AL-KUFA JOURNAL FOR BIOLOGY
**ISSN (Print):** 2073-8854 **ISSN (online):** 2311-6544

Original Research Paper

# Molecular Detection of fimA Gene and Its Association with Serum Resistance and Proteolytic Activity in Clinical Isolates of Enterobacter cloacae

**Mays alreem A. Aljebori[1], Oruba k. Al-Bermani[2], Ekhlas Al shareefi**

[1]*Department of Biology, College of Science For Women, University of Babylon Iraq.*

*Article history*
Received: 11/5/2025
Revised: 5/7/2025
Accepted: 14/7/2025

*Corresponding Author*: Mays alreem A. Aljebori, Department of Biology , College of Science For Women, University of Babylon Iraq*

**Abstract: Background :** Bacterial virulence factors are essential components of microorganisms' ability to cause disease. This study aims to investigate the prevalence of candidate virulence-associated gene (fimA )in clinical *Enterobacter cloacae* isolates , and to assess the potential correlation between the presence of this gene with proteolytic activity, and their resistance to human serum.
**Methods and materials :** Polymerase chain reaction (PCR) was used to detect virulence gene ( fimA). Serum resistance was also tested in an in vitro experiment using human serum. A portion of the serum was heated at 56°C for 30 minutes to inactivate the complement system and compared with untreated serum to assess the isolates' ability to survive under both conditions. The proteolysis ability of Enterobacter cloacae was assessed using skim milk agar. **Result:** Molecular analyses using PCR revealed variations in the distribution of virulence factor gene among the studied bacterial isolates. The fimA gene was detected in 13 out of 20 isolates (65%). 15(75%) were resistant to serum and 5(25%) were sensitive. Strong proteolytic activity was seen in 6 resistant isolates. Moderate activity appeared in 11 isolates, mostly from the resistant group. Weak activity was found in 3 isolates, all of which were serum-sensitive. This suggests potential correlation between higher proteolytic activity and serum resistance showed the highest survival rates in unheated-serum. The role of FimA gene is associated with bacterial adhesion to host tissue and it does not play significant role in serum resistance and proteolytic activity.

**Keywords:** FimA gene , serum resistance, proteolysis activity , Enterobacter cloacae

## 1. Introduction

The respiratory tract, surgical wounds, urinary tract, and bloodstream infections are all linked to the Enterobacter cloacae complex (ECC), a collection of important pathogenic bacteria that cause nosocomial infections, which are characterized by virulence properties [1]. Adhesion and other virulence-related genes in ECC, including *fimA*, are involved in its infection. [2]. A variety of Gram-negative uropathogens were found to contain FimA according to many scientific truths. Bacteria bind to mannosylated receptor molecules on the uroepithelium because of this stickiness. FimH is the molecule found at the fimbriae tip, and it is encoded by the fimACDFIHZ locus [3]. For the infection to successfully colonize and establish itself, it must be able to stick to the surfaces of host cells, such as epithelial tissues, as a way to survive. Gram-negative bacteria

**DOI:** https://doi.org/10.36320/ajb/v17.i3.20336

therefore frequently express adherence factors, which are in charge of identifying and attaching to particular host cell receptors to allow the bacteria to infiltrate the host cells and colonize the corresponding tissue. [4]. Bacterial resistance to innate immunity is a critical factor contributing to pathogenicity and infection severity. Among the key mechanisms that support bacterial survival within the host is serum resistance, which allows pathogens to evade the bactericidal activity of the complement system. Previous studies have demonstrated that certain bacteria produce proteolytic enzymes (proteases) capable of degrading essential complement proteins such as C3 and C5, thereby impairing opsonization and complement-mediated killing [5]. Bacterial proteases are pivotal in facilitating immune evasion by degrading key components of the host's immune system. In Enterobacteriaceae, serine protease auto transporters (SPATEs) such as Pic and EspP cleave complement proteins (e.g., C2, C3, C4, C5), impairing complement-mediated lysis and promoting serum resistance [6]. The study aim is to elucidate the occurrence of FimA adhesion factor, serum resistance, and proteolytic activity in *E.cloacae* and its role in bacterial pathogenicity and immune evasion.

