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Prevalence of plasmid-mediated quinolone resistance and 16S rRNA methylase genes among *Escherichia coli* clinical isolates in a hospital in Saudi Arabia

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Abstract: The plasmid-mediated quinolone resistance (PMQR) and 16S ribosomal RNA (rRNA) methylase genes can lead to high-level bacterial resistance to various antibiotics, including quinolones and aminoglycosides. This study determined the prevalence of PMQR and 16S rRNA methylase determinants in clinical *Escherichia coli*. Non-duplicate *E. coli* resistant to quinolones (nalidixic acid, ciprofloxacin, or norfloxacin) and/or aminoglycosides (amikacin or gentamicin) were collected from patients at King Abdullah Hospital, Bisha, Saudi Arabia. A multiplex PCR was performed to identify the targeted genes. Out of 107 screened isolates, 44 (41%) were found to carry resistance genes, individually or in combination, including 41 PMQR and 14 16S rRNA methylase. The *qnrS* gene had the highest prevalence (23.4%) among PMQR, followed by *aac(6')-Ib* (16.8%). Of the 14 identified 16S rRNA methylase, 8 were *rmtB* and 6 were *armA*. Out of 44 positive isolates, 72.7% carried only one resistance gene, 25% had co-existing resistance genes, with *qnrS* and *rmtB* or *aac(6)-Ib* and *armA* being the most common pairs. A single isolate was carried three genes (*qnrS*, *aac(6')-Ib* and *rmtB*). As antibiotic resistance continues to become more prevalent, there is a need for extensive research to identify the genetic determinants of resistance and to develop new antibiotic therapies.

Keywords: PMQR, 16S rRNA methylase genes, *Escherichia coli,* Saudi Arabia.

INTRODUCTION

Escherichia coli is one of the major causes of human infections, including urinary tract infections, gastrointestinal infections, wound infections, pneumonia and meningitis (Nsofor *et al*., 2021). Quinolones and amino glycosides are widely used antibiotics for treating Gram-negative bacterial infections, including those caused by *E. coli* (Balkhy *et al*., 2020; Mutair *et al*., 2021; Nsofor *et al*., 2021). The rapid dissemination of antibiotic-resistant bacteria, especially against these therapeutic agents, poses challenges in daily clinical practice (Alshahrani *et al*., 2022; Ibrahim, 2023; Qamar *et al*., 2019).

In 2014, the World Health Organization (WHO) identified fluoroquinolone resistance in *E. coli* and related gram-negative bacteria as a principal public health threat (Kibwana *et al*., 2023). Saudi Arabia has witnessed an increasing trend of resistance to quinolones and amino glycosides, which has varied over time (Balkhy *et al*., 2020; Mutair *et al*., 2021). Over a decade now (2007-2016), the overall resistance rates in pathogenic *E. coli* within a multi-hospital healthcare system were 34.8% for aminoglycosides and 43.9% for fluoroquinolones (Balkhy *et al*., 2020). A recent study conducted between 2015 and 2019 reported an elevated resistance rate of 64.9% among *E. coli* isolates against aminoglycosides (Mutair *et al*., 2021).

Plasmid-mediated quinolone resistance (PMQR) genes have been recognized as key determinants that confer resistance not only to quinolones but also to other antibiotics, including β-lactams and amino glycosides, in *Enterobacteriaceae*. These PMQR determinants include five variants of the *qnr* gene family (*qnrA, qnrB, qnrS, qnrC and qnrD*), which protect DNA gyrase from inhibition by quinolones, amino glycoside-modifying enzymes such as *aac (6')-Ib* and antibiotic efflux pump-encoding genes such as *oqxAB* and *qepA* (Kibwana *et al*., 2023; Salah *et al*., 2019). The *qnrA* gene family comprises six variants (*qnrA1* to *qnrA6*) and has been identified in *Enterobacteriaceae* (Cattoir *et al*., 2007). Additionally, *qnrB* (with six variants) and *qnrS* (with two variants) are well-documented in gram-negative bacteria (Cattoir *et al*., 2007). The *aac (6')-Ib* is the most prevalent amino glycoside-modifying enzyme that confers resistance to amikacin, tobramycin and kanamycin (Ahmed *et al*., 2023).

