Resistance Pattern and Plasmid Profile of E. coli Isolated from Diarrhoeic Children in Selected Health Centres in Sokoto, Nigeria

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Authors’ contributions
This work was carried out in collaboration between all authors. Authors BRA, KM and SAO designed the study. Author ZN performed the statistical analysis, wrote the protocol and first draft of the manuscript. Author JO managed the analyses of the study. Author SLK managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The resistance pattern and plasmid profile of E. coli was evaluated in this study.

Study Design: A cross-sectional study was carried out.

Place and Duration of Study: The research was conducted in Specialist Hospital; Maryam Abacha Women and Children Hospital; and Women and Children Welfare Clinic, Sokoto State, Nigeria from May to October, 2017.

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Methodology: A total of 236 stool samples were collected from diarrheic children of age ≤5 years from selected Hospitals in Sokoto, Nigeria. Isolation and identification of E. coli strains were carried out using standard methods and procedures. Antibiotic susceptibility testing was performed using disc-diffusion method. Plasmid extraction was carried out using alkaline lysis method while curing of the plasmid harbouring strains was done using standard curing technique.

Results: The result showed that 96 (41%) of the bacteria were E. coli, all of which were resistant to ampicillin and augmentin. The resistance to antibiotics shows 19 different resistance patterns. Sixty-one point five (61.5%) were multidrug resistant (MDR). Majority 9/10 (90%) of the MDR isolates harbour plasmids, with size ranging from 6.0 to 20 kb. All the cured strains were susceptible to ciprofloxacin, ofloxacin and ceftazidime indicating that resistance to these antibiotics was plasmid mediated.

Conclusion: E. coli isolates from diarrheic children in selected Health centres, demonstrated a significant antibiotic resistance and they harboured plasmids of diverse sizes.

Keywords: Plasmid profile; E. coli; diarrhoea; antibiotic resistance.

1. INTRODUCTION

Diarrhoea is defined as the passage of loose or watery stool at least three times in every twenty-four hours or more frequently than normal for an individual [1]. A high rate of morbidity and mortality in developing countries has been attributed to diarrhoea especially among children below the age of five [2]. Escherichia coli is a common cause of diarrhoea among children [3]. E. coli has been implicated as a causative agent of some disease conditions which include urinary tract infection, pylonephritis, endocarditis, septicaemia and meningitis [4]. The emergence of antimicrobial resistance among bacteria is a significant health issue that poses serious threat to public health especially in developing countries [5]. This is mainly due to misuse and abuse of antibiotics in the treatment of symptomatic and asymptomatic illnesses. Researchers have reported a high prevalence of resistance to many available antibiotics by enteric bacteria, especially E. coli [3,6]. Resistance genes are usually transferred from more virulent pathogenic bacteria to non-pathogenic bacteria of the human gastrointestinal tract [7]. Plasmids are an extrachromosomal piece of DNA that can replicate independently of the host genome [8]. They play a significant role in resistance to many antibiotics [9]. Multidrug resistance (MDR) in bacteria has been reported to be linked to plasmids which may harbour one or more resistance gene and can transfer these genes horizontally to a new host [10]. Antibiotic resistance plasmids confer resistance to some antibiotics and they have the capacity to transfer resistance genes between bacterial species by conjugation [11]. There is a paucity of information on the resistance pattern of E. coli and its plasmid profile in Sokoto, Northwestern Nigeria.

2. MATERIALS AND METHODS

2.1 Sample Collection and Analysis

Faecal specimens were collected from two hundred and thirty-six children of five years old and below in age who had diarrhoea and who attended selected health centres in Sokoto, Nigeria. These included children who as at the time of reporting to the hospital had not taken any antibiotics and whose parents consented to participate in the study. The children who did not meet the above criteria were excluded. Standard procedures were used in the isolation and identification of E. coli as follows: About 1 gram of stool samples was inoculated onto 10ml Selenite F broth and incubated at 37°C for 16 hours after which it was sub-cultured onto Xylose lysine deoxycholate agar (XLD). Stool samples were also inoculated onto MacConkey agar and culture plates were incubated aerobically at 37°C for 18-24 hours. Aseptic techniques, purity testing and quality control were strictly maintained throughout the procedures. Isolates were examined for characteristic colonial morphology, were Gram stained and identified as E. coli using a conventional biochemical system which included Kligler iron agar, Citrate, Urease, Indole, Oxidase and Motility medium [12].

Written informed consent was obtained from each of the parents of the children enrolled for study. Also, an ethical approval (SKHREC/026/017) was obtained from the State Ministry of Health, Sokoto, Nigeria.

