https://doi.org/10.54361/ajmas.261124

Original article

Prevalence and Distribution of *bla*-$_{CTX-M}$ Genogroups Among Cephalosporin-Resistant Clinical Isolates of *Escherichia coli* and *Klebsiella pneumoniae* in Misrata, Libya: A Cross-Sectional Study

Mohamed Shallouf[1,3]*, Reyad Latrish-Bitlmal[2], Mohamed Eshlak[1,3], Eltaher Elshagmani[3,4]

[1]*Biomedical and Biosciences Research Unit, Faculty of Medical Technology, Misrata, Libya*
[2]*Medical Laboratory Department, Faculty of Medical Technology, Misrata, Libya*
[3]*Medical Microbiology Department, Faculty of Medical Technology, Misrata, Libya*
[4]*Department of Microbiology, National Cancer Institute, Misrata, Libya*
Email. *mohamed.shallouf@gmail.com*

Abstract
CTX-M enzymes are the most prevalent extended-spectrum β-lactamase (ESBL) family worldwide, yet genogroup-level data from Libya in general, and from Misrata in particular, remain limited. This laboratory-based cross-sectional study aimed to investigate the prevalence and distribution of *bla*-$_{CTX-M}$ genogroups among cephalosporin-resistant clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* in Misrata, Libya, and to explore the differences between the two bacterial species. From December 2024 to May 2025, 120 non-duplicated cephalosporin-resistant isolates (43 *E. coli* and 77 *K. pneumoniae*) were collected from public and private diagnostic laboratories across Misrata. Species identification and disc-diffusion susceptibility testing had been performed at the source laboratories. DNA was extracted using a standard heat-lysis method. All isolates were initially screened for *bla*-$_{CTX-M}$ genes by singleplex PCR with universal primers. Positive isolates were subsequently assigned to *bla*-$_{CTX-M}$ genogroups 1, 2, 8, 9 and 25 by multiplex PCR. Proportions were compared using the chi-squared or Fisher's exact test. Overall, *bla*-$_{CTX-M}$ was detected in 90.0% (95% CI 83.3–94.2), with no significant difference between *E. coli* (90.7%) and *K. pneumoniae* (89.6%; p = 1.00). Among the five targeted *bla*-$_{CTX-M}$ genogroups, only *bla*-$_{CTX-M}$ genogroups 1 and 9 were detected. Genogroup 1 was the predominant genotype, accounting for 78.7% (85/108), and genogroup 9 accounted for 57.4% (62/108). Co-carriage of both genogroups 1 and 9 was detected in 36.1% (39/108) of the positive isolates and was significantly more frequent in *K. pneumoniae* than in *E. coli* (43.5% versus 23.1%, p = 0.034). This difference was sensitive to the statistical test used (p = 0.056 with continuity correction) and may be partly confounded by specimen source, as E. coli isolates were more often obtained from urine. Overall, *bla*-$_{CTX-M}$ genes were highly prevalent among cephalosporin-resistant *E. coli* and *K. pneumoniae* investigated in this study, with *bla*-$_{CTX-M}$ genogroup 1 and 9 accounting for all detected *bla*-$_{CTX-M}$ genogroups. The considerable frequency of co-carriage, particularly among *K. pneumoniae* isolates, highlights the importance of continued molecular surveillance. Further investigation through variant-level identification and plasmid characterisation would help clarify the genetic basis and potential dissemination of these resistance determinants, providing valuable information to support local infection prevention and control measures and antimicrobial stewardship strategies.
Keywords. Bla-$_{CTX-M}$; Genogroups; *Escherichia coli*; *Klebsiella pneumoniae*; Cephalosporin Resistance; Libya