## 2. Methodology

### *Bacterial strains.*

A total of 20 E. cloacae strains were included in this study 10 from urine, 5 from semen and 5 from the intestine biopsy. All of the strains had been isolated recently in the Discipline of Microbiology, College of science for women, University of Babylon during the period September2024 to February2025, Iraq. The clinical history of each case and full information was taken directly from patients. The strains were identified by vitek II compact system at the biological number 0637735753173010. These bacteria have been investigated for gene (FimA ) for its important in virulence factors.

### *Serum resistance*

The cellular bacteria that surviving following incubation in diluted serum were assessed to determine the bacterial susceptibility to the bactericidal activity of serum [7]. In Luria-Bertani (LB) broth, bacterial cultures were growth until the mid-log phase (OD600 of 0.5). After a single wash with 1× phosphate buffered saline (PBS), 100 microliters of culture were reached to a concentration of roughly 2×106 bacteria per milliliter of 1× PBS. 75 microliters of pooled normal human serum (NHS; Innovative Research, MI, United States) and 25 microliters of the bacterial suspension were combined, and the mixture was shaken for three hours. Following incubation, the mixture was spotted onto an LB agar plate and rinsed once with 1×PBS. A comparison was made between the number of colony-forming units (CFUs) that survived NHS treatment and the number of CFUs that survived treatment with serum heat-inactivated for half hour at 56°C.

### *Proteolytic Activity Assay Using Skim Milk Agar*

The proteolytic activity assay was carried out according to Paudel et al. in order to assess the bacterial proteolytic activity among different species of ECC [8]. To put it briefly, two microliters of the over-night cultures were placed on a skim milk agar plate (prepared by dissolving 5 grams of skim milk powder in 100 ml of sterile distilled water) and left to incubate for the entire night at 37°C. The development of a lysis loop encircling the bacterial colonies was then used to gauge the proteolytic activity.

### *Genetic Study*

### *Genomic DNA Extraction and PCR* Amplification

A single colony of 20 isolates of bacteria under interest isolates was inoculated in Nutrient agar (Hi media, India) and incubated at 37°C for 24 hours to obtained new and fresh colony.

Bacterial genomic DNA was extracted according to the guidelines provided by the manufactured scientific institutions (Favrogene Kit). Primers were dissolved and prepared according to the manufacturer's instructions (Biotech Corp., Pingtung, Taiwan). The forward primer sequence is FimA-F (5′TGCTGTCGAGGATCTCAATG3′) and the reverse primer is FimA-R (5′ ACGGTTAATCTCGGCCAGTA3′) [9] Initial denaturation at 95°C for 5 minutes, 35 cycles of denaturation at 94°C for 30 seconds, annealing at 58°C

**DOI:** https://doi.org/10.36320/ajb/v17.i3.20336

for 30 seconds, extension at 72°C for 1 minute, and a final extension at 72°C for 5 minutes all comprised the PCR cycling conditions. Dissolved in the free $ddH_2O$ to produce a stock solution with a final concentration of 100 pmol/µl. Maintain a stock at -20 to create a work primer suspension with a concentration of 10 pmol/µl. Mix 10 µl of the stock solution with 90 µl of the free $ddH_2O$ water to achieve a final volume of 100 µl. the PCR mixture volume was 50µ includes 25 µl of 2× PCR Master Mix (containing Taq DNA polymerase, dNTPs, $MgCl_2$ , and reaction buffer), 1 µl of forward primer (10 pmol/µl), 1 µl of reverse primer (10 pmol/µl), 2 µl of DNA template, and 21 µl of nuclease-free water. Amplified products were resolved in 1.5% agarose gel, and stained with safe red stain. Agarose gel electrophoresis has been prepared at 80V, 65Amp for 1 hour. The DNA samples were detected by viewing under UV-trans illuminator. The PCR mixture volume was 50µl include

*FimA Amplicon Sequencing for PCR*

The clarified PCR of FimA gene was done for 20 bacterial isolates ( amplicons were commercially sequenced from the termini, forward, and reverse, as per instructions from the sequencing company (Macrogen Inc., Geumchen, Seoul, South Korea). discrepancies were not the result of PCR or artifact sequencing. Virtual positions and other details of the PCR pieces acquired were determined by comparing the samples' observed nucleic acid sequences to the bacterial database conserved standard sequences . The sequencing outcomes of the PCR products of various samples were modified, coordinated, and assessed with "Bio Edit Sequence Alignment Editor Software Version 7.1 (DNASTAR, Madison, WI, USA)". Each sequenced sample's PCR amplicons was numbered, as were the alterations that were found and where in the referring genome they were found [10].