2.2 Antibiotic Susceptibility Testing

The antibiotic susceptibility test of all identified E. coli was done using disc diffusion method as
described by Kirby-Bauer [13] using standard antibiotics (Rapid Labs, Uk and Oxoid, UK) following the recommendations in clinical and laboratory standard Institute (CLSI) document [14]. The following antibiotics were tested; ofloxacin (5 µg/disc), ciprofloxacin (5 µg/disc), gentamycin (10 µg/disc), cefuroxime (30 µg/disc), ceftazidime (30 µg/disc), ampicillin (10 µg/disc), cotrimoxazole (5 µg/disc), augmentin (30 µg/disc), chloramphenicol (30 µg/disc), ceftriaxone (30 µg/disc). America Type Control Culture (ATCC) strain of E. coli 25922 was used as control.

2.3 Isolation and Separation of Plasmid DNA

Isolates that were resistant to at least one agent in three or more categories of antibiotics were regarded as multidrug resistant. Ten MDR E. coli were grown overnight in Luria broth (Himedia, India) at 37°C with mild agitation and plasmid DNA were extracted by alkaline lysis method described by Birnboim and Doly [15] using ZymoPURE™ plasmid miniprep kit (Zymo Research Corporation U.S.A). A 0.8% agarose gel was used. The extracted plasmids were run on horizontal apparatus (EDVOTEK-U.S.A) at 90v for 1 hour with pH 8.0 Tris-borate EDTA buffer. The gel was stained with 5 µl ethidium bromide and bands were visualized using a gel documentation system (Wealtec Corp USA). Quick Load® 1 KB Extend DNA ladder (New England BIOLABS (U.K.) LIMITED) was used as standard DNA marker.

2.4 Plasmid Curing

MDR isolates that harboured plasmid were cured by subjecting them to modified standard curing method of Ahrne et al. [16]. Each of the isolates were sub-cultured on Luria broth (LB) for 24 hours at 37°C and 9 ml of freshly prepared nutrient broth was then inoculated with an aliquot from the overnight culture on LB medium. It was incubated at 37°C for 4 hours with mild agitation to allow for minimal growth of the organisms. Curing agent, 1 ml of Sodium dodecyl sulphate (SDS) was added to the mixture to sufficiently bring the concentration to 1%. It was incubated for 48 hours at 37°C. Inoculation of 1 ml of cured culture into 9 ml of freshly prepared nutrient broth was carried out and this was incubated at 37°C for 24 hours. The product of the curing experiment was subjected to plasmid isolation and gel electrophoresis as already described. Isolates found to have lost their plasmid were considered cured. Post curing susceptibility test was carried out as already described using the same set of antibiotics. The result of pre and post-curing susceptibility was then compared.

3. RESULTS

3.1 Prevalence and Antibiotic Resistance Patterns of E. coli

Out of the two hundred and thirty six faecal samples collected, 96 (40.7%) yielded growth of E. coli all of which were resistant to ampicillin and augmentin. Table 1 reveals the distribution of resistance among isolates showing 19 different antibiotic resistance patterns. The resistance pattern ranged from pattern containing two antibiotics AMP and AUG, (7.3%) to a pattern containing all 10 antibiotics tested (16.7%). Majority (20.9%) were resistant to AMP, AUG, SXT only and 61.5% of the isolates were MDR.

3.2 Plasmid Studies

A total of ten (10) MDR isolates were screened for plasmid. Table 2 highlights the plasmid profile of the selected isolates and their resistance patterns, 9(90%), of the MDR, harbour plasmid (6-20 kb). E141 did not harbour plasmid despite been resistant to all ten antibiotics tested. The resistance level observed in this 10 range from four-drug resistance (E124) to ten-drug resistance (E145). The plasmid size did not correlate with the number of antibiotics an isolate was resistant to, as isolates were found with higher molecular weight and were resistant to fewer antibiotics while some had lower molecular weight and were resistant to more antibiotics. Plasmid curing procedure was carried out to determine if the antibiotic resistance observed in the study was plasmid mediated. Plasmid bands were not seen after electrophoretic separation of SDS treated isolates. Table 3 demonstrates the susceptibility pattern of the plasmid harbouring isolates before and after curing. Loss of plasmids corresponds to loss of resistance in all (100%) isolates previously resistant to ceftazidime, ciprofloxacin and ofloxacin prior to SDS treatment. Loss of resistance was also observed to cotrimoxazole (in E125, E025 and E134) and augmentin (in E145, E025 and E083).
Table 1. Resistance pattern of *E. coli* isolates