Introduction

Multidrug resistance to broad-spectrum β-lactams among members of Enterobacteriaceae represents a major global public health concern, posing significant therapeutic challenges. Extended-spectrum β-lactamases (ESBLs) hydrolyse the β-lactam ring, rendering these antibiotics ineffective against ESBL-producing bacteria [1]. ESBL production in Enterobacteriaceae is clinically significant because it frequently co-exists with other resistance mechanisms, which further restrict treatment options [2]. *Escherichia coli* and *Klebsiella pneumoniae* are among the most important ESBL producers [3], because of their high clinical prevalence, their ability to acquire and disseminate antibacterial-resistant genes, and their contribution to difficult-to-treat infections in both community and healthcare settings [4]. TEM- and SHV-type enzymes were once the dominant ESBL mechanisms in Enterobacteriaceae. Over the past two decades, however, this pattern has shifted towards the predominance of CTX-M-type enzymes [5,6]. CTX-M enzymes are now the most prevalent ESBL family in various parts of the world [7]. Their prevalence and dissemination are attributed to horizontal gene transfer, generally via plasmids and other mobile genetic elements [2,6]. Based on the sequencing of amino acids, CTX-M has been classified into five lineages across pathogens: CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, and CTX-M-25 [6,8]. Regional studies show that this trend is well recognised across Africa and the Middle East countries [4,7]. The distribution of *bla*-$_{CTX-M}$ genogroups varies geographically, reflecting differences in the epidemiology and dissemination of CTX-M-producing bacteria. In North Africa, *bla*-$_{CTX-M}$ is widely reported among ESBL-producing Enterobacterales, particularly in *E. coli* and *K. pneumoniae* [7]. Studies from various countries in Asia, Africa, the Middle East, and neighbouring regions have shown that the distribution of *bla*-$_{CTX-M}$ genogroups varies across geographical regions and study settings [5,9–14]. Characterising the distribution of *bla*-$_{CTX-M}$ genogroups is therefore important for understanding the molecular epidemiology of ESBL-mediated resistance and identifying differences in the occurrence of these resistance determinants between bacterial species. Third-generation cephalosporin-resistant Enterobacterales are classified as a critical priority pathogen group by the World Health Organisation [15], emphasising the need for continuing molecular surveillance of the resistance determinants involved. Despite the global predominance of *bla*-$_{CTX-M}$ genes, molecular data from Libya are limited. A study from Tripoli reported a

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Received*: 05-09-2026 -* ***Accepted****: 04-11-2026 -* ***Published****: 12-11-2026*

high prevalence of *bla*-$_{CTX\text{-}M\text{-}15}$ among urinary isolates [16]. In addition, a previous study from Misrata detected *bla*-$_{CTX\text{-}M}$ in urine samples without determining genogroups [17]. A recent meta-analysis has also highlighted gaps in the surveillance of ESBL-producing Enterobacterales in North Africa [7]. Genogroup-level data for cephalosporin-resistant *E. coli* and *K. pneumoniae* from Misrata, including a comparison between the two species, are therefore lacking. This study aimed to determine the prevalence and distribution of *bla*-$_{CTX\text{-}M}$ genogroups among cephalosporin-resistant clinical isolates of *E. coli* and *K. pneumoniae* collected from diagnostic laboratories in Misrata, and to compare the distribution of the detected genogroups between the two species. These local molecular data may support improved antimicrobial resistance surveillance and strengthen infection prevention and control practices by healthcare teams and public health authorities.

Materials and Methods

Study Period and Design

This laboratory-based cross-sectional study was conducted over six months from December 2024 to May 2025 at the Biomedical and Biosciences Research Unit (BBRU), Faculty of Medical Technology, Misrata, Libya.

Sample Size and Collection

A total of 120 non-duplicated cephalosporin-resistant isolates were included: 43 *E. coli* and 77 *K. pneumoniae* collected from public and private medical laboratories in Misrata during the study period. The source of the isolates was wound swabs, surgical wound swabs, diabetic foot swabs, respiratory specimens, aspirated pus, blood and urine. Species identification and antimicrobial susceptibility testing (AST) had been performed at the medical laboratories before the isolates were collected for this study. Identification was based on standard microbiological and biochemical tests, and AST was performed by the disc diffusion method in accordance with the 2024 Clinical and Laboratory Standards Institute (CLSI) guidelines [18]. Isolates were considered cephalosporin-resistant if they demonstrated resistance to at least one of the third-generation cephalosporins.

DNA extraction

Genomic DNA was extracted using the heat lysis method as previously described [19]. Briefly, two bacterial colonies grown overnight on nutrient agar were picked with a sterile wire loop and suspended in 200 µL of molecular-grade water. The suspension was boiled to 100 °C for 10 min in a water bath, then immediately transferred to an ice bath for 10 min to rapidly cool and stabilise the nucleic acids. The tubes were then centrifuged at 12,000 x g for 10 min at 4 °C in a table-top high-speed refrigerated centrifuge (BKC-TH16RD; Biobase Co., Ltd., Shandong, China), and the supernatant was transferred to a new sterile 1.5 mL microcentrifuge tube. DNA concentration and purity were assessed using a Microvolume UV/VIS (NanoDrop) spectrophotometer (SP-MUV2000F; Bioevopeak, Co., Ltd., Shandong, China). DNA electrophoresis on a 1% agarose gel was used to assess the extracted DNA integrity. The extracted DNA was stored at −20 °C until use in the PCR reaction.

