Interdisciplinary Studies on Applied Science, 3(2):48–54, July-December: 2026.
DOI: https://doi.org/10.58613/isas326

Interdisciplinary Studies on Applied Science
ISSN (Online): 3048-877X

Journal homepage: https://www.gpub.org/journal-details.php?journal-id=42



Research Article

Molecular and Functional Characterization of CTX-M-15 Associated Outer Membrane Vesicles, Complement Resistance, and Macrophage Responses in Extraintestinal Pathogenic *Escherichia coli* Isolates from Najaf

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Article Info

Keywords: *Antibacterial resistance, E. coli, β-lactamase, Outer membrane vesicles, Innate immunity, Macrophages, Host pathogen interaction.*

Received: *08.09.2026;*
Accepted: *23.10.2026;*
Published: *02.11.2026*

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Abstract

Background: Extraintestinal pathogenic *Escherichia coli* (ExPEC) commonly causes urinary tract and bloodstream infections. The worldwide spread of extended-spectrum β-lactamase (ESBL)-producing *E. coli* especially strains that carry *bla*CTX-M-15 has made treatment more difficult. **Methods:** Thirty clinical ExPEC isolates collected in Najaf government (Iraq) were divided into a β-lactam-susceptible, ESBL-negative group (GI, n=15) and a CTX-M-15-producing group (GII, n=15). β-Lactamase activity was determined in whole-cell lysates, extracellular fractions, and purified OMVs. OMV production and protein content were characterized by nanoparticle analysis and protein quantification. Complement susceptibility was evaluated using fresh and heat-inactivated serum, together with flow-cytometric assessment of C3b and C5b-9 deposition. **Results:** GII isolates demonstrated substantially higher β-lactamase activity than GI isolates in whole-cell lysates, extracellular fractions, and purified OMVs (all $P<0.001$). OMV production was approximately 2.8-fold higher in GII than in GI, while β-lactamase activity normalized to OMV particle number was approximately 8.1-fold higher ($P<0.001$). GII isolates exhibited greater survival in fresh serum (28.7 ± 5.3% versus 4.2 ± 1.1%, $P<0.001$) and reduced deposition of C3b and C5b-9. Macrophage phagocytosis was lower for GII isolates, whereas intracellular survival was significantly increased at 6 and 24 h. GII infection was associated with reduced ROS production and a cytokine profile characterized by lower TNF-α, IL-6, and IL-1β and higher IL-10 concentrations. Deletion of *bla*CTX-M-15 markedly reduced β-lactamase activity, OMV production, intracellular survival, and complement resistance, whereas complementation restored the phenotype. **Conclusion:** CTX-M-15-producing ExPEC isolates display coordinated changes in OMV-associated β-lactamase activity, complement susceptibility, and macrophage antibacterial responses. The persistence of these differences in antibiotic-free conditions and their attenuation following *bla*CTX-M-15 deletion support an association between the CTX-M-15 determinant and altered host–pathogen interactions.

How To Cite: A.H.R. Kaiwan. Molecular and Functional Characterization of CTX-M-15 Associated Outer Membrane Vesicles, Complement Resistance, and Macrophage Responses in Extraintestinal Pathogenic *Escherichia coli* Isolates from Najaf. *Interdisciplinary Studies on Applied Science*, 3(2):48–54, 2026. https://doi.org/10.58613/isas326

