Molecular Detection of blaNDM, qnrA, and mcr-1 Clinical *Escherichia Coli* Isolates From Baqubah Teaching Hospital, Diyala, Iraq.

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Abstract

Antimicrobial-resistant genes such as blaNDM, qnrA, and mcr-1 have emerged as a major global threat, with their prevalence increasing in many countries. International travel, hospital outbreaks, and the food chain have all contributed to their global dissemination. The aim of the study is to assess the prevalence of antimicrobial-resistant genes blaNDM, qnrA, and mcr-1, among *E. coli,* to compare these levels with the global estimates, and to identify risk factors associated with the carriage of these genes. Over seven months, clinical samples were collected from. Inpatients at Baqubh teaching hospital. *E. coli* isolates were identified by routine culture. Molecular diagnosis was performed by extracting genomic DNA and using validated primers for blaNDM, qnrA, and mcr-1 in a single Multiplex PCR reaction. Data management and prevalence estimation were performed with binomial exact Clopper–Pearson confidence intervals. The prevalence of blaNDM (6.9%) and qnrA (3.4%) appeared low relative to other countries' reports. Clopper–Pearson upper bounds were 22.7% and 18.1%, respectively, reflecting considerable statistical uncertainty due to the limited sample size. Regarding the mcr-1 gene, the point prevalence was 0%.

1. Introduction

Antimicrobial resistance in *Escherichia coli* poses a serious threat to global public health, particularly in healthcare settings where the overuse and inappropriate use of antibiotics exert strong selective pressure. Among the most clinically concerning determinants are the New Delhi metallo-beta-lactamase (blaNDM), which confers carbapenem resistance. The mobile colistin and resistance gene (mcr-1), which compromises the effectiveness of colistin as a last-resort treatment, and the plasmid-mediated quinolone resistance gene qnrA, the first PMQR gene discovered. The blaNDM family was first identified in 2008 and spread quickly in Europe via overseas healthcare exposure, with hospital-acquired infections and travel to the Indian subcontinent being major risk factors [1], [2].

Global studies conducted between 2000 and 2024 have shown an increasing and uneven burden of these genes in clinical *E. coli* cases. Meta-analyses indicate significant geographic variation and report a pooled prevalence of blaNDM of approximately 9.4% in hospital isolates of *E. coli [3]*[3]. Colistin resistance mediated by mcr-1 was first reported in China in 2015 in animal, food, and human sources. Human bloodstream infections and contaminated imported chicken meat contributed to its rapid global dissemination [4]. In many parts of the world, mcr-1 has been detected in clinical *E. coli* cases, with an estimated prevalence of approximately 4.2% in clinical settings [15].

In recent years, qnrA has continued to be carried on class 1 integrons and conjugative plasmids, where it alone can provide low-level resistance but facilitates the emergence of mutations in fluoroquinolone target sites and increased efflux activity [6]. Its spread is often mediated by IncX3 plasmids and insertion sequences such as ISAba125, which act as essential promoters [7].

The epidemiology of blaNDM, mcr-1, and qnrA in the health systems of many countries is strikingly underrepresented. To date, most large-scale genomic surveys derive only from high-income regions, leaving significant gaps in our understanding of resistance gene prevalence and plasmid diversity in Iraq and other Middle Eastern countries [8], [9].

The aim of this study is to (1) assess the prevalence of three antimicrobial resistance genes (blaNDM, mcr-1, qnrA) among clinical E. coli isolates obtained from Baqubah Teaching Hospital in Diyala, Iraq; (2) benchmark these levels against global average prevalence; and (3) identify patient and risk factors associated with carriage of these genes, thereby generating data for infection control and antibiotic management programs

2. Materials and Methods

2.1 Study Design and Setting

This partial study was conducted at Baqubah Teaching Hospital, a tertiary referral center serving urban and peri-urban districts of Diyala Governorate, over 7 months (February–November 2025). A total of 67 non-duplicate clinical samples were collected from routine clinical specimens (urine) during the study period. Inclusion criterion: culture-confirmed *E. coli* from inpatients or outpatients. The objectives were to estimate the prevalence of blaNDM, mcr-1, and qnrA among clinical *E. coli* isolates and to compare the findings with published global prevalence data.

