Molecular Detection of Plasmid-Mediated Quinolone Resistance Genes (qnrA and \textit{aac(6')-Ib-cr}) in Drug Resistant \textit{Escherichia coli}, Sudan

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Authors' contributions
This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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**ABSTRACT**

**Background:** The quinolone group, a synthetic antimicrobial, is widely used worldwide to treat many diseases, including those caused by Gram-negative bacteria. *Escherichia coli* and others are among the bacteria that produce quinolone resistance genes (qnr) such as qnrA and aac(6')-lb-cr.

**Objective:** The present study aimed to isolate *Escherichia coli* from patients attending some hospitals in Wad Medani city, identification of drug resistance patterns and detection of the frequency of quinolones resistance genes; qnrA and aac(6')-lb-cr among isolated strains.

**Methods:** A cross-sectional descriptive, hospital-based study included 119 *Escherichia coli* strains was conducted. A designed questionnaire used for demographic data collection and the attitude toward antimicrobials usage. Clinical specimens were processed for aerobic bacterial isolation and identification. Antimicrobial sensitivity performed by Kirby Bauer disc diffusion technique according to the CLSI guidelines. Presence of qnrA and aac(6')-lb-cr genes was assessed by multiplex PCR.

**Results:** Most strains of *Escherichia coli* originated from urine 53.8% (64/119) and wounds 42.9% (51/119) specimens. Meropenem had the best effect against tested strains with susceptibility of 85% (101/119). Multiplex PCR assay, using specific primers, demonstrated that 41.2% (49/119) specimens possessed qnrA and aac(6')-lb-cr genes respectively.

**Conclusion:** The high rate of qnrA and aac (6)-lb-cr genes among *Escherichia coli* necessitate the usage of molecular tools in detecting the genetic determinants of drug resistance microorganisms in countries such as Sudan.

**Keywords:** *Escherichia coli*; quinolone resistance; qnrA; aac(6')-lb-cr; multiplex-PCR; Sudan.

1. INTRODUCTION

Pathogenic microbes that are becoming resistant to antimicrobials treatment is a growing global health dilemma. The drug resistance in Sudan is of particular concern; Self-medication, use of antimicrobials without clinical guidance, and inadequate regulation of the distribution and sale of prescription are also contributing factors [1]. Diseases caused by *Escherichia coli* include systemic opportunistic infections such as inflammation of the urinary and respiratory tracts and local infections such as wound infections as well as bacteremia [2]. Resistance of bacteria to quinolones has been studied earlier in the family Enterobacteriaceae, particularly in *K. pneumoniae* strains [3]. It is worth noting that the dilemma of antibacterial resistance, which results in the failure of treatment, pervades all types of antimicrobials used at the medical and veterinary levels [4]. As a result of all of the above, it is difficult and limited to choose successful treatment for many pathological conditions [5].

The quinolone group, a synthetic antimicrobial, is widely used worldwide to treat many diseases, including those caused by Gram-negative bacteria [6]. The three known ways by which bacterial resistance to quinolone occurs are first, chromosomal mutations in responsible encoding genes, second, mutations that reduce the concentration of the drug inside the cytoplasm of cells, and third, plasmid mediated-resistance genes [7,8]. Chromosomal mediated resistance in gram-negative bacteria is associated with specific mutation in DNA gyrase enzyme, while plasmid-mediated resistance resulted in altered metabolism, interaction and increase quinolones efflux [9]. *Escherichia coli* and others are among the bacteria that produce quinolone resistance genes (qnr) which are often found on plasmids. qnrA is one of resistance genes that has not been identified much in *Escherichia coli*. It is believed that the presence of aminoglycoside acetyltransferase gene such as aac(6')-lb-cr related to prevalence of quinolone resistance genes [10,11].

The aims of the current study was isolate *Escherichia coli* from various clinical samples, determine the drug resistance patterns and assess the frequency of quinolones resistance genes (qnrA and aac(6')-lb-cr) in different hospitals in Wad Medani city.

