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 multidrug resistant Escherichia coli having single sized plasmids of the same weight 854bp and were all resistant to erythromycin, cefuroxime, 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 antibiotics. Urinary tract infections (UTIs) are among the most common infectious diseases of humans and a major cause of morbidity and mortality. It is estimated that 40–50% of healthy adult women have experienced at least one UTI episode.

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. 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.

Occurrence of urinary pathogens varies among different age groups, sex, catheterization, hospitalization and previous exposure to antimicrobials. Signs and symptoms of burning sensation during urination, frequent or intense urges to urinate, back or lower abdominal pain, fever or chills, 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
Escherichia coli is the most common organism associated with asymptomatic bacteruria (ABU)\(^1\). 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\(^2\). Escherichia coli is responsible for more than 80\% of all UTIs and causes both ABU and symptomatic UTI\(^3\). The main factor pre-disposing to urinary tract infection has been attributed to poor personal hygiene and culture habit imposition\(^4\). Bacterial adherence conferred by specific surface-associated adhesins is normally considered as a prerequisite for colonization of the urinary tract\(^5\).

Multiple drug resistance isolates causing UTI has serious implications for the empire therapy against pathogenic isolates and for the possible co-selection of antimicrobial resistant mediated by multi drug resistant plasmids\(^6\). E. coli from clinical isolates are known to harbour plasmids of different molecular sizes\(^7\). It has been widely reported that bacteria harbour antibiotic resistant genes which can be horizontally transferred to other bacteria\(^8\).

According to Aibinu et al.\(^9\), 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\(^10\).

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\(^11\)\(^-\)\(^13\). 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 2\,ml were collected from patients attending inpatient and outpatient Clinics in public health facilities in Abokuta 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\(^\circ\)C. Samples that showed pure growth of isolate in a count of \(>10^5\) colony-forming units (CFU) per ml of urine after overnight incubation were considered to indicate significant bacteriuria\(^14\). 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, ptyocin production, hydrolysis of arginine, nitrate production and growth on acetamide agar was carried out according to Cheadle\(^15\).

#### 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 (Gm), 10\,µg/disc; Erythromycin (Ery), 15\,µg/disc; Levofloxacin (Lev), 5\,µg/disc; Ampicillin (Amp), 10\,µg/disc; Augmentin (Aug), 10\,µg/disc; Cotrimoxazole (Cot), 25\,µg/disc; Ofloxacin (Oft), 5\,µg/disc; Tetracycline (Tet), 30\,µg/disc; Streptomycin (Str), 10\,µg/disc; Ciprofloxacin (Cip), 5\,µg/disc; Clexacillin (Cae), 5\,µg/disc; Amoxicillin (Amx), 25\,µg/disc; Cefuroxime (Cfx), 30\,µg/disc and Cefazidime (Caf), 30\,µg/disc. The antimicrobial susceptibility test of each isolate was carried out as described by the Kirby – Bauer disc diffusion method\(^16\) using 0.5 Macfarland’s standard turbidity and interpreted according to the National Committee for Clinical Laboratory Standards (NCCLS)\(^17\) 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 100\,mA at 30 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.\(^18\). 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.\(^19\).

#### Data analysis

Significance of the multi-drug resistant E. coli with their respective plasmid weights was determined by X2 at p<0.05.

### Results

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

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Strains of Antibiotics</th>
<th>No. Resistant (%)</th>
<th>No. Susceptible (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aminoglycoside</td>
<td>Streptomycin</td>
<td>84(70.0)</td>
<td>36(30.0)</td>
</tr>
<tr>
<td></td>
<td>Gentamycin</td>
<td>17(14.2)</td>
<td>102(85.8)</td>
</tr>
<tr>
<td>Cephalosporin</td>
<td>Cefazidime</td>
<td>19(15.8)</td>
<td>101(84.2)</td>
</tr>
<tr>
<td></td>
<td>Ceftriaxone</td>
<td>28(23.3)</td>
<td>92(76.7)</td>
</tr>
<tr>
<td></td>
<td>Cefuroxime</td>
<td>52(43.3)</td>
<td>68(56.7)</td>
</tr>
<tr>
<td>Cotrimoxazole</td>
<td>Cotrimoxazole</td>
<td>84(70.0)</td>
<td>36(30)</td>
</tr>
<tr>
<td>Penicillin</td>
<td>Ampicillin</td>
<td>109(90.8)</td>
<td>11(9.2)</td>
</tr>
<tr>
<td></td>
<td>Amoxicillin</td>
<td>109(90.8)</td>
<td>11(9.2)</td>
</tr>
<tr>
<td></td>
<td>Augmentin</td>
<td>53(44.2)</td>
<td>67(55.8)</td>
</tr>
<tr>
<td></td>
<td>Cloxacin</td>
<td>111(92.5)</td>
<td>9(7.5)</td>
</tr>
<tr>
<td>Quinolone</td>
<td></td>
<td>33(27.5)</td>
<td>87(72.5)</td>
</tr>
</tbody>
</table>

| Ofoxacin      | 47(39.2)               | 73(60.8)          |
| Levofloxacin  | 24(20.0)               | 96(80.0)          |
| Macrolide     | 91(75.8)               | 29(24.2)          |

| Tetracycline  | 826(68.3)              | 383(31.7)         |

### Table 2. 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 ceftriaxin, ampicillin, amoxicillin, streptomycin, erythromycin and tetracycline (Table 2).
analyses revealed that there were detectable plasmids of otic resistant isolates. Lane M, is the standard molecule.