Resistance to aminoglycoside in gram-negative bacteria is attributed to aminoglycoside-modifying enzymes and 16S ribosomal RNA (rRNA) (Ghafoor *et al*., 2021). In *E. coli*, methylation of 16S rRNA by methylases reduces binding to aminoglycosides, leading to increased resistance to amikacin, gentamicin, tobramicin and kanamycin (Yang and Hu, 2022). Currently, seven 16S rRNA methylase genes are known (*armA, rmtA, rmtB, rmtC, rmtD, rmtE, rmtF and npmA*), with *armA* and *rmtB* being the most commonly spreading genes (Nasiri *et al*., 2018).

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The PMQR and 16S rRNA methylase genes have been observed to foster high-level bacterial resistance to quinolones, aminoglycosides and other antibiotics, potentially leading to treatment failures in clinical settings worldwide (Cirit *et al*., 2019; Ghafoor *et al*., 2021; Kibwana *et al*., 2023). Numerous studies have investigated the molecular mechanisms associated with aminoglycoside and quinolone resistance in *E. coli* in Saudi Arabia. In the capital city of Riyadh, a survey found that among the PMQR-tested genes in *E. coli* isolated from hospitalized patients, only *qnr* and *aac(6')-Ib-cr* were prevalent (Al-agamy *et al*., 2018). Another report from Makkah city detected that 43% of uropathogenic *E. coli* carried the *aac(6')-Ib* gene (Alyamani *et al*., 2017). Conversely, the 16S rRNA methylase genes, predominantly *rmtD,* have been documented in *Klebsiella pneumoniae* collected from tertiary hospitals in Makkah (Ahmed *et al*., 2023). However, data regarding the emergence of aminoglycoside and quinolone resistance genes remains limited. Molecular characterization of diverse antimicrobial resistance genes offers essential insights for controlling the spread of multi-drug-resistant pathogens and facilitating the selection of appropriate treatments for bacterial infections (Fatima *et al*., 2018; Ibrahim *et al*., 2021). The present study examined 107 clinical *E. coli* isolates collected from a referral hospital in Saudi Arabia, screening for PMQR and 16S rRNA methylase resistance genes.

MATERIALS AND METHODS

Bacteria isolates

A total of 107 non-duplicate clinical *E. coli* isolates were collected at King Abdullah Hospital, located in Bisha, southern Saudi Arabia, from June to September 2023. These isolates were obtained from clinical samples gathered at the hospital's microbiology laboratory during routine investigations of infectious diseases. The majority of isolates were sourced from urine (56.1%; 60), followed by wound pus (21.5%; 23), sputum (15.9%; 17) and blood (6.5%; 7). Selection for inclusion in the study was based on their resistance to at least one antimicrobial agent, either a quinolone (nalidixic acid, ciprofloxacin, or norfloxacin) or an aminoglycoside (amikacin, or gentamicin). Additional inclusion criteria were patients aged ≥15 years and the presence of a single significant growth of *E. coli* in their clinical sample. Patients with insufficient clinical data or those previously admitted during the last three months were excluded from the study.

Antimicrobial susceptibility testing

The antibacterial susceptibility testing of *E. coli* isolates was conducted using Vitek 2 and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guideline breakpoints (CLSI, 2023). The tested antibiotics included amikacin (30µg), amoxicillin/clavulanate (20/10 µg), ampicillin (10µg), aztreonam (30µg), cefotaxime (30µg), ceftazidime (30µg), cefuroxime (30µg), ciprofloxacin (5µg), colistin (10µg), co-trimoxazole (25 µg), gentamicin (10µg), imipenem (10µg), meropenem (10µg), nalidixic acid (30µg), norfloxacin (5µg), piperacillin (100µg) and piperacillin/ tazobactam (100/10 µg). *E. coli* American Type Culture Collection (ATCC) 25922 served as the control strain for antibiotic susceptibility testing. The final antibiotic susceptibility results for the isolates were interpreted and categorized as either susceptible or resistant.