2. METHODS

2.1 Study Setting and Population

This was a survey of major hospitals in Wad Madani city (Wad Madani Teaching Hospital, National Cancer Institute, Wad Madani Pediatrics Hospital, Wad Medani Teaching Hospital for Obstetrics and Gynecology). Study periode was from August 2019 to March 2020. Enrolled participants were admitted patients suffer from clinical presentation of an infection.
2.2 Criteria of Inclusion
In the current study, bacterial isolates of different samples including urine, wound swabs, throat swabs, ear swabs and sputum were identified as *E. coli* using routine biochemical tests.

2.3 Isolation, Identification and Susceptibility Testing
119 *Escherichia coli* strains were isolated. Subjects were given a sample questionnaire containing demographic information such as age, gender, marital status and attitudes towards antimicrobials use. Obtained samples were processed within one hour after collection [12]. Samples were inoculated on MacConkey, chocolate, CLED and blood agar media, and incubated aerobically at 37°C for 24 hours. Bacterial isolates were identified according to group characteristics, Gram stain and biochemical tests [13]. Isolates strains of *E. coli* were examined for antimicrobial sensitivity by disk diffusion method according to CLSI guidance [14]. Commonly used antimicrobials; ciprofloxacin, gentamicin, cefixime, Amoxicillin/clavulanate, ceftriaxone, meropenem were used (Bioanalysis Co. Italy).

2.4 Extraction of Genomic DNA
Isolate of DNA was accomplished using boiling lysis technique. A boiling for 30 minutes at 100 °C was achieved for 400 μL of bacteria suspension. After careful centrifugation for about 10 minutes at 14000 rpm, the supernatant was taken to a new tube. About 800 μL of anhydrous cold ethanol used for DNA precipitation for 20 minutes. Bacterial DNA pellet obtained by centrifugation for 15 minutes at 14,000 rpm. Finally the pellet was washed in 1000μL of 70% ethanol, dried and re-suspended in 100μL of sterile water.

2.5 Multiplex PCR
Primers used for target genes amplification shown in (Table 1).

"The following volumes were used; (total reaction volume was 20 μl) in 0.2 ml PCR tube; 2.2 μl deionized sterile water. 3 μl Master mix (Solis Biodyne, Korea). 0.2 μl QnrA forward primer (Macrogen Company, Seoul, Korea). 0.2 μl QnrA reverse primer (Macrogen Company, Seoul, Korea). 0.2 μl AcrA forward primer (Macrogen Company, Seoul, Korea). 0.2 μl AcrA reverse primer (Macrogen Company, Seoul, Korea). 0.3 μl KPC forward primer (Macrogen Company, Seoul, Korea). 0.3 μl KPC reverse primer (Macrogen Company, Seoul, Korea). 0.2 μl K16RNA forward primer (Macrogen Company, Seoul, Korea). 0.2 μl K16RNA reverse primer (Macrogen Company, Seoul, Korea). 3 μl DNA (template DNA)" [15].

For amplification by thermal cycling conditions; 5 minutes of initial denaturation at 94°C, followed by 38 cycles of amplification at 94°C for 45 seconds, 58°C for 45 seconds, and 72°C for 45 seconds. Final extension at 72 °C for 3 minutes.

2.6 Preparation of Primers
Primers were prepared according to manufacure instructions [14].

2.7 Statistics
Data analysis was performed using a computer original software SPSS version 16 (SPSS Inc., Chicago, IL, USA) with descriptive statistics including frequency, competitiveness and population characteristics of microbiological and clinical conditions.

Table 1. Primers used for detection of quinolones resistant genes and confirmation of *Escherichia coli* species

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sequence</th>
<th>Product Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>AcrA</td>
<td>F TCTGATCGACGGTGACATCC</td>
<td>157</td>
</tr>
<tr>
<td>aac(6')-Ib</td>
<td>R TCGAGCAATTGTTTGCTGCG</td>
<td>516</td>
</tr>
<tr>
<td><em>Escherichia coli</em> 16 rRNA</td>
<td>F ATTTTCTACGCGCAGGATTTG</td>
<td>340</td>
</tr>
<tr>
<td></td>
<td>R GATCGGCAAAGGTAGATTCGA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R ACGACGGCATAGTCATTTCG</td>
<td></td>
</tr>
</tbody>
</table>
3. RESULTS

3.1 Baseline Data

The 119 strains of *Escherichia coli* were recovered from a total of 819 clinical specimens in different hospitals in Wad Medani city, the overall bacterial growth revealed 57.8% (473/819) while *Escherichia coli* constituted 25.2% (119/473). Table 1 represented number of *Escherichia coli* isolates according to sample types, most strains were from Wad Medani Teaching Hospital for Obstetrics and Gynecology and Gezira Hospital for Renal Diseases and Surgery, and urine was the most frequent sample. Number of strains isolated from male were patients were 33% (40/119) and female patients were 77% (79/119). The study subjects distributed to three age groups; children (1 – 15 years), adults (16-55 years) and geriatric (above 56 years) represented 20% (24/119), 38% (45/119) and 42% (50/119) respectively.