2.2 Microbiological Culture

Species identification was performed using MacConkey agar, and colonies were confirmed by the VITEK system [10], [11].

2.3 Molecular Detection of Resistance Genes

Multiplex PCR was performed targeting blaNDM, mcr-1, and qnrA, as well as the 16S rRNA gene for *E. coli* confirmation. Primers and conditions followed established protocols [12]. Primers are listed in Table 1.

Table 1: Genes Target

Gene Target	Primer Name	Sequence (5'→3')	Product Size (bp)	Annealing Temp. (°C)
blaNDM	blaNDM-F	GGTTTGGCGATCTGGTTTTC	1538	55
	blaNDM-R	CGGAATGGCTCATCACGATC		
mcr-1	mcr-1-F	CGGTCAGTCCGTTTGTTC	309	
	mcr-1-R	CTTGGTCGGTCTGTAGGG		
qnrA	mcr-1-F	ATTTCTCACGCCAGGATTTG	621	51
	mcr-1-R	GACCTCGGTTTAGTTCACAGA		
16S rRNA	16S Rrna-F	GACCTCGGTTTAGTTCACAGA	585	53
	16S Rrna-R	CACACGCTGACGCTGACCA		

2.4 DNA Quantification

Quantus Fluorometer was used to detect the concentration of extracted DNA. For 1 µl of DNA, 200 µl of diluted Quant Fluor Dye was mixed. After 5 min incubation at room temperature, DNA concentration values were recorded [13].

2.5 Primer Preparation

Lyophilized primers were dissolved in nuclease-free water to a final concentration of 100 pmol/µl as a stock solution. A working solution (10 pmol/µl) was prepared by adding 10 µl of stock solution to 90 µl of nuclease-free water. Primer preparation volumes are listed in Table 2. Reaction Setup and Thermal Cycling Protocol are shown in Tables 3 and 4.

Table 2*:* Primer preparation volumes

Primer Name	Volume of Nuclease-Free Water (µl)	Concentration (pmol/µl)
blaNDM-F	300	100
blaNDM-R	290	100
mcr-1-F	300	100
mcr-1-R	320	100
qnrA-F	300	100
qnrA-R	300	100
16S Rrna-F	320	100
16S Rrna-R	300	100

Table 3: PCR Reaction Setup

Parameter	Details
Number of reactions	10 rxn
Reaction volume per run	20 µl
Annealing temperature (°C)	55,51,53
PCR product length (bp)	1538,309,621,585

Table 4: PCR Master Mix Components (per 1 reaction, 20 µl total

Component	Stock Concentration	Final Concentration	Volume (µl)
Master Mix (2X)	2X	1X	10
Forward Primer	10 µM	0.5 µM	1
Revers Primer	10 µM	0.5 µM	1
Nuclease-Free Water	-----	----	6
DNA Template	ng/µl	ng/µl	2
Total Volume			20

Aliquoting: Add 18 µl of the prepared master mix to each PCR tube, then add 2 µl of DNA template to reach a final volume of 20 µl. Note: The appropriate annealing temperature (55°C, 51°C, or 53°C) was used depending on the primer pair and target gene, as presented in Table 5.

Table 5: PCR Thermal Cycling Program

Step	Temperature (°C)	Time (min: sec)	Cycles
Initial Denaturation	95	05:00	1
Denaturation	95	00:30	30
Annealing	55/ 51 / 53	00:30	30
Extension	72	00:30	30
Final Extension	72	07:00	1
Hold	10	10:00	1

2.7 Statistical Analysis

Gene-specific prevalence was calculated as the number of positive isolates divided by the total number of isolates tested. Exact 95% confidence intervals were estimated using the binomial (Clopper–Pearson) method [9]. Prevalence (95% CI) = (Positive isolates / Total isolates) with Clopper–Pearson bounds.