Multiplex PCR for the detection of resistance genes

For the detection of PMQR (*qnrA*, *qnrB*, *qnrS,* and *aac (6')-Ib*) and 16S rRNA methylase (*armA* and *rmtB*) resistance genes, *E. coli* isolates underwent screening using multiplex PCR assays. The screening employed a set of oligonucleotide primers (table 1), following previously established protocols (Cattoir *et al.,* 2007). To prepare DNA templates for PCR reactions, 1 to 2 pure colonies of the isolates were suspended in test tubes containing 1000ml of sterile, free RNA-distilled water. These suspensions were subjected to heating in a water bath at 95°C for 10 minutes, followed by cooling to room temperature and subsequent centrifugation at 15,000 rpm for 5 minutes to pellet the cell debris. The resulting supernatants were transferred into sterile Eppendorf tubes and utilized as DNA templates for the PCR reaction mixture. The PCR reaction was conducted in the Eppendorf Master Cycler Gradient instrument, with a total volume of 50µL for each reaction. Each reaction mixture comprised 25µL of Hot Star Taq Plus Master Mix (Qiagen GmbH, Hilden, Germany), 4µL of DNA template, a variable volume of a specific primer group, and 9µL of nuclease-free water. The PCR methods consisted of initial heat activation at 95◦C for 10 minutes, followed by 35 cycles of denaturation at 94◦C for 45 seconds, annealing (for quinolone genes optimized at 55◦C for 45 seconds and for aminoglycoside genes optimized at 54.5◦C for 45 seconds) and extension at 72◦C for 1 minute. The cycling concluded with a final extension step at 72◦C for 10 minutes. Then, 7 to 10µl of the PCR products were loaded onto a 2% agarose gel containing ethidium bromide (1µg/mL) for rapid gel electrophoresis, performed at 90 volts for 30 minutes. Positive controls, consisting of *Klebsiella pneumoniae* strains with known *qnr*, *armA* and *rmtB* genes from our previous study (Ahmed *et al*., 2023), were utilized for validation. The resulting amplification products were visualized under ultraviolet light at a wavelength of 312 nm to determine the presence of target genes. To ascertain the size of the PCR products, a 100-bp DNA ladder (Bioline, London, UK) was used as a standard molecular weight reference.

Ethical approval

Ethical approval for this study was granted by the Research Ethics Local Committee at the College of Medicine, University of Bisha, Saudi Arabia (UB-RELOC

H-06-BH-087/(0606.23) and it adhered to the ethical principles outlined in the Helsinki Declaration. Informed consent was not sought for this study, as the isolates and their associated data were collected as part of the routine clinical sample processing at the hospital laboratory.

STATISTICAL ANALYSIS

Statistical analyses were performed using Statistical Package for Social Sciences (IBM SPSS) version 28.0 (SPSS Inc., Chicago, IL, USA). The Fisher's exact test and the chi-square tests were employed to compare the demographic variables and the presence of resistance genes. All results with p-value < 0.05 were considered statistically significant.

RESULTS

A total of one hundred and seven *E. coli* isolates were recovered from clinical samples from 107 patients. Among these, 60 (56.1%) were males, and 47 (43.9%) were females. The patient's ages ranged from 18 to 97 years old, with a mean age of 59.2 years old (SD = 18.7 years old). The majority of the patients (48.6%; 52) were aged ≥65 years old, followed by those aged 45–64 years old (31.8%; 34), 25-44 years old (11.2%; 12), and 15-24 years old (8.4%; 9). Additionally, twenty-seven (25.2%) of patients had one or more chronic illnesses.

E. coli isolates were retrieved from various hospital wards, including ICU (22.4%; 24), surgery (18.7%; 20), urology (17.8%; 19), medical (15%; 16), chronic high-dependency unit (12.1%; 13), critical care unit (7.5%; 8) and emergency (6.5%; 7). Most of the isolates were obtained from urine samples (56.1%; 60), followed by wound pus (21.5%; 23), sputum (15.9%; 17) and blood (6.5%; 7).

