

Resistant plasmid profile analysis of multidrug resistant *Escherichia coli* isolated from urinary tract infections in Abeokuta, Nigeria

O Akingbade¹, S Balogun¹, D Ojo¹, P Akinduti², P O Okerentugba³, J C Nwanze⁴, I O Okonko³

1. Department of Microbiology, Federal University of Agriculture, Abeokuta, Nigeria
2. Department of Medical Microbiology & Parasitology, Olabisi Onabanjo University, Ago-Iwoye, Nigeria.
3. Medical Microbiology Unit, Department of Microbiology, University of Port Harcourt, East- West Road, P.M.B. 5323, Choba, Port Harcourt, Rivers State, Nigeria
4. Department of Pharmacology and Therapeutics, Igbinedion University, Okada, Edo State, Nigeria

Abstract

Background: Multi-drug resistant *Escherichia coli* has become a major threat and cause of many urinary tract infections (UTIs) in Abeokuta, Nigeria.

Objectives: This study was carried out to determine the resistant plasmids of multidrug resistant *Escherichia coli* isolated from (Urinary tract infections)UTIs in Abeokuta.

Methods: A total of 120 *Escherichia coli* isolates were obtained from urine samples collected from patients attending inpatient and outpatient clinics presenting UTI; with their biodata. Antibiotics susceptibility was performed and multi-drug resistant isolates were selected for plasmid profiling. Plasmids were extracted by the alkaline lysis method, electrophoresed on 0.8% agarose gel and profiled using a gel-photo documentation system gel.

Results: *Escherichia coli* isolates obtained shows high resistance to cloxacillin (92.5%), amoxicillin (90.8%), ampicillin (90.8%), erythromycin (75.8%), cotrimoxazole (70.0%), streptomycin (70.0%) and tetracycline (68.3%) while 85.8% and 84.2% were susceptible to gentamycin and ceftazidime respectively. Sixteen *Escherichia coli* strains were observed to be resistant to more than two classes of antibiotics. The resistant plasmid DNA was detectable in 6(37.5%) of the 16 multi-drug resistant *Escherichia coli* having single sized plasmids of the same weight 854bp and were all resistant to erythromycin, cefuroxime, cloxacillin, amoxicillin, ampicillin and cotrimoxazole.

Conclusion: This study has highlighted the emergence of multidrug resistant R-plasmids among *Escherichia coli* causing urinary tract infections in Abeokuta, Nigeria. There is a high level of resistance to many antimicrobials that are frequently used in Abeokuta, Nigeria.

Keywords: *Escherichia coli*, UTI, R-plasmid, multidrug resist

DOI: <http://dx.doi.org/10.4314/ahs.v14i4.8>

Introduction

Urinary tract infection is one of the significant illnesses that cause burden on national exchequer. Due to widespread and injudicious use of antibiotics at community level we are encountered more and more resistance pattern of micro-organisms to common antibiotics¹. Urinary tract infections (UTIs) are among the most common infectious diseases of humans and a major cause of morbidity and mortality²⁻³. It is estimated

that 40–50% of healthy adult women have experienced at least one UTI episode²⁻⁴.

UTI has become the most common hospital-acquired infection, accounting for as many as 35.0% of nosocomial infections, and it is the second most common cause of bacteraemia in hospitalized patients⁵. Previous reports have also suggested that UTI can occur in both males and females of any age, with bacterial counts as low as 100 colony forming units (CFU) per millimeter in urine⁵⁻¹⁰.

Occurrence of urinary pathogens varies among different age groups, sex, catheterization, hospitalization and previous exposure to antimicrobials¹¹. Signs and symptoms of burning sensation during urination, frequent or intense urges to urinate, back or lower abdominal pain, fever or chills¹², frequently characterize severe UTI. The leading causes of acute and uncompli-

Corresponding author:

Iheanyi Omezuruike Okonko
Medical Microbiology Unit,
Department of Microbiology, University of
Port Harcourt, PMB 5323 Choba, East-West Road, Port
Harcourt, Rivers State, 500102 Nigeria;
E-mail: mac2finney@yahoo.com,
iheanyi.okonko@uniport.edu.ng;
Tel.: +234 803 5380891

cated UTI in ambulatory patients have been reported to be due to *Escherichia coli*, *Staphylococcus aureus*, *Proteus spp*, *Klebsiella spp* and *Pseudomonas aeruginosa*¹³⁻¹⁵. In Nigeria, *E. coli*, *Proteus spp* and *Klebsiella spp* have been isolated in 90.0% of UTI reported cases^{5,9,10,16-18}.

Escherichia coli is the most common organism associated with asymptomatic bacteriuria (ABU)¹⁹. In contrast to uropathogenic *E. coli* (UPEC), which causes symptomatic urinary tract infections (UTI), very little is known about the mechanisms by which these strains colonize the human urinary tract¹⁹. *Escherichia coli* is responsible for more than 80.0% of all UTIs and causes both ABU and symptomatic UTI¹⁹. The main factor pre-disposing to urinary tract infection has been attributed to poor personal hygiene and culture habit imposition^{15,20}. Bacterial adhesion conferred by specific surface-associated adhesins is normally considered as a prerequisite for colonization of the urinary tract³.