3. Results

From 67 urine specimens, the distribution of bacterial isolates is shown in Table 6. E. coli constituted 43.2% (29 specimens), Klebsiella spp. 14.2% (10 specimens), Pseudomonas aeruginosa 5.9% (4 specimens), and 35.8% (24 specimens). Amplification of the 16S rRNA gene confirmed that all 29 isolates were E. coli (Fig. 1). Prevalence of blaNDM was 2/29 = 6.9% (Fig. 2). Prevalence of qnrA was 1/29 = 3.4% (Fig. 3). No isolate carried mcr-1 (0/29, 0%) (Fig. 4). Prevalence of Resistance genes with Binomial Confidence (Clopper – Pearson) in Table 7.

All patients had long hospitalization periods (1–3 weeks), received various antibiotics during hospitalization, used invasive devices, and were exposed to contaminated hospital environments. These factors likely contributed to the transmission of these resistance genes

Table 6: Distribution of bacterial isolates from urine samples

Sample size positive culture	Escherichia coli	Klebsiella spp.	Pseudomonas aeruginosa	Others
67	29 (43.2 %)	10 (14.2%)	4 (5.9 %)	24 (35.8%)

Table 7: Prevalence of Resistance genes with Binomial Confidence (Clopper – Pearson)

Gene	Prevalence	Exact 95% CI (Clopper – Pearson)	Positive / Total
blaNDM	6.9 %	0.8-22.7	2/29
qnrA	3.4%	0.1-18.1	1/29
mcr-1	0.0%	0.0- 9.8	0/9

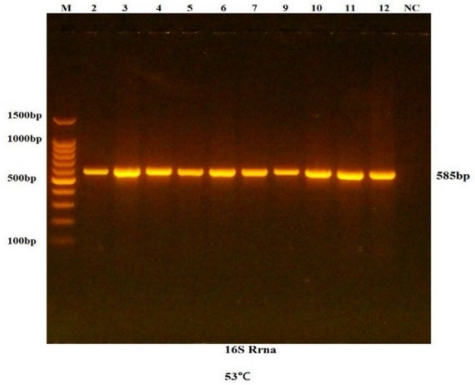

Fig. 1 Results of amplification of 16S rRNA gene of *Escherichia coli* samples were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes 2-12 resemble 585 bp PCR products, NC: negative control.

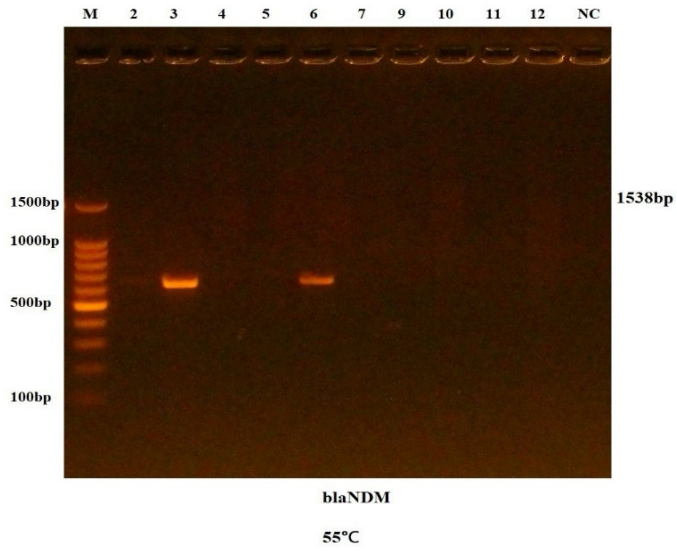

Fig. 2 Results of the amplification of **the** blaNDM gene of *Escherichia coli* samples species were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes 2-12 resemble 1538 bp PCR products, NC: negative control.

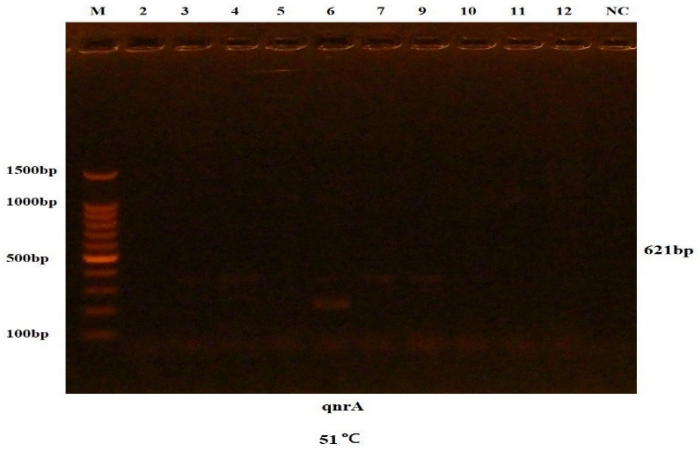

Fig. 3 Results of the amplification of qnrA gene of *Escherichia coli* samples species were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes 2-12 resemble 621 bp PCR products, NC: negative control.