Prevalence of PMQR and 16S rRNA methylase genes

Fig. 1 depicts a representative image of the agarose gel electrophoresis that shows different types of PMQR and 16S rRNA methylase genes. Out of the 107 tested isolates, 44 (41.1%) were found to carry at least one PMQR or 16S rRNA methylase gene. PMQR genes were identified in 38.3% (41/107) of *E. coli* isolates, either individually or in combination. The *qnrS* (*qnrS1* to *qnrS2*) gene was identified in 25 (23.4%) isolates, while *aac(6')-Ib* was detected in 18 (16.8%) isolates. However, none of the screened PMQR genes were found in other isolates. figs. 1A and 1B show the amplification products of the *qnrS* and *aac (6')-Ib* genes. The 16S rRNA methylase genes were identified in 13.1% (14/107) isolates, with 8 (7.5%) harboring the *rmtB* gene and 6 (5.6%) containing the *armA* gene. Fig. 1 (C and D) illustrates the PCR products of the *rmtB* and *armA* genes.

Table 2 presents the frequency of PMQR and 16S rRNA methylase resistance genes in *E. coli* isolates. Out of the isolates that were found to carry PMQR and 16S rRNA methylase genes, 72.7% (32 out of 44) were observed to have a single resistance gene, with *qnrS* (n=18) being the most common, followed by *aac(6')-Ib* (n=11). Co-existing two resistance genes were identified in 25% (11/44) of isolates. The following gene pairs were found: *qnrS* and *rmtB* (n=4); *aac(6')-Ib* and *armA* (n=4); *aac(6')-Ib* and *rmtB*; (n=1); *qnrS* and *armA* (n=1); *qnrS* and *aac(6')-Ib* (n=1). Only one isolate carried a triple gene combination (*qnrS*, *aac (6')-Ib* and *rmtB*). A representative image of the agarose gel electrophoresis displaying two co-existing genes in screened isolates is illustrated in figs. 1C and 1D.

Antibiotic resistance patterns

The antibiotic resistance patterns of 107 *E. coli* isolates against tested antibiotics are shown in fig. 2. Of the isolates examined, 71 (66%) were found to be resistant to the quinolone antibiotics. Specifically, 77.6% of the isolates were resistant to nalidixic acid, 66.4% were resistant to ciprofloxacin, and 54.2% were resistant to norfloxacin. of the quinolone-resistant isolates, PMQR genes were present in 50% (29/58) of the norfloxacin-resistant isolates, 43.4% (36/83) of the nalidixic acid-resistant isolates and 42.3% (30/71) of the ciprofloxacin-resistant isolates.

Of the 107 *E. coli* isolates tested, 50 (46.8%) were resistant to the screened aminoglycoside antibiotics. Specifically, 49 (45.8%) isolates were resistant to amikacin and 51 (47.7%) were resistant to gentamicin. Among the isolates resistant to aminoglycosides, 10 out of 49 (20%) amikacin-resistant isolates and 9 out of 51 (18%) gentamicin-resistant isolates carried 16S rRNA methylase genes. The frequency of PMQR and 16S rRNA methylase gene variants among quinolone and aminoglycoside-resistant isolates is detailed in table 3.

Association between resistance genes and the source of isolates

Table 4 presents the distribution of *E. coli* isolates containing various resistance genes (n = 44) based on the general information of the patients. The proportion of resistance genes was almost equal in both male and female patients (41.7% vs. 40.4%). Most of the resistance gene-carrying isolates were found in sputum samples (64.7%; 11), patients aged 65 years or older (53.8%; 52), and patients in the Chronic High-Dependency Unit (CHDU) (76.9%; 10). Significant differences were observed between the presence of isolates carrying genes and the age group of the patients (p=0.038) and patients with comorbidities (p=0.027).

DISCUSSION

This study presents the first report on the existence of PMQR and 16S rRNA methylase genes in pathogenic *E.*

Table 1: Oligonucleotide primers used for amplification of PMQR and 16S rRNA methylases genes in *E. coli* isolates