Multiple drug resistance isolates causing UTI has serious implications for the empiric therapy against pathogenic isolates and for the possible co-selection of antimicrobial resistant mediated by multi drug resistant plasmids^{21,22}. *E. coli* from clinical isolates are known to harbour plasmids of different molecular sizes²³. It has been widely reported that bacteria harbour antibiotic resistant genes which can be horizontally transferred to other bacteria²⁴.

According to Aibinu et al.²⁵, *E. coli* is highly resistant to ampicillin, amoxicillin, tetracycline and trimethoprim - sulfamethoxazole. The widespread occurrence of drug resistant *E. coli* and other pathogens in our environment has necessitated the need for regular monitoring of antibiotics susceptibility trends to provide the basis for developing rational prescription programs, making policy decisions and assessing the effectiveness of both^{26,27}.

In recent years, the application of molecular techniques for isolation and differentiation of bacterial isolates in hospitals have provided a set of powerful new tools that can augment both epidemiological investigations and patient treatment²⁷⁻²⁹. Therefore, this study was carried out to determine the resistant plasmids of multi-drug resistant *Escherichia coli* isolated from Urinary tract infections in Abokuta, Nigeria.

Materials and methods

Collection of Samples: Clean-voided, mid-stream urine samples of about 20ml were collected from patients attending inpatient and outpatient Clinics in public health facilities in Abeokuta with their respective bio-data. The following signs and symptoms of UTI including frequency of micturation, retention of urine, burning micturation, fever and chills were obtained.

Microbiological analysis: All urine samples were cultured within one hour of collection onto MacConkey agar, Blood agar, Heated Blood Agar, and Eosin Methylene Blue Agar; and were incubated aerobically overnight at 37°C. Samples that showed pure growth of isolate in a count of $\geq 10^5$ colony-forming units (CFU) per ml of urine after overnight incubation were considered to indicate significant bacteriuria³⁰. Cultural characterisation was carried out on *Escherichia coli* using a combination of colonial morphology, Gram stain characteristics, motility tests and pigmentation.

Biochemical test: oxidation-fermentation tests, catalase, oxidase activity tests, pyocyanin production, hydrolysis of arginine, nitrate production and growth on acetamide agar was carried out according to Cheesbrough³⁰.

Antimicrobial sensitivity testing: Commercially available antimicrobial discs (Abtek Biological Ltd., UK) were used to determine the drug sensitivity and resistance pattern of the isolates. The 15 different antibiotics disc concentrations such as Gentamycin (Gen), 10µg/disc; Erythromycin (Ery), 15µg/disc; Levofloxacin (Lev), 5µg/disc; Ampicillin (Amp), 10µg/disc; Augmentin (Aug), 10µg/disc; Ceftriaxone (Cef), 30µg/disc; Cotrimoxazole (Cot), 25µg/disc; Ofloxacin (Of), 5µg/disc; Tetracycline (Tet), 30µg/disc; Streptomycin (Str), 10µg/disc; Ciprofloxacin (Cip), 5µg/disc; Cloxacillin (Cxc), 5µg/disc; Amoxicillin (Amx), 25µg/disc; Cefuroxime (Cxm), 30µg/disc and Cefazidime (Caz), 30µg/disc. The antimicrobial susceptibility test of each isolate was carried out as described by the Kirby – Bauer disc diffusion method³¹ using 0.5 Macfarland's standard turbidity and interpreted according to the National Committee for Clinical Laboratory Standards (NCCLS)³² to be recommendation and the control was ATCC 25922 *E. coli* strain.

Plasmid isolation and profiling: Pure isolates of *Escherichia coli* strains were inoculated on Nutrient agar and incubated overnight. Resistant Plasmid DNA was

extracted using alkaline lysis method (Zymogen, UK). The extracted DNA was electrophoresed on a 0.8% agarose gel stained with ethidium bromide. DNA molecular weight marker was loaded and electrophoresed in a horizontal tank at 100mA at 30v for 30 minutes. After electrophoresis, plasmid DNA bands were visualized by fluorescence ultraviolet light transilluminator and analysed using a photo documentation system. The molecular weights of the plasmid were calculated using an online molecular weight calculator described by Bikandi et al.³⁴. Plasmid sizes were estimated by comparing with previously characterized plasmids.

Plasmid Curing: The curing of the resistant plasmids of the clinical *Escherichia coli* isolates was done according to Vivyan et al.³⁵.

Data analysis: Significance of the multi-drug resistant *E. coli* with their respectively plasmid weights was determined by X2 at $p < 0.05$.

Results

Table 1 showed the antibiotic resistance and susceptibility pattern of the 120 *Escherichia coli* isolates obtained from the urine samples. The result showed that *Escherichia coli* isolates had highest resistance (92.5%) to cloxacillin followed by amoxicillin (90.8%), ampicillin (90.8%), erythromycin (75.8%), cotrimoxazole (70.0%), streptomycin (70.0%) and tetracycline (68.3%). *E. coli* isolates were most susceptible to gentamycin an aminoglycoside (85.8%), ceftazidime a third generation cephalosporin (84.2%), levofloxacin a quinolone (80.0%), ceftriaxone another third generation cephalosporin (76.7%), ciprofloxacin, ofloxacin (quinolone) (72.5%) and (60.8%) respectively.