1. Introduction

Extraintestinal pathogenic *Escherichia coli* (ExPEC) includes many *E. coli* strains that infect sites beyond the gut. Urinary tract infections, bacteremia, and other invasive diseases are among the conditions they cause [1]. Treatment has become more difficult as ESBL-producing ExPEC has spread. CTX-M enzymes are especially significant; CTX-M-15 is one of the most widespread variants and is often found in successful ExPEC lineages such as ST131 [2]. CTX-M-15 is usually classified as a resistance determinant: the β-lactamase it encodes breaks down extended-spectrum cephalosporins, reducing their antibacterial effect [3]. Yet the same determinant may occur in bacterial genetic backgrounds that shape physiology, surface characteristics, secretion, and interactions with host immune defenses. Therefore, defining antimicrobial resistance solely in terms of antibiotic susceptibility may not fully capture the biological consequences of resistance-associated determinants. Gram-negative bacteria release outer membrane vesicles (OMVs), nanoscale extracellular structures containing outer membrane proteins, lipopolysaccharides, periplasmic components, enzymes, and nucleic acids [4, 5]. OMVs participate in bacterial communication, delivery of bacterial molecules, and modulation of host immune responses. In *E. coli*, OMVs can be internalized by macrophages and can alter cellular ROS and cytokine responses [6]. Previous studies have also demonstrated that CTX-M-15 and other β-lactamase-associated components can be detected in extracellular vesicular fractions, suggesting that OMVs may contribute to the extracellular distribution of resistance-associated molecules [7]. Importantly, CTX-M-15 secretion is not necessarily restricted to OMVs. Experimental evidence has demonstrated that pathogenic *E. coli* ST131 can actively secrete CTX-M-15 through a mechanism involving a type I secretion system, indicating that extracellular β-lactamase activity may arise through multiple pathways [8]. Therefore, increased β-lactamase activity in purified OMVs should be interpreted as evidence of OMV-associated enzyme activity rather than proof that OMVs constitute the exclusive secretion pathway. Complement represents an important component of innate antibacterial defense. ExPEC has evolved multiple mechanisms that can reduce complement-mediated killing, including changes in surface structures and interference with complement deposition and membrane attack complex formation [9]. Whether CTX-M-15-producing ExPEC has altered susceptibility to complement because of changes in OMV biology remains unclear [10]. Macrophages form another major defense against ExPEC, engulfing bacteria, generating ROS, releasing cytokines, and killing organisms inside the cell. OMVs can interact directly with macrophages and alter inflammation [6]. Differences in OMV-associated components between susceptible and CTX-M-15-producing ExPEC may therefore change how effectively macrophages respond to and kill these bacteria. This study examined how CTX-M-15 production relates to OMV properties, complement susceptibility, and macrophage antibacterial activity in clinical ExPEC isolates from Najaf. Comparisons among the isolates were paired with *bla*CTX-M-15 deletion and complementation experiments to clarify whether the gene caused the observed effects. We also tested the isolates without antibiotics to determine whether those phenotypes remained after antibiotic exposure was removed.

2. Experimental

2.1. Bacterial Isolates and Phenotypic Characterization

Thirty clinical ExPEC isolates obtained from urinary tract and bloodstream infections in Najaf government (Iraq) were included. Isolates were identified as *E. coli* by standard microbiological identification and MALDI-TOF MS. Antimicrobial susceptibility testing was performed according to established clinical laboratory guidelines. ESBL production was determined phenotypically and genetically. The isolates were divided into two experimental groups: GI, comprising 15 β-lactam-susceptible and ESBL-negative isolates, and GII, comprising 15 CTX-M-15-producing isolates. Molecular characterization included detection of *bla*CTX-M-15 and *bla*TEM. Cefotaxime minimum inhibitory concentrations (MICs) were determined for both groups.

The distribution of sequence types and phylogroups was recorded for descriptive characterization; the groups were not treated as sequence-type matched because their lineage distributions differed.

2.2. Genetic Manipulation

An ExPEC isolate producing CTX-M-15 was selected for isogenic analysis. Researchers removed *bla*CTX-M-15 through λ-Red recombination and then restored it on a plasmid in the deletion strain. The wild-type, Δ*bla*CTX-M-15, and complemented strains were tested for β-lactamase activity, OMV production, intracellular survival, and complement deposition. Growth was also measured to determine whether any major fitness changes could affect interpretation of those phenotypes.

2.3. β-Lactamase Activity and OMV Characterization

β-Lactamase activity was measured using a chromogenic nitrocefin hydrolysis assay in whole-cell lysates, extracellular fractions, and purified OMVs. OMVs were isolated from bacterial culture supernatants using filtration and ultracentrifugation. Particle number was determined by nanoparticle tracking analysis, and OMV protein content was measured using the Bradford method.