Gene's name	Primer	Sequence	Size (bp)	Ref.
PMQR gene				
*qnrA1*to *qnrA6*	*qnrA*-F *qnrA*-R	AGAGGATTTCTCACGCCAGG TGCCAGGCACAGATCTTGAC	580	(Cattoir *et al*., 2007)
*qnrB1*to *qnrB6*	*qnrB*-F *qnrB*-R	GGMATHGAAATTCGCCACTG TTTGCYGYYCGCCAGTCGAA	264	(Cattoir *et al*., 2007)
qnrS1 to *qnrS2*	*qnrS*-F *qnrS*-R	GCAAGTTCATTGAACAGGGT TCTAAACCGTCGAGTTCGGCG	428	(Cattoir *et al*., 2007)
aac(6')-Ib	*aac(6')-Ib*-F *aac(6')-Ib*-R	TTGCGATGCTCTATGAGTGGCTA CTCGAATGCCTGGCGTGTTT	482	(Cirit *et al*., 2019)
16S rRNA methylase				
armA	*armA*-F *armA*-R	ATTCTGCCTATCCTAATTGG ACCTATACTTTATCGTCGTC	315	(Nasiri *et al*., 2018)
rmtB	*rmtB*-F *rmtB*-R	GCTTTCTGCGGGCGATGTAA ATGCAATGCCGCGCTCGTAT	173	(Nasiri *et al*., 2018)

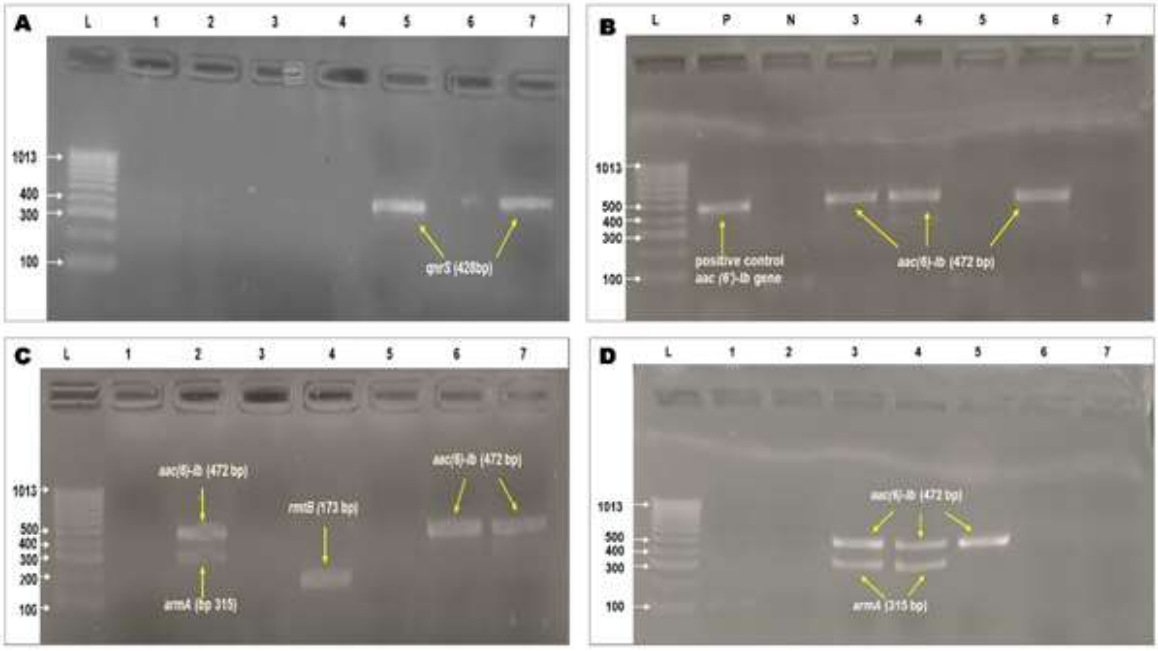

Fig. 1: A representative agarose gel electrophoresis displays PCR products using primers designed to detect PMQR and 16S rRNA methylase genes in *Escherichia coli*. The lanes (L) are: 100 bp DNA ladder (A) Lanes 5 and 7 represent the *qnrS* gene. (B) Lane P is a positive control of *aac (6')-Ib* gene, lane N is a negative control, Lanes 3, 4, and 6 represent the *aac (6')-Ib* gene. (C). Lane 2 represents the *armA* gene, and Lane 4 represents the *rmtB* gene. Lanes 6 and 7 represent the *aac (6')-Ib* gene. Lane 2 demonstrates the co-existence of two genes (*aac (6')-Ib* and *armA*). (D) Lanes 3 and 4 show the co-existence of two genes.

coli isolated from a referral hospital in southern Saudi Arabia. The current findings revealed a 38.3% prevalence of PMQR genes among 107 *E. coli* isolates.