Resistance of *Escherichia coli* strains to penicillins [cloxacillin (92.5%), amoxicillin (90.8%) and ampicillin (90.8%)] is too high. There is relatively high susceptibility of strains to ceftriaxone, ciprofloxacin and ofloxacin. Very high susceptibility was registered to gentamycin and ceftazidime. Detail analysis of

Table 1. Antibiotic resistant and susceptibility pattern of the 120 *Escherichia coli* isolates

Classes of Antibiotics	Types of Antibiotics	No. Resistant (%)	No. Susceptible (%)
Aminoglycoside	Streptomycin	84(70.0)	36(30.0)
	Gentamycin	17(14.2)	103(85.8)
Cephalosporin	Ceftazidime	19(15.8)	101(84.2)
	Ceftriaxone	28(23.3)	92(76.7)
	Cefuroxime	52(43.3)	68(56.7)
	Cotrimoxazole	84(70.0)	36(30)
Penicillin	Ampicillin	109(90.8)	11(9.2)
	Amoxicillin	109(90.8)	11(9.2)
	Augmentin	53(44.2)	67(55.8)
	Cloxacillin	111(92.5)	9(7.3)
Quinolone		33(27.5)	87(72.5)
	Ofloxacin	47(39.2)	73(60.8)
	Levofloxacin	24(20.0)	96(80.0)
Macrolide	Erythromycin	91(75.8)	29(24.2)
	Tetracycline	82(68.3)	38(31.7)

antibiotic resistance profiles of strains show that a vast majority of the strains were resistant to more than one antibiotic (72.0%). Sixteen strains (13.3%) show resistance to antibiotics from three and more classes and this define them as multidrug resistant.

Table 2 shows antibiotic resistance profile of *Es-*

cherichia coli isolated from urine samples. The sixteen *Escherichia coli* isolates that were resistant to three or more classes of antibiotics in this study were E2, E6, E25, E27, E39, E46, E58, E67, E78, E81, E86, E90, E99, E105, E113 and E117. Most of these strains were resistant to cloxacillin, ampicillin, amoxicillin, streptomycin, erythromycin and tetracycline (Table 2).

Table 2: Antibiotic profile of multi drug resistant *Escherichia coli* isolates isolated from patients with urinary tract infections

Isolates	Resistant antibiotics
E 2	Amp, Amx, Cxm, Cot, Cip, Cxc, Ery, Lev, OfI, Str, Tet
E 6	Amp, Amx, Aug, Cxm, Cip, Cxc, Ery, OfI, Tet
E 25	Amp, Amx, Cot, Aug, Cef, Cxm, Gen, Lev, OfI, Str, Lev
E 27	Amp, Aug, Cef, Cxm, Ery, Gen, Lev, OfI, Str
E 39	Amp, Amx, Cot, Cef, Caz, Cxm, Cxc, Ery, Gen, Lev
E 46	Amp, Amx, Cot, Aug, Cxm, Cip, Cxc, Ery, Lev, Str, Tet
E 58	Amp, Amx, Cot, Cef, Caz, Cxm, Cxc, Ery, OfI, Str, Tet
E 67	Amx, Aug, Cot, Cxm, Cxc, Ery, OfI, Str, Tet, Lev
E 78	Amx, Aug, Cot, Cxm, Cip, Cxc, Ery, OfI, Str, Tet, Lev
E 81	Amp, Amx, Cot, Cef, Cxm, Cxc, Ery, Gen, OfI, Str, Tet
E 86	Amp, Amx, Cxm, Cip, Cxc, Ery, OfI, Str, Tet, Lev
E 90	Amp, Amx, Cot, Aug, Cef, Cxm, Cip, Cxc, Ery, Gen
E 99	Amp, Amx, Cot, Cxm, Aug, Cef, Cxc, Ery, Gen, Lev,
E 105	Amp, Amx, Cot, Aug, Cef, Gen, Lev, OfI, Str, Tet
E 113	Amp, Amx, Cef, Caz, Cxm, Cxc, Ery, Str, Tet, Lev
E 117	Amp, Amx, Cot, Aug, Cxm, Cxc, Ery, OfI, Tet

E = *Escherichia coli*

Figure 1 showed the Agarose gel electrophoretic analysis of the plasmids extracted from the multiple antibiotic resistant isolates. Lane M, is the standard molecular marker used (1000bp DNA ladder). The plasmid analyses revealed that there were detectable plasmids

in 6(37.5%) of the 16 selected multi-drug resistant *Escherichia coli* isolates. Ten of the isolates possessed no plasmid. The six isolates possessed single sized plasmids of the same weight 854bp. The six resistant plasmid bands were obtained from *Escherichia coli* isolates (E2, E27, E58, E67, E90 and E113).