PCR Detection of bla-$_{CTX\text{-}M}$

Cephalosporin-resistant *E. coli* and *K. pneumoniae* isolates were initially screened for the presence of *bla*-$_{CTX\text{-}M}$ by singleplex PCR using the universal primers CTX-MU1 and CTX-MU2 (Table 1). Crude genomic DNA obtained by heat lysis was used as the template. Each 25 µL reaction contained 2 µL of DNA template, 12.5 µL (1× final concentration) of 2X Flash Hot Start Master Mix (Cat. CW3007M, CWBIO, China), 0.5 µL of each primer (10 µM; 0.2 µM final concentration), and 9.5 µL of molecular-grade water. Amplification was performed in a thermal cycler (Biobase BK-AI; Biobase Co., Ltd., Shandong, China) with the following conditions: initial activation at 95 °C for 12 min; 30 cycles of denaturation at 95 °C for 15 s, annealing at 60 °C for 30 s, and extension at 72 °C for 45 s; and a final extension at 72 °C for 5 min. Chimeric positive control and no template control (NTC) were included in each PCR reaction.

Table 1. Oligonucleotides used in this study

Target	Primer name	Sequence (5'-3')	Size bp	Ref.
Universal *bla*-$_{CTX\text{-}M}$ primers				
bla-$_{CTX\text{-}M}$	CTX-MU1	5'-ATG TGC AGY ACC AGT AAR GTK ATG GC-3'	593	[20]
	CTX-MU2	5'-TGG GTR ARR TAR GTS ACC AGA AYC AGC GG-3'		
bla-$_{CTX\text{-}M}$ group-specific primers				
bla-$_{CTX\text{-}M}$*group-1*	CTX-M gp1F	5'-AAA AAT CAC TGC GCC AGT TC-3'	415	[21]
	CTX-M gp1R	5'-AGC TTA TTC ATC GCC ACG TT-3'		
bla-$_{CTX\text{-}M}$*group-2*	CTX-M gp2F	5'-CGA CGC TAC CCC TGC TAT T-3'	522	
	CTX-M gp2R	5'-CCA GCG TCA GAT TTT TCA GG-3'		
bla-$_{CTX\text{-}M}$*group-9*	CTX-M gp9F	5'-CAA AGA GAG TGC AAC GGA TG-3'	205	
	CTX-M gp9R	5'-ATT GGA AAG CGT TCA TCA CC-3		
bla-$_{CTX\text{-}M}$*group-8*	CTX-M gp8F	5'-TCG CGT TAA GCG GAT GAT GC-3'	666	
	CTX-M gp25F	5'-GCA CGA TGA CAT TCG GG-3'		
bla-$_{CTX\text{-}M}$*group-25*	CTX-M gp8/25R*	5'-AAC CCA CGA TGT GGG TAG C-3'	327	

** This primer was used as a reverse primer for genogroup-8 and genogroup-25.*

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Received*: 05-09-2026 -* ***Accepted****: 04-11-2026 -* ***Published****: 12-11-2026*

Multiplex PCR Detection of bla-$_{CTX-M}$ Genogroups

Bla-$_{CTX-M}$ genogroups 1, 2, 8, 9, and 25 were investigated in all *E. coli* and *K. pneumoniae* isolates that tested positive for *bla*-$_{CTX-M}$ in the singleplex PCR. Multiplex PCR was performed using group-specific primers (Table 1) in the same thermal cycler. Each 20 µL reaction contained 2 µL of DNA template, 4 µL (1× final concentration) of 5× Hot FIREPol Multiplex Master Mix (Cat. No. 43600120, Solis BioDyne, Tartu, Estonia), and each primer at 0.2 µM, with molecular-grade water up to 20 µL volume. The cycling conditions were: initial activation at 95 °C for 12 min; 30 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30 s; and a final extension at 72 °C for 5 min. To assess non-specific amplification, a *bla*-$_{CTX-M}$ negative isolate (confirmed negative by singleplex PCR) and a no-template control (NTC) were included in each multiplex PCR run. Multiplex results were compared with those of the singleplex PCRs, and amplicon sizes were compared with the expected sizes for each genogroup.

