) for another while transversion involved a change from pyrimidine to purine and vice versa. Usually a single mutation at the appropriate genetic locus produces a small increase in resistance while the level of resistance build up with each successful alteration on the bacterial genome (Franklin, 1987). Such genotype alternation may be expressed through various physiological (phenotypic) mechanisms which may be strain dependent (Franklin, 1987).

1.11.4 Plasmid mediated antibiotic resistance.

In addition to the bacterial chromosome that contains all the genes necessary for the growth and replication of cell, some bacteria possess elements of DNA which are capable of replicating and transferring independent of the chromosome (Power, 1998). This extra chromosomal element is referred to as plasmid. The clinical problem of bacterial drugs resistance is mainly as a result of the remarkable ability of many bacteria to acquire such additional genetic materials (Power, 1998). Plasmid can code for number of properties including antibiotic resistance. The plasmid are largely transmissible (conjugative) in Gram negative bacteria and transducible in Gram-positive bacteria and are know to mediate the production of various drugs inactivating enzymes.

Common examples of such enzymes are the β - lactamases that open the β - lactam ring present in penicillin, cephalosporin, and related drugs, converting them into inactive products (Franklin, 1987; Olayinka, 1998).

1.11.5 Transfer of plasmid antibiotic resistance

The ability of bacterial cells to genetically acquire antibiotic resistance is through the process of conjugation, transduction and transformation (Franklin¹, 1987). This ability of plasmid to transfer information within and between species made plasmid acquired antibiotic resistance much more threatening in terms of spread of antibiotic resistance than the chromosomal acquired resistance (Ntiejumokwu, 1998).

a. Conjugation

This is a process of indiscriminate transfer of genetic information including drugs resistance by sexual recombination in Gram- negative bacteria either of the same or completely unrelated species (Power, 1998). The process of conjugation require cell to cell contact and involve the transfer of DNA from donor cell to recipient cell through conjugate tube and always initiated by plasmid (Koh,1986). The process of conjugation as illustrated by Olayinka (1998) is:-

- 1) Wall to wall contact between the two bacteria that want to exchange genetic information.
- 2) Formation of mating bridge (Trans - membrane pore)
- 3) The helical structure of DNA is broken at specific sites (depending on the bacteria strain).

- 4) The broken DNA moves to the recipient cell through the trans-membrane pores.
- 5) Formation of complementary strands in the two cells.
- 6) The linear extra chromosomal DNA (plasmid) is then turned into the helical structure so that each cell has its own copy of the DNA.

The first clinical evidence of this type of transfer was encountered in Japan during a dysentery epidemic caused by *Shigella* spp. The use of sulfonamide initially proved effective but strains of resistant *Shigella* spp were soon isolated. The isolation of both sensitive and resistant *Shigella* and also of strains of another enteric *Bacillus* and *Escherichia coli* with multiple drug resistance from the same patient led Japanese researcher to suggest inter-genus transfer of multiple antibiotic resistance markers (Franklin, 1987).

b. Transduction

It is unidirectional transfer of DNA using bacteriophage as the vector and plays an active role in the transfer of antibiotic resistance in Gram positive bacteria such as *Staphylococcus aureus*, *Streptococcus pyogenes* and enterococci (Power, 1998). The process is initiated by a bacteriophage infecting a susceptible bacteria cell. The virus may either replicate immediately resulting in the cell lyses and release of many virus cell or enter into lysogenic state and be integrated into the host gene which may be chromosomal or plasmid mediated (Franklin, 1987). The virus in this latent state may be induced to replicate by

changes in environmental conditions causing bacterial cell lyses and subsequent release of virus particles (Franklin, 1987; Al-Masaudi *et al*, 1991). Genetic material transfer will be as a result of the replicating bacteriophage DNA picking up a piece of the host DNA incorporating into its complete phage particle and infect a new host cell. In this case all the genetic markers acquired in the original host are incorporated into the new host genome and expressed. Although transduction is a known mean of transferring bacterial drugs resistance in gram positive cells, the process is limited to organisms of the same species and therefore its role in the transfer of antibiotic resistance is less significant in clinical practice than conjugation. Olayinka (1998) reported that a phage-mediated conjugation which unlike generalized transduction needs viable cell to cell contact as well as the presence of lysogenic phage in either the donor or recipient. The transduction transfer can be achieved without the death of the donor or the risk of the recipient being destroyed by normal phage particles (Al-Masundi *et al*, 1991). The precise sequence of events in this transfer is not known but high cell densities and calcium iron are both required for high rate of gene transfer (Witte, 1981). Due to the high frequency of transfer it has been suggested that transfer of small plasmid and chromosomal genes between staphylococci is likely to occur under natural conditions by this mechanism (Al-Masaudi *et al*, 1991; Olayinka, 1998).

c. Transformation.

Transformation is the ability of microorganism to transfer genetic materials through soluble naked DNA (Power, 1998). It is mostly

confined to Gram-positive bacteria notably *Neisseria gonorrhoea* which have the ability to recognize their own species. Transformation takes place between closely related species and its thought to involve a recombination event in which the transforming DNA rather than addition of transforming DNA replace part of the recipient gene (Franklin, 1987; Olayinka, 1998).

d Transposon

They are minute mobile genetic element of DNA capable of transferring independently from one DNA molecule to another (Power, 1998). Transposon is often referred to as “Jumping gene” with the ability of indiscriminately inserting themselves into many genomic sites with which they are not homologous (Franklin, 1987; Power, 1998). This process of genetic transfer is common among staphylococci, gonococci and pneumococci. A transposon can mediate single or multiple antibiotic resistant and can, after entering a bacterial cell, become incorporated into its plasmid or chromosome (Simon *et al*, 1993).

1.11.6. Biochemical mechanism of bacterial resistance

Bacterial resistance (acquired or inherent) manifested through some basic biochemical mechanisms as identified and described by Quintiliani and Courvalin (1995).

a. Enzymatic inactivation of antibiotic

Antimicrobial-resistant bacteria can produce enzymes that are capable of destroying the drugs or converting them into inactive compounds (Power, 1998). These enzymes are produced extracellularly by Gram positive bacteria like *Staph aureus* and *Bacillus cereus* or

intracellularly in Gram negative bacteria like *Klebsiella aerogenes* and *Pseudomonas aeruginosa* (Franklin, 1987; Simon *et al*, 1993). The intracellularly enzymes are well placed within the periplasmic space to intercept any antibiotic that find its way into the cell (Brown, 1975). One of such enzymes is β -lactamase, which is capable of opening the β -lactam ring, hydrolyzing the cyclic amide bond in the lactam antibiotic molecules converting them into inactive products (Franklin, 1987; Power 1998). They are constitutively produced in small quantities and controlled by plasmid in Gram negative bacteria. The synthesis is initiated by the presence of toxic agents (antibiotic) and controlled by transducible plasmid in Gram-positive organisms. Genetic difference is responsible for the differences in the β -lactam antimicrobial resistance pattern of Gram positive and negative organisms (Franklin, 1987). The β -lactamase produced by Gram positive resistant strains consist of penicillinase and cephalosporinase enzymes and are capable of attacking penicillin and cephalosporin antibiotics to produce penicilloic and cephalosporoic acid respectively, which have no antimicrobial activities (Power,1998). Another penicillin inactivating enzymes (penicillin amylase) produced by a number of species of *Streptomyces* fungi and some Gram negative bacilli are of commercial importance in the production of precursor (6-Amino-penicillanic acid) for semi synthetic production of penicillin (Russel, 1998). Another important bacterial drug resistant due to inactivation of the antibiotic is the plasmid-mediated acetylation of Chloramphenicol by Gram positive and negative bacteria e.g. *Staph aureus* and *Escherichia coli* (Quintiliani and Courvalin, 1995). Other groups of enzyme that are capable of

inactivating aminoglycoside are phosphotransferase, adenylyltransferase and acetyltransferase (Brown, 1975). These enzymes are specifically produced from periplasmic zone of the cell wall and the underlying biochemical basis of the inactivation of aminoglycoside falls into one of these three classes;

- N-Acetylation of amino groups in which acetyltransferase enzyme uses acetyl co-enzyme as constituent (Power,1998).
- Phosphorylation reaction catalyses by phosphotransferase enzymes using ATP as source of energy (Franklin,1987).
- Adenylylation of hydroxyl group in which ATP is use as a Source of energy.

The products of the reactions have no antimicrobial activities.

b. Decrease cellular permeability

Bacterial cells can develop resistance to antimicrobial agents by the loss of cellular permeability to antibiotics (Nikaido, 1994). This diminished uptake of antibiotic is due to ultra structural alteration of the cell envelope particularly slime and outer cell membrane. The slime or capsule layer of bacterial cell consist of polyanion hydrophilic hence repulses hydrophobic drug molecules, resulting in impairment of drug penetration to deeper lipophyllic layer (Franklin, 1987; Olayinka, 1998). On the other hand, the outer membrane is composed of hydrophobic properties inhibiting the penetration and interaction of hydrophilic drugs e.g. penicillin and streptomycin (Franklin, 1987). Bacterial drug resistance can be brought about by the disruption of transport across the membrane thereby preventing drugs accumulation at the target site (Nikaido, 1994). The resistance

to tetracycline antibiotic in both Gram-positive and negative bacteria is by the reduction of accumulation of the drug due to the inhibition of transport system (Simon *et al*, 1993) or through the plasmid encoded tetracycline efflux. Resistance to cycloserine is due to the reduced efficiency of one or more transport systems for D-alanine which is commonly mediated through the plasmid (Power, 1998).

c. Moderation of target sites

Often, mutation can result to the change in the antibiotic target site leading to resistance of the organism to a particular drug. This was seen when a single amino acid replacement in either one or two specific position of protein S₁₂ of the 30s subunit of the bacterial 70s ribosome resulted in the resistance of *E coli* to streptomycin (Franklin, 1987). The alteration of three of the four major penicillin binding proteins (PBP) found in the sensitive bacterial cells are responsible for the resistance of *Streptomyces pneumoniae* to penicillin derivatives (Power, 1998). The process of resistance to quinolone is by subtle conformational changes on DNA gyrase that reduce binding site of quinolone (Franklin, 1987). Resistance of bacteria to polymyxin antibiotic is through modification in the cell envelope resulting in the decrease of affinity or quantity of membrane content of lipopolysaccharide and phospholipid (Brown, 1975). Other important example of this mechanism is the bacterial resistance to nucleic acid synthesis inhibitor of *Streptococcus pneumoniae*, which is by the alteration of dihydropteroate synthetase enzymes (DHPS) thereby reducing the affinity for the antibiotic (Lamkanra and Ndep, 1989; Power, 1998).

d. Alteration of metabolic pathway

Some bacterial cells can develop resistance as a result of enhancement of an alternative metabolic pathway by-passing the inhibited one or by decreasing the requirement of the product of the inhibited pathway (Franklin, 1987). Example of this mechanism is the bacterial resistance to dihydrofolate reductase inhibitor (Trimethoprim) through the hyper-production of dihydrofolate reductase (DHFR) to overcome the inhibited pathway (Power, 1998).

1.11.7 Factors facilitating microbial resistance

The occurrence and wide spread of bacterial resistance in the world has been attributed to many reasons; among them are:-

- * Free availability of antibiotic which allow uncontrolled or irrational and excessive use of drug that provides selective pressure for the emergent of resistant strains (Paul *et al*, 1982, Murray *et al*, 1985; Johnson *et al*, 1999).
- * Inadequate infrastructure and other relevant diagnostic facilities in the hospital to ensure detailed laboratory investigation have been suggested as another contributing factor.
- * Inadequate control procedure in the hospital resulting to the spread of infectious diseases and resistant strain is also identified as a factor.

- * The excessive antimicrobial therapy of catheter urinary tract infection (CUTI) provides selective pressure for the emergence of more highly resistant organisms (Johnson *et al*, 1999).
- * Inadequate information and counseling on the use of antibiotic especially at the rural areas gives rise to irrational prescription and usage of antibiotics which in turn support the emergence of resistant strains (Ngwai, 1999).

1.12 **Statement of research problem**

Urinary tract infections (UTIs) are among the most common extra-intestinal bacterial infections in human (John *et al*, 1999). They are the major types of infection in acute and long-term care setting (Johnson *et al*, 2002). The infections of urinary tract are probably second only to infections of the respiratory tract in frequency and are more important because they often result in progressive and irreversible impairment of kidney functions (Cohen and Irew, 1972). They contribute substantially to enormous aggregate of morbidity and mortality, increased health care cost and antibiotic usage. The significant increased in risk of bladder cancer in men and women have been associated with persistent chronic infection of the urinary tract (Howe *et al*, 1980). In Abuja, the unpublished statistical report of Department of health and social services has shown a steady increase in the number of bacteriuria cases in the hospitals.