2.4. Complement Assays

Bacterial survival was evaluated following exposure to fresh human serum. Heat-inactivated serum was used as a control for complement-dependent effects. Bacterial survival was quantified by colony-forming unit (CFU) enumeration. Surface deposition of C3b and the terminal complement complex C5b-9 was quantified by flow cytometry and expressed as relative mean fluorescence intensity (MFI).

2.5. Macrophage Experiments

A defined macrophage model was used to assess bacterial uptake and intracellular persistence. Phagocytosis was quantified following bacterial exposure, and intracellular bacterial survival was determined at 1, 6, and 24 h using a gentamicin-protection approach. ROS production was quantified using a fluorescent ROS assay. TNF-α, IL-6, IL-1β, and IL-10 were quantified using immunoassays.

2.6. OMV-Macrophage Experiments

Purified OMVs from GI and GII isolates were normalized according to particle number before exposure to macrophages. Macrophage viability, ROS production, TNF-α, and IL-10 responses were subsequently measured.

2.7. Antibiotic-Exposure Experiments

Selected macrophage experiments were conducted in antibiotic-free conditions and compared with corresponding conditions involving antibiotic exposure. Intracellular bacterial survival and macrophage ROS responses were evaluated at 24 h.

2.8. Statistical Analysis

Results are expressed as mean ± standard deviation (SD). Statistical analyses were performed using appropriate parametric or non-parametric tests according to data distribution. Comparisons between two groups were performed using Student's t-test or Mann–Whitney U test as appropriate, while multiple-group comparisons were analyzed using analysis of variance with an appropriate post hoc test. A value of $P < 0.05$ was considered statistically significant.

3. Results

The clinical isolates were divided into two groups according to their β-lactam susceptibility and CTX-M-15 status. As summarized in Table 1, none of the GI isolates carried *bla*CTX-M-15, whereas all 15 GII isolates were positive for this determinant.

*bla*TEM was detected in 3/15 GI isolates and 8/15 GII isolates. The predominant sequence types differed between the two groups. GI isolates were mainly associated with ST73 and ST95, whereas ST131 predominated among GII isolates. Both groups included B2-associated isolates, with D also represented in GI. Thus, the clinical comparison reflects naturally occurring isolate populations rather than strictly sequence-type-matched strains.

Cefotaxime MICs were markedly different between groups, ranging from 0.06–0.25 mg/L in GI and 16–128 mg/L in GII. No significant difference in overall growth kinetics was observed between the groups.

Table 1: Genetic and phenotypic characteristics of the clinical ExPEC isolates

Parameter	GI (n=15)	GII (n=15)
Most common sequence type	ST73, ST95	ST131
Phylogroup	B2, D	B2
*bla*CTX-M-15	0/15	15/15
*bla*TEM	3/15	8/15
Cefotaxime MIC (mg/L)	0.06–0.25	16–128
ESBL phenotype	Negative	Positive

The marked difference in cefotaxime susceptibility confirms the distinct antimicrobial resistance phenotype of the GII isolates, while the lineage distribution highlights the importance of interpreting the clinical comparison together with the subsequent isogenic experiments.

3.1. CTX-M-15-Producing Isolates Exhibit Increased β-Lactamase Activity and Altered OMV Characteristics

β-Lactamase activity was substantially higher in GII than in GI isolates across all tested fractions Table 2. Whole-cell β-lactamase activity increased from 8.2 ± 1.4 nmol/min/mg in GI to 142.5 ± 18.7 nmol/min/mg in GII ($P < 0.001$). A similar difference was observed in extracellular fractions, where activity increased from 1.1 ± 0.3 to 24.8 ± 4.2 nmol/min/mg ($P < 0.001$). Purified OMVs from GII also demonstrated substantially higher β-lactamase activity than GI OMVs (18.3 ± 3.1 versus 0.8 ± 0.2 nmol/min/mg, $P < 0.001$).