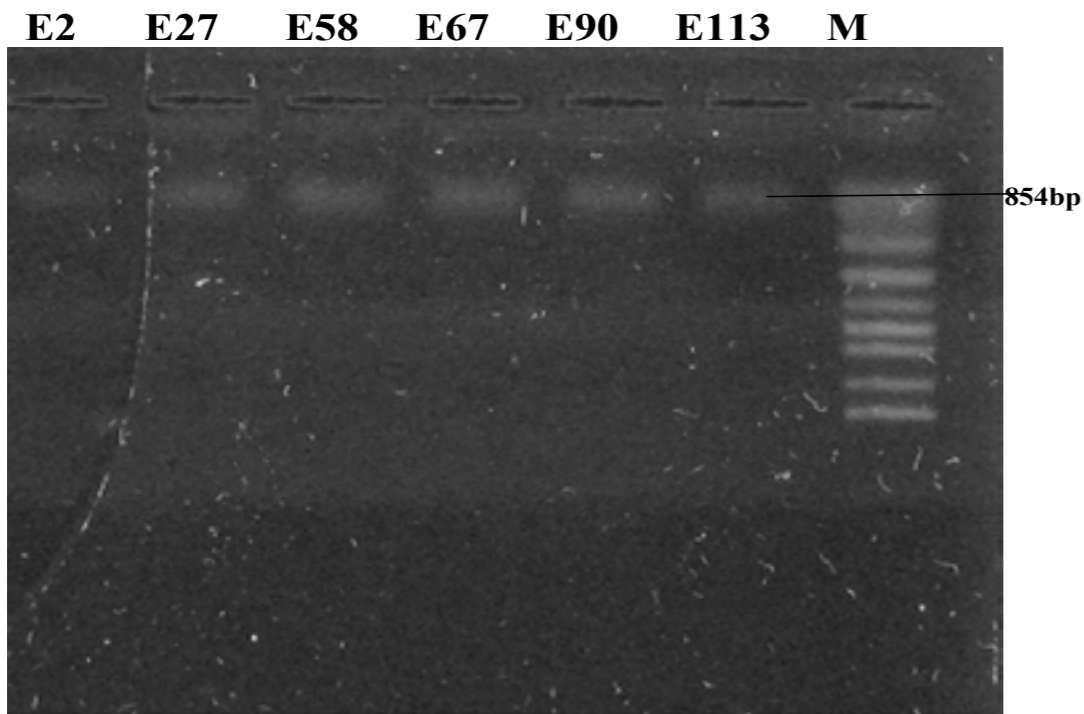


Figure 1: Agarose gel electrophoretic analysis of plasmids extracted from multiple antibiotic resistant *Escherichia coli* isolates. Lane M, 1000bp ladder.

Discussion

Escherichia coli has been reported as the most common cause of urinary tract infections^{10,36-37}. The overall incidence of antibiotic resistant of *Escherichia coli* in this study was high.

Escherichia coli isolates had high resistant to cloxacillin, amoxicillin, ampicillin, erythromycin, cotrimoxazole, streptomycin and tetracycline. This high level of resistance of the *E. coli* to cloxacillin, amoxicillin and ampicillin was in agreement with the findings of Aibinu et al.²⁵, Daini and Adesemowo³⁸, Ogbolu et al.³⁹ and Stelling et al.⁴⁰. It has been reported that pathogenic isolates of *E. coli* have relatively high potential for developing resistance⁴¹⁻⁴². Besides, amongst the enteric pathogens, resistant of *E. coli* was observed to be increasing, especially to first line, broad spectrum antibiotic. The high resistance of *E. coli* to numerous antimicrobial agents (antibiotics) observed in this present research may be due to indiscriminate and widespread use of these antibiotics in Abeokuta, Nigeria. Roos et al.^{3,19} stated that drug resistance in pathogens is a serious medical problem because of very fast turn over and spread of mutant strains, insusceptible of medical treatment.

The resistance of urinary *E. coli* isolates to ampicillin in this study is consistent with reports from previous studies in Pakistan (78.4%), showing high degree of resistance to *E. coli* ranging from 58.0% in 1989 to 74.0% in 2001⁴³⁻⁴⁴. These results are congruent to the results reported by Aibinu et al.²⁵ who found 100.0% resistance of *E. coli* isolates to ampicillin. Such multi drug resistance has serious implications for the empiric therapy of infections caused by *E. coli*²¹.

Resistant of *E. coli* from urinary tract infection to cotrimoxazole was 70.0% in this study and is in contrast to results obtained elsewhere. Cotrimoxazole resistance was approximately 30.0% in a study by Oteo et al.⁴⁵ and similar to the 27.0% reported by Alos et al.⁴⁶ in urinary tract infection isolates in Spain in 1993. Aiyegoro et al.⁴⁷ reported that 66.7% of the pathogens were resistance to cotrimoxazole and that resistance of *E. coli* to cotrimoxazole was 57.9%. From the result of this study, it is obvious that cotrimoxazole is no longer effective against uropathogens. Previously, cotrimoxazole was used as the drug of choice for empirical treatment of UTI.