The high prevalence of bacteriuria necessitated the prescription and unrestricted usage of a wide range of anti-microbial agents. In the

recent years, antibiotics resistance among bacteria pathogenic to man and animals has been recognized world-wide, as a major problem in the management of health-care delivery services (Kunin, 1993). These resistance, which are easily transferred between the organisms of the same, or different genera through plasmid lead to therapeutic failure with associated increase in the rate of hospitalization and duration of symptoms.

1.13. **Specific aim and objectives of the research**

In view of the outlined statement of research problem, the investigations were aimed at;

1. Carrying out a pre-study survey to verify the prevalence of symptomatic bacteriuria in Abuja and environ.
2. Collecting “mid-stream” non-repetitive urine samples from suspected bacteriuric patients to determine the state of bacteriuria using microbiological culture method and then characterize the causative organisms.
3. Evaluating the antibiotic susceptibility profile of the isolates and further determining the minimum inhibitory concentration (MIC) and multiple antibiotic resistance indices (MARI) of the resistant isolates.
4. Determining whether the antibiotic resistance of the isolates (representative) was plasmid or chromosome mediated using the conventional plasmid curing method.

5. Investigating the ability of the multiple antibiotic resistance isolates (representative) to produce β -lactamase enzymes.

CHAPTER TWO

2.0. MATERIALS AND METHODS

2.1. Materials

2.1.1 Samples

Urine samples from male and female suspected UTI patients that visited both in-and out-patient departments of the hospitals.

2.1.2 Hospitals

- Asokoro is a sixty- bed secondary health institution in Abuja. It provides secondary health services to inhabitants of Asokoro, Nyanya, Karu and part of Nasarawa state.
- Maitama is a sixty-bed General hospital located in Maitama district of Abuja and serves the people of Maitama, Mpape and Bwari Districts of Federal capital territory.
- Wuse is a General hospital located in central part of Wuse. It provides secondary health services to inhabitants of Wuse, Mpape, Dutse and Kubwa Districts of Abuja.
- National hospital is a tertiary health institution in Abuja. It is located in the central area of the city and takes referrals from any part of Abuja and the neighboring states.

- Gwagwalada specialist hospital is a tertiary health institution situated in Gwagwalada district of the capital city. It takes referrals from any part of Abuja and the neighboring states.
- Divine Medical and Diagnostic Centre Durumi is a private health centre that provides medical and laboratory diagnostic services for the people of Durumi and neighboring villages in Abuja.

2.1.3 Culture Media

- Nutrient agar, Nutrient broth, MacConkey agar and Peptone water (Lab M. Topley Bury Lancashire, UK)
- Mueller-Hinton agar (Biotec Laboratories Ltd. England)
- Mannitol Salt agar and Urea broth (Difco Laboratories, UK)
- Kligler Iron agar (Oxoid Ltd, England).

2.1.4 Reagent and Chemicals

- Glucose, Lactose, Mannitol, Iodine Crystal, Hydrochloric acid and Maize starch (BDH Chemical Poole, England)
- Urea and Ethanol (E. Merk, Darmstadt, Germany)
- Crystal Violet (Fluka Buchs, Switzerland)
- Hydrogen peroxide (20%) (Nova Pharm Ltd. Nigeria)
- Acridine Orange dye (Williams Head well Health , England)
- Dettol^R (Rechit and Colman Ltd. Nigeria)

- Andrade Indicator, aqueous Tetramethyl phenylenediamine hydrochloride, Bhomes reagents , Barium Chloride, Distilled water, sulphuric acid and normal Saline (laboratory stock)
- Ampicilin powder (Huayuan Zhangheng Pharmaceutical)
- streptomycin powder (Laborate Pharmaceutical India)
- Crystalline penicillin G powder (Carion medical Lagos).

2.1.5 Equipment

- Autoclave (Adelphi manufacturing Company Ltd. UK)
- Balance (Top Loading) (W&T Ltd. Birmingham England)
- Incubator and inoculating wire loop (Baird and Tat lock Essex)
- Light microscope (Wild mill Switzerland)
- Membrane filter equipment (Millipore Cooperation Mass USA)
- Oven (Hot Air) (Baird and Tat lock Ltd. Essex)
- Micropipette (Gilson, France)
- Colonies counter (Gallen Kamp Ltd. England)
- Vortex mixer and Refrigerator (Gallen Kamp Ltd. England)

2.1.6 Glassware and other accessories

Glass and Cover slide, , Durham tubes, Universal bottles, McCartney bottles, Petri dishes, Test tubes, Beaker, Measuring cylinder, Bunsen burner, Disc dispenser, Filter paper and Forceps.

2.1.7 Antibiotic discs.

Ampicillin (10µg), Gentamicin (10µg) and Ceftriaxone (30µg) were products of Oxoid; Multi disc antibiotics (Optun laboratory Nigeria Ltd.) containing, Streptomycin (30µg), Chloramphenicol (30µg), Cephalexin (10µg), Ciprofloxacin (5µg), Tetracycline (30µg), Nitrofurantoin (300ug), Rifampicin (10µg), Co-trimoxazole (30µg), Norfloxacin (30µg), Erythromycin (30µg), Flucloxacillin (30µg), Amoxicillin + Clavulanic acid (30µg), Ampicillin + cloxacillin (30µg), Nalidixic acid (30µg), Lincomycin (30µg), Ofloxacin (10µg) and Pefloxacin (10 µg).

2.2 METHOD

2.2.1 Designing, distribution and analysis of questionnaires

In order to justify the necessity for the research work, the incidence of symptomatic UTI was assessed through questionnaires. The questionnaire was structured to collect data such as age, sex, marital and financial status, common clinical symptoms and diagnostic laboratory investigation used to confirm UTI infection, drug therapy and the likely source of contracting the infection (Appendix A). Five hundred (500) copies of the questionnaires were printed and distributed as follows: Seventy-one copies of the questionnaire were distributed to the underlisted Centre except National hospital that has seventy-four copies. The hospitals and clinic are;

- * Asokoro General hospital
- * Maitama General hospital
- * Wuse General hospital
- * Gwagwalada specialist hospital
- * National Assembly clinic
- * Divine Medical and Diagnostic Centre Durumi.

Secondly, with the approval of the relevant Authority Medical records of bacteriuria patients that visited the above-mentioned hospitals and clinic in the year 2003 were assessed. From the records of the patients, the following data were collected; age, sex, marital status, the most common clinical symptoms, confirmatory test and instituted drugs therapy.

2.2.2 Collection of urine specimen and treatment

Suspected bacteriuria cases from in- and out-patients departments of National, Asokoro, Maitama and Gwagwalada specialist hospitals and Divine Medical and Laboratory Centre, Durumi were screened for bacteriuric pathogens.

With the consent of the staff of the laboratories and the patients, a total of three hundred and five samples of urine were collected from patients presumptively diagnosed of having significant bacteriuria and referred to microbiological laboratories for confirmation. Well-clean universal bottles were given to patients and instructed on how to collect early morning mid-stream urine. The collected urine samples

were immediately taken to a laboratory in Winners Medical and laboratory Research Centre Abuja for processing.

2.2.3 Preparation of culture media.

Specified quantities of dehydrated media were reconstituted in freshly distilled water according to the instructions of the manufacturers. They were dispensed in specified volumes and sterilized in an autoclave, cooled and stored in the refrigerator.

2.2.4 Cultivation, isolation and characterization of isolates

On arrival at the laboratory, a standard bacteriological loopful of each urine sample was spread on the dried surface of MacConkey and nutrient agar plates and incubated aerobically at 37°C for 18 hours. The presence or absence of growth, number and characteristics of the colonies were properly recorded. The growth were sub-cultured onto the surfaces of selective media such as MacConkey and Mannitol salt agar plates using sterile loops and incubated at 37°C for 18 hours. The characteristics of the colonies were compared with those stated in the individual manual and also sub-cultured onto Nutrient agar slants for preservation in the refrigerator.

2.2.5 Gram staining

The Gram staining method described by Cheesbrough (1991) was adopted. The culture was made on the glass slide and heat fixed. Crystal violet was used to stain the slides and fixed with Lugol iodine solution. They were decolorized with 95% ethanol and counter-

stained with diluted carbol fuchsin solution. The slides were subsequently examined under the microscope.

2.2.6. Motility

Test for motility of the isolates was carried out using hanging drop method as described by Baker and Silverton (1998). In this method, a ring of Vaseline was made on a clean glass slide and was gently and neatly pressed onto the drop of the culture on the surface of the cover slide. The slide was quickly inverted and examined under the microscope, focusing on the edge of the drop for the presence or absence of motile cells.

2.2.7. Biochemical Tests

a. Sugar fermentation test

The ability of the isolates to ferment sugars was tested using the method described by Adedare (1997). According to the method, sugar solution (1% w/v) and Andrade's solution (1% v/v) were added to the prepared peptone water which was divided into aliquots of 5mls in the test tubes. Into each of the test tubes, Durham tube was introduced in inverted position and sterilized at 115⁰C for 30 min. The aliquots were inoculated with the isolates using sterile loop and incubated at 37⁰C for 18 hours along with negative control. The presence or absence of reddish color and gas production were noted.

b. Indole fermentation test

Peptone water was used for this test. The isolates were sub-cultured into the peptone water broth and incubated at 37⁰C for 5 days. To the culture, xylene with shaking was added and allowed to settle on the

surface. Bhome's reagent solution A was added by the side of the test tube. If no color changed within a minute, Bhome's reagent solution B was added and the color change was noted and recorded.

c. Citrate utilization test

Koser citrate medium was prepared according to the manufacturer. The medium was inoculated with the isolates and incubated at 37°C for 18 hours and the color change was noted.

d. Oxidase test

Agar slant culture of the organisms was used for the test. Drops of 1% aqueous solution of tetramethyl-p-phenylenediamine hydrochloride were placed on Whatman paper No 1 filter paper on a white tile. The colonies from the agar slant were smeared onto the impregnated filter paper. Production of purple color within 10-16 seconds was considered positive to oxidase.

e. Catalase test

Few colonies of the organism were introduced into about 5mls of Hydrogen peroxide solution in the test tube and the evolution of gas bubbles was noted for catalase positive.

f. Test for urea hydrolysis

The basal urea broth medium was constituted and distributed in aliquot of 9ml into the test tubes. Twenty percent (20%) urea solution was prepared with distilled water and sterilized by membrane filter

(Pore size of 0.45 micron). One milliliter of this urea solution was added to sterile basal medium, inoculated with the isolates and incubated at 37°C for 5-days with control basal medium which contained no organisms. The production of pink color is noted for positive urease enzymes possession.

g. Test for hydrogen sulfide production

Kligler iron agar which contained double sugars (glucose and lactose) and ferric iron was used in determining the ability of the isolated organisms to produce hydrogen sulfide according to the method described by Adedare (1997). With the use of sterile loops the surface and the butt of the agar were inoculated and incubated with control at 37°C for 48 hours. The production of black coloration on the surface and the butt of the agar as well as production of gas within the butt portion was recorded.

2.2.8. Antibiotics susceptibility test

The antibiogram of isolates to commonly used antibiotics was determined using Kirby-Bauer technique (Bauer *et al*, 1966) as modified by National committee for Clinical Laboratory standard (Lennette *et al*, 1993) .The culture were standardized by transferring four to five similar colonies of overnight culture with sterile wire loop into separate test tubes containing 5ml of nutrient broth and incubated at 37°C for 18 hours. The turbidity was adjusted to match that of barium sulfate by diluting the culture using sterile normal saline (NaCl. 0.9g, distilled water to 100ml). The barium sulfate suspension

was prepared by adding 1.7gm barium chloride dihydrate (BaCl₂.2H₂O) solution to 99.51gm of 0.36 M (1.0%) sulfuric acids. Sterile plates of Mueller- Hinton agar (MHA) were prepared according to the Manual and their dried surface inoculated with the standardized culture. The plate was allowed to dry for 5 minutes at room temperature before antibiotic sensitivity discs were evenly placed on the dried surface of the plates using multi-disc dispenser. The plates were left at room temperature for pre- incubation diffusion time of 2 hours and thereafter incubated at 37°C for 18 hours. The diameter of zone of inhibitions (including diameter of the discs) were measured with plastic ruler.