Gel Electrophoresis

PCR products (5 µL each) were separated by electrophoresis on a 2% agarose gel prepared in 1X TBE buffer, stained with ethidium bromide (EtBr, 0.5 µg/mL), alongside a 100 bp DNA ladder (CWBIO, China), at 80 V for 90 min. Bands were visualised using a gel documentation system (Biobase Biodustry, Shandong Co., Ltd., China).

Statistical Analysis

Data were analysed using Statistical Package for Social Sciences (SPSS, version 23, SPSS, Chicago, IL, U.S.A.). Categorical variables are presented as frequencies and percentages, and the prevalence of *bla*-$_{CTX-M}$ is reported with 95% confidence intervals (Wilson score method). Proportions were compared using the chi-squared test or Fisher's exact test (Fisher-Freeman-Halton for tables larger than 2x2) when expected counts were below 5. A *p*-value below 0.05 was considered statistically significant. Genogroup distribution and co-carriage were calculated among *bla*-$_{CTX-M}$-positive isolates unless stated otherwise.

Results

Study Isolates

A total of 120 cephalosporin-resistant isolates were included: 43 (35.8%) *E. coli* and 77 (64.2%) *K. pneumoniae*. Urine was the commonest source (46/120, 38.3%), followed by wound swabs (19, 15.8%), respiratory tract (19, 15.8%), surgical wound swabs (16, 13.3%), blood (8, 6.7%), diabetic foot swabs (7, 5.8%) and aspirated pus (5, 4.2%). Urine accounted for a higher proportion of *E. coli* isolates (60.5%) than of *K. pneumoniae* isolates (26.0%) ($p < 0.001$).

Prevalence of bla-$_{CTX-M}$

Overall, 108 of 120 isolates (90.0%; 95% CI 83.3–94.2) carried *the bla*-$_{CTX-M}$ gene, and 12 (10.0%) were negative for *bla*-$_{CTX-M}$ (Figure 1). Prevalence was 90.7% (39/43; 95% CI 78.4–96.3) in *E. coli* and 89.6% (69/77; 95% CI 80.8–94.6) in *K. pneumoniae*, with no significant difference ($p = 1.00$).

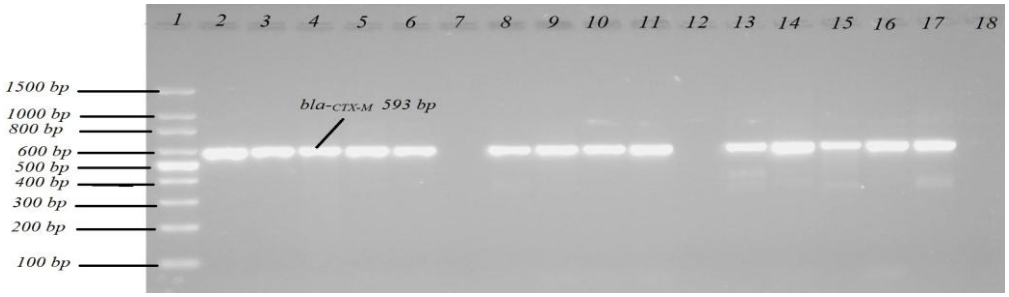


Figure 1. Agarose gel electrophoresis of PCR products amplified with universal bla-CTX-M primers.

Lane 1: 100 bp DNA ladder; lane 2: Chimeric positive control; lanes 3–6, 8–11 and 13–17: bla-$_{CTX-M}$-positive isolates (593 bp); lane 7 and 12: bla-$_{CTX-M}$-negative isolates; lane 18: NTC.

Distribution of bla-$_{CTX-M}$ genogroups

Only *bla*-$_{CTX-M}$ genogroups 1 and 9 were detected (Figure 2); *bla*-$_{CTX-M}$ genogroups 2, 8 and 25 were not found. Genogroup 1 alone was present in 46 isolates (42.6%), genogroup 9 alone in 23 (21.3%), and both genogroups together in 39 (36.1%). In total, 85 isolates (78.7%) carried a genogroup 1 gene and 62 (57.4%) carried a group 9 gene (Table 2). Among *bla*-$_{CTX-M}$-positive isolates, the distribution of genogroup patterns did not differ significantly between species ($p = 0.106$). Co-carriage of genogroups 1 and 9 was more frequent in *K. pneumoniae* (30/69, 43.5%) than in *E. coli* (9/39, 23.1%) ($p = 0.034$, chi-squared test).