Escherichia coli isolates obtained from this research were susceptible to gentamycin (85.8%), ceftazidime

(84.2%), levofloxacin (80.0%), ceftriaxone (76.7%), ciprofloxacin (72.5%) and ofloxacin (60.8%) respectively. It has been observed that antibiotic susceptibility of bacterial isolates is not constant, but dynamic and varies with time and environment⁴⁸. Akinjogunla et al.⁴⁹ reported low percentage resistance of *E. coli* to ciprofloxacin, ceftazidime and ceftriaxone.

Escherichia coli are most susceptible to gentamycin, an aminoglycoside in this study. Ceftazidime, a 3rd generation cephalosporin was the second most effective antibiotic. In a study carried out by Iqbal et al.⁵⁰ in Islamabad, *Escherichia coli* recorded high resistance to third generation cephalosporins. Levofloxacin, a quinolone, was the third most effective antibiotic against *Escherichia coli* followed by ciprofloxacin however study conducted by Khan and Ahmed in 2001, resistance of *Escherichia coli* to quinolones was reported to be 50.0%, which is much higher than reported by Farooqi et al.⁵¹ (25.0% in 1997). Quinolones (LEV, OFL, and CIP) have a broad spectrum antimicrobial activity as well as a unique mechanism of action. Studies on virulence of *E. coli* have demonstrated that quinolone-resistant *E. coli* strains have fewer virulence factors than quinolone-susceptible strains⁴. The difference in susceptibility or resistance pattern demonstrated in different geographic locations may be attributable to factors like exposure to antibiotics. From the results of this study, gentamycin may be considered as empirical therapy of first choice for *Escherichia coli* urinary tract infections in south west, Nigeria followed by ceftazidime and levofloxacin.

According to Umolu et al.²⁷, consistent stepwise increase in *E. coli* resistance to ciprofloxacin was observed from 1995 (0.7%) to 2001 (2.5%) by Bolon et al.⁵². Ciprofloxacin resistance in Portugal was 25.8% and Italy 24.3% while in Germany and Netherlands it was 15.2% and 6.8% respectively^{27,53}. In previous years, *E. coli* was 100% susceptible to the fluoroquinolones. Similar high resistance of *E. coli* to ofloxacin has also been documented by Alex et al.⁵⁴; they observed that 24% of 189 *E. coli* isolates were resistant to ofloxacin. Umolu et al.²⁷ also reported very high resistance levels (>75%) against tetracycline, augmentin and amoxicillin while nitrofurantoin and ofloxacin recorded the least resistance levels of 6.0% and 19.0% respectively among the *E. coli* isolates.

Six multi drug *Escherichia coli* possessed plasmids with similar molecular weight of 854bp and were all resistant

to erythromycin and cefuroxime. All the six isolates with the plasmids weight of 854bp were also resistance to cloxacillin, amoxicillin, ampicillin and cotrimoxazole. The emergence of R-plasmids in this study could be ascribed to the indiscriminate and widespread use of antibiotics caused by over-the-counter availability of antibiotics as well as the higher exposure of people to enteric flora in places with poor sanitation^{25,39,55,56}. Clinical isolates of *E. coli* which showed multiple drug resistance were also found to harbour plasmids with molecular sizes ranging from 2kb to 6.5 kb and a maximum 26kb. This agrees favourably with previous studies. Umolu et al.²⁷ reported that *E. coli* Isolates with high multi-drug resistance profiles were found to possess multiple plasmids with large sizes in the range of 6.557 – 23.130kb. This is also similar to what was observed by Smith et al.⁵⁷ who reported that 47 of the *E. coli* isolated from animals in Lagos harboured detectable plasmids which ranged in sizes from 0.564kb to >23kb. Danbara et al.²³ also reported plasmids of sizes between 3.9kb and 50kb in *E. coli* strains isolated from Traveller's diarrhoea. Similarly, Todorova et al.⁵⁸ showed that 92% of *E. coli* serotype 0164 strain possessed two small plasmids of molecular sizes 9.06kb and 7.248kb.

Conclusion

This study showed that *Escherichia coli* were most susceptible to gentamycin, ceftazidime Levoxin and ciprofloxacin. It also *Escherichia coli* used in this study all resistant to erythromycin, cefuroxime, cloxacillin, amoxicillin, ampicillin and cotrimoxazole. This study has also highlighted the emergence of multidrug resistant R-plasmids among *Escherichia coli* causing urinary tract infections in Abeokuta, Southwestern Nigeria. The uncontrolled use of antibiotics has contributed largely to this situation. Thus government should make considerable effort to establish an antibiotic policy for the country. Also the finding demonstrates an increasing incidence of urinary tract infections with multidrug resistant *E. coli*. Some of the isolates were harbouring plasmids. High resistance rates to penicillin were observed also among these strains. It is therefore recommended that extending mandatory surveillance to include urinary tract infections not only at hospitals but in the community to gain a better understanding of plasmids mediated resistance *E. coli* be carried out. Monitoring of plasmids mediated resistance and antimicrobial susceptibility testing was necessary to avoid treatment failure in patients with urinary tract infections.