<table>
<thead>
<tr>
<th>Number of antibiotics</th>
<th>Antibiotics to which isolates where resistant</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>AMP AUG</td>
<td>7 (7.30)</td>
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<tr>
<td>3</td>
<td>AMP AUG SXT</td>
<td>20 (20.90)</td>
</tr>
<tr>
<td>3</td>
<td>AMP AUG CRX</td>
<td>2 (2.10)</td>
</tr>
<tr>
<td>4</td>
<td>AMP AUG CRX CAZ</td>
<td>2 (2.10)</td>
</tr>
<tr>
<td>4</td>
<td>AMP AUG CRX SXT</td>
<td>8 (8.30)</td>
</tr>
<tr>
<td>5</td>
<td>AMP AUG CRX SXT CHL</td>
<td>6 (6.30)</td>
</tr>
<tr>
<td>6</td>
<td>AMP AUG CRX SXT GEN CHL</td>
<td>3 (3.10)</td>
</tr>
<tr>
<td>5</td>
<td>AMP AUG CRX CAZ SXT</td>
<td>5 (5.20)</td>
</tr>
<tr>
<td>6</td>
<td>AMP AUG CRX CAZ SXT CHL</td>
<td>2 (2.10)</td>
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<tr>
<td>4</td>
<td>AMP AUG SXT GEN</td>
<td>4 (4.20)</td>
</tr>
<tr>
<td>4</td>
<td>AMP AUG CAZ SXT</td>
<td>4 (4.20)</td>
</tr>
<tr>
<td>5</td>
<td>AMP AUG SXT CHL</td>
<td>2 (2.10)</td>
</tr>
<tr>
<td>6</td>
<td>AMP AUG SXT CHL OFL CAZ</td>
<td>1 (1.00)</td>
</tr>
<tr>
<td>4</td>
<td>AMP AUG SXT OFL</td>
<td>1 (1.00)</td>
</tr>
<tr>
<td>8</td>
<td>AMP AUG CRX CAZ SXT CHL CPR OFL</td>
<td>1 (1.00)</td>
</tr>
<tr>
<td>10</td>
<td>AMP AUG CRX CAZ CTR SXT CHL CPR OFL GEN</td>
<td>16 (16.70)</td>
</tr>
<tr>
<td>7</td>
<td>AMP AUG CRX CAZ CTR SXT CHL</td>
<td>3 (3.10)</td>
</tr>
<tr>
<td>7</td>
<td>AMP AUG CRX CAZ CTR SXT OFL</td>
<td>1 (1.05)</td>
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<tr>
<td>0</td>
<td>nil</td>
<td>6 (6.30)</td>
</tr>
</tbody>
</table>

AMP=Ampicillin, AUG=Augmentin, CRX=Cefuroxime, CAZ=Ceftazidime, CTR=Ceftriaxone, SXT=Cotrimoxazole, CHL=Chloramphenicol, CPR=Ciprofloxacin, OFL=Ofloxacin, GEN=Gentamycin

Table 2. Plasmid profile of selected multidrug-resistant isolates and their resistance pattern

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Plasmid size (kb)</th>
<th>Number of antibiotics</th>
<th>Combination of antibiotics</th>
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<td>20</td>
<td>10</td>
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</tr>
<tr>
<td>E124</td>
<td>20</td>
<td>4</td>
<td>AMP AUG CRX SXT</td>
</tr>
<tr>
<td>E025</td>
<td>15</td>
<td>7</td>
<td>AMP AUG CRX CAZ SXT OFL GEN</td>
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<tr>
<td>E019</td>
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<td>7</td>
<td>AMP AUG CRX CAZ SXT CHL GEN</td>
</tr>
<tr>
<td>E141</td>
<td>0</td>
<td>10</td>
<td>AMP AUG CRX CAZ CTR SXT CHL CRP OFL GEN</td>
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<tr>
<td>E134</td>
<td>10</td>
<td>6</td>
<td>AMP AUG CRX CAZ SXT GEN</td>
</tr>
<tr>
<td>E129</td>
<td>10</td>
<td>5</td>
<td>AMP AUG CRX CAZ SXT</td>
</tr>
<tr>
<td>E083</td>
<td>8</td>
<td>6</td>
<td>AMP AUG SXT CHL CTR OFL</td>
</tr>
<tr>
<td>E028</td>
<td>6</td>
<td>7</td>
<td>AMP AUG CRX SXT CHL CRP OFL</td>
</tr>
<tr>
<td>E173</td>
<td>6</td>
<td>8</td>
<td>AMP AUG CRX CTR SXT CHL CRP OFL</td>
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</tbody>
</table>

AMP=Ampicillin, AUG=Augmentin, CRX=Cefuroxime, CAZ=Ceftazidime, SXT=Cotrimoxazole, CHL=Chloramphenicol, CTR=Ceftriaxone, CPR=Ciprofloxacin, OFL=Ofloxacin, GEN=Gentamycin

4. DISCUSSION

The present study reveals that there is a high rate of resistance to antibiotics among *E. coli* in the subject studied. All the isolates were resistant to ampicillin and augmentin. It is worrisome, knowing that ampicillin is the first line antibiotic of choice in the treatment of diarrhoea in Nigeria and most other developing countries.