Design of a Comprehensive Phylogenetic Tree

A specific and comprehensive tree was built in this study using the neighbor-joining Mega-6 procedure as described by the NCBI-BLAST, server was used to compare the detected variations to their neighbors' homologous reference sequences. After that, a fully inclusive tree was created using the neighbor-joining strategy and aligned with the observed version of the E. cloacae strains as well as the reference strain NM-004053.1 [11].

## Statistical Analysis

The statistical analysis was performed using SPSS software version 26. To compare bacterial survival in normal and heat-inactivated serum, the mean optical density (OD) values and standard deviations were calculated for each Enterobacter cloacae isolate. A paired t-test was applied to determine whether the differences between the two conditions were statistically significant.

Additionally, the association between proteolytic activity and serum resistance was analyzed using the Chi-square ($\chi^2$) test. A P-value of less than 0.05 was considered statistically significant.

## Result and discussion

*Bacterial Isolation and Identification*

The initial identification of Enterobacter cloacae isolates was based on colonial morphology, pigmentation, and growth patterns on selective and differential media (such as MacConkey and EMB agar), as well as microscopic characteristics and conventional biochemical tests they are catalase-positive, oxidase- and DNAase-negative, fermentative and non-pigmented.

In this study, a total of 20 E. cloacae isolates were recovered from three clinical sources: urine (10 isolates), intestinal biopsies (5 isolates), and seminal fluid samples (5 isolates). The isolates from urine represented 50% (10/20) of the total, followed by 25% (5/20) from biopsies, and 25% (5/20) from semen.

The presence of E. cloacae was significantly associated with patients suffering from genitourinary or intestinal infections, particularly those with recurrent urinary tract infections and male infertility complaints. Notably, the isolation rate was less in males (35%) than in females(65%), and the bacterium exhibited notable phenotypic variability depending on the source of isolation.

**DOI:** https://doi.org/10.36320/ajb/v17.i3.20336

*Resistance of Enterobacter cloacae to the microbicidal action of human serum*

The current study result reveals that E.cloacae isolates showed resistance to the lethal action of serum components at a percentage (75%) while the sensitive to the lethal action of serum was (25%). Table1 showed Resistance to the bactericidal action at serum it indicated by the absence of different bacterial growth in both type of serum (normal serum and inactive serum).

(Table – 1) Comparison of optical density reading at 620nm for Enterobacter cloacae isolates in normal and heated-in activated human serum .

| Isolate No. | Mean (Normal Serum) ± SD | Mean (Activated Serum) ± SD | P-value |
|---|---|---|---|
| 1 | 1.47 ± 0.01 | 1.24 ± 0.01 | .002* |
| 2 | 1.59 ± 0.01 | 1.46 ± 0.01 | .004* |
| 3 | 1.58 ± 0.04 | 1.33 ± 0.05 | .003* |
| 4 | 1.37 ± 0.01 | 1.37 ± 0.01 | .583$^{NS}$ |
| 5 | 3.54 ± 1.82 | 1.26 ± 0.00 | .161$^{NS}$ |
| 6 | 1.27 ± 0.00 | 1.35 ± 0.01 | .005* |
| 7 | 1.44 ± 0.00 | 1.24 ± 0.13 | .108$^{NS}$ |
| 8 | 1.01 ± 0.01 | 1.19 ± 0.00 | .001$^{*}$ |
| 9 | 1.82 ± 0.01 | 0.30 ± 0.00 | .000* |
| 10 | 0.88 ± 0.00 | 1.47 ± 0.06 | .003* |
| 11 | 1.57 ± 0.00 | 1.62 ± 0.01 | .003* |
| 12 | 1.77 ± 0.01 | 1.25 ± 0.01 | .000* |
| 13 | 0.33 ± 0.00 | 0.33 ± 0.00 | .184$^{NS}$ |
| 14 | 1.65 ± 0.18 | 0.68 ± 0.00 | .011* |
| 15 | 2.32 ± 0.00 | 1.46 ± 0.17 | .013* |
| 16 | 1.60 ± 0.01 | 1.21 ± 0.00 | .000* |
| 17 | 1.29 ± 0.00 | 1.46 ± 0.00 | .000* |
| 18 | 1.53 ± 0.04 | 1.02 ± 0.50 | .242$^{NS}$ |
| 19 | 1.52 ± 0.04 | 0.47 ± 0.00 | .001* |
| 20 | 1.87 ± 0.00 | 0.97 ± 0.01 | .000* |
| Overall | 1.57 ± 0.69 | 1.13 ± 0.40 | .161$^{NS}$ |
| P-value | .000* | .000* | |
| *Significant difference at the 0.05 level by T- test NS:Non-significant | | | |