References

1. Paryani JP, Memon, SR, Rajpar ZH, Shah SA. Pattern and Sensitivity of Microorganisms Causing Urinary Tract Infection at Teaching Hospital. *JLUMHS*, 2012; 11(02): 97-100
2. Roos V, Klemm P. Global gene expression profiling of the asymptomatic bacteriuria *Escherichia coli* strain 83972 in the human urinary tract. *Infect Immun*. 2006;74:3565–3575.
3. Roos V, Schembri MA, Ulett GC, Klemm P. Asymptomatic bacteriuria *Escherichia coli* strain 83972 carries mutations in the *foc* locus and is unable to express F1C fimbriae. *Microbiology*, 2006a, 152:1799–1806.
4. Soto SM, Jimenez de Anta MT, Vila J. Quinolones induce partial or total loss of pathogenicity islands in uropathogenic *Escherichia coli* by SOS-dependent or -independent pathways, respectively. *Antimicrob. Agents Chemother*. 2006; 50(2):649-53.
5. Kolawole AS, Kolawole OM, Kandaki-Olukemi YT, Babatunde SK, Durowade KA, Kolawole CF. Prevalence of urinary tract infections (UTI) among patients attending Dalhatu Araf Specialist Hospital, Lafia, Nasarawa State, Nigeria. *Int. J. Medicinal Med. Sci.* 2009, 1(5):163-167
6. Azubuike JC, Nkeaniginieme KEO. *Paediatrics and Applied Health in Nigeria* 1999, pp. 236-239.
7. Omonigho SE, Obasi EE, Akukalia RN. In vitro Resistance of Urinary Isolates of *Escherichia coli* and *Klebsiella* species to Nalidixic Acid. *Niger. J. Microbiol.*, 2001, 15(1):25-29.
8. Ebie, M. Y., Kandakai-Olukemi, Y. T., Ayanbadejo, J. and Tanyigna, K.B. Urinary tract infections in a Nigerian military hospital. *Nigerian Journal of Microbiology*, 2001, 15, 31-37.
9. Obiogbolu CH, IO Okonko, CO Anyamere, AO Adedeji, AO Akanbi, AA Ogun, J. Ejembi, TOC Faleye. Incidence of Urinary Tract Infections (UTIs) among Pregnant Women in Akwa Metropolis, Southeastern Nigeria. *Scientific Research and Essay*, 2009, 4(8):820-824
10. Okonko IO, Ijandipe LA, Ilusanya AO, Donbraye-Emmanuel OB, Ejembi J, Udeze AO, Egun OC, Fowotade A, Nkang AO. Incidence of Urinary Tract Infection (UTI) among Pregnant Women in Ibadan, South-Western Nigeria. *African Journal of Biotechnology*, 2009; 8(23):6649-6657
11. Moges, F., Genetu, A., and Mengstu, G. Antibiotics Sensitivity of Common Bacterial Pathogens in Urinary Tract Infections at Gondar Hospital, Ethiopia. *East Afr. Med. J.* 2002, 79:140-142.
12. National Kidney and Urologic Diseases Information Clearinghouse (NKUDIC). Fact sheet: Urinary

Tract Infections in Adults. *NIH Publication* No. 06-2097, 2005

13. Foxman B. urinary tract infection: Incidence and risk factors. *Am J Pub Health*. 1990; 80:331-333.
14. Eva T, Bendt M, Jens K. Studies on betalactamases from *Escherichia coli* Isolated from urinary tract infection. *AMPIS*. 1990; 98: 345-52.
15. Ehinmidu, J.O. Antibiotics susceptibility patterns of urine bacterial isolates in Zaria, Nigeria. *Tropical Journal of Pharmaceutical Research*, 2003; 2 (2): 223-228
16. Olanipekun KA, Montefiore D. Bacterial infections, sensitivity patterns and chemotherapy among hospital patients in the tropics. *Scand J Infect Dis*. 1978; 10:295-302.
17. Sever JL, Ellenberg JH, Edmonds D. Urinary Tract Infection During Pregnancy: Maternal and Paediatric Finding. In: Kass EH, Brumfitt W (eds). *Infections of Urinary Tract*. Chicago, USA: *Chicago Press*, 1985 pp 19-21.
18. Obaseiki-Ebor EE. Trimethoprim Sulphamethiazole resistance in *Escherichia coli* and *Klebsiella* spp of urinary isolates. *Afri J Med Sci*. 1988; 17: 175-179.
19. Roos V, Ulett GC, Schembri MA, Klemm P. The asymptomatic bacteriuria *Escherichia coli* strain 83972 out-competes UPEC strains in human urine. *Infect Immun*. 2006b, 74:615–624.
20. Johnson JR. Urinary tract infection in women; Diagnosis and treatment. *An Int Med*.1989; 111: 109-17
21. Salm, D.F., C. Thornsbery, D.C. May field, M.E. Jones, J.A. Karlowsky. Antimicro. *Agent chemother*, 2001, 41, 15-22
22. Sherley, M., Gardon, D.M., Collingnon, P.J. Evolution of multi-resistance plasmids in Australia clinical isolates of *Escherichia coli*. *Microbiology*, 2004, 150: 1539–1546.
23. Danbara, H., Komase, K Ivli, Y., Shinohawa, M., Arita, H., Makino, A. and Yoshikawa, M. Analysis of the plasmids of *Escherichia coli* 0148:H28 from travellers with diarrhoea. *Microbiol. Path.* 1987, 3 (4): 269 - 278.
24. Piddock, L.J.V. Clinically relevant chromosomally encoded multi drug resistance efflux pumps in bacteria. *Clin. Microbiol. Rev.*, 2006, 19(2): 382-402.
25. Aibinu, I.E., Adenipekun, E.A., Odugbemi, T.O. Emergence of Quinolone Resistance amongst *E. coli* Strains Isolated from Clinical Infections in Some Lagos State Hospitals, in Nigeria. *Nig. J. Health Biomed. Sci.*, 2004, 3: 73-78.
26. Omigie, O., Enweani, I.B., Ohenhen, R.E., Umolu, I.P. and BenEdo-Osagie, O. Bacteriological survey of wound infections in Benin City, Nigeria. *Nig. Ann.*