2.2.9. Multi -antibiotic resistant (MAR) determination

The multi- antibiotic resistant (MAR) index was determined for each isolates. The MAR Index is the number of antibiotic to which the organism is resistant divided by the total number of antibiotic tested (Krumperman, 1983, Paul *et al*, 1997).

It is mathematically calculated as:

$$\text{MAR Index} = \frac{\text{No of antibiotic to which isolates were resistant}}{\text{Total no of antibiotic tested.}}$$

2.2.10. Minimum inhibitory concentration (MIC) determination

The minimum inhibitory concentration (MIC) of ampicillin and streptomycin against multiple antibiotic resistant isolates were determined using the macrobroth dilution protocol of Lennette *et al*

(1974) and Barry (1976). In this experiment twelve clean test tubes were labeled 1-12 and eleven of them (2-12) were each filled with 1ml of nutrient broth and sterilized. Thereafter tube 1 and 2 were aseptically filled with one milliliter of Ampicillin stock solution (10mg/ml). The content of Test tube 2 was serially diluted up to tube 10 from which 1ml was discarded. From standardized inoculum 1ml was transferred to all the test tubes except tube 12 which served as a check for the sterility of the medium (medium sterility control MSC) and tube 11 served as control for viability of the organism (organism viability control OVC). Tubes were incubated at 37⁰C for 18 hours and checked for the presence or absence of growth and the lowest antibiotic concentration which produced no visible growth as seen with the unaided eye were taken as MICs. The same procedure was repeated for streptomycin

2.2.11. Test for the presence of plasmid resistant determinant

Sub-MIC of acridine orange (625.00ug/ml) as determined using the method above was used in the plasmid curing experiment (Ngwai, 1999). Overnight inoculum of each isolate was prepared in sterile nutrient broth. Seventeen test tubes each containing 1ml of nutrient broth were prepared and autoclave at 121⁰C for 15 minutes. One milliliter of stock solution of the acridine orange in sterile water (1,250ug/ml) was dispensed into each of the test tubes. The contents of the test tube were vortexed for few seconds. Into each of the test tube drops of the overnight inoculum earlier prepared (each isolate for each test tube) was added. They were incubated at 37⁰C for 18 hours

and the resulted colonies were assessed for change in resistant pattern and MIC values as describe under 2.2.8 and 2.2.10.

2.2.12. Test for β - lactamase enzyme production

The experiment was carried out according to the method described by Olayinka (1998) in which suspensions of the isolates were prepared in triplicate by emulsifying bacterial colonies (from an overnight nutrient agar culture) in 0.5ml of phosphate buffer solution containing 0.06mg/ml (10,000 units/ml) Penicillin G. They were incubated at room temperature for at least 1 hour. Thereafter two drops of freshly prepared 1% aqueous starch solution were added to the bacterial suspension with gentle shaking, followed by one drop of Iodine solution and allowed to stand for 10 minutes at room temperature.

β -lactamase producing organisms changed the color of the mixture from blue-black to colorless immediately.

2.2.13 Statistical method

1 The prevalence rate of bacteriuria among the hospitals in Abuja and environ were compared using X^2 test (Singha, 2002). The statistical significance was defined as $p < 0.05$.

2 Two-way classification analysis of variance (F) test (Singha, 2002) was carried out to test the statistical significance of the difference in the sensitivity among the organisms with the view to determining the effect of variation due to isolates and antibiotics on the sensitivity at a significance level of $\alpha_{0.05}$.

3 Statistical two-tail t-test was used to determine the significance in the mean difference between the match- pairs of MICs values of streptomycin, ampicillin and the values of MARIs before and after plasmid curing processes with the view to identifying the effect of acridine orange dye on the MICs and MARIs values. The significance level was defined as α 0.05.

CHAPTER THREE

3.0 RESULT

3.1. Analysis of response to questionnaire on UTI cases

Table 3.1 shows the results of the distribution of questionnaires to the suspected UTI patients that visited some health institutions in Abuja. It showed that of the five hundred (500) copies of questionnaire sent out, only 320 copies were retrieved. A high percentage of the respondent gave inconsistent or rather contradicting responses even some were completely filled incorrectly hence only one hundred and seventy one (171) copies were adjudged satisfactory for the purpose of this work. Of these number 125 (73.09%) of them gave information that was indicative of the occurrence of bacteriuria as shown in the table.

The age, sex and marital distribution of positively adjudged respondents were reported in Table 3.2 below. According to the response to the questionnaires, bacteriuria was more prevalent among young adults of the age group of 21-30years, accounting for 43.30%. Children of less than 10 years had lowest percentage of occurrence of bacteriuria accounting for 2.4% of the respondents. Female respondents reported higher percentage of 56% of significant bacteriuria and a relatively higher percentage (56.80%) of unmarried respondents had bacteriuria.

The distribution of the respondents based on their previous history of self medication in relation to their financial status as shown in Table 3.3 revealed that a large proportion of the bacteriuric respondents have practiced higher self-medication before reporting to the hospitals. The highest proportion

(60.00%) of the incidence is among the middle income earner and is lowest (25.70%) with low-income earner respondents.

Table 3.4 shows the distribution of respondents according to the sex and source of contracting the infections. As shown in the table 61 (48.80%) of the bacteriuria respondents were of the opinion that they contracted the infection through unknown source. To a considerable level 45 (36.00%) of the respondents claimed to have contracted the infection through sexual intercourse while 19 (15.20%) of them believed they contracted the infection through the sharing of underwear.

The report of antibiotic management and gender distribution among the bacteriuric respondents is depicted in Table 3.5. The result indicated that 28.80% of the presumptively positive UTI respondents were at one time or the other treated with the Penicillin group of antibiotic. Sixteen percent of them have been administered with aminoglycoside antibiotic, while 12.00% claimed to have being treated with the quinolone antibiotic.

3.2 Analysis of Retrospective Study of bacteriuria cases in Abuja

The incidence of bacteriuria in some health institutions in Abuja is reported in Table 3.6. The results showed that, of the 7,352 available records in the five centres, 5805 cases were adjudged positive bacteriuria. The highest number of the records of 2215 obtained from National hospital had 2015 bacteriuria positive while Wuse with the lowest number of cases of 1121 had 580 as presumed positive. The proportion of the records that were adjudged bacteriuria positive was of the order National hospital > Gwagwalada specialist Hospital > Asokoro Hospital > Maitama Hospital > Wuse Hospital.

Table 3.1: Distribution of questionnaires to suspected UTI patients in several health institutions in Abuja in the year 2003

Distribution	Hospital / clinic							Total
	Asokoro	Maitama	National Hosp	G/Lada	Wuse	Nass clinic	Durumi	
No. Distributed	71	71	74	71	71	71	71	500
No. Retrieved	30	51	64	30	55	44	46	320
No adjudged positive	21	27	29	20	28	22	24	171
No. (%) with significant Bacteriuria.	12(57)	18(67)	25(86)	15(75)	23(82)	17(77)	15(63)	125 (73)

Key:

Asokoro ; Asokoro General Hospital
Maitama; Maitama General Hospital
National Hosp; National Hospital
G/lada; Gwagwalada Specialist Hospital
Wuse; Wuse General Hospital
Nass Clinic; National Assembly Clinic
Durumi; Divine Clinic and Diagnostic Centre Durumi

Table 3.2: Age, Gender, and Marital Distribution of adjudged positive Respondents

Age		No with significant bacteriuria				
(Years)	No of Respondent	Males		Females		Total
		Married	Single	Married	Single	
0-10	19	-	1	-	2	3
11-20	22	1	2	6	8	17
21-30	68	10	16	13	15	54
>30	62	12	13	12	14	51
TOTAL	171	23	32	31	39	125

Table 3.3: Economic and antibiotic therapy of bacteriuric respondents.

Financial status	No of Respondents	Self- Medication (%)
Low income earner	48	18 (25.70)
Middle income earner	66	42 (60.00)
High income earner	11	10 (14.29)
TOTAL	125	70 (100.00)

Table 3.4: Distribution of respondents according to sex and possible source of contracting the infection

Sources	Males	Females	Total
Sexual Intercourse	28	17	45 (36.00)
Sharing underwear	7	12	19 (15.20)
Unknown causes	20	41	61 (48.80)
Total	55	70	125 (100.00)

Table 3.5: Antibiotic Management and gender distribution among the bacteriuric respondents

Antibiotics	No of respondents		Total
	Male	Female	
Ampicillin	4	10	14
Ampicillin / Cloxacillin	2	11	13
Procaine penicillin	1	8	9
Streptomycin	1	7	8
Erythromycin	4	5	9
Tetracycline	6	7	13
Co-trimoxazole	6	4	10
Nalidixic Acid	2	4	6
Nitrofurantoin	4	2	6
Gentimicin	4	3	7
Ofloxacin	3	1	4
Pefloxacin	2	3	5
Cefuroxime.	4	2	6
Togomycin	5	-	5
Ceftiazime	2	3	5
Ceftriaxone.	5	-	5
Total	55	70	125

Table 3.6: Prevalence of significant bacteriuria in Abuja and Environ.

S/No	Hospitals	No of records investigated	No with bacteriuria (%)
1	National Hospital	2215	2,015 (90.9)
2	G/Lada Specialist	1610	1,021 (63.4)
3	Asokoro Hospital	1231	1,114 (90.5)
4	Maitama Hospital	1175	1,075 (91.5)
5	Wuse Hospital	1121	580 (51.7)
Total		7352	5805 (79.00)

Table 3.7 shows the age and sex distribution of UTI cases in National, Gwagwalada Specialist, Maitama District, Asokoro District and Wuse District hospitals in Abuja and environs in the year 2003. From the records of the retrospective study of 5805 bacteriuria cases, 2484 (42.79%) were in the age group of 21-30 years, constituting the highest category of bacteriuria cases. Generally the proportion of bacteriuria cases increased with age from infancy to adulthood, peaking at a young adulthood and thereafter slightly decreased. At a lower age bracket (11-20 years), the prevalence of bacteriuria cases among the sexes was very similar. At higher age brackets, a considerably large proportion of bacteriuria cases was among the females constituting 56.76% and 97.60% in 21-30 years and over 30 years age groups respectively.

3.3 Analysis of Specimen from Presumptively adjudged positive bacteriuria cases

A total of 305 urine samples were collected from suspected UTI patients in five collection centres namely Asokoro and Maitama District Hospitals, National Hospital, Gwagwalada Specialist Hospital and Divine Clinic and Diagnostic Centre, Durumi.

The age, sex and hospital distribution of the specimens as shown in Table 3.8 indicated that, of the 305 urine samples, 298 (99.3%) were from adults. Specimen from female bacteriuria patients accounted for 67.50% which is more than twice those from male bacteriuria patients.

Table 3.7: Ages and gender distribution of bacteriuria cases in Abuja in 2003.

Age (years)	Sex		Total (%)
	Male	Female (%)	
0-10	305	281 (47.95)	586 (10.09)
11-20	371	366 (49.66)	737 (12.69)
21-30	1074	1410 (56.76)	2484 (42.79)
> 30	948	1050 (97.60)	2001 (34.47)
Total	2698	3107 (53.57)	5805 (100.00)

Table 3.8: The age, sex and hospital distribution of urine specimens

Hospitals	Bacteriuria cases				
	Age(yrs)		Sex		
	Adult	Children	Male	Female	Total
Asokoro Hospital	67	3	21	49	70
Maitama Hospital	65	-	29	36	65
National Hospital Gwagwalada Specialist Hospital	94	2	29	67	96
Durumi	11	-	2	9	11
Total (%)	298	7	99	206	305

The proportion of urine samples with significant bacteriuria as shown in Table 3.9 revealed that, Asokoro hospital had the highest proportion of samples with significant bacteriuria followed by Specialist Hospital Gwagwalada and National Hospital. Maitama District hospital and Durumi recorded relatively low proportions. A total of 100 bacterial isolates were obtained from 96 samples of significant bacteriuria as shown in the Table.

The distribution of uropathogenic isolates from bacteriuria cases in health institutions in Abuja is reported in Table 3.10. The table showed that of 100 uropathogenic organisms of different types isolated from the 96 significant bacteriuric samples, *Enterobacteriaceae* constituted 79% with *E. coli* and *Proteus* spp constituting the highest number of the species. Large proportion of isolates from National hospital was *E. coli*, while *Proteus* spp constituted the largest percentage of isolates in the urine samples from Asokoro. In general six species of uropathogenic organisms were isolated from the samples from all the hospitals except samples from Divine Clinic and Diagnostic Centre Durumi in which *E. coli* was the only organism isolated.

