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

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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 in-patient 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

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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 antibiotics[1]. Urinary tract infections (UTIs) are among the most common infectious diseases of humans and a major cause of morbidity and mortality[2-3]. It is estimated that 40–50% of healthy adult women have experienced at least one UTI episode[2-4].

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 patients[5]. 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 urine[5-10].

Occurrence of urinary pathogens varies among different age groups, sex, catheterization, hospitalization and previous exposure to antimicrobials[11]. Signs and symptoms of burning sensation during urination, frequent or intense urges to urinate, back or lower abdominal pain, fever or chills[12], frequently characterize severe UTI. The leading causes of acute and uncompli-

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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 tract[19]. Escherichia coli is responsible for more than 80.0% of all UTIs and causes both ABU and symptomatic UTI[19]. 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 tract[3].

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 sizes[23]. It has been widely reported that bacteria habour antibiotic resistant genes which can be horizontally transferred to other bacteria[24].

According to Aibinu et al.[25], E. coli is highly resistant to ampicillin, amoxicillin, tetracycline and trimethoprim - sulfamethoxazole. The widespread occurence 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 treatment[27-29]. Therefore, this study was carried out to determine the resistant plasmids of multidrug 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 ≥105 colony-forming units (CFU) per ml of urine after overnight incubation were considered to indicate significant bacteriuria[30]. 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 Cheesbrough[30].

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; Levoxin (Lev), 5µg/disc; Ampicillin (Amp), 10µg/disc; Augmentin (Aug), 10µg/disc; Ceftriaxone (Cef), 30µg/disc; Cotrimoxazole (Cot), 25µg/disc; Ofloxacin (Ofl), 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 Ceftazidime (Caz), 30µg/disc. The antimicrobial susceptibility test of each isolate was carried out as described by the Kirby – Bauer disc diffusion method[31] 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 electrophoresied 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%), levoxin 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 ampicllin (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 ceftazidine. Detail analysis of 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 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	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)
	Levoxin	24(20.0)	96(80.0)
Macrolide	Erythromycin	91(75.8)	29(24.2)
Tetracycline	Tetracycline	82(68.3)	38(31.7)

Table 2 shows antibiotic resistance profile of Escherichia 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, Ofl, Str, Tet
E 6	Amp, Amx, Aug, Cxm, Cip, Cxc, Ery, Ofl, Tet
E 25	Amp, Amx, Cot, Aug, Cef, Cxm, Gen, Lev, Ofl, Str, Lev
E 27	Amp, Aug, Cef, Cxm, Ery, Gen, Lev, Ofl, 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, Ofl, Str, Tet
E 67	Amx, Aug, Cot, Cxm, Cxc, Ery, Ofl, Str, Tet, Lev
E 78	Amx, Aug, Cot, Cxm, Cip, Cxc, Ery, Ofl, Str, Tet, Lev
E 81	Amp, Amx, Cot, Cef, Cxm, Cxc, Ery, Gen, Ofl, Str, Tet
E 86	Amp, Amx, Cxm, Cip, Cxc, Ery, Ofl, 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, Ofl, Str, Tet
E 113	Amp, Amx, Cef, Caz, Cxm, Cxc, Ery, Str, Tet, Lev
E 117	Amp, Amx, Cot, Aug, Cxm, Cxc, Ery, Ofl, 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).

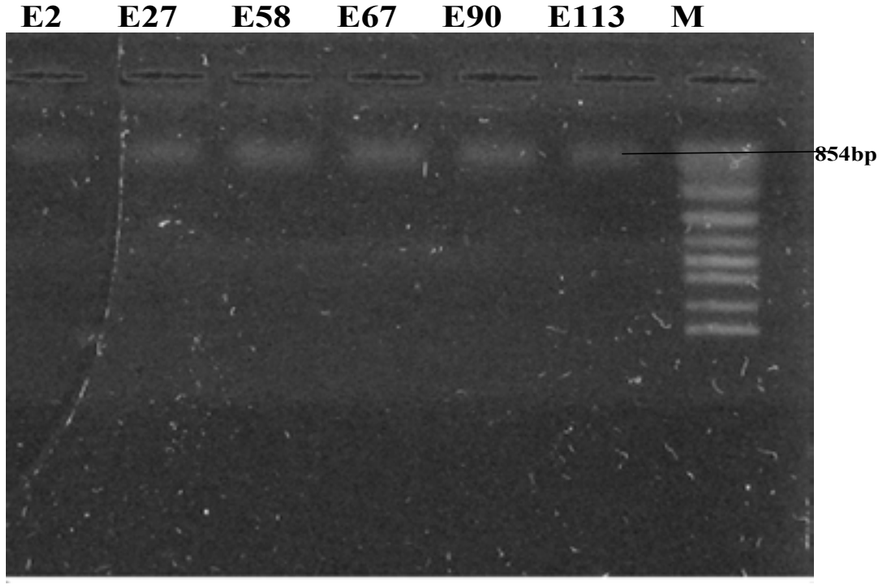

Figure 1: Agarose gel electrophoretic analysis of plasmids extracted from multiple antibiotic resistant *Escherichia coli* isolates. *Lane* M, 1000bp ladder.

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, amoxcillin 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 relativity high potential for developing resistance[41-42]. 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 2001[43-44]. 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%), levoxin (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 environment[48]. 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. Levoxin, 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 strains[4]. 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 levoxin.

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 harboure 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.

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