Out of 20 E.cloacae clinical isolates 15 (75%) resistance to human serum , while only 5 (25) sensitive .this high prevalence of serum resistance indicate the majority of isolates possess mechanism the ability to evade bactericidal action of the complement system

They variability in serum resistance observed in this study aligns with findings reported by (Lee et al., 2023)[2], who evaluated 183 isolates of the Enterobacter cloacae complex and found significant differences in serum susceptibility among species. In their study, E. hormaechei and E. kobei were more resistant to human serum and exhibited higher virulence in Galleria mellonella infection models, while E. asburiae and E. roggenkampii showed greater sensitivity.

*Proteolysis ability*

The proteolytic ability of E.cloacae isolates exhibited varying levels of activity . Among the twenty isolates tested, 30% (6/20) demonstrated strong proteolytic activity(clear zone diameter ≥10 mm), while 55% (11/20) showed moderate proteolytic activity(5–9 mm), and 15% (3/20) exhibited weak proteolytic activity(<5 mm) based on visual assessment of clear halo zones on skim milk agar(Table-2). This classification aligns with methodologies described in previous studies such as Saran et al. (2007)[13]. .It is generally consistent with the previous results of Masiello et al. (2016), where the percentage of proteolytic bacteria was 71%, which indicates the role of this factor in enhancing the ability of bacteria to grow in different environments.[14]

Table 2: Proteolytic Activity of Enterobacter cloacae Isolates Based on Hydrolysis Zone Diameter

| Isolates | Diameter zone | Proteolytic ability |
|---|---|---|
| 1 | 10mm | Strong |
| 2 | 5mm | Moderate |
| 3 | 5mm | Moderate |
| 4 | 6mm | Moderate |
| 5 | 12mm | Strong |
| 6 | 3mm | Weak |
| 7 | 10mm | Strong |

**DOI:** https://doi.org/10.36320/ajb/v17.i3.20336

| | | |
|---|---|---|
| 8 | 2mm | Weak |
| 9 | 11mm | Strong |
| 10 | 7mm | Moderate |
| 11 | 5mm | Moderate |
| 12 | 10mm | Strong |
| 13 | 9mm | Moderate |
| 14 | 6mm | Moderate |
| 15 | 8mm | Moderate |
| 16 | 5mm | Moderate |
| 17 | 4mm | Weak |
| 18 | 7mm | Moderate |
| 19 | 6mm | Moderate |
| 20 | 11mm | Strong |

*Genotyping Assay*

*PCR Amplification of FimAGene and Sequencing*

The local sample was included by using a locus-specific primer FimA (65%) to generate amplicons of approximately base pairs in length. The 229 bp region of the FimA gene was amplified by PCR using the isolated bacterial genomic DNA as a template. Each ribosomal amplicon was checked to make sure it had distinct bands before it was sent for sequencing. Prior to sending these gene amplicons for sequencing, it was ensured that every amplified amplicon displayed clear, distinct and crisp bands. (Figure1).


**Fig 1:** 1.5% agarose gel Electrophoresis image at 80V for 2 hours, that show PCR products of FimA gene in Enterobacter cloacae complex isolates ; M : is DNA marker (100-2000pb) lane (1,3,5,6,9,10,11,12,13,14,15,16,17) are amplified gene products at 229pb stained with safe red stain .

*FimA Amplicons sequence of E.cloacae complex*

Regarding 229-bp PCR amplicons of the FimA gene, the sequenced samples and their targets showed complete sequences of similarities, according to analysis by the NCBI BLASTN search engine .Nucleotide composition analysis was compared to those derived using the more standard alignment-based method. The Fim A gene region was covered by approximately 99% of the predicted target, according to the NCBI BLASTN engine. Through contrasting the returned DNA sequences with the recognized DNA sequences of the samples being tested (Gene Bank accession number NZ_OW968328.1), as shown in Figure 2, it was possible to determine the precise positions and other details of the retrieved PCR fragments.