The distribution of the isolates according to the gender as reported in Table 3.11 showed that, 84 of the 100 isolates were obtained from urine samples of female bacteriuria patients. This represents 40.77% of the 206 female urine samples examined. On the other hand the 99 urine samples from male UTI patients yielded 16 isolates representing 16.20% of the samples from male patients. Only two of the 100 isolates were obtained from the 7 urine samples from children. Ninety one percent of *Staph aureus* isolates, 78.57%

of *Klebsiella* spp and 78.12% of *Proteus* spp were recovered from female bacteriuria cases.

Table 3.9: Prevalence of significant bacteriuria in Abuja and environ.

Districts/ Hospitals	No of urine specimen	No with significant bacteriuria (%)	No of Isolates
Asokoro	70	31 (44.30)	33
Maitama	65	11 (17.00)	11
National hospital	96	30 (31.12)	30
Gwagwalada	63	22 (35.00)	24
Durumi	11	2 (18.20)	2
Total	305	96 (31.50)	100

Table 3.10: Distribution of uropathogenic isolates from bacteriuric specimen in Abuja and Environ.

Collection							
Centre	Isolates						
	<i>E .coli</i>	<i>Proteus</i>	<i>Klebsiella</i>	<i>Pseudomonas</i>	<i>Staphylococcus</i>	<i>Streptococcus</i>	<i>Total</i>
	Spp.	Spp.	spp.	<i>Aeruginosa</i>	<i>aureus</i>	spp	
Asokoro	9	12	2	1	7	2	33
Maitama	5	3	1	-	1	1	11
National							
Hosp	13	7	5	1	2	2	30
G/Lada	4	10	6	2	1	1	24.
Durumi	2	-	-	-	-	-	2
Total	33	32	14	4	11	6	100

Table 3.11: Distribution of isolate according to the gender

Isolates	Gender	
	Male (n = 99)	Female (n=206)
<i>Escherichia.coli</i>	5	28
<i>Proteus spp.</i>	7	25
<i>Klebsiella spp.</i>	3	11
<i>Pseudomonas aeruginosa</i>	0	4
<i>Staphylococcus aureus</i>	1	10
<i>Streptococcus spp</i>	0	6
Total	16 (16.20%)	84 (40.77%)

The result of the tests for antibiotic sensitivity profile of the uropathogenic isolates is reported in Table 3.12. It showed that a total of 70% - 85% of the isolates were sensitive to Gentimicin, Ciprofloxacin, Ceftriaxone, Pefloxacin and Lincomycin. However less percentage (37-59%), were susceptible to Erythromycin, Norfloxacin, Streptomycin, Nalidixic acid, Ampicillin/cloxacillin, Cephalexin, Flucloxacillin, Ampicillin and Tetracycline.

3.4: Multiple antibiotic resistance of the isolates

Table 3.13 shows the multiple antibiotic resistance indices of the isolates. The result revealed that 100.00% of *Pseudomonas aeruginosa*, 33.33% of *Streptococcus* spp, 54.54% of *Staphylococcus.aureus*, 50.00% of *Klebsiella* spp, 59.37% of *proteus* spp and 36.36% of *Escherichia. coli* exhibited MAR indices greater than 0.2. Generally, the result depicted that 50% of the isolates had MAR index greater than 0.2 (resistant to at least four antibiotics), indicating a high prevalence of multi-antibiotic resistance among bacteriuria pathogens in Abuja.

Table 3.12: Antibiotic sensitivity profile of uropathogenic isolates

Antibiotics	Percentages of isolate sensitive to antibiotic (%)					
	<i>E.coli</i> (n=33)	<i>Proteus</i> (n=32)	<i>Klebsiella</i> (n=14)	<i>Staph aureus</i> (n=11)	<i>Streptococcus</i> (n=6)	<i>Ps.aeruginosa</i> (n=4)
Ampicillin	57.60	40.63	42.86	27.27	50.00	50.00
Ampicillin + Cloxacillin	63.64	50.00	71.43	45.45	83.33	25.00
Amoxicillin + Clav Acid	69.70	59.38	57.14	63.64	100.00	25.00
Flucloxacillin	63.64	46.88	64.29	36.36	83.33	50.00
Erythromycin	66.67	50.00	64.29	72.2	100.00	50.00
Gentimicin	78.79	78.13	92.86	90.91	100.00	75.00
Streptomycin	60.61	46.88	64.29	63.64	66.67	25.00
Ciprofloxacin	72.73	81.25	92.57	90.91	100.00	75.00
Pefloxacin	75.76	78.13	78.57	63.64	100.00	75.00
Ofloxacin	75.76	71.88	71.43	90.91	100.00	50.00
Norfloxacin	60.61	59.38	50.00	72.72	83.33	50.00
Nalidixic Acid	66.67	43.75	50.00	72.72	83.33	75.00
Co-trimoxazole	66.67	59.38	78.57	63.64	83.33	50.00
Ceftriaxone	72.73	78.13	92.86	90.91	100.00	75.00
Cephalexin	66.67	40.63	64.29	54.55	100.00	25.00
Tetracycline	48.48	43.75	28.57	54.55	66.67	25.00
Nitrofurantoin	69.70	50.00	64.29	81.81	100.00	50.00
Rifampicin	63.64	50.00	64.29	72.72	83.33	75.00
Lincomycin	63.64	59.38	64.29	72.72	100.00	75.00
Chloramphenicol	72.72	62.50	78.57	72.72	100.00	75.00

Table 3.13: Multiple antibiotic resistant Index (MARI) of the isolates.

MARI	Organisms						Total
	<i>E.coli</i>	<i>Proteus</i> Spp	<i>Klebsiella</i> spp	<i>Staph</i> aureus	<i>Streptococcus</i> spp	<i>Ps aeruginosa</i>	
0.0	8	5	2	1	0	0	16
0.1	11	7	2	3	2	0	25
0.2	2	1	3	1	2	0	9
0.3	0	1	1	1	1	3	7
0.4	0	2	1	2	0	0	5
0.5	0	0	2	0	0	0	2
0.6	2	4	0	1	0	0	7
0.7	1	2	1	0	0	0	4
0.8	1	4	0	0	0	0	5
0.9	3	3	2	0	0	0	8
1.0	5	3	0	2	1	1	12
TOTAL	33	32	14	11	6	4	100

3.5. **Minimum inhibitory concentration of the antibiotic resistant isolates**

Table 3.14 depicts the results of the MIC test on 17 of the isolates (most resistant isolates) before and after plasmid curing. It showed that majority of the isolates had high MIC values for the two antibiotics (ampicillin and streptomycin) used in the study. With ampicillin three of the 17 isolates had MIC of 78.125 µg /ml, two had MIC of 156.25 µg/ml, six had MIC of 312.5µg/ml and six gave MIC greater than 625.00µg/ml. The values of MIC of streptomycin against the isolates were considerably higher with only one having an MIC of 156.25 µg/ml and two with MIC of 312.50 µg/ml. The remaining isolates gave MIC of 625.00µg/ml and above. The MIC significantly changed (reduced) when the isolates were subjected to plasmid curing treatment with acridine dye. Twelve (71%) and fifteen (88%) of the isolates had reduced MIC values against ampicillin and streptomycin respectively. With respect to ampicillin five of the isolates had their MIC reduced by one geometric dilution, one by 2 geometric dilution, four by 3 geometric dilutions and two by 4 geometric dilutions. In the case of streptomycin, seven isolates had their MIC reduced by one geometric dilution, five by 2 geometric dilutions, two by 3 geometric dilutions and only one by 4 geometric dilution.

Table 3.15 shows the MAR indices of isolates before and after plasmid curing. The results following the performance of antibiotics sensitivity testing of the cured isolates revealed that all the 17 resistant isolates had increased sensitivity to the drugs with substantial reduction in their MAR indices. On the average, a MAR index of 0.95 was reduced to 0.3 and sensitivity of the isolates was increased from 2 to 14 antibiotics.

Table 3.14: Minimum inhibitory Concentration (MIC) of resistant isolates before and after plasmid curing.

Codes	Isolates	MIC ($\mu\text{g/ml}$)			
		Streptomycin		Ampicillin	
		Before Curing	After Curing	Before Curing	After Curing
AK 10	<i>Klebsiella</i> spp	312.50	78.125	156.25	78.125
AK 53	<i>Proteus</i> spp	625.00	156.25	625.00	625.00
NH 91	<i>Klebsiella</i> spp	156.25	78.125	78.125	19.53
NH 94	<i>E coli</i>	1250.00	625.00	625.00	312.50
NH 98	<i>Proteus</i> spp	625.00	312.50	312.50	312.50
AK 115	<i>Staph aureus</i>	1250.00	312.50	312.50	312.50
NH 150	<i>E coli</i>	1250.00	78.125	78.125	39.06
GH 161	<i>Proteus</i> spp	625.00	312.50	312.50	156.25
GH 166	<i>Proteus</i> spp	1250.00	625.00	625.00	78.125
GH 168	<i>E coli</i>	1250.00	312.50	312.50	156.25
GH 169	<i>E coli</i>	2500.00	1,250.00	1250.00	78.125
GH 170	<i>Proteus</i> spp	1250.50	1250.00	1250.00	78.125
AK 194	<i>E coli</i>	1250.00	312.50	312.50	39.06
AK 233	<i>Proteus</i> spp	312..50	312.50	312.50	39.06
NH 297	<i>Staph aureus</i>	1250.00	625.00	625.00	78.125
NH 298	<i>Ps aeruginosa</i>	625.00	78.125	78.125	78.125
NH 302	<i>Streptococcus</i> spp	1250.00	156.25	156.25	156.25

Table 3.15: Multiple antibiotic resistance indices (MARI) of isolates before and after plasmid curing

Codes	Isolates	MARI	
		Before curing	After curing
AK 10	<i>Klebsiella</i> spp	0.90	0.30
AK 53	<i>Proteus</i> spp	0.85	0.20
NH 91	<i>Klebsiella</i> Spp	0.85	0.25
NH 94	<i>E. coli</i>	1.00	0.20
NH 98	<i>Proteus</i> spp	0.85	0.30
AK 115	<i>Staph aureus</i>	1.00	0.25
NH 150	<i>E. coli</i>	0.95	0.25
GH 161	<i>Proteus</i> spp	0.85	0.10
GH 166	<i>Proteus</i> spp	1.00	0.30
GH 168	<i>E. coli</i>	0.95	0.55
GH 169	<i>E. coli</i>	0.95	0.15
GH 170	<i>Proteus</i> spp	1.00	0.25
AK 194	<i>E. coli</i>	1.00	0.20
AK 233	<i>Proteus</i> spp	1.00	0.50
NH 297	<i>Staph. aureus</i>	0.95	0.40
NH 298	<i>Ps. aeruginosa</i>	0.95	0.30
NH 302	<i>Streptococcus</i> spp	1.00	0.30

Table 3.16: β -lactamase enzyme production by resistant isolates.

Isolates	No of Isolates	No (%) positive
<i>Escherichia coli</i>	5	3 (60.00)
<i>Proteus spp</i>	6	4 (67.00)
<i>Klebsiella spp</i>	2	1 (50.00)
<i>Ps.aeruginosa</i>	1	1 (100.00)
<i>Staphylococcus aureus</i>	2	2 (100.00)
<i>Streptococcus spp</i>	1	-
Total	17	11(65)

Table 3.16 shows that eleven of the multiple antibiotic resistance isolates tested were positive to β - lactamase enzyme production. Two (2) species each of *E.coli* and *Proteus* spp isolates, one of *Klebsiella* spp and the isolate of *Streptococcus* species selected for this test were negative.

3.6 Statistical analysis

Chi-square statistical test was used to determine the relationship between the prevalence of bacteriuria and the various health care institutions in Abuja where urine samples were obtained. Using the Null Hypothesis that there is relationship between prevalence of bacteriuria and sampling centre, the observed and expected frequencies were computed as shown in Appendix F1 and Table F1. With observed χ^2 of 12.95, as against the critical limit of χ^2 of 9.488 (for $p < 0.05$), the null hypothesis was rejected. It implied that difference in the prevalence of bacteriuria among the hospitals was significant.

The 2 – way analysis of variance (F-test) which was used to test the differences in the sensitivity of the isolates to the antibiotics, revealed that there was significant differences in the susceptibilities among the various isolates to the antibiotics (Appendix F2 and table F2, F3). This is because the critical value of $p < 0.05$ at Degrees of freedom of 5 and 95 is 2.29 and that of 19 and 95 degrees of freedom is 1.66, while the corresponding observed values of F are 27.65 and 8.23 which are much larger than the critical values.