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***Received**: 05-09-2026 - **Accepted**: 04-11-2026 - **Published**: 12-11-2026*

https://doi.org/10.54361/ajmas.261124

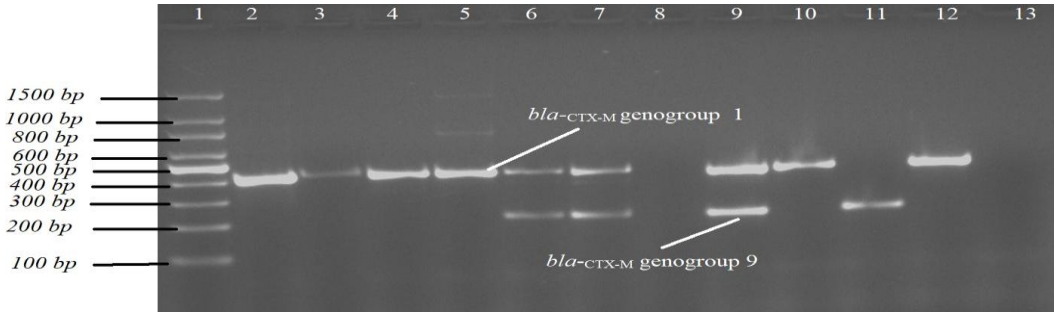


Figure 2. Representative agarose gel electrophoresis of multiplex PCR products for bla-CTX-M genogroup identification.

Lane 1: 100 bp DNA ladder; genogroup 1 is 415 bp and group 9 is 205 bp. Lanes 2-5, 10, 12: genogroup 1 only; lane11: genogroup 9 only; lanes 6, 7, 9: both genogroups; lane:8 bla-$_{CTX-M}$ negative isolate; lane 13: NTC.

Table 2. bla-$_{CTX-M}$ status and group distribution by organism

Result	*E. coli*	*K. pneumoniae*	Total
Isolates tested	43	77	120
bla-$_{CTX-M}$ negative	4 (9.3%)	8 (10.4%)	12 (10.0%)
bla-$_{CTX-M}$ positive	39 (90.7%)	69 (89.6%)	108 (90.0%)
Among positive isolates			
Genogroup 1 only	20 (51.3%)	26 (37.7%)	46 (42.6%)
Genogroup 9 only	10 (25.6%)	13 (18.8%)	23 (21.3%)
Genogroups 1 and 9	9 (23.1%)	30 (43.5%)	39 (36.1%)

Percentages for the genogroup rows are of *bla*-$_{CTX-M}$-positive isolates; percentages for the negative and positive rows are of all isolates tested.

Distribution by specimen source

bla-$_{CTX-M}$ positivity did not differ significantly among the specimen sources (p = 0.43; Fisher-Freeman-Halton exact test; (Table 3). It was 84.8% (39/46) in urine and 93.2% (69/74) in non-urine specimens (p = 0.21, Fisher exact test). All 8 blood isolates were positive. Among *bla*-$_{CTX-M}$-positive isolates, co-carriage of *bla*-$_{CTX-M}$ genogroup 1 and 9 was less frequent in urine isolates (9/39, 23.1%) than in non-urine isolates (30/69, 43.5%) (p = 0.034, chi-squared test). Of note, the urine versus non-urine and *E. coli* versus *K. pneumoniae* comparisons yielded identical counts (9/39 vs. 30/69) by coincidence, reflecting the fact that urine isolates were predominantly *E. coli*.