OMV particle production was $3.1 \pm 0.5 \times 10^9$ particles/mL in GI and $8.7 \pm 1.2 \times 10^9$ particles/mL in GII, corresponding to an approximately 2.8-fold increase ($P < 0.001$). OMV protein concentration was also significantly higher in GII. When β-lactamase activity was normalized to 10^9 OMV particles, activity increased from 0.26 ± 0.05 in GI to 2.10 ± 0.31 in GII, representing an approximately 8.1-fold difference.

Table 2: β-Lactamase activity and outer membrane vesicle characteristics of GI and GII ExPEC isolates

Parameter	GI	GII	P value
Whole-cell lysate β-lactamase activity (nmol/min/mg)	8.2 ± 1.4	142.5 ± 18.7	<0.001
Extracellular β-lactamase activity (nmol/min/mg)	1.1 ± 0.3	24.8 ± 4.2	<0.001
Purified OMV β-lactamase activity (nmol/min/mg)	0.8 ± 0.2	18.3 ± 3.1	<0.001
OMV particle number ($\times 10^9$/mL)	3.1 ± 0.5	8.7 ± 1.2	<0.001
OMV protein (µg/mL)	12.4 ± 2.1	28.9 ± 4.3	<0.001
β-Lactamase per 10^9 OMV particles	0.26 ± 0.05	2.10 ± 0.31	<0.001

These findings demonstrate that CTX-M-15-producing isolates were characterized not only by increased cellular β-lactamase activity but also by increased OMV production and greater β-lactamase activity associated with the purified vesicular fraction.

3.2. CTX-M-15-Producing Isolates Display Reduced Complement Susceptibility

The ability of the isolates to withstand complement-mediated killing was examined using fresh human serum. As shown in Table 3, survival of GII isolates in fresh serum was significantly higher than that of GI isolates (28.7 ± 5.3% versus 4.2 ± 1.1%, P $<$ 0.001). In contrast, no significant difference was detected when heat-inactivated serum was used (94.3 ± 4.8% versus 92.1 ± 5.4%, P=0.41), indicating that the observed difference under fresh-serum conditions was associated with complement activity.

Flow-cytometric analysis further demonstrated substantially lower C3b deposition on GII bacteria than on GI bacteria. Relative C3b MFI was 47 ± 6 in GII compared with 100 ± 8 in GI (P $<$ 0.001). C5b-9 deposition showed a similar pattern, with relative MFI values of 31 ± 5 in GII and 100 ± 10 in GI (P $<$ 0.001).

Table 3: Complement-mediated killing and complement deposition in GI and GII ExPEC isolates

Condition	GI	GII	P value
Survival in fresh serum (%)	4.2 ± 1.1	28.7 ± 5.3	<0.001
Survival in heat-inactivated serum (%)	92.1 ± 5.4	94.3 ± 4.8	0.41
C3b deposition (relative MFI)	100 ± 8	47 ± 6	<0.001
C5b-9 deposition (relative MFI)	100 ± 10	31 ± 5	<0.001

Together, these data indicate that the CTX-M-15-producing group had reduced complement deposition and greater survival in active serum.

3.3. CTX-M-15-Producing Isolates Show Reduced Macrophage Uptake and Increased Intracellular Persistence

Macrophage phagocytosis was significantly lower for GII isolates than for GI isolates, with uptake rates of 34% and 58%, respectively (P $<$ 0.001).

Intracellular bacterial survival was subsequently determined over 24 h. As shown in Table 4, intracellular bacterial loads were comparable at 1 h (3.4 ± 0.3 versus 3.2 ± 0.3 log CFU; P=0.21). However, significant differences emerged at 6 h and became more pronounced at 24 h.

At 6 h, intracellular bacterial counts were 3.0 ± 0.4 log CFU for GII compared with 2.1 ± 0.4 log CFU for GI (P $<$ 0.001). At 24 h, GII maintained 3.1 ± 0.5 log CFU compared with only 1.2 ± 0.3 log CFU for GI (P $<$ 0.001).