Nat. Sci. 2006, Vol. 6 (In press).

27. Umolu P. Idia, Omigie O., Tafeng Y., Omorogbe F.I., Aisabokhale F, Ugbodagah O. P. Antimicrobial Susceptibility and Plasmid Profiles of *Escherichia coli* Isolates Obtained from Different Human Clinical Specimens in Lagos – Nigeria. *The Journal of American Science*, 2006; 2(4):70-75
28. Villari P, Iacuzio L, Torre I, Scarcella A. Molecular epidemiology as an effective tool in the surveillance of infection in neonatal intensive care unit. *J. Infect.*, 1998, 37: 274- 281.
29. Gakuya, F., Kyule M. and Gathura, P. Antimicrobial susceptibility and plasmids from *Escherichia coli* isolates from rats East *Afr. Med. J.* 2001, 78: 518 - 522.
30. Cheesbrough, M. District laboratory practice in tropical countries. *Cambridge University Press*. 2004, pp. 357.
31. Bauer AW, Kirby MM, Sherris JC, Truck M. Antibiotic susceptibility testing by a standardized single disk method. *American J. Clinical Pathology* 1966; 45: 493-496.
32. National Committee for Clinical Laboratory Standards (NCCLS). Performance Standards for Antimicrobial Disk Susceptibility Testing, 10 *Information Supplement Approved Standard*, M100-S10, 2002, Wayne P. A. U.S.A.
33. Johnson, P. Plasmid analysis. In: Johnson A.P., Woodford, N. (eds.) *Molecular Bacteriology Protocols and Clinical Applications*. *Humana Press Inc., Totowa*, 1998, pp. 51- 62.
34. Bikandi, J., R. San Millán, A. Rementeria and J. Garaizar. In silico analysis of complete bacterial genomes: PCR AFLP-PCR and endonuclease restriction. *Bioinformatics*, 2004, 20: 798-799.
35. Vivyan E, Hedges RW, Datta N. Two modes of curing transmissible bacterial plasmids. *J.Gen.Microbiol.*1972, 70:443-452.
36. Andrade, S.S., Sader, H.S., Jones, R.N., Pereira, A.S., Pignatari, A.C. and Gales, A.C. Increased resistance to first-line agents among bacterial pathogens isolated from urinary tract infections in Latin America: time for local guidelines? *Mem. Inst. Oswaldo Cruz.*, 2006, 101:741–748.
37. Dash N, AL-Zarouni, M., Al-Kous N, Al-Shehhi F, Al-Najjar J, Senok A and Debadatta Panigrahi. Distribution and Resistance Trends of Community Associated Urinary Tract Pathogens in Sharjah, UAE. *Microbiology Insights*, 2008:1 41–45
38. Daini, O.A., Adesemowo, A. Antimicrobial Susceptibility Patterns and R-Plasmids of Clinical strains of *Escherichia coli*. *Aust. J. Basic Appl. Sci.*, 2008, 2: 397-400.