Table 3. bla-$_{CTX-M}$ status and group pattern by specimen source

Source	n	Negative	G1 only	G9 only	G1 + G9	Positive (%)
Urine	46	7	20	10	9	39 (84.8%)
Wound swabs	19	1	6	3	9	18 (94.7%)
Respiratory specimens	19	3	4	4	8	16 (84.2%)
Surgical wound swabs	16	0	7	5	4	16 (100%)
Blood	8	0	6	1	1	8 (100%)
Diabetic foot	7	1	1	0	5	6 (85.7%)
Aspirated pus	5	0	2	0	3	5 (100%)
Total	120	12	46	23	39	108 (90.0%)

Discussion

In the current study, *bla*-$_{CTX-M}$ genes were detected in 90% (108/120) of the tested isolates, including 90.7% (39/43) of *E. coli* and 89.6% (69/77) of *K. pneumoniae* isolates. Of the five *bla*-$_{CTX-M}$ genogroups investigated, only *bla*-$_{CTX-M}$ genogroups 1 and 9 were detected. Co-carriage of *bla*-$_{CTX-M}$ genogroups 1 and 9 was also detected in 36.1% (39/108) of the *bla*-$_{CTX-M}$-positive isolates. *K. pneumoniae* exhibited a statistically significantly higher percentage of isolates co-carrying both genogroups compared to *E. coli* (43.5% vs. 23.1%, respectively; p = 0.034). The high detection rate of *bla*-$_{CTX-M}$ observed in this study could be explained by its selective nature, as only cephalosporin-resistant isolates were included. Similarly, a Malaysian study of *E. coli* and *K. pneumoniae* bacteremia isolates found *bla*-$_{CTX-M}$ in 89.2% (33/37) of ESBL phenotype-positive isolates, compared with 38% (38/100) of ESBL phenotype-negative isolates [22]. These results highlight the strong influence of phenotypic selection criteria on the observed molecular detection rate. In line with the present study, high *bla*-$_{CTX-M}$ detection rates were reported among ESBL-producing isolates from Bir Hospital, Nepal, with rates of 93.81% in *E. coli* and 78.94% in *K. pneumoniae* [23]. Likewise, a study from India reported *bla*-$_{CTX-M}$ in 93.8% of a subset of ESBL-producing

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Received*: 05-09-2026 -* ***Accepted****: 04-11-2026 -* ***Published****: 12-11-2026*