3.2 Antimicrobial Susceptibility Profile

It was noted that 41.3% (338/819) of study participants reported that they had received antimicrobials without medical prescription at least one time. From collected data, the most frequently used antimicrobial for self-medication was azithromycin, followed by ceftriaxone and amoxicillin. Also, 55.9% (458/819) of the participants reported having a drug in the home. Meropenem against *Escherichia coli* had the best effect in antimicrobial susceptibility tests. The ciprofloxacin resistance rate among the isolates was 55%. Antibiotic susceptibility testing results for clinical isolates of *Escherichia coli* is shown in Table 3.

Table 2. Distribution of *Escherichia coli* isolate in subject hospitals, and sample types. No 119

<table>
<thead>
<tr>
<th>Hospital</th>
<th>Wound Swab</th>
<th>Urine</th>
<th>Throat Swab</th>
<th>Ear Swab</th>
<th>Sputum</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wad Medani Emergency Hospital</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>Wad Medani Teaching Hospital (Surgery department)</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>Wad Medani Teaching Hospital for Obstetrics and Gynecology</td>
<td>4</td>
<td>26</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td>Gezira Hospital For Renal Diseases and Surgery</td>
<td>4</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>24</td>
</tr>
<tr>
<td>National Cancer Institute</td>
<td>08</td>
<td>06</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>Wad Medani Pediatric Hospital</td>
<td>4</td>
<td>04</td>
<td>0</td>
<td>03</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>National Center for Pediatric Surgery</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Wad Medani Teaching Hospital (ENT department)</td>
<td>04</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>51</strong></td>
<td><strong>64</strong></td>
<td><strong>2</strong></td>
<td><strong>3</strong></td>
<td><strong>2</strong></td>
<td><strong>119</strong></td>
</tr>
</tbody>
</table>

Table 3. Antimicrobial susceptibility testing of isolated *Escherichia coli* against commonly used drugs

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Resistance %</th>
<th>Intermediate %</th>
<th>Sensitive %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin</td>
<td>55</td>
<td>05</td>
<td>40</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>30</td>
<td>03</td>
<td>67</td>
</tr>
<tr>
<td>Cefixime</td>
<td>61</td>
<td>12</td>
<td>27</td>
</tr>
<tr>
<td>Ceftriazone</td>
<td>68</td>
<td>20</td>
<td>12</td>
</tr>
<tr>
<td>Meropenem</td>
<td>13</td>
<td>01</td>
<td>85</td>
</tr>
<tr>
<td>Amoxicillin/Clavulanic Acid</td>
<td>85</td>
<td>03</td>
<td>12</td>
</tr>
</tbody>
</table>

Table 4. Frequency and distributed of genes out of isolated *Escherichia coli*

<table>
<thead>
<tr>
<th>No of Genes detected</th>
<th>Type of Genes</th>
<th>No of isolated organisms</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 Genes</td>
<td>qnrA + aac (6)-lb-cr</td>
<td>21</td>
<td>17.6 %</td>
</tr>
<tr>
<td>1 Gene</td>
<td>qnrA</td>
<td>28</td>
<td>23.5 %</td>
</tr>
<tr>
<td>1 Gene</td>
<td>aac (6)-lb-cr</td>
<td>24</td>
<td>20.1 %</td>
</tr>
<tr>
<td>Zero gene</td>
<td>No genes</td>
<td>43</td>
<td>36.1 %</td>
</tr>
</tbody>
</table>
3.3 Multiplex PCR Assay

“The distribution of the antibiotic resistance genes in the *Escherichia coli* isolates is shown in (Table 4). The multiplex PCR assay, using specific primers, demonstrated that among the 119 isolates, 49 (41.2%) and 45 (37.8%) isolates were positive for the qnrA and *aac* (6)-*lb-cr* gene, respectively, showing that qnrA was circulating with a high frequency” [15].