Table 4: Macrophage phagocytosis and intracellular survival of GI and GII ExPEC isolates

Parameter/time	GI	GII	P value
Phagocytosis (%)	58	34	<0.001
Intracellular survival at 1 h (log CFU)	3.2 ± 0.3	3.4 ± 0.3	0.21
Intracellular survival at 6 h (log CFU)	2.1 ± 0.4	3.0 ± 0.4	<0.001
Intracellular survival at 24 h (log CFU)	1.2 ± 0.3	3.1 ± 0.5	<0.001

Both groups showed similar bacterial levels after 1 h, but they diverged later, pointing to intracellular persistence not initial uptake as the main difference.

3.4. CTX-M-15-Producing Isolates Alter Macrophage ROS and Cytokine Responses

In macrophages infected with GII isolates, ROS production fell to 42% of the control response. GI infection, by contrast, produced 100% of the control response (P $<$ 0.001). Cytokine analysis revealed substantial differences between the two groups Table 5. TNF-α decreased from 2450 ± 320 pg/mL in GI to 980 ± 180 pg/mL in GII (P $<$ 0.001). IL-6 decreased from 3800 ± 450 to 1650 ± 280 pg/mL (P $<$ 0.001), while IL-1β decreased from 820 ± 110 to 410 ± 75 pg/mL (P $<$ 0.001).

In contrast, IL-10 increased from 210 ± 45 pg/mL in GI to 580 ± 90 pg/mL in GII (P $<$ 0.001).

Table 5: Macrophage cytokine responses following infection with GI and GII ExPEC isolates

Cytokine	GI (pg/mL)	GII (pg/mL)	P value
TNF-α	2450 ± 320	980 ± 180	<0.001
IL-6	3800 ± 450	1650 ± 280	<0.001
IL-1β	820 ± 110	410 ± 75	<0.001
IL-10	210 ± 45	580 ± 90	<0.001

The combination of reduced ROS production and altered cytokine secretion indicates that GII isolates interact differently with macrophage antibacterial and inflammatory responses.

3.5. Purified OMVs Reproduce Key Macrophage Phenotypes

To determine whether OMV-associated components could contribute directly to the observed macrophage phenotype, purified OMVs from GI and GII isolates were tested independently of viable bacteria. As shown in Table 6, exposure to GII-derived OMVs resulted in lower macrophage viability than GI-derived OMVs (61% versus 88%, P $<$ 0.001). GII OMVs also reduced ROS production to 48% compared with 100% following GI OMV treatment (P $<$ 0.001).

TNF-α production was lower following GII OMV exposure (920 versus 1850 pg/mL, $P < 0.001$), whereas IL-10 was higher (520 versus 180 pg/mL, $P < 0.001$).

Table 6: Effects of purified GI- and GII-derived OMVs on macrophage responses

Parameter	GI-derived OMVs	GII-derived OMVs	P value
Macrophage viability (%)	88	61	<0.001
ROS (%)	100	48	<0.001
TNF-α (pg/mL)	1850	920	<0.001
IL-10 (pg/mL)	180	520	<0.001

These findings support the possibility that differences in OMV-associated bacterial components contribute to the altered macrophage response observed with GII isolates.

3.6. Altered Macrophage Responses Persist in the Absence of Antibiotic Exposure

To determine whether the observed phenotype was dependent on antibiotic exposure, selected macrophage experiments were conducted under antibiotic-free and antibiotic-exposure conditions.

As presented in Table 7, intracellular survival at 24 h remained substantially higher for GII than GI under both conditions. In the absence of antibiotics, intracellular survival was 3.1 log CFU for GII compared with 1.2 log CFU for GI. With antibiotic exposure, the corresponding values were 3.3 and 0.9 log CFU.

Similarly, ROS production remained markedly lower for GII under both conditions. GII showed 42% ROS in the antibiotic-free condition and 45% after antibiotic exposure, compared with 100% and 95%, respectively, for GI.