As also shown in Appendix F3, Tables F4, F5 and F6 paired t-test analysis revealed that there was significant differences in the MICs of Streptomycin and Ampicillin and MAR indices against isolates subjected to plasmid curing, when compared with the values before plasmid curing. The Calculated t- values were 4.92, 3.21 and 22.12 for MICs of Streptomycin, Ampicillin and MAR indices as against the critical value of 2.12.

CHAPTER FOUR

4.0 DISCUSSION AND CONCLUSION

4.1. Discussion

Although urinary tract infection is not usually thought of as a disease associated with community-wide outbreak, certain multi drug resistant uropathogenic lineage of *E coli* has exhibited epidemic behavior. For instance *E coli* 015: k52: H1 caused an out-break of community acquired cystitis, pyelonephritis and septicemia in south London in 1987 and 1988 and it is an endemic cause of urinary tract infection in Barcelona, Spain (Ameo *et al*, 2001). It was equally reported that acute pyelonephritis in about thirty percent (30%) of women is due to untreated bacteriuria (UTI) and the incidence is irrespective of race and socio-economic difference in the individuals (William *et al*, 1972).

It is on the strength of the above, that pre-study survey was carried out to determine the prevalence of bacteriuria in Abuja and the surrounding towns/settlements. As the result of the survey showed, a relatively high proportion of respondents exhibited significant bacteriuria indicating a high rate of prevalence of bacteriuria in the study area. This might be due to the status of Abuja as a new and growing capital city of Nigeria that is witnessing great influx of people of different socio-economic background. It should however be realized, that this high proportion only reflects the proportion of

respondents that was suspected of bacteriuria and sent to the laboratory for confirmation. This figure did not take into consideration specific time frame and individual with asymptomatic bacteriuria. The underlying objective of the questionnaire was to determine the extent of significant bacteriuria among the symptomatic bacteriuria patients that visited the hospitals base on clinical symptoms.

From the result the adult constituted the most vulnerable group of contracting urinary tract infection probably because the group is the most sexually active in the study area. This observation is in agreement with the report of Ehinmidu (2004) that showed that the numbers of bacteria isolated from the urine of unmarried commercial sex hawkers was highest among the study groups in Zaria. Although it was observed that a large number of respondents were involved in drug self- medication of their UTIs, it is not surprising because this category of respondents was middle-income earners who have fairly comfortable financial position which enabled them to purchase antibiotic over the counter without resorting to qualified medical personnel. The preponderance of drugs such as ampicillin, tetracycline ampicillin-cloxacillin combination, procaine penicillin, co-trimoxazole and streptomycin among the most misused antibiotics might be due to their low cost and ready availability over the counter, compared to the costly newer drugs such as ofloxacin, pefloxacin and injectable ones like gentimicin which though cheap, required professional skills for its administration.

The proportion of individuals that exhibited significant bacteriuria (31.4%) in the study area is very much similar to figures obtained by

some earlier workers (Rosalind and Polak, 1972). From the statistical point of view, the difference between the rates of prevalence of bacteriuria among the hospitals was significant therefore the observed high proportion of samples of bacteriuria in Asokoro and Gwagwalada hospitals compared with those of Maitama and National hospitals was not unexpected. This is because a large proportion of patients that patronized Asokoro and Gwagwalada hospitals are low income earners and resident in areas that are associated with poor environmental hygiene and structures compared with Maitama and National hospitals that are patronized largely by inhabitants who are more educated, enlightened and financially more affluent to bear the cost of medical treatment of the hospitals.

The uropathogenic bacterial species in this study are in agreement with reports of Johnson *et al* (1995), Jill *et al* (1998) and Olanipekun and Montefiore (1978). In the study by Johnson *et al* (1995), 140 bacterial species that comprised among others *E.coli* (121), *Klebsiella pneumoniae* (6), *Proteus mirabilis* (5), *Pseudomonas aeruginosa* (2), and *Staphylococcus saprophyticus* (2) were isolated from UTI patients. Jill *et al* (1998) also reported that in children and adults without complicating factor, *Escherichia coli* is by far the most common organism to cause infections of urinary tract with *Klebsiella spp* and *proteus Spp* running a distant second and third respectively. Olanipekun and Montefiore (1978) also reported that approximately 70% of UTI in Nigeria were due to *E. coli*, *Staph. aureus*, *Proteus* and *Klebsiella spp*. The predominance of *E.coli*, *Proteus spp* and *Klebsiella spp* in this study and previous works is perhaps a reflection

of the presence in certain *E.coli* and other bacterial strains of specific virulence factors that interact with the host to overcome host defense in the urinary tract and to stimulate an inflammatory response. This has been confirmed by Johnson *et al* (2002), who reported that most extra intestinal infection due to *E coli* including urinary tract infections and neonatal meningitis are caused by distinctive “virulent clones” of *E.coli*. Such virulent clones exhibit diverse specialized virulence factors (VFs) that enable the organisms to overcome or subvert host defenses, injure or invade host cells, and incite a noxious inflammatory response, thereby producing disease. These virulent clones can be recognized by their characteristic surface antigens (O.K.H) which are globally distributed and collectively accounted for the majority of significant extra intestinal *E.coli* (Johnson *et al*, 2002). These virulent properties that are of documented important in the pathogenesis of extra intestinal *E coli* infection include P-fimbrial adhesins, the toxins: alpha-hemolysin and cytotoxin, necrotizing factor, the aerobactin, iron sequestration system, *guaA* and *guaC*, group II & III polysaccharide capsule and lipopolysaccharide. Of these, the evidence for a central role in uropathogenesis is strongest for P-fimbriae, which mediate attachment to host intestinal, vaginal, and urinary epithelial surfaces (Johnson *et al*, 1997.)

The higher prevalence of uropathogenic bacteria in females suggests that females are at greater risk of urinary tract infections throughout most of their lives time than are males. This finding is in agreement with earlier reports indicating that bacteriuric cases are more prevalent

in females than males (Rosalind and Polak, 1972; Akinkugbe *et al*, 1973).

This gender difference might be attributed to:

- The greater proximity of the female urethral orifice to the colonic bacterial reservoir (Jill *et al*, 1998; <http://familydoctor.org/190.xml>, 2005).
- The shorter female urethra means that bacteria have a very short distance to travel before entering the bladder.
- The vagina serves as an additional reservoir for uropathogenic bacteria in females (Jill *et al*, 1998).
- The non-production of antibacterial prostatic secretion to protect the urinary tract of the females from bacterial colonization (Odutola, 1986).
- The use of spermicide-based contraceptive method is often linked to the high risk of UTI in such females (Jill *et al*, 1998).
- During sexual intercourse, microbes can be pushed more easily into the urethra in women than men. (Jill *et al*, 1998).

The study also showed that adults are at the greater risk of UTIs than children. This may not be unconnected with the assertion that sexual intercourse promotes bacteriuria in both sexes by facilitating the entering of intestinal and virginal bacteria into the urinary tract. This is supported by the result of the pre-study survey and that of Johnson

and Parissa (2002), who established that direct person to person transmission of pathogenic bacteria through sexual contact occurs among heterosexual. This report was based on sequential or simultaneous occurrence of UTI episode due to the same strains in both members of the sexually active couples and colonization with the strains responsible for the women's UTI in male patients. Among homosexual men the occurrence of acute UTIs due to bacterial species that exhibit characteristic of extra intestinal pathogenic species also suggested the possibility of urethral inoculation by pathogens from the partners' faecal flora via anal intercourse. Jill *et al* (1998) also reported that complicating factors such as indwelling catheters, nephrostomy tube, urinary stone, surgical reconstructions of the urinary tracts and impaired bladder emptying, which are very common with adults, not only help micro organisms enter and persist within the urinary tract, but also alter the spectrum of bacteriuria pathogens.

Antibiotic resistance among bacterial strains, pathogenic to man and animal has been recognized world wide as a major problem in the management of health care delivery system (Kunin, 1993).

The observed differences in the sensitivities of the isolates were confirmed by the statistical significant of the difference between susceptibility of the bacterial pathogens and also revealed that these differences were due to variation in organisms and the tested antibiotics. The proportion of isolates sensitive to the respective antibiotic was listed in descending order of ; gentimicin, ciprofloxacin, ceftriaxone, pefloxacin, lincomicin, chloramphenicol,

co-trimoxazole, ofloxacin, amoxicillin + clavulanic acid, nitrofurantoin, rifampicin, erythromycin, streptomycin, norfloxacin, nalidixic acid, ampicillin + cloxacillin combination, cephalixin, flucloxacillin, ampicillin and tetracycline .

The efficacy of gentamicin against the isolates might be due to its limited use and reduced abuse arising from the inconvenience of its administration by injection. Reports have also indicated that newer and expensive drugs like fluoroquinolones and third generation cephalosporins are generally effective with few cases of bacterial resistance (Ball, 1990; Ekweozor and Onyemenem, 1996; Olayinka, 1998), hence the high activity of ceftriaxone, ciprofloxacin, ofloxacin and pefloxacin, are not unexpected. It is also possible that the cost might have prevented the ready availability of the drugs and subsequent abuse that would have led to the “selective pressure” with its attendant development of resistant strains in the community. Bacterial resistance to fluoroquinolones antibiotics are not plasmid mediated (Courvalin, 1986), hence R-factor resistant cannot be easily transferred from one organism to another, thereby reducing the epidemiological problem of bacterial drug resistance. Ceftriaxone is a third generation cephalosporin which is extremely stable to beta-lactamase enzymes hence the level of resistance is reduced. The relatively high efficacy of lincomycin, co-trimoxazole and cephalixin in this study may be due to the fact that despite the relative low cost of the drugs, some (cephalexin and lincomycin) are not readily available, thereby limiting their routine use. Several other factors that might be responsible for those antibiotics that exhibited moderate activities

such as nitrofurantoin, norfloxacin streptomycin, rifampicin, and Ampicillin + cloxacillin are:

Gastrointestinal upset and inconveniences in oral administration associated with nitrofurantoin have limited its use in clinical or Veterinary medicine and multiple mechanisms of action of nitrofuran derivatives (inactivation of ribosomal proteins and other macromolecules with consequent inhibition of protein, DNA, RNA, cell wall synthesis and aerobic energy metabolism) may provide an intrinsic barrier to the development of resistance to the drug (Johnson *et al*, 1999).

The resistance to norfloxacin is not plasmid mediated (Janknegt, 1986) thus reduces the likelihood of transferring resistant factors from one organism to another as such the emergence of norfloxacin-resistant strains would likely be slower and limited process.

Streptomycin and rifampicin are currently restricted to the treatment of life threatening infections (tuberculoses) hence reduces their use in clinical treatment of other infections and emergence of resistance.

Ampicillin/cloxacillin combination produces synergistic effect, delaying the emergence of resistance (Maple *et al*, 1989).

The relative ineffectiveness of ampicillin and tetracycline might be due to wide use and high level of adulteration associated with the drugs.

The percentage of isolates simultaneously resistant to a good number of antibiotics is of great epidemiological significance in the study area. More than 60% of the isolates are resistant to a considerable number of antibiotics. The screening for multiple antibiotic resistant in bacteria has become an epidemiological tool for monitoring and ensuring adequate therapy. Existence of multiple antibiotic resistant strains among the microorganisms is now a world-wide problem (Kingman, 1994). Koh (1986) isolated *Enterobacteriaceae* from patients in Peninsular, Malaysia which were resistant to a good number of antibiotics. In Nigeria, multiple antibiotic resistant bacteria have been isolated by several researchers (Rotimi *et al*, 1984; Adeyemi-Doro and Rotowa, 1986; Lamikanra and Ndep, 1989; Montefiore *et al*, 1989; Ntiejumokwu, 1998; Ngwai, 1999; Agina *et al*, 2000; Ehinmidu, 2004).

Occurrence of MAR bacteria in developing countries has often been attributed to uncontrolled antibiotic usage caused by the over the counter availability of antibiotic as well as exposure of people to enteric flora in places where sanitation is poor (Ngwai, 1999). These are facts that apply to the area under the study. The result of multiple antibiotic resistant screening indicated that most of the MAR isolates has multi-antibiotic resistant index values above 0.2 suggesting that most of the isolates originated from an area where several antibiotics were often used and abused (Krumperman, 1983) or such bacteria acquired resistance factor from antibiotics trained isolates (Ehinmidu, 2004). This has a serious clinical implication because it provides selective pressures on the isolates, selecting more MAR isolates

within the population. The high MIC values obtained in this study for most of the isolates, with 82.35% of them having MIC values greater than 150µg/ml for ampicillin and above 500µg/ml for streptomycin indicated a high level of resistance which makes the continuous use of these drugs in the treatment of urinary tract infections unsafe and economic waste in the study area.