The sequence result that 99% compatibility 2 transition (A/G and A\G) and 1 transversions (G/C) when compared Query with Subject, as shown in Figure 2. Query represents DNA of the samples, while Subject represents DNA of the NCBI database.

**DOI:** https://doi.org/10.36320/ajb/v17.i3.20336

Table 3: Determinate of DNA sequencing of E.cloacae complex FimA gene .

| Enterobacter cloacae complex | | | | | | | |
|---|---|---|---|---|---|---|---|
| No. Of sample | **Type of substitution** | **Location** | **Nucleotide** | **Gene** | **Sequence ID with compare** | **Sequence ID with submission** | Identities |
| 1 | **Transvertion** | 1132348 | G\C | **FimA** | **ID: CP091486.1** | | 99 % |
| | Transition | 1132395 | A\G | | | | |
| | **Transvertion** | 1132434 | G\C | | | | |
| 2 | Transition | 1132395 | A\G | FimA | ID: CP091486.1 | | 99 % |

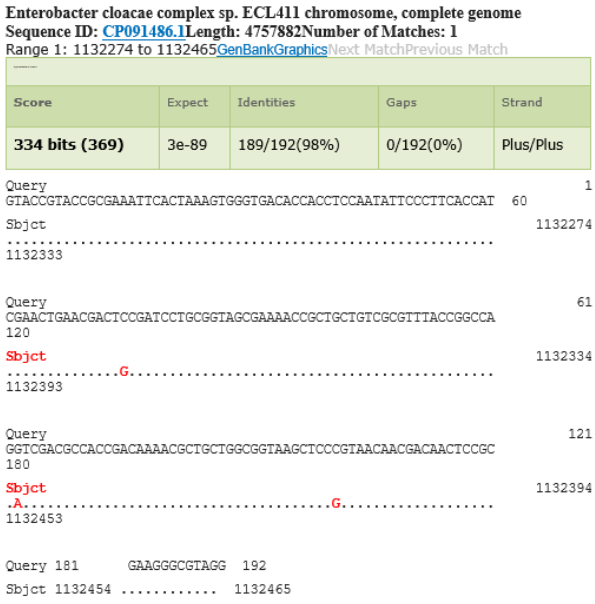


**Fig 2A:** Alignment analysis of E. cloacae gene bank at NC The other bacterial isolates were also compared to subject of NCBI in the same method

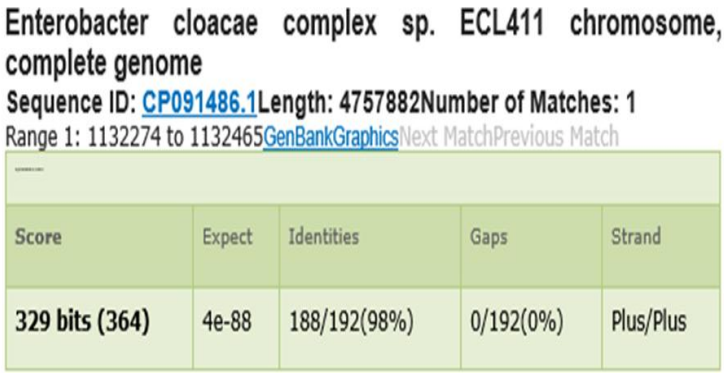


**Fig 2B:** Alignment analysis of *E. cloacae* gene bank at NC The other bacterial isolates were also compared to subject of NCBI in the same method.

*A Phylogenetic Tree Based on the Sequence of FimA gene*

**DOI:** https://doi.org/10.36320/ajb/v17.i3.20336

The current investigation, FimA PCR amplicons was used to construct an accurate phylogenetic tree. The evolutionary history was inferred using the UPGMA method [15]. The ideal tree is displayed with a branch length sum of 0.01606125 and is shown to scale, with branch lengths placed in the same units as the evolutionary distances used to estimate it. The evolutionary distances are expressed in terms of the number of base substitutions per site and were calculated using the Maximum Composite Likelihood approach [16]. Five nucleotide sequences were analyzed. The positions of the first, second, third, and noncoding codons were discussed. All positions with gaps or insufficient information were disqualified. A total of 189 locations were included in the final dataset. Evolutionary analyses were performed with MEGA6 [17].