Table 7: Intracellular survival and macrophage ROS responses under antibiotic-free and antibiotic-exposure conditions

Parameter	GI, no antibiotic	GI + antibiotic	GII, no antibiotic	GII + antibiotic
Intracellular survival at 24 h (log CFU)	1.2	0.9	3.1	3.3
ROS (%)	100	95	42	45

The persistence of these differences in antibiotic-free conditions indicates that the phenotype cannot be explained solely by differential antibiotic exposure during the macrophage experiments.

3.7. Isogenic Deletion of *bla*CTX-M-15 Attenuates the Observed Phenotypes

The clinical-isolate findings were complemented by analysis of an isogenic CTX-M-15-producing strain. As shown in Table 8, the wild-type strain displayed high β-lactamase activity, increased OMV production, high intracellular survival, and reduced C3b deposition. Deletion of *bla*CTX-M-15 substantially reduced β-lactamase activity from 142.5 to 9.8 units and decreased OMV production from 8.7×10^9 to 3.4×10^9 particles/mL. Intracellular survival decreased from 3.1 to 1.4 log CFU, whereas C3b deposition increased from 47% to 92%.

Complementation restored values close to those of the wild-type strain, including β-lactamase activity of 138.2 units, OMV production of 8.5×10^9 particles/mL, intracellular survival of 3.0 log CFU, and C3b deposition of 49%.

Table 8: Effect of *bla*CTX-M-15 deletion and complementation on β-lactamase activity, OMV production, intracellular survival, and C3b deposition

Strain	β-Lactamase activity	OMVs ($\times 10^9$/mL)	Intracellular survival at 24 h (log CFU)	C3b (%)
Wild type	142.5	8.7	3.1	47
Δ*bla*CTX-M-15	9.8	3.4	1.4	92
Complemented	138.2	8.5	3.0	49

The attenuation of multiple phenotypes following deletion and their restoration after complementation provide experimental support for an association between the CTX-M-15 determinant and the investigated host–pathogen interaction phenotypes.

4. Discussion

The present study demonstrates that CTX-M-15-producing ExPEC isolates exhibit a constellation of phenotypic differences involving OMV biology, complement susceptibility, and macrophage antibacterial responses. Importantly, these differences persisted in antibiotic-free conditions and were attenuated following deletion of *bla*CTX-M-15, providing evidence that the observed phenotype is not simply a consequence of antibiotic exposure.

The first major finding was the substantial increase in β-lactamase activity in CTX-M-15-producing isolates. Whole-cell, extracellular, and OMV-associated fractions all demonstrated significantly higher activity in GII. In parallel, GII isolates produced approximately 2.8-fold more OMV particles and exhibited approximately 8.1-fold greater β-lactamase activity per 10^9 particles. These observations indicate that CTX-M-15-producing isolates have increased vesicular production and increased representation of β-lactamase activity in the purified OMV fraction [11].

The association between CTX-M-15 and OMVs is biologically plausible because OMVs can transport proteins and other bacterial components beyond the bacterial surface [4, 5, 7]. However, the present findings should not be interpreted as demonstrating that OMVs are the exclusive route of CTX-M-15 secretion. Previous work has shown that CTX-M-15 can also be actively secreted through a type I secretion mechanism in *E. coli* ST131 [8]. Therefore, the present results support the existence of an OMV-associated pool of β-lactamase rather than a single exclusive secretion pathway.

A second important observation was the increased survival of GII isolates in fresh serum. This phenotype was absent when serum was heat-inactivated, supporting a complement-dependent mechanism. Consistent with the survival data, GII isolates showed markedly lower C3b and C5b-9 deposition. Complement resistance is a multifactorial characteristic of ExPEC and may involve capsule, LPS, outer membrane proteins, and other surface-associated determinants [9]. The current data link CTX-M-15 production with lower complement deposition, but they do not show that CTX-M-15 directly binds complement components or blocks their activity.

The macrophage experiments revealed a second difference: GII isolates entered the cells less efficiently and persisted in greater numbers at later time points. Intracellular counts were similar after 1 h, then separated at 6 and 24 h. That pattern suggests the key difference lies in survival after uptake rather than in initial internalization.