More than 50% of the *E. coli* isolates were MDR and some were resistant to all the antibiotics tested. The prevalence of resistance to ampicillin, augmentin, cotrimoxazole and cefuroxime was very high in this work. This high prevalence may be due to indiscriminate use of oral antibiotics that are readily available over the counter. Resistance to ampicillin, augmentin may be due to the action of beta-lactamases, and inhibitor-resistant TEM (IRT) enzymes respectively [10] while resistance to cotrimoxazole may be due to a different form of diamino-pyrimidine folate reductase enzymes all of which may be borne on the plasmid and are transferable [17]. The emerging high rate of resistance to third-generation cephalosporins
calls for serious concern as evident in this study [18]. The marked resistance attributed to this work will leave the treatment of diarrhoea caused by E. coli to more expensive antibiotics and this may pose a major challenge to the management of bacterial diarrhoea putting into cognizance the burden of poverty in this part of the world.

Plasmids that encode multiple antimicrobial resistances bestow on their host, the capacity to survive in the presence of antibiotics. Although multiple drug resistance in bacteria is commonly linked with the presence of plasmids which may contain resistance gene that encodes antibiotic resistance features, some multiple antibiotic resistances are associated with the chromosome [10]. George and Levy, [19] have shown that chromosomal multiple antibiotic resistance systems were present in E. coli. Majority of the selected MDR isolates tested for plasmid harboured plasmid which may confer resistance to antibiotics. This is comparable to 86% reported by Clarence et al. [20] and 75% reported by Abdel Nasser et al. [21] and is high compared to 64% in the findings of Uma et al. [18], and 59% reported by Khalid et al. [22]. The plasmid size ranges from 6-20 kb, predicting that the size is diverse. This is in agreement with the previous report [21] of plasmid size ranging from 1-25 kb; it, however, contradicted the report by Chigor et al. [23] of sizes less than 2.1 kbp, 1.2-5.3 kbp reported by Abdel Nasser [20] and 0.55 - 1.14 kbp reported by Clarence et al. [19]. Unlike the findings of other researchers [18,21-24] who reported multiple plasmids within a single isolate, this research found a single band in each plasmid positive isolates analysed. The strains derived from SDS treatment of plasmid harbouring MDR isolates were susceptible to some antibiotics they were previously resistant to. This suggests that a part of their resistance gene must have been removed by SDS. Loss of plasmid correlated to the loss of resistance to ciprofloxacin, ofloxacin and ceftazidime in all the cured strains. This, therefore, confirms that MDR to these antibiotics was plasmid mediated.

5. CONCLUSION

It can be concluded that E. coli isolates from diarrhoeic children in Sokoto demonstrated a wide range of antibiotic resistance pattern and harboured plasmids of diverse size. The entire cured strains of E. coli were susceptible to ciprofloxacin, ofloxacin and ceftazidime indicating that resistance to these antibiotics was plasmid mediated.

**ETHICAL APPROVAL**

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore

### Table 3. Pre and post-curing susceptibility pattern of the plasmid containing isolates of E. Coli

<table>
<thead>
<tr>
<th>Isolate</th>
<th>AMP</th>
<th>AUG</th>
<th>CRX</th>
<th>CTR</th>
<th>CAZ</th>
<th>SXT</th>
<th>CHL</th>
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<th>OFL</th>
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<tbody>
<tr>
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**Key:** AMP= Ampicillin, AUG= Augmentin, CRX= Cefuroxime, CAZ= Ceftazidime, CTR= Ceftriaxone, SXT= Cotrimoxazole, CHL= Chloramphenicol, CPR= Ciprofloxacin, OFL= Ofloxacin and GEN= Gentamycin.

R=Resistant, S= Sensitive and I=Intermediate
been performed by the ethical standards laid down in the 1964 Declaration of Helsinki.

**COMPETING INTERESTS**

Authors have declared that no competing interests exist.

**REFERENCES**


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