

## **Molecular Detection of Plasmid-mediated Quinolone Resistance Genes (*qnrA* and *aac(6')-Ib-cr*) in Drug Resistant *Escherichia coli*, Sudan**

### **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')-Ib-cr*. **Objective:** The present study aimed to the 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')-Ib-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')-Ib-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) and 37.8% (45/119) of isolated *Escherichia coli* possessed *qnrA* and *aac(6')-Ib-cr* genes respectively. **Conclusion:** The high rate of *qnrA* and *aac(6')-Ib-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.

**Key Words:** *Escherichia coli*, Quinolone resistance, *qnrA*, *aac(6')-Ib-cr*, Multiplex-PCR, Sudan

## **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 (Aldred KJ, Kerns RJ, Osheroff N. Mechanism of quinolone action and resistance. *Biochemistry*. 2014 Mar 18;53(10):1565-74. doi: 10.1021/bi5000564. Epub 2014 Mar 7. PMID: 24576155; PMCID: PMC3985860). *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')-Ib-cr* related to prevalence of quinolone resistance genes [9, 10].

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

## **Methods**

### **Study setting and population**

This was a survey of major hospitals in Wad Madani city (Wad Madani Teaching Hospital, National Cancer Institute, Wad Medani Pediatrics Hospital, Wad Medani Teaching Hospital for Obstetrics and Gynecology). Study period was from August 2019 to March 2020. Enrolled participants were admitted patients suffer from clinical presentation of an infection.

### **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

### **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 (11). 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 (12). Isolates strains of *E.coli* were examined for antimicrobial sensitivity by disk diffusion method according to CLSI guidance (13). Commonly used antimicrobials; ciprofloxacin, gentamicin, cefixime, Amoxicillin/clavulanate, ceftriaxone, meropenem were used (Bioanalysis Co. Italy).

### **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.

### **Multiplex PCR**

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

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

Gene	Sequence	Product Size
<b>AcrA</b>	F TCTGATCGACGGTGACATCC R TCGAGCAATGATTTCTGCG	157
<b>aac(6')-Ib</b>	F ATTTCTCACGCCAGGATTTG R GATCGGCAAAGGTTAGGTCA	516
<b><i>Escherichia coli</i> 16 rRNA</b>	F TGTTGCTGAAGGAGTTGGGC R ACGACGGCATAGTCATTTGC	340

“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)”. [33]

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.

### **Preparation of primers**

Primers were prepared according to manufacture instructions (13).

### **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.

**Results:**

**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.

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

Hospital	Sample type					Total
	wound Swab	Urine	Throat Swab	Ear Swab	Sputum	
Wad Medani Emergency Hospital	0	8	0	0	2	10
Wad Medani Teaching Hospital (Surgery department)	15	0	0	0	0	15
Wad Medani Teaching Hospital for Obstetrics and Gynecology.	4	26	0	0	0	30
Gezira Hospital For Renal Diseases and Surgery	4	20	0	0	0	24
National Cancer Institute	08	06	0	0	0	14
Wad Medani Pediatric Hospital	4	04	0	03	0	8
National Center for Pediatric	10	0	0	0	0	10

<b>Surgery</b>						
<b>Wad Medani Teaching Hospital (ENT department)</b>	04	0	2	0	0	4
<b>Total</b>	51	64	2	3	2	119

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

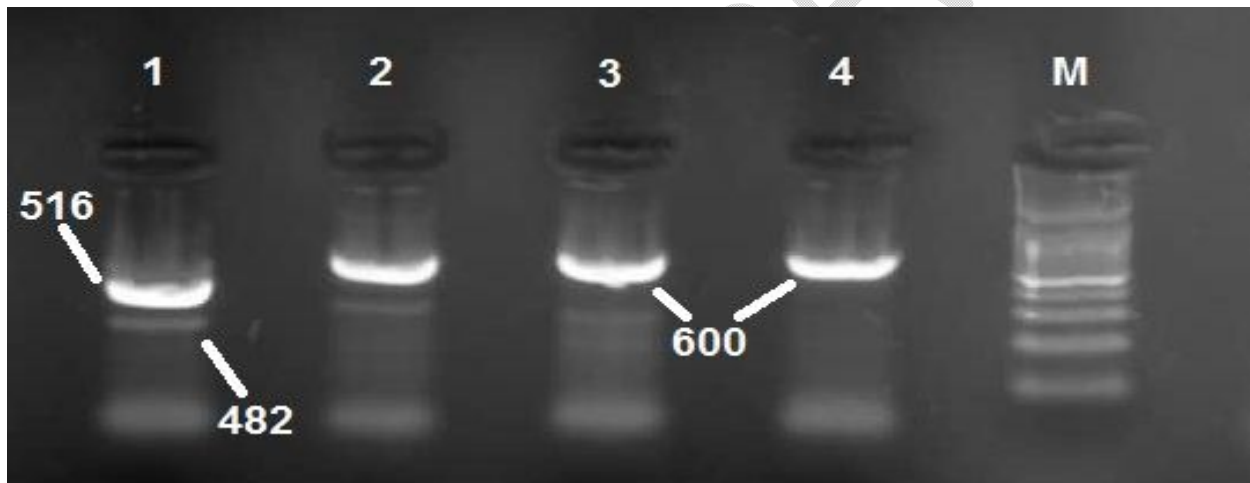
<b>Antibiotic</b>	<b>Resistance %</b>	<b>Intermediate %</b>	<b>Sensitive %</b>
<b>Ciprofloxacin</b>	55	05	40
<b>Gentamicin</b>	30	03	67
<b>Cefixime</b>	61	12	27
<b>Ceftriaxone</b>	68	20	12
<b>Meropeneme</b>	13	01	85
<b>Amoxicillin/Clavulanic Acid</b>	85	03	12

### 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)-Ib-cr* gene, respectively, showing that qnrA was circulating with a high frequency”. [33]

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

No of Genes detected	Type of Genes	No of isolated organisms	Percentage %
2 Genes	qnrA + aac (6)-Ib-cr	21	17.6 %
1 Gene	qnrA	28	23.5 %
1 Gene	aac (6)-Ib-cr	24	20.1 %
Zero gene	No genes	43	36.1 %



**Figure 1.** Genotyping of resistant genes of *Escherichia coli* using multiplex PCR. Lane 1: The 482 bp band for *aac (6)-Ib-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).

### 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')-Ib-cr* [14].

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 [15]. In contrast, in another study conducted by Deshmukh *et al.*, male were more frequent [16]. However, risk factors such sexual activities and personal hygiene have been attributed to the high infection rates among female patients [17, 18]. 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.*, 2018 (19). A study discussed the reasons for using over-the-counter treatment and stated that the main reason is the long distance to health services [19]. It is a problem mainly in developing countries where the health system is weak [20]. 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 % [21]. Rate of ciprofloxacin among the isolates was 55%, similar results were obtained in studies conducted in Sudan [22, 23]. Results from Sudan were in agreement with studies conducted in Thailand where high resistance to ciprofloxacin, ceftazidime and cefotaxime were reported [24, 25]. It can be said that, geographical variation of ciprofloxacin resistance could be an emerging problem in both developed and developing countries [26].

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* [27]. 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 [28]. 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* [29]. Other findings were in accordance observed in Niger and Togo in which the rating of qnr gene was 47% and 44.4% respectively [30, 31].

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% [32].

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.

### **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.

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