However, among the quinolone-resistant isolates, 46.8% were positive for either *qnrS* or *aac (6')-Ib,* indicating the predominance of these gene types. Other tested PMQR gene determinants were absent. These results align with previous reports from Saudi Arabia. The *qnrS* gene was initially detected in *E. coli* and *K. pneumoniae* isolated from hospitalized patients in 2014 in Saudi Arabia (Al-agamy *et al*., 2018). Al-Agamy *et al.* found that *qnrS* was the most frequently encountered PMQR gene (61.3%; 19/31), followed by the *aac (6')-Ib-cr* gene (51.6%; 16/31) (Al-agamy *et al*., 2018). Another study in Riyadh reported a high prevalence of the *aac(6')-Ib-cr* variant, conferring resistance to ciprofloxacin in extended-spectrum beta-lactamase (ESBL)-producing *E. coli* and other *Enterobacteriaceae* (Shibl *et al*., 2012). The dissemination of PMQR gene determinants has been

documented in Mediterranean countries, with prevalence varying relatively widely depending on the sources, population, selection criteria of the strains studied, and geographical locations (Yanat *et al*., 2017).

Table 2: Frequency of PMQR and 16S rRNA methylase resistance genes among *Escherichia coli* isolates

Gene combination	Number of isolates
Single	
qnrS1 to *qnrS2*	18
aac(6')-Ib	11
rmtB	2
armA	1
Total	32
Two genes	
qnrS & *rmtB*	4
aac(6')-Ib & *armA*	4
aac(6')-Ib & *rmtB*	1
qnrS & *armA*	1
qnrS & *aac(6')-Ib*	1
Total	11
Three genes	
qnrS1 to *qnrS2* & *aac(6')-Ib* & *rmtB*	1
Overall	44

Consistently, a high prevalence of *aac(6')-Ib-cr* (66.7%) and *qnr* (61.1%) has been detected in 134 clinical *E. coli* isolates in Egypt, contributing to the widespread quinolone resistance in the country (Abdel-Rhman *et al*., 2021). Iraq has also reported the predominance of *aac(6')-Ib-cr*, *qnrS* and *qnrB* among uropathogenic *E. coli* (Alm'amoori *et al*., 2020). Reports from developed countries also indicate the global spread of PMQR gene variants. In the United States, *qnrA, qnrB, and qnrS* have been identified as emerging in non-typhoidal Salmonella-causing human diseases(Karp *et al*., 2018). In Spain, PMQR was detected in 31.8% of clinical Enterobacteriaceae isolates producing AmpC β-lactamase and/or carbapenemase genes (Machuca *et al*., 2017). These findings, along with our present results, underscore the global distribution of PMQR gene variants, emphasizing the continued importance of surveillance programs for monitoring the occurrence of PMQR genes among bacterial pathogens.

The present study revealed associations between the presence of *qnrS* and *aac(6')-Ib* in *E. coli* isolates and reduced susceptibility to norfloxacin, nalidixic acid and ciprofloxacin. The role of PMQR genes in reducing the susceptibility of gram-negative bacteria to quinolones and other antibiotics has been well-documented in various studies (Alm'amoori *et al*., 2020; Nsofor *et al*., 2021; Yanat *et al*., 2017). Research evidence indicates that the *qnrS* gene confers low-level resistance to fluoroquinolones, facilitating the selection of mutants under antibiotic-selective pressure and contributing significantly to treatment failures (Babosan *et al*., 2022; Salah *et al*., 2019). PMQR resistance genes are usually carried by the same plasmids that encode resistance to various antibiotic agents, such as extended-spectrum β-lactamase agents, trimethoprim, aminoglycosides, sulphonamides, and tetracycline (Al-agamy *et al*., 2018; Kibwana *et al*., 2023). The emergence of bacterial pathogens harboring such plasmids is of great concern since these elements can transfer between bacterial species in hospital settings, leading to nosocomial outbreaks and challenging infection control measures (Abdalhamid *et al*., 2017). Future studies focusing on plasmid analysis will be necessary to gain a deeper understanding of the successful dissemination of PMQR genes (Yanat *et al*., 2017).