The result of plasmid curing experiment used in demonstrating the role of genetic elements and its carriage by bacterial culture (Crosa *et al*, 1994) indicates that six (35.29%) and three (17.65%) of the plasmid cured isolates exhibited reduction in MIC values by above three geometric dilution with respect to ampicillin and streptomycin respectively. The lower proportion in the reduction of MIC values exhibited among the resistance isolates was an indication that the genes responsible for the resistant in these organisms were not largely resided within the extrachromosomal element (plasmid) which suggested the partial involvement of chromosome in the resistance. This observation is in conformity with the works of other researchers in Nigeria that reported the involvement of plasmid and chromosomal genes in bacterial resistance (Obaseki – Ebor *et al*, 1986; Lamikanra and Ndep, 1989). The reductions in the MAR indices of isolate for instance GH166 and AK233 also indicated that resistance to the antibiotics was both plasmid and chromosome mediated. The fairly improved sensitivity of the plasmid cured isolates as reflected in the reduction in the values of minimum inhibitory concentration (MIC) and multi-antibiotic resistant indices (MARI) was due to the plasmid curing processes using acridine orange dye. This was confirmed by

the statistical significant of the mean difference between the match pairs of the values of minimum inhibitory concentration (MIC) and multi-antibiotic resistant indices (MARI) of the plasmid cured and uncured isolates.

The detection of β -lactamase enzymes in 11 (65.00%) of the 17 screened multiple antibiotic resistant isolates points to the possible role of the enzymes in mediating resistance to β -lactam antibiotics used in this study. This is because production of the enzymes coded for by genes is a major mechanism of resistant to β -lactam antibiotics (Zhou *et al*, 1994).

Those other isolates that did not show the evidence of β -lactamase production might have developed resistance to β -lactam antibiotics through mechanism other than enzymatic detoxification like alteration of penicillin binding protein, impermeability/efflux of drugs and moderation of target sites (Nikaido, 1994).

4.2 **Conclusion and recommendations**

Results of the pre-study survey and analysis of urine samples obtained from presumptively diagnosed patients showed that, there is relatively high prevalence of bacteriuria in Abuja and environ. Although the occurrence cut across age, gender and socio-economic status it is more prevalent among women, particularly those in the active child bearing age. *Escherichia coli* are the most incriminating bacteria closely followed by *Proteus* spp in bacteriuric cases in Abuja and its environ.

The isolated uropathogens were generally resistance to ampicillin and tetracycline within the study environment whereas antibiotics like gentamicin, ceftriaxone, ciprofloxacin, ofloxacin, pefloxacin, nalidixic acid and chloramphenicol exerted profound inhibitory activities against the bacterial isolates. Multiple antibiotic indexing of the isolates showed that a very large proportion of the isolates were multiple- antibiotic resistant, indicating the need for reappraisal in the management of bacteriuria and accessibility of the public to antibiotics in this environment. The resistance was to some extent mediated and spread by plasmid.

The high incidence of urinary tract infection coupled with the wide spread antibiotic resistance among the various isolates is of a great concern as it poses epidemiological problem to the health care delivery system and the populace. To ensure a successful control and management of urinary tract infections so as to minimize the associated epidemiological and health problems, some degree of prevention and corrective measures need to be undertaken. Such measures are:-

- Educating the populace on maintaining personal and environmental hygienic conditions especially proper washing of colonic sites and hand after urination or bowel movement. The wiping must be from front to back to avoid the spread of bacteria from the rectal area to the urethra.
- Enlightening the public on the avoidance of unnecessary retention of urine to aid the frequent flushing of the urinary

tract to prevent the attachment and colonization of the epithelial lining (<http://familydoctor.org/190.xml>, 2005).

- Advising the relevant authority on the establishment of adequately equipped clinics with qualified personnel in suburb areas of Abuja like Nyanya, Karu, Mpape, and Gwagwalada and charge with adequate surveillance of common infection within the populace.
- Educating the public on the impact of unprotected sex and sharing of underwear on the prevalence of bacteriuria.
- Encouraging the restriction of antibiotics to the populace through the implementation of the relevant laws.
- Supporting urinary tract infection research with human and financial resources which can be through the creation of opportunities for training, conferences, improved research management and effective research output.
- Encouraging the strict compliance with the World health organization (WHO, 1981) recommendations so as to address the problem of the high incidence of antibiotic resistance.

The recommendations are: -

- Restriction of sale of antibiotics and making them available only on the authorization of a medically qualified person.

Ensuring that antibiotics are prescribed correctly and establishing guidelines and regulations for their appropriate use, including the selective use of certain antibiotics.

- Furnishing the prescriber of antibiotic up to date information on antibiotic profile.
- Provision of laboratory facilities locally or regionally for the rapid diagnosis of common infectious diseases and the reliable antibiotics sensitivity testing of pathogens which are frequently antibiotic resistant.
- Using more than one drug in the treatments of certain diseases but ensuring that antibiotics selected for combined therapy do not give cross resistant.
- Improving control procedure in hospitals to prevent the spread of resistant strains.
- Recording and reporting microbial resistance so that health authorities can monitor the emergence of resistance bacteria and plan the effective use of antibiotics.

The hope of man in the control and treatment of urinary tract infection lies in the control and judicious use of antibiotics by all that use them in medical or other fields. In this way, the tide of resistance would be halted and even to some extent reversed.

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Appendix F
Statistical calculation

1. Chi-test analysis of Prevalence of Bacteriuria

Chi-square (k^2) statistical test (Singha, 2002) was used to determine the relationship between the prevalence rates of bacteriuria in the various hospitals in Abuja and environ.

Null hypothesis H_0 : prevalence rates of bacteriuria are related in various healthcare institutions in Abuja.

H_1 :- No relationship between the prevalence rates of bacteriuria in the healthcare institutions in Abuja.

Table F1: observed and expected frequencies

Factor B (prevalence)	Factor A (hospitals)					Total
No. of bacteriuric samples	39 (47.97)	54 (44.54)	66 (65.78)	41 (43.17)	9 (7.54)	209
No. of Non-bacteriuric samples	31 (22.03)	11 (20.46)	30 (30.21)	22 (19.83)	2 (3.46)	96
Total	70	65	96	63	11	305

Key: O= observed frequency
(E)= Expected frequency

The test statistic is:

$$\begin{aligned} X^2 &= \frac{(\text{observed frequency} - \text{Expected frequency})^2}{\text{Expected frequency}} \\ X^2 &= \frac{(39 - 47.97)^2}{47.97} + \frac{(54 - 44.54)^2}{44.54} + \frac{(66 - 65.78)^2}{65.78} \\ &+ \frac{(41 - 43.17)^2}{43.17} + \frac{(9 - 7.54)^2}{7.54} + \frac{(31 - 22.03)^2}{22.03} + \frac{(11 - 20.46)^2}{20.46} \\ &+ \frac{(30 - 30.21)^2}{30.21} + \frac{(22 - 19.83)^2}{19.83} + \frac{(2 - 3.46)^2}{3.46} \qquad = 12.95 \end{aligned}$$

$$\text{Degree of freedom} = (C-1)(r-1) = 4 \times 1 = 4$$

Decision

The critical limit of x^2 with degree of freedom of 4 at significance level of $p < 0.05$ is 9.48, and that of observed $x^2 = 12.95$ hence the x^2 value falls within the region of rejection as such the null hypothesis (H_0) is hereby rejected.

2. F-test analysis of antibiotics susceptibilities of isolates

Table F2: percentage of sensitivity of the organisms

(Factor B) antibiotic	factor A (organisms)						Total
	<i>E.coli</i> (n=33)	<i>Proteus sp</i> (n=32)	<i>Klebsiela</i> (n=14)	<i>Staph</i> <i>Aureus</i> (n=11)	Streptococcus (n=6)	<i>Ps.</i> <i>aeruginosa</i> n=4)	
Ampicillin	57.60	40.63	42.86	27.27	50.00	50.00	268.36
Amp/clox	63.64	50.00	71.43	45.45	83.33	25.00	338.85
Amox/clav	69.70	59.38	57.14	63.64	100.00	25.00	374.86
Flocloxacillin	63.64	46.88	64.29	36.36	83.33	50.00	344.50
Erythromycin	66.67	50.00	64.29	72.72	100.00	50.00	403.68
Gentimicin	78.79	78.13	92.86	90.91	100.00	75.00	515.69
Streptomycin	60.61	46.88	64.29	63.64	66.67	25.00	327.09
Ciprofloxacin	72.73	81.25	92.86	90.91	100.00	75.00	512.75
Pefloxacin	75.76	78.13	78.57	63.64	100.00	75.00	471.10
Ofloxacin	75.76	71.88	71.43	90.91	100.00	50.00	459.98
Norfloxacin	60.61	59.38	50.00	72.72	83.33	50.00	376.04
Nalidixic acid	66.67	43.75	50.00	72.72	83.33	75.00	391.04
Cotrimoxazole	66.67	59.38	78.57	63.64	83.33	50.00	401.59
Ceftriaxone	72.73	78.13	92.86	90.91	100.00	75.00	509.63
Cephalexin	66.67	40.63	64.29	54.55	100.00	25.00	351.14
Tetracycline	48.48	43.75	28.57	54.55	66.67	25.00	267.02
Nitrofurantoin	69.70	50.00	64.29	81.81	100.00	50.00	415.80
Rifampicin	63.64	50.00	64.29	72.72	83.33	75.00	408.98
Lincomycin	63.64	59.38	64.29	72.72	100.00	75.00	435.03
Chloranphenicol	72.72	62.50	78.57	72.72	100.00	75.00	461.51
Total (T)	1336.43	1150.06	1335.75	1354.50	1783.32	1075.00	8035.07

Null hypothesis: H_0 : The means sensitivity are the same for all the isolates and antibiotics.

H_1 : Any two or more means are not equal

$$\text{Total sum of square (TSS)} = \left\{ T^2 - \frac{T^2}{rc} \right\}$$

$$\therefore \text{TSS} = 580730.36 - 538019.58$$

$$\text{TSS} = 42710.78$$

$$\text{Sum of square due to A (ASS)} = \left\{ \frac{T_a^2}{r} - \frac{T^2}{rc} \right\}$$

$$\text{ASS} = 553171.83 - 538019.58$$

$$\text{ASS} = 15152.25$$

$$\text{Sum of square due to B (BSS)} = \left\{ \frac{T_b^2}{C} - \frac{T^2}{rc} \right\}$$

$$\text{BSS} = 555165.89 - 538019.58$$

$$\text{BSS} = 17146.31$$

$$\text{Sum of square due to error} = \text{TSS} - \text{ASS} - \text{BSS}$$

$$= 42710.78 - 15152.25 - 17146.31$$

$$\text{ESS} = 10412.22$$

Degree of freedom; $(c-1) = 5$ for factor A, $(r-1) = 19$ for factor B,

$(c-1)(r-1) = 5 \times 19 = 95$ for error, $(c-1) + (r-1) + (c-1)(r-1) = 119$ for total.

Table F3: Analysis of variance for the antibiotic sensitivity of the organism

Variation	Degree of freedom	Sum of square	Mean square	Observed F
Among factor A (organism)	5	15152.25	3030.45	27.65
Among factor B (Antibiotic)	19	17146.35	902.45	8.23
Error or unexplained	95	10412.18	109.60	
Total	119	42710.78	4042.50	

Decision

The critical values of $p < 0.05$ at Degree of freedom of 5 and 95 is 2.29 and that of 19 and 95 degree of freedom is 1.66, while the corresponding observed values of F are 27.65 and 8.23 which are much larger than the critical value hence the hypothesis H_0 is rejected.

3 Paired student t- test Analysis of MIC and MAR of plasmid cured and uncured isolates

Table F4: Calculation of matched-pairs difference for MICs of streptomycin against plasmid cured and uncured isolates.