39. Ogbolu, D.O., Daini, O.A., Ogunledun, A., Alli, A.O., Webber, M.A. High Levels of Multidrug Resistance in Clinical isolates of Gram-negative Pathogens from Nigeria. *Int. J. Antimicrob. Agents*, 2011, 37, 62-66.
40. Stelling, J.M., Travers, K., Jones, R.N., Turner, P.J., O'Brien, T.F., and Levy, S.B. Integrating Escherichia coli antimicrobial susceptibility data from multiple surveillance programs *Emerg. Infect. Dis.*, 2005, 11 (6): 873 – 882.
41. Karlowsky, J.A., Jones, M.E., Draghi, D.C., Thornsbury, C., Sahm, D.F., Volturo, G.A. Prevalence of antimicrobial susceptibilities of bacteria isolated from blood cultures of hospitalized patients in the United States in 2002. *Ann. Clin. Microbiol. Antimicrob.* 2004, 3: 7. doi:10.1186/1476-0711-3-7
42. Nkang AO, Okonko IO, Mejeha OK, Adewale OG, Udeze AO, Fowotade A, Fajobi EA, Adedeji AO, Babalola ET. Assessment of antibiotics susceptibility profiles of some selected clinical isolates from laboratories in Nigeria. *Journal of Microbiology and Antimicrobial*, 2009, 1(2):019-026
43. Khan, S.W., Ahmed, A. Uropathogens and their Susceptibility Pattern: A Retrospective Analysis. *J. Pak. Med. Assoc.*, 2001, 5 1:98-100.
44. Livermore, D.M., Brown, D.F. Detection of β -lactamase-mediated Resistance. *J. Antimicrob. Chemother*, 2001, 48:59-64.
45. Oteo, J., J. Campos and F. Baquero. Antibiotic resistance in 1962 invasive isolates of Escherichia coli in 27 Spanish hospitals participating in the European Antimicrobial Resistance Surveillance System. *J. Antimicrob. Chemother*, 2002. 50: 945-52.
46. Alos, J.I., J.L. Gómez-Garcés, I. García-Bermejo, J.J. García-Gómez, R. Gonzalez-Palacios and B. Padilla. The prevalence of Escherichia coli susceptibility to quinolone and other antibiotics in community-acquired bacteriurias in Madrid. *Med. Clin. (Barc)*, 1993, 101:87-90.
47. Aiyegoro OA, Igbinosa OO, Ogunmwonyi IN, Odjadjare EE, Igbinosa OE, Okoh AI. Incidence of urinary tract infections (UTI) among children and adolescents in Ile-Ife, Nigeria. *Afr. J. Microbiol. Res.*, 2007, 013-019
48. Hassan, S.H. Sensitivity of Salmonella and Shigella to antibiotics and chemotherapeutic agents in Sudan. *J. Trop. Med. Hyg.*, 1995, 88: 243 - 248.
49. Akinjogunla, O. J., Odeyemi, A. T. and Olasehinde, G. I. Epidemiological Studies of Urinary Tract Infection (UTI) among Post-menopausal Women in Uyo Metropolis, South-South, Nigeria. *Journal of American Science* 2010; 6(12):1674-1681
50. Iqbal, M., I.K. Patel, Q. Ain, N. Barney, Q. Kiani, K.Z. Rabbani, G. Zaidi, B. Mehdi and S. H. Shah. Susceptibility Patterns of Escherichia coli: Prevalence of Multidrug-resistant Isolates and Extended Spectrum Beta-Lactamase Phenotype. *JPMA*, 2002, 52:407.
51. Farooqi BJ, Shareeq F, Rizvi QK, Qureshi HS, Ashfaq MK. Changing pattern of antimicrobial susceptibility of organisms causing community acquired urinary tract infections. *J Pak Med Assoc.* 2000; 50:369-373.
52. Bolon, M.K., Wright, S.B., Gold, H.S., Cermeli, Y. The magnitude of the association between fluoroquinolone use and quinolone-resistant Escherichia coli and Klebsiella pneumoniae may be lower than previously reported. *Antimicrob. Agents Chemother.*, 2004; 48: 1934 - 1940.
53. Oteo, J., Lazaro, E., de Abjo, F.J., Baquero, F., Campos, J and Spanish members of EARSS. Antimicrobial ---resistant invasive Escherichia coli, Spain. *Emerg. Infect. Dis.* 2005, 11 (4): 546 – 553
54. Alex, B., Goesseri, W., Schee, C.V., Margreet, C.V., Cornelissen, J., Hubert, E. Rapid emergence of Ofloxacin resistant Enterobacteriaceae containing multiple Gentamicin resistant associated integron in a Dutch hospital. *Emerg. Infect. Dis.*, 2001; 7 (5):862–871.
55. Daini, O. A., Ogbolu, D. O., Ogunledun, A. (2006). Plasmid Determined Resistance to Quinolones in Clinical isolates of Gram-negative Bacilli. *Afr. J. MD. Med Sci.*, 2006, 35: 437-441.
56. Marquez, C., Lasbate, M., Raymondo, C., Fernandez, J., Gestol, A.M., Holley, M., Borthgaray, G., Stokes, H.W. Urinary Tract Infections in a South American Population: Dynamic Spread of Class 1 Integrons and Multidrug Resistance by Homologous and Site –Specific Recombination. *J. Clin. Microbiol.*, 2008, 46: 3417-3425.
57. Smith, S.I., Aboaba, O.O., Odeigha, P., Shodipo, K., Adeyeye, J. A., Ibrahim, A., Adebiyi, T., Onibokun, H. and Odunukwe, N.N. Plasmid profile of Escherichia coli 0157:H7 from apparently healthy animals. *Afr. J. Biotechnol.*, 2003, 2 (9): 322 – 324.
58. Todorova, K., Bratoera, M., and Danera, M. Characterization of enteroinvasive Escherichia coli serotype 0164 by means of plasmid analysis and virulence assay. *J. Basic Microbiol.*, 1990, 30 (6): 451 - 454.