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

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Resistant antibiotics</th>
</tr>
</thead>
<tbody>
<tr>
<td>E 2</td>
<td>Amp, Amx, CxM, Ctx, Cpe, Ery, Lev, Ofl, Str, Tet</td>
</tr>
<tr>
<td>E 6</td>
<td>Amp, Amx, Aug, CxM, Cip, Ctx, Ery, Ofl, Tet</td>
</tr>
<tr>
<td>E 25</td>
<td>Amp, Amx, Cot, Aug, Cef, CxM, Gen, Lev, Ofl, Str, Lev</td>
</tr>
<tr>
<td>E 27</td>
<td>Amp, Aug, Cef, Cxm, Ery, Gen, Lev, Ofl, Str</td>
</tr>
<tr>
<td>E 39</td>
<td>Amp, Amx, Cot, Cef, Caz, CxM, Ctx, Ery, Gen, Lev</td>
</tr>
<tr>
<td>E 46</td>
<td>Amp, Amx, Cot, Aug, CxM, Cip, Cx, Ery, Lev, Str, Tet</td>
</tr>
<tr>
<td>E 58</td>
<td>Amp, Amx, Cet, Caz, CxM, Ctx, Ery, Ofl, Str, Tet</td>
</tr>
<tr>
<td>E 67</td>
<td>Amp, Aug, Cot, CxM, Ctx, Ery, Ofl, Str, Tet, Lev</td>
</tr>
<tr>
<td>E 78</td>
<td>Amp, Amx, Cot, Aug, CxM, Cip, Cx, Ery, Ofl, Str, Tet</td>
</tr>
<tr>
<td>E 81</td>
<td>Amp, Amx, Cot, Cef, Cxm, Ctx, Ery, Gen, Ofl, Str, Tet</td>
</tr>
<tr>
<td>E 86</td>
<td>Amp, Amx, Cip, Ctx, Ery, Ofl, Str, Tet, Lev</td>
</tr>
<tr>
<td>E 90</td>
<td>Amp, Amx, Cot, Aug, Cef, CxM, Cip, Cx, Ery, Gen</td>
</tr>
<tr>
<td>E 99</td>
<td>Amp, Amx, Cot, CxM, Aug, Cef, Cx, Ery, Gen, Lev,</td>
</tr>
<tr>
<td>E 105</td>
<td>Amp, Amx, Cot, Aug, Cef, Gen, Lev, Ofl, Str, Tet</td>
</tr>
<tr>
<td>E 113</td>
<td>Amp, Amx, Cef, Caz, CxM, Ctx, Ery, Str, Tet</td>
</tr>
<tr>
<td>E 117</td>
<td>Amp, Amx, Cot, Aug, CxM, Cmx, Ery, Ofl, Tet</td>
</tr>
</tbody>
</table>

E = Escherichia coli

Figure 1 showed the Agarose gel electrophoretic analysis of the plasmids extracted from the multi 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 854-bp. The six resistant plasmid bands were obtained from Escherichia coli isolates (E2, E27, E58, E67, E90 and E113).

**Discussion**

Escherichia coli has been reported as the most common cause of urinary tract infections in children. The overall incidence of antibiotic resistant of Escherichia coli in this study was high. Escherichia coli isolates that had high resistant to cefoxitin, amoxicillin, ampicillin, erythromycin, cotrimoxazole, streptomycin and tetracycline. This high level of resistance of the E. coli to cefoxitin, amoxicillin and ampicillin was in agreement with the findings of Albinu et al., Daini and Adesemowo, Ogolue et al. and Stelling et al. It has been reported that pathogenic isolates of E. coli have relative high potential for developing resistance. Besides, amongst the enteric pathogens, resistant of E. coli were 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. stated that drug resistance in pathogens is a serious medical problem because of very fast turn over and spread of mutant strains, insensitive 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. These results are congruent to the results reported by Albinu et al. 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.

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. and similar to the 27.0% reported by Alos et al. in urinary tract infection isolates in Spain in 1993. Ayegoro et al. reported that 66.7% of the pathogens were resistant 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 gentamicin (85.8%), cefazidime (84.2%), levosin (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. Akinogunla et al. reported low percentage of E. coli to ciprofloxacin, cefazidime and ceftriaxone. Escherichia coli are most susceptible to gentamicin, an aminoglycoside in this study. Cefazidime, a 3rd generation cephalosporin was the second most effective antibiotic. In a study carried out by Iqbal et al. in Islamabad, Escherichia coli recorded high resistance to third generation cephalosporins. Levokin, 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. (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. 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, gentamicin may be considered as empirical therapy of first choice for Escherichia coli urinary tract infections in south west, Nigeria followed by cefazidime and levokin.

According to Umolu et al., consistent stepwise increase in E. coli resistance to ciprofloxacin was observed from 1995 (0.7%) to 2001 (2.5%) by Bolon et al. Ciprofloxacin resistance in Portugal was 25.8% and Italy 24.3% while in Germany and Netherlands it was 15.2% and 6.8% respectively. 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.; they observed that 24% of 189 E. coli isolates were resistant to ofloxacin. Umolu et al. 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
References


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African Health Sciences Vol 14 Issue 4, December 2014

African Health Sciences Vol 14 Issue 4, December 2014