SN	Codes	Isolate	MICs(($\mu\text{g/ml}$)			
			before curing (x_1)	after curing (X_2)	difference ($x_2 - x_1$) d	($x_2 - x_1$) ² d ²
1	AK10	<i>Klebsiella spp</i>	312.125	78.125	-234.00	54756.00
2	AK53	<i>Proteus spp</i>	625.00	156.250	-468.75	219726.56
3	NH91	<i>Klebsiella spp</i>	156.250	78.125	-78.125	6103.52
4	NH94	<i>E.coli</i>	1250.00	625.00	-625.00	390625
5	NH98	<i>Proteus spp</i>	625.00	512.50	-312.50	97344
6	AK115	<i>Staph.aureus</i>	1250.00	312.50	-937.00	877969.00
7	NH150	<i>E.coli</i>	1250.00	78.125	-1171.875	1373302.73
8	GH161	<i>Proteus spp</i>	625.00	312.50	-312.50	97344
9	GH166	<i>Proteus spp</i>	1250.00	625.00	-625.00	390625
10	GH168	<i>E.coli</i>	1250.00	312.50	-937.00	877969.00
11	GH169	<i>E.coli</i>	2500.00	1250.00	-1250.00	1562500
12	GH170	<i>Proteus spp</i>	1250.00	1250.00	-0.00	0.00
13	AK194	<i>E.coil</i>	1250.00	312.50	-937.00	877969
14	AK233	<i>Proteus spp</i>	312.50	312.50	0	0.00
15	NH297	<i>Staph.aureus</i>	1250.00	625.00	-625.00	390625
16	NH298	<i>Ps.aeruginosa</i>	625.00	78.125	-533.875	283022.52
17	NH302	<i>Streptococcus spp</i>	1250.00	156.25	-1091.75	1191918.06
Total					$\Sigma d = -9436.63$	$\Sigma d^2 = 8693799.39$

Null Hypothesis; $H_0: X_1 = X_2$

$H_1: X_1 \neq X_2$

Mean (d) = -555.09

$$Sd = \frac{\sqrt{\frac{Ed^2 - nd^2}{n-1}}}{\sqrt{n}} = \frac{\sqrt{\frac{8693799.39 - 5238123.44}{16}}}{\sqrt{17}}$$

Sd = 464.73

$$t = \frac{d}{\frac{sd}{\sqrt{n}}} = \frac{-555.09}{\frac{464.73}{\sqrt{17}}} = \frac{-555.09}{112.7} = -4.92$$

$t_{0.05} = 2.12$

$t_{cal} = -4.92$

Table F5: Calculation of match–pairs difference for MICs of ampicillin against plasmid cured and uncured isolates.

SN	Codes	Isolate	MICs (ng/mc)			
			before curing (X_1)	After curing (X_2)	Differences ($x_1 - x_2$)	($x_2 - x_1$) ²
1	AK10	<i>Klebsiella spp</i>	156.250	78.125	-78.125	6103.52
2	AK53	<i>Proteus spp</i>	625.00	625.00	0	0
3	NH91	<i>Klebsiella spp</i>	78.125	19.53	-58.595	3433.37
4	NH94	<i>E.coli</i>	625.00	312.50	-312.50	97676.25
5	NH98	<i>Proteus spp</i>	312.50	312.50	0	0
6	AK115	<i>Staph.aureus</i>	312.50	312.50	0	0
7	NH150	<i>E.coli</i>	78.125	39.06	-39.06	1525.68
8	GH161	<i>Proteus spp</i>	312.50	156.125	-156.125	24375.01
9	GH166	<i>Proteus spp</i>	625.00	78.125	-546.875	299066.79
10	GH168	<i>E.coli</i>	312.50	156.125	-156.125	24375.01
11	GH169	<i>E.coli</i>	1250.00	78.125	-1171.875	1373279.29
12	GH170	<i>Proteus spp</i>	1250.00	78.125	-1171.875	1373279.29
13	AK194	<i>E.coil</i>	312.50	39.06	-273.440	74769.43
14	AK233	<i>Proteus spp</i>	312.50	39.06	-273.440	74769.43
15	NH297	<i>Staph.aureus</i>	625.00	78.125	-546.875	299066.29
16	NH298	<i>Ps.aeruginosa</i>	78.125	78.125	0	0
17	NH302	<i>Streptococcus spp</i>	78.125	78.125	0	0
TOTAL					$\Sigma d = 4784.91$	$\Sigma d^2 = 3650699.891$

Null Hypothesis; $H_0: X_1 = X_2$

$H_1: X_1 \neq X_2$

$$\begin{aligned} \bar{d} &= \frac{\sum d}{n} = \frac{4784.91}{17} \\ &= 281.46 \end{aligned}$$

$$sd = \sqrt{\frac{\sum d - nd^2}{n-1}}$$

$$Sd = \sqrt{3650699.89 - 1346786.1} = 379.46$$

$$t = \frac{\bar{d}}{\frac{Sd}{\sqrt{n}}} = \frac{281.46}{\frac{379.46}{\sqrt{17}}} = -3.06$$

$$t_{0.05} = 2.12 \quad t_{cal} = -3.06$$

$$\begin{aligned}\Sigma d &= -11.25 \\ d &= -0.66\end{aligned}$$

$$Sd = 0.12$$

$$t = \frac{\underline{d}}{\underline{Sd} \sqrt{n}} \quad t = \frac{\underline{-0.66}}{\underline{0.12} \sqrt{17}}$$

$$t = -22.68$$

$$t_{0.05} = 2.12 \quad t_{cal} = 22.68$$

Decision

The critical value of $p < 0.05$ at degree of freedom of 16 is 2.12, the calculated value of t for MIC of streptomycin; Ampicillin and MARI are -4.92, 3.06 and 22.12 respectively. This shows that the probability of the observed values are much less than the critical value hence H_0 is rejected in all the cases.

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APPENDIX C

Susceptibility profile of the isolates

S/N	Code	Age	Sex	Isolates	PN	COT	NA	CP	CN	PEN	AC	Zones of inhibition (m)				
												OF	S	CEP	CNC	PXN
1	MT3	A	M	<i>E. coli</i>	S	S	S	S	S	S	S	S	S	S	S	S
2	MT4	A	F	<i>Klebsiella spp</i>	R	S	S	S	S	S	S	S	S	S	S	S
3	MT8	A	F	<i>E. coli</i>	S	S	S	S	S	S	S	S	S	S	S	S
4	MT9	A	F	<i>Strept spp</i>	S	S	S	S	S	S	S	S	S	S	S	S
5	AK9	A	F	<i>Staph aureus</i>	R	R	R	I	S	R	R	I	R	R	R	R
6	AK10	A	F	<i>Klebsiella Spp</i>	S	S	S	S	S	I	R	R	R	I	S	S
7	AK11	A	F	<i>Ps. aeruginosa</i>	R	R	S	S	S	S	S	S	R	S	I	S
8	AK17	A	F	<i>Strept spp</i>	R	S	S	S	S	S	I	I	S	S	I	R
9	AK20	A	F	<i>Staph aureus</i>	S	S	S	S	S	S	S	S	S	S	S	S
10	AK21	A	F	<i>Proteus SPP</i>	R	S	R	S	S	S	R	S	S	R	I	R
11	AK22	A	F	<i>Staph aureus</i>	R	R	R	S	S	T	R	I	R	R	R	R
12	AK23	A	F	<i>Proteus SPP</i>	R	R	R	S	I	R	I	R	R	R	R	R
13	AK25	A	F	<i>E. coli</i>	R	R	R	S	S	T	R	I	R	R	S	R
14	AK26	A	F	<i>E. coli</i>	S	S	R	S	S	S	S	S	R	S	I	R
15	MT35	AD	F	<i>E. coli</i>	S	S	S	S	S	S	S	S	S	S	I	S
16	MT36	A	F	<i>E. coli</i>	R	R	R	R	S	S	S	I	R	S	R	R
17	MT43	A	F	<i>Proteus SPP</i>	S	S	S	S	I	S	S	S	S	S	S	S
18	AK53	A	F	<i>Proteus SPP</i>	R	R	R	I	S	S	R	R	R	R	R	R
19	AK58	A	M	<i>Protens</i>	R	S	I	S	S	I	I	S	S	I	S	R
20	AK59	C	F	<i>Staph aureus</i>	R	I	I	S	S	S	I	S	S	I	S	R
21	AK60	A	M	<i>Proteus SPP</i>	I	S	I	S	S	I	S	S	S	I	I	S
22	AK61	A	F	<i>Staph aureus</i>	I	S	I	S	I	I	S	I	S	I	S	S
23	AK62	A	F	<i>Proteus SPP</i>	I	R	S	I	S	S	I	I	S	S	R	S
24	AK63	A	M	<i>E. coli</i>	R	S	I	S	I	I	I	I	R	R	R	R
25	AK65	A	M	<i>Staph aureus</i>	R	S	S	I	S	I	R	S	S	R	S	R
26	AK67	A	F	<i>Proteus SPP</i>	R	S	S	S	S	S		I	S	S	I	R
27	NH75	A	F	<i>E. coli</i>	S	S	S	S	S	S	S	S	S	S	S	S
28	NH83	F	A	<i>E. coli</i>	S	S	I	S	S	S	S	I	S	S	S	S
29	NH84	F	A	<i>E. coli</i>	R	R	I	S	S	S	S	S	I	I	S	S
30	NH84	F	A	<i>Proteus SPP</i>	S	S	S	S	S	S	S	S	S	S	S	S
31	NH87	F	A	<i>Staph aureus</i>	S	S	S	S	S	S	S	S	S	S	S	S
32	NH91	F	A	<i>Klebsiella SPP</i>	R	R	R	R	S	R	R	R	R	R	I	R
33	NH92	A	M	<i>Proteus SPP</i>	R	R	R	S	S	I	R	S	R	R	S	R
34	NH94	F	A	<i>E. coli</i>	R	R	R	R	R	R	R	R	R	R	R	R
35	NH96	F	A	<i>Klebsiella SPP</i>	R	R	R	S	S	I	R	S	S	S	S	S
36	NH97	M	A	<i>Proteus SPP</i>	S	S	S	S	S	S	S	S	S	S	S	S
37	NH98	F	A	<i>Proteus SPP</i>	R	R	I	R	R	R	R	R	R	S	R	R
38	NH103	F	A	<i>E. coli</i>	R	R	R	R	S	R	R	R	R	R	S	R
39	AK115	F	A	<i>Staph aureus</i>	R	R	R	R	R	R	R	R	R	S	R	R
40	AK116	F	A	<i>E. coli</i>	R	S	S	S	S	S	S	S	S	S	S	S
41	AH135	M	A	<i>E. coli</i>	S	S	S	S	S	S	S	S	R	S	S	S
42	MT142	F	A	<i>Staph aureus</i>	R	R	S	S	S	S	S	S	I	S	S	S
43	MT143	F	A	<i>Proteus SPP</i>	S	S	S	S	S	S	S	S	S	S	S	S
44	NH144	F	A	<i>E. coli</i>	S	S	S	S	S	S	S	S	S	S	S	S
45	NH145	M	A	<i>E. coli</i>	S	S	S	S	S	S	S	S	S	S	S	S
46	NH147	F	A	<i>E. coli</i>	S	S	S	S	S	S	S	S	S	S	S	S
47	NH148	M	A	<i>Proteus SPP</i>	S	S	S	S	S	S	S	S	S	S	S	S
48	NH150	F	A	<i>E. coli</i>	R	R	R	R	R	R	R	R	R	R	R	R
49	GH160	F	A	<i>Proteus SPP</i>	R	S	R	R	S	I	R	R	R	R	R	R