4. DISCUSSION

Limiting the prevalence of antimicrobial resistance shown by many pathogenic bacteria based primarily on early detection and accurate molecular diagnosis. Resistance to quinolones occurs in both gram- positive and gram-negative bacteria in several ways, including the production of resistance genes such qnrA and *aac*(6′)-*lb-cr* [16].

From the results and consider the gender classification, the frequency of female was more compared to male, as a source of *Escherichia coli*, and this is in agreement with other study carried out in hospitals of Anyigba, Nigeria by Mofolorunsho *et al.*, in 2021 [17]. In contrast, in another study conducted by Deshmukh *et al.*, male were more frequent [18]. However, risk factors such sexual activities and personal hygiene have been attributed to the high infection rates among female patients [19,20]. In addition with regard to women, the shortness of the urinary tract and the nature of the topography of the genital tract help germs to rise into the urinary tract.

Misconceptions such as buying antimicrobials without a prescription from a specialist doctor (self-medication) in some communities resulted in improper use of these drugs. It was found that antimicrobials most frequently used for self-medication included broad spectrum agent such as ceftriaxone which may have many side effects. This study found that females are 1.6 times higher than males in self-medication, which was observed by a second study in Sudanese community by Elmahi *et al.*, [21]. A study discussed the reasons for using over-the-counter treatment and stated that the main reason is the long distance to health services [21]. It is a problem mainly in developing countries where the health system is weak [22]. The practice of self-medication recorded in this study can be attributed to patients’ inability to afford medical professionals, in addition to the poor drug control by the government.

Meropenem against *Escherichia coli* as demented in this study had the best effect, similar result for this drug was reported in 2017 in Sudan, Khartoum by Elbadawi *et al.* whom observed resistance of 9 % [23]. Rate of ciprofloxacin among the isolates was 55%, similar results were obtained in studies conducted in Sudan [24,25]. Results from Sudan were in agreement with studies conducted in Thailand where high resistance to ciprofloxacin, ceftriaxime and cefotaxime were reported [26,27]. It can be said that, geographical variation of ciprofloxacin resistance could be an emerging problem in both developed and developing countries [28].

**Fig. 1. Genotyping of resistant genes of Escherichia coli using multiplex PCR**

Lane 1: The 482 bp band for *aac* (6)-*lb-cr* and 516 bp for qnrA genes. Lanes 2, 3 and 4: 600 bp band for 16 RNA gene. Lane M: DNA ladder (100-1000 bp).
From the current results, multi-drug resistant *E.coli* accounting to 94.8%. Similar high frequencies of 74.4%, 90.1% and 97.5% recorded in Sudan, Egypt and KSA respectively by Azab *et al.* [29]. Factors such as behavior of antimicrobials usage and geographic proximity may explain result similarity obtained from Egypt and Sudan.

The presence of drug resistance genes such as qnr and aac (6) is an indicator of the increase in the number of quinolone-resistant bacterial strains [30]. This study demonstrated 41.2% of *E.coli* isolates as qnrA gene producer, this rate is higher than that documented in Egypt, among *Klebsiella pneumoniae* [31]. Other findings were in accordance observed in Niger and Togo in which the rating of qnr gene was 47% and 44.4% respectively [32,33].

In this study also aac (6’)-Ib-cr gene was observed in 37.8% of isolated *E.coli*, which is higher than that found in Iran where Zahra reported the frequency of 25% [34].

Essentially, the drug resistance gene is produced due to selective pressures for the use of antibiotics in the medical field or due to the horizontal transmission. Then genes are usually transported through plasmid and reach different or related members of bacterial species.

It should be noted in our study that a significant part of the bacterial strains showed resistance to ciprofloxacin and the absence of target genes, the discrepancy of the genotype-phenotype association could be explained by presence of other resistance genes or certain factors that have not been addressed in this study.

5. CONCLUSION

The study found high percentages of quinolone resistance genes; qnrA and aac(6’)-Ib-cr among *E. coli* strains obtained from Sudan.

CONSENT

All caregivers were recruited to participate with parental written consent.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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