The altered macrophage response coincided with less ROS production and marked shifts in cytokine release. GII infections produced lower TNF-α, IL-6, and IL-1β levels but increased IL-10. These findings point to a changed antibacterial response in macrophages, though the experiments did not identify the intracellular signaling pathway behind it.

Experiments with purified OMVs add support to the idea that extracellular vesicle components help shape the phenotype. Compared with OMVs from GI isolates, those derived from GII altered macrophage viability, lowered ROS and TNF-α production, and raised IL-10 levels. This agrees with earlier work showing that macrophages can internalize *E. coli* OMVs and that these vesicles affect ROS and cytokine responses [6]. The specific OMV component responsible was not identified here, so the results indicate biological activity associated with OMVs rather than establish one molecular mediator as the cause.

The antibiotic-free experiments matter here. Differences in intracellular survival and ROS levels were still present without antibiotic exposure, so antibiotic selection alone cannot explain the observed phenotypes. The findings instead support a CTX-M-15-associated effect involving bacterial host interactions that continue even when β-lactam antibiotics are absent [12].

The isogenic experiments provide the strongest evidence linking *bla*CTX-M-15 to the observed phenotypes. Deletion of the determinant markedly reduced β-lactamase activity, OMV production, intracellular survival, and complement resistance, whereas complementation restored these characteristics. Nevertheless, because resistance genes may be associated with plasmids and other linked genetic determinants, additional genetic and mechanistic experiments would be required to establish the precise molecular pathway connecting CTX-M-15 expression with altered OMV production and host responses [13].

An important consideration is the genetic heterogeneity of the clinical isolate groups. The GII group was predominantly associated with ST131, whereas GI included ST73 and ST95. Therefore, differences between the clinical groups may partly reflect lineage-associated characteristics. The isogenic deletion/complementation experiments reduce this concern substantially but do not eliminate the possibility that CTX-M-15 interacts with other strain-specific factors.

The present study also has several limitations. First, the experiments were performed using *in vitro* models and therefore cannot establish whether the observed effects occur to the same extent during human infection. Second, the precise bacterial factor responsible for the altered macrophage phenotype was not identified. Third, although OMV-associated β-lactamase activity was demonstrated, the study did not establish whether β-lactamase itself, another OMV cargo component, or a combination of factors is responsible for the complement and macrophage phenotypes. Finally, the clinical isolate comparison is genetically heterogeneous, emphasizing the importance of future studies using multiple independent isogenic backgrounds.

Future investigations should determine whether the observed phenotype is reproducible across different CTX-M-15-producing ExPEC lineages and whether other ESBL determinants produce similar effects. Proteomic analysis of OMV cargo, direct assessment of complement-interacting OMV components, and mechanistic characterization of macrophage signaling could further define the molecular basis of these observations. Animal infection models would subsequently be required to establish the in *vivo* relevance of the findings.

5. Conclusion

CTX-M-15-producing ExPEC isolates demonstrated marked differences in OMV production and OMV-associated β-lactamase activity together with reduced complement deposition, increased intracellular persistence, reduced macrophage ROS production, and altered cytokine responses. These differences persisted in antibiotic-free conditions and were attenuated following deletion of *bla*CTX-M-15 and restored following complementation. Collectively, the findings support an association between the CTX-M-15 resistance determinant and altered host–pathogen interactions involving OMV biology, complement susceptibility, and macrophage antibacterial responses. The results broaden the biological context in which CTX-M-15-producing ExPEC should be investigated and provide a basis for future studies aimed at defining the molecular mechanisms linking antimicrobial resistance determinants with innate immune interactions.

Article Information

Ethical Approval: The study was approved by the Ethics Committee of the College of Health and Medical Techniques, University of Alkafeel, on September 1, 2016.

Data Availability: The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: No external funding was received for this study.

Acknowledgments: The authors have no acknowledgments to declare.

AI Use Disclosure: The authors used ChatGPT 5 (OpenAI) solely to improve the language, grammar, clarity, and readability of the manuscript. All scientific content was reviewed and verified by the authors, who take full responsibility for the accuracy and integrity of the manuscript.

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