50	GH161	F	A	Proteus spp	I	R	R	R	R	R	R	R	R	R	I	I
51	GH162	F	A	Klebsiella spp	S	S	I	I	S	I	R	S	R	S	S	R
52	GH163	F	A	Proteus SPP	S	S	S	S	S	S	I	s	s	s	S	S
53	GH165	M	A	Proteus SPP	S	S	S	S	S	S	I	S	S	S	R	S
54	GH166	F	A	Proteus SPP	R	R	R	R	R	R	R	R	R	R	R	R
55	GH168	M	A	E. coli	R	R	R	R	R	R	R	R	R	R	R	R
56	GH169	F	A	E. coli	R	R	R	R	S	R	R	R	R	R	R	R
57	GH170	F	A	Proteus SPP	R	R	R	R	R	R	R	R	R	R	R	R
58	GH175	F	A	Klebsiella SPP	R	S	R	S	S	S	R	S	R	S	S	S
59	GH176	F	A	Strept spp	R	S	R	S	S	S	I	S	S	S	S	S
60	GH179	F	A	Klebsiella SPP	I	S	S	S	S	S	I	S	S	S	R	R
61	GH181	F	A	E. coli	S	S	S	S	S	S	S	S	S	S	S	S
62	GH183	F	A	Proteus SPP	R	R	I	S	S	S	S	S	S	S	S	S
63	NH185	F	A	Ps. Aeruginosa	I	I	S	S	S	S	S	S	R	R	S	R
64	NH188	F	A	Strept spp	R	S	S	S	S	S	S	S	R	S	S	R
65	NH189	F	A	E. coli	S	S	S	S	S	S	S	S	S	S	S	S
66	AK192	F	A	E. coli	S	S	S	S	S	S	S	S	S	R	I	S
67	AK194	F	A	E. coli	R	R	R	R	R	R	R	R	R	S	R	R
68	AK196	F	A	E. coli	S	S	S	S	S	S	S	S	S	S	S	S
69	GH211	F	A	E. coli	S	S	S	S	S	S	S	S	S	S	S	S
70	NH218	M	A	Klebsiella SPP	S	S	S	S	S	S	S	S	S	S	S	S
71	NH224	F	A	E. coli	S	S	S	S	S	S	S	S	S	S	S	S
72	NH230	F	A	E. coli	R	R	R	R	S	R	R	I	R	R	S	R
73	NH232	F	A	E. coli	R	S	R	R	R	R	R	R	R	R	R	R
74	AK233	F	A	Proteus SPP	R	R	R	R	S	R	R	R	R	R	R	R
75	AK234	F	A	Proteus SPP	R	R	R	R	S	R	R	R	R	R	S	S
76	AK235	F	A	Klebsiella SPP	S	S	S	S	S	S	S	S	S	R	R	S
78	AK237	F	C	Strept spp	S	S	S	S	S	S	S	S	S	S	S	S
79	AK238	M	A	Proteus SPP	R	R	R	S	S	S	S	I	R	R	R	R
80	AK240	M	A	E. coli	R	R	S	I	I	I	I	I	S	I	R	R
81	AK241	M	A	Proteus SPP	R	S	R	S	S	S	R	S	S	R	S	R
82	NH242	F	A	Proteus SPP	R	S	R	S	S	S	S	S	R	R	R	I
83	NH244	F	A	Klebsiella SPP	R	S	R	S	S	S	S	S	R	S	S	S
84	NH245	F	A	Proteus SPP	R	S	R	S	S	S	S	S	R	R	R	S
85	NH248	M	C	Klebsiella SPP	R	S	R	S	S	R	S	R	R	R	R	S
86	GH249	F	A	Proteus SPP	S	S	S	S	S	S	S	S	S	S	S	S
87	GH250	M	A	Klebsiella SPP	R	S	R	S	S	S	R	S	S	R	S	S
88	GH251	F	A	Ps. Aeruginosa	R	I	S	S	S	S	R	S	S	R	S	R
89	GH252	M	A	Klebsiella SPP	I	S	S	S	S	S	S	S	S	S	S	S
90	GH253	F	A	Klebsiella SPP	S	S	S	S	S	S	S	S	S	S	S	S
91	AK254	F	A	E. coli	S	S	S	S	S	S	S	S	S	S	S	S
92	GH265	F	A	Staph aureus	S	S	S	S	S	S	S	S	S	S	S	S
93	GH271	F	A	Proteus SPP	R	R	R	S	S	S	R	S	R	R	R	R
94	GH273	F	A	Proteus SPP	R	R	R	I	S	I	R	S	R	R	R	R
95	GH273	F	A	E. coli	I	S	S	S	S	S	I	S	S	S	S	I
96	DL294	F	A	E. coli	R	S	R	S	S	S	R	S	S	S	S	I
97	NH297	F	A	Staph aureus	R	R	R	R	R	R	R	R	R	R	R	R
98	NH298	F	A	Ps. Aeruginosa	R	R	R	R	R	R	R	R	R	R	R	R
99	NH299	F	A	A Proteus	R	R	R	S	S	I	S	I	R	R	R	R
100	NH302	F	A	A Strept spp	R	R	R	R	R	R	R	R	R	R	R	R

APPENDIX E

SUMMARY OF ANTIBIOTIC RESISTANCE AND MULTIPLE ANTIBIOTIC RESISTANCE INDICES (MARI)

S/N	CODES	ANTIBIOTICS	MARI
1	MT3	--	0.0
2	MT4	PN	0.05
3	MT6	TCN	0.05
4	MT8	--	0.0
5	AK9	PN, COT, NA, PEN, AC, S, CEP, CNC, PXN, FLO, NO, TCN	0.60
6	AK10	PN, COT, NA, CN, PEN, AC, OF S, CEP, CNC, PXN, E, FLO, LC, NO, RF, N, TCN	0.90
7	AK11	AC, OF S, RF, TCN	0.25
8	AK17	PN, COT, S, NO	0.20
9	AK20	PN, PXN, FLO	0.15
10	AK21	--	0.0
11	AK22	PN, NA, AC, CEP, PXN, FLO, TCN	0.35
12	AK24	PN, COT, NA, AC, S, CEP, CNC, PXN, E, FLO, LC	0.55
13	AK25	PN, COT, NA, AC, CP, CN, PEN, OF, S, CEP, CNC, PNX, E, FLO, LC, NO, RF, N	0.90
14	AK28	NA, S	0.10
15	AK35	--	0.0
16	AK37	PN, NA, AC, S, CEP, CNC, PXN, E, FLO, LC, RF, N, TCN	0.65
17	AK43	--	0.0
18	AK53	PN, COT, NA, PEN, AC, OF, S, CEP, CNC, PXN,	

		E, FLO, LC, NO, RF, N, TCN	0.85
19	AK58	PN, FLO	0.10
20	AK59	PN, PXN, E, FLO, NO, TCN	0.30
21	AK60	--	0.0
22	AK61	--	0.0
23	AK62	COT, CNC, E, TCN	0.20
24	AK63	PN, CN, S, CEP, CNC, PXN, E, FLO, LC, NO, RF, TCN	0.60
25	AK65	PN, PXN, CEP, AC, FLO, RF, TCN	0.35
26	AK67	PN, PXN	0.10
27	NH75	--	0.0
28	NH83	--	0.0
29	NH84	PN, COT	0.10
30	NH86	--	0.0
31	NH87	--	0.0
32	NH91	PN, COT, NA, CP, PEN, AC, OF, S, CEP, PXN, FLO, LC, NO, RF, N, TCN, REF	0.85
33	NH92	PN, COT, NA, AC, S, CEP, PXN, FLO, NO, RF, TCN	0.55
34	NH94	PN, COT, NA, CP, CN, PEN, AC, OF, S, CEP, E, CNC, PXN, FLO, LC, NO, RF, N, TCN, CEF.	1.0
35	NH96	PN, COT, NA, AC	0.20
36	NH97	--	0.0
37	NH98	PN, COT, NA, CN, PEN, AC, OF, S, CEP, PXN, E, FLO, LC, NO, N, TCN, CEF	0.85
38	NH103	PN, COT NA, CP, PEN, AC, OF, S, CEP, PXN, FLO, LC, NO, RF, TCN	0.75
39	AK115	PN, COT, NA, CP, PEN, CN, AC, OF, S, CEP, CNC, PXN, FLO, LC, E, NO, RF, N, TCN, CEF	1.0
40	AK116	TCN	0.05
41	NH135	S	0.05
42	MT142	PN, COT	0.10

43	MT143	--	0.0
44	NH144	--	0.0
45	NH145	--	0.0
46	NH147	--	0.0
47	NH148	--	0.0
48	NH150	PN, COT, NA, CP, CN, PEN, AC, OF, S, CEP, CNC, PXN, E, FLO, LC, NO, RF, N, TCN	0.95
49	NH160	PN, NA, CP, AC, OF, S, CEP, CNC, PXN, E, FLO, LC, NO, RF, N, TCN	0.80
50	GH161	COT, NA, CP, CN, PEN, AC, OF, S, CEP, E, FLO, LC, NO, RF, N, TCN, CEF	0.85
51	Gh162	AC, S, PXN, TCN	0.20
52	GH163	RF, TCN	0.10
53	GH165	CNC, TCN	0.10
54	GH166	PN, COT, NA, CP, CN, PEN, AC, OF, S, CEP, CNC, PXN, E, FLO, LC, NO, RF, N, TCN, CEF	1.0
55	GH168	PN, COT, NA, CP, PEN, AC, OF, S, CEP, CNC, PXN, E, FLO, LC, NO, RF, N, TCN, CEF	0.95
56	GH169	PN, COT, NA, CP, CN, PEN, AC, OF, S, CEP, CNC, PXN, E, FLO, LC, NO, RF, TCN, CEF	0.95
57	GH170	PN, COT, NA, CP, CN, PEN, AC, OF, S, CEP, CNC, PXN, E, FLO, LC, NO, RF, N, TCN, CEF	1.0
58	GH175	PN, NA, AC, S, E, FLO, NO, N, TCN	0.45
59	GH176	PN, NA, TCN	0.15
60	GH176	PXN, E, FLO	0.15
61	GH181	--	0.0
62	GH183	PN, COT, E, RF, N	0.25
63	GH185	S, CEP, PXN, E, FLO	0.25
64	NH188	PN, S, PXN, FLO, TCN	0.25
65	NH189	--	0.0

66	AK192	FLO, NO	0.10
67	AK190	PN, COT, NA, CP, CN, PEN, AC, OF, S, CEP, CNC, PXN, E, FLO, LC, NO, RF, N, TCN, CEF	1.0
68	AK196	NO, N, TCN	0.15
69	GH211	--	0.0
70	NH218	--	0.0
71	NH224	--	0.0
72	NH230	PN, COT, NA, CP, PEN, OF, S, CEP, PXN, E, FLO, LC, NO, RF, N, TCN, CEF	0.85
73	NH232	PN, NA, CP, CN PEN, AC, OF, S, CEP, CNC, PXN, E, FLO, LC, NO, RF, N, TCN	0.90
74	AK233	PN, COT, NA, CP, CN, PEN, OF, AC, S, CEP, CNC, PXN, E, FLO, LC, NO, RF, N, TCN, CEF	1.0
75	AK234	PN, COT, NA, CP, CN, PEN, AC, OF, S, CEP, E, LC, NO, N, TCN, CEF	0.80
76	AK235	CEP, CNC, E LC, NO, RF, TCN	0.35
77	AK236	PN, NA, AC CEP, CNC, PXN, FLO, NO, RF, N, TCN	0.55
78	AK237	RF	0.05
79	AK238	PN, COT, NA, AC, S, CEP, PXN, E, FLO, LC NO, RF, N, TCN	0.70
80	AK 240	PN, COT, CNC, PXN, E, FLO, NO, RF, N, TCN, CEF	0.55
81	AK241	PN, NA, CN, CEP, PXN, E, FLO, NO, RF, N, TCN, CEF	0.60
82	MT242	PN NA, S, CEP, CNC, RF, N, TCN	0.40
83	NH244	PN, NA, OF, LC, NO, TCN	0.30
84	NH245	PN, NA, S, CEP, CNC, RF, N, TCN	0.40
85	MH248	PN, NA, PEN, OF, S, CEP, CNC, FLO, LC, NO, RF, N, TCN	0.65
86	GH249	--	0.0

87	GH250	PN, NA, AC, CEP, E, NO, RF, N, TCN	0.45
88	GH251	AC, CEP, PXN, N, TCN	0.25
89	GH252	TCN	0.05
90	GH253	TCN	0.05
91	AK254	TCN	0.05
92	GH265	RF	0.05
93	GH271	PN, COT, NA, AC, S, CEP, CNC, PXN, E, FLO, LC, NO, RF, N, TCN	0.75
94	GH27	PN, COT, NA, AC, OF, S, CEP, CNC, PXN, E, FLO, LC, NO, RF, N, TCN	0.80
95	DL285	TCN	0.05
96	DL294	PN, NA, AC, TCN	0.20
97	NH297	PN, COT, NA, CP, CN, PEN, AC, OF, S, CEP, CNC, PXN, E, FLO, LC, NF, RF, N, TCN	0.95
98	NH298	PN, COT, NA, CP, CN, PEN, AC, OF, S, CEP, CNC, PXN, E, FLO, LC, NO, RF, N, TCN	0.95
99	HN299	PN, NA, S, CEP, CNC, PXN, E, FLO, LC, NO, RF, N, TCN	0.65
100	NH300	PN, COT, NA, CP, CN, PEN, AC, OF, S, CEP, CNC, PXN, E, FLO, LC, NO, RF, N, TCN, CEF	1.0