Enterobacteriaceae [24]. A previous study from Misrata detected *bla*-$_{CTX-M}$ in 53.8% of urinary isolates [17] compared with 84.8% (39/46) of urinary isolates in the present study. This difference may reflect differences in selection criteria, as only cephalosporin-resistant isolates were included here. In contrast, *bla*-$_{CTX-M}$ detection rates were markedly lower in studies of unselected Gram-negative organisms. For example, in a study of 66 hospitals across the United States, *bla*-$_{CTX-M}$ was found in only 16% of *E. coli* and 14% of *K. pneumoniae* isolates from blood cultures [25]. These findings support previous studies showing that *bla*-$_{CTX-M}$ genes have become one of the main drivers of cephalosporin resistance [2,26,27]. They also indicate that the high detection rate observed in the present study should not be generalised to unselected clinical isolates of *E. coli* and *K. pneumoniae*. Twelve isolates (10%, 12/120) did not carry any of the five *bla*-$_{CTX-M}$ genogroups. The absence of *bla*-$_{CTX-M}$ genogroups in these cephalosporin-resistant isolates does not rule out the presence of other ESBL genes or other resistant mechanism. Cephalosporin resistance in *bla-CTX-M-negative* isolates has also been attributed to ESBL variants of *bla*-$_{TEM}$ and *bla*-$_{SHV}$, AmpC β-lactamases, Carbapenemases, or downregulation of porin expression [22,24,25,28,29]. The exclusive detection of *bla*-$_{CTX-M}$ genogroups 1 and 9 reflects their global molecular epidemiology, as they are two of the most widely distributed genogroups worldwide. Among the 108 *bla*-$_{CTX-M}$-positive isolates, *bla*-$_{CTX-M}$ genogroup 1 was detected in 78.7% (85/108) and *bla*-$_{CTX-M}$ genogroup 9 was detected in 57.4% (62/108), including isolates co-carrying both genogroups. According to Bevan et al. [30], *bla*-$_{CTX-M}$ genogroup 1 predominates in most geographical regions, including Africa and the Middle East, whereas *bla*-$_{CTX-M}$ genogroup 9 is more prevalent in East and Southeast Asia and Spain. The same authors reported that *bla*-$_{CTX-M}$ genogroup 2 remains common in South America, while *bla*-$_{CTX-M}$ genogroups 8 and 25 have more restricted geographical distribution and are reported less frequently, which is consistent with the absence of *bla*-$_{CTX-M}$ genogroups 2, 8 and 25 in the present study. Recent studies have likewise identified *bla*-$_{CTX-M}$ genogroups 1 and 9 as among the most prevalent *bla*-$_{CTX-M}$ genogroups in clinical isolates [31,32]. The predominance of *bla*-$_{CTX-M}$ genogroup 1 over *bla*-$_{CTX-M}$ genogroup 9 in the present study agrees with reports from Libya and other North African countries. A study from Tripoli reported a high prevalence of CTX-M-15 (genogroup 1) among urinary isolates [16], and similar results were reported from Egypt [33] and Tunisia [34]. CTX-M variants were not determined in our isolates; therefore, these comparisons are indirect. Nevertheless, the relatively high proportion of *bla*-$_{CTX-M}$ genogroup 9 indicates that it contributes considerably to the CTX-M profile of clinical isolates in Misrata. A similar finding was reported in a community-based study of cefotaxime-resistant Enterobacteriaceae in Thailand [35], where genogroup 9 accounted for 57.4% of *bla*-CTX-M-positive isolates, identical to the proportion observed in the present study. However, genogroup 1 was less frequently detected in the Thai study than in the present study (51.5% vs. 78.7%). Co-carriage of *bla*-$_{CTX-M}$ genogroups 1 and 9 was present in 36.1% (39/108) of *bla-CTX-M-positive* isolates, and it was more frequent in *K. pneumoniae* than in *E. coli* (43.5% [30/69] vs. 23.1% [9/39]; $p = 0.034$). Co-carriage of *bla*-$_{CTX-M}$ group 1 and *bla*-$_{CTX-M}$ group 9 has been documented in the community-based study from Thailand, where it accounted for 10.1% (17/169) of *bla*-$_{CTX-M}$-positive isolates [35]. The proportion observed in the current study was considerably higher, which may reflect differences in setting and population. The dissemination of *bla*-$_{CTX-M}$ is closely associated with mobile genetic elements, mainly insertion sequences such as IS*Ecp1* and IS*CR1*, that facilitate the expression and mobilisation of *bla*-$_{CTX-M}$ genes. In addition to the insertion sequences, the association of *bla*-$_{CTX-M}$ with conjugative plasmids such as IncF and IncN contributes to the spread of these genes through horizontal gene transfer between bacterial species, including *E. coli* and *K. pneumoniae* [26,36]. Consequently, the dissemination and co-carriage of multiple *bla*-$_{CTX-M}$ genes may result from the acquisition of insertion sequences and multiple plasmids, or of a multi-resistance plasmid carrying more than one *bla*-$_{CTX-M}$ genogroup. However, because plasmids and insertion sequences were not characterised in this study, the precise genetic basis driving this high rate of co-carriage observed in *K. pneumoniae* remains to be explained. The significance of this difference was sensitive to the test used ($p = 0.034$ by chi-squared test and 0.039 by Fisher's exact test, but 0.056 with continuity correction); it should therefore be interpreted cautiously, particularly as *E. coli* isolates were more often from urine (60.5% vs. 26.0% of all isolates), a source in which co-carriage was less frequent (23.1% vs. 43.5% of positive isolates).

Conclusion

Among cephalosporin-resistant *E. coli* and *K. pneumoniae* isolates from Misrata, Libya, *bla*-$_{CTX-M}$ was highly prevalent (90%), and only genogroups 1 and 9 were detected, with genogroup 1 predominating. Co-carriage of both genogroups was common (36.1% of positive isolates) and significantly more frequent in *K. pneumoniae* than in *E. coli*. Variant identification, plasmid characterisation, and genomic sequencing are needed to investigate the genetic basis of co-carriage and the mobile genetic elements involved. Ongoing surveillance is highly recommended to monitor and control the dissemination of these resistant strains, and to inform local infection prevention and control and antimicrobial stewardship policies.

Acknowledgement

The authors would like to acknowledge the public and private medical laboratories in Misrata, Libya, for providing the bacterial isolates used in this study. Their cooperation and support are gratefully appreciated.

Conflicts of Interest

The authors declare that they have no conflicts of interest related to the publication of this study.

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Received: *05-09-2026 -* ***Accepted:*** *04-11-2026 -* ***Published:*** *12-11-2026*

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Received*: 05-09-2026 -* ***Accepted****: 04-11-2026 -* ***Published****: 12-11-2026*

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Received*: 05-09-2026 -* ***Accepted****: 04-11-2026 -* ***Published****: 12-11-2026*