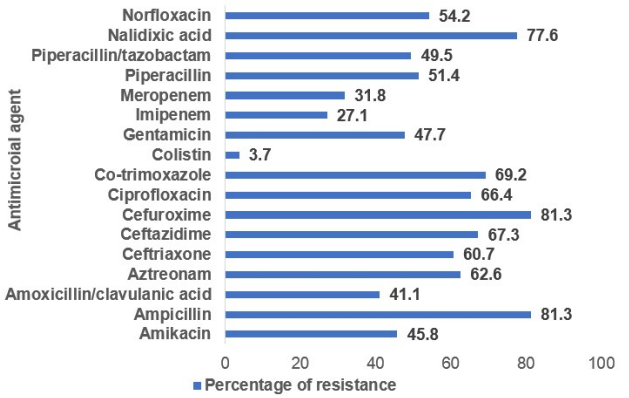

Fig. 2: Antibiotic-resistant patterns of *Escherichia coli* isolates

In the present study, the overall prevalence of 16S rRNA methylase genes in the examined *E. coli* isolates was 13.1%, with *rmtB* and *armA* types being the most common. A previous study conducted in Riyadh, the central region of Saudi Arabia, reported that 48% (24 out of 50) of *E. coli* isolates carried *rmtB*, *armA,* and *rmtC* genes (Sheikh *et al*., 2014). Similarly, the existence of *rmtB* and *armA* genes in members of the *Enterobacteriaceae* family, with over half of them being *E. coli* isolates, has been documented in the eastern region of Saudi Arabia (Abdalhamid *et al*., 2017). A study conducted in 11 Chinese teaching hospitals found a high prevalence of the plasmid-mediated *rmtB* gene among clinical *K. pneumoniae* isolates associated with bloodstream infections in China (Shen *et al*., 2020). On the contrary, a Turkish study found that the occurrence of 16S rRNA methylases is limited among clinical isolates of *E. coli* and *K. pneumoniae* (Cirit *et al*., 2019).

In the present study, although only two types of 16S rRNA methylase (*armA* and *rmtB*) were screened among the isolates, the findings revealed that 12.2% of amikacin resistance and 11.8% of gentamicin resistance could be attributed to the *armA* gene. Additionally, *rmtB* gene-carrying isolates were detected in 8.2% amikacin-resistant and 5.9% gentamicin-resistant isolates. Fortunately, the

Table 3: Distribution of PMQR and 16S rRNA methylase genes among quinolone and aminoglycosides-resistant *Escherichia coli*

Quinolone	No. of resistance isolates	n(%)PMQR	*qnrS*	*aac(6')- Ib*	*qnrS+ aac(6')- Ib*
Ciprofloxacin	71	30 (42.3)	18 (25.3)	11 (15.5)	1(1.4)
Nalidixic acid	83	36 (43.4)	22 (26.5)	12 (14.5)	2 (2.4)
Norfloxacin	58	29 (50)	19 (32.8)	8 (13.8)	2 (3.4)
Aminoglycoside	No of resistant isolate	n(%)16S rRNA methylase	*armA*	*rmtB*	*armA + rmtB*
Amikacin	49	10 (20.4)	6 (12.2)	4 (8.2)	0
Gentamicin	51	9 (17.6)	6 (11.8)	3 (5.9)	0

Table 4: Association between the presence of PMQR and 16S rRNA methylase carrying *E. coli* and baseline information of the patients.