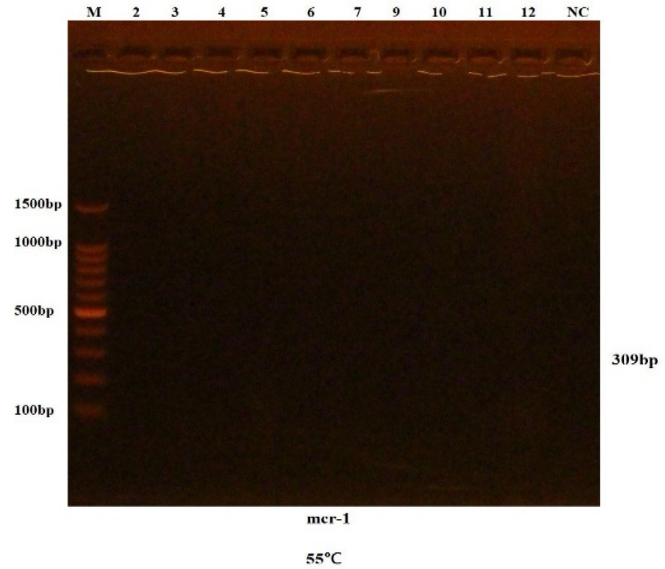

Fig. 4 Results of the amplification of the MCR-1 gene of *Escherichia coli* samples were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes 2-12 resemble 309 bp PCR products, NC: negative control.

4. Discussion

The point prevalence of blaNDM (6.9%) and qnrA (3.4%) appears low relative to reports from other countries. However, the Clopper–Pearson upper bounds (22.7% and 18.1%, respectively) reflect considerable statistical uncertainty due to the limited and small sample size. For mcr-1, the point prevalence was 0%, but the 95% CI widens to 9.8%, indicating that up to ~1 in 10 clinical isolates might carry this gene in the population.

Comparison with other countries – blaNDM: Reports from Iran and Turkey show proportionally low carriage of carbapenemase genes among clinical *E. coli* isolates with blaNDM prevalence commonly below 5% [14], [15]. In China, prevalence is mid-range (4%–12%) [16], [17]. India reports prevalence up to 20%, reflecting endemic NDM-bearing plasmids [17]. Pakistan, Bangladesh, and Egypt have high prevalence (10%–25%, 5%–15%, and 5%–10%, respectively) [18], [19], [20]. The rate in this study (6.9%) falls within the average range, but the wide CI overlaps both low and high -burden sitting.

Comparison qnrA: The prevalence of qnrA in this study (3.4%) is nearly equal to or lower than regional and international reports. The CI (0.1%–18.1%) extends across the entire regional spectrum [21], [22]. In China, Bangladesh, Pakistan, and Egypt, qnrA prevalence generally ranges from 3% to 12% [19], [20], [23], and [24]. India has reported rates up to 15%. In China, Bangladesh, Pakistan, and Egypt, qnrA Prevalence generally ranges from 3% to 12%. India has reported rates up to 15%.

Comparison – mcr`-1: Human clinical sample surveys detect mcr-1 at rates of (0-7%) [25], [26]..The zero percent result in this study aligns with the lower end, although the CI indicates that a moderate prevalence cannot be ruled out.

5. Conclusions

The prevalence of blaNDM and qnrA was moderate, while mcr-1 was not detected. However, the right confidence intervals and inherent mobility of these genes highlight the need for ongoing surveillance. Standardized molecular surveillance and rigorous antimicrobial stewardship should be established to limit further spread.

Conflict of interests

The authors declare no conflict of interest.

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