Baseline	n(%) of positive resistance gene(s) carrying isolates (either alone or in combination)	PMQR		16S rRNA methylase		P value
		qnrS1 to qnrS2	*aac(6)-Ib*	*armA*	*rmtB*	
Gender						.897
Male (n=60)	25 (41.7)	15	9	3	3	
Female (n=47)	19 (40.4)	10	9	3	5	
Sample						.091
Urine (n=60)	23 (38.3)	12	10	3	3	
Wound (n=23)	9 (39.1)	6	3	0	3	
Sputum (n=17)	11 (64.7)	6	5	3	2	
Blood (n=7)	1 (14.3)	1	0	0	0	
Age group						.038
15-24 (n=9)	4 (44.4)	4	1	0	1	
25-44 (n=12)	4 (33.3)	4	0	0	1	
45-64 (n=34)	8 (23.5)	2	5	0	1	
≥65 (n=52)	28 (53.8)	15	12	6	5	
Wards						.083
ICU (n=24)	10 (41.7)	4	5	4	0	
Medical (n=16)	5 (31.3)	3	2	0	0	
Surgery (n=20)	9 (45.0)	4	4	0	4	
Urology (n=19)	4 (21.1)	4	0	0	0	
CHDU (n=13)	10 (76.9)	7	4	2	2	
CCU (n=8)	3 (37.5)	1	2	0	0	
Emergency (n=7)	3 (42.9)	2	1	0	1	
Comorbidities						.027
Yes (n=27)	16 (59.3)	7	7	5	3	
No (n=80)	28 (35.0)	18	11	1	5	

low prevalence of aminoglycoside resistance among *E. coli* isolates carrying 16S rRNA methylase genes may suggest that this agent could be a suitable choice for treating infections caused by such gene-producing isolates in our clinical setting.

However, global studies have indicated that the newer 16S rRNA methylase determinant is growing and contributing to the dissemination of aminoglycoside-resistant pathogens (Nasiri *et al*., 2018; Shen *et al*., 2020; Yang and Hu, 2022). Microorganisms carrying 16S rRNA methylase determinants are likely to exhibit resistance to multiple antibiotics, including beta-lactams (Ahmed *et al*., 2023). According to a study conducted in China, it was found that the majority of microorganisms producing 16S rRNA methylase genes were extended-spectrum β-lactamase (ESBL) producers and each isolate carried at least one carbapenemase gene. As a result of this, the use of combinations of aminoglycoside and beta-lactam antibiotics may become ineffective in treating pathogens carrying 16S rRNA methylase genes (Shen *et al*., 2020). It has been well documented that bacteria resistant to aminoglycosides and carrying genes such as *aac(6')-Ib/Ib-cr*, *rmtB,* and *armA* are often resistant to multiple other antibiotics.(Ahmed *et al*., 2023; Bodendoerfer *et al*., 2020; Cirit *et al*., 2019) Therefore, implementing

effective infection-control measures and early identification of these resistance mechanisms will help in optimizing antimicrobial therapy.

In the present study, it was observed that the majority of isolates carried only one PMQR gene. This is consistent with a previous report, which found that a single PMQR was present in 83.7% of Enterobacteriaceae isolates (Machuca *et al*., 2017). Nevertheless, the current study also revealed the co-existence of two PMQR and 16S rRNA methylase genes in 11 isolates. This co-existence was particularly common between *qnrS* and *rmtB* (n=4) and between *aac(6')-Ib* and *armA genes* (n=4). This phenomenon can be attributed to the frequent occurrence of PMQR gene determinants on the same resistance plasmids that confer resistance to aminoglycoside agents (Kibwana *et al*., 2023).

The present study has several limitations. Firstly, it was conducted as a single-center study; therefore, a multicenter evaluation is needed to generalize these findings. Secondly, the study included only the isolates that were resistant to aminoglycoside or quinolone antibiotics. However, it has been suggested that there is a strong association between susceptible *E. coli* isolates and the presence of PMQR and 16S rRNA methylase-resistant genes, which were determined in this study. Thirdly, the study screened isolates for the prevalence of only two variants of 16S rRNA methylase genes. Thus, the findings regarding the prevalence of 16S rRNA methylase among *E. coli* isolates represent a minimum estimation.

CONCLUSION

This study offers important information about how common and where certain genes that cause resistance to antibiotics are found in pathogenic *E. coli* collected from southern Saudi Arabia. The predominant PMQR genes identified in *E. coli* were *qnrS* and *aac (6')-Ib,* while *the* 16S rRNA methylase genes *rmtB* and *armA* were also detected. The detection of these resistance genes is a matter of concern as they can contribute to the spread of resistance across various antibiotic classes. These results underscore the importance of ongoing, long-term surveillance efforts to monitor the spread of resistant pathogens and assess the use and outcome of empirical therapy in clinical settings. As antibiotic resistance continues to become more prevalent, there is a need for extensive research to identify the genetic determinants of resistance and to develop new antibiotic therapies.

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