The isolates originated from China and the USA, with sources including human hosts and environmental drainage. genetic similarity was 98%, indicating that the FimA gene sequence is highly conserved across different geographic locations and isolation sources. The 98% similarity indicates a very close evolutionary relationship between the isolates and suggests that the FimA gene is evolutionarily conserved.

Total branch length = 0.01606, a relatively small value, supports the conclusion that genetic divergence is minimal, reflecting a high degree of sequence identity among the isolates (Table3).

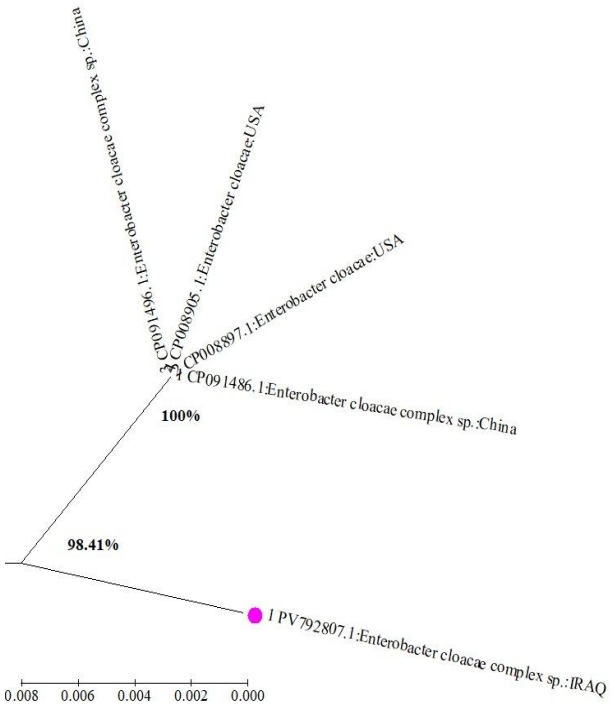


**Fig 3:** Construction Phylogeny tree of in NCBI on Neighbor-Joining tree Mega 6 software FimA gene of Enterobacter cloacae complex and alignment with reference data based

The phylogenetic analysis based on the fimA gene showed that this gene is useful in identifying and distinguishing the bacterial isolates. the primers used for the fimA gene are effective in detecting differences sub situation of nucleotides between the studied strains.

Table 4: Comparative sequence analysis of FimA gene in Enterobacter cloacae complex isolated from human and environmental source based on NCBI data

| Enterobacter cloacae complex sp.; FimA gene | | | | |
|---|---|---|---|---|
| Accession | Geographic location | Source of Isolation | date of registration | Matching |
| ID: CP091486.1 | China | Homo sapiens | 2022 | 98% |
| ID: CP008897.1 | USA | Homo sapiens | 2014 | 98% |
| ID: CP091496.1 | China | Drainage | 2022 | 98% |
| ID: CP008905.1 | USA | Homo sapiens | 2014 | 98% |

*Correlation Between Virulence Determinants and Phenotypic Traits in E. cloacae*

To better understand possible relationships between genetic and phenotypic virulence factors in Enterobacter cloacae, a comparative analysis was carried out to examine the connection between the presence of three virulence-related gene (fimA), proteolytic activity, and resistance to human serum. The findings were organized for 20 clinical isolates, allowing a clear evaluation of how these genes may relate to enzyme production and the ability to survive in serum. This comparison helps to explain the different aspects involved in the pathogenic behavior of E. cloacae, as shown in (Table-5) show a correlation between proteolytic activity and serum

**DOI:** https://doi.org/10.36320/ajb/v17.i3.20336

resistance in E. cloacae isolates. Among the strong proteolytic isolates (6 isolates), 100% were resistant to serum, indicating that high protease production may enhance bacterial resistance to host defense mechanisms. Conversely, the weak proteolytic isolates (3 isolates) were all sensitive to serum, suggesting that lower proteolytic activity correlates with reduced serum resistance. For the moderate proteolytic isolates (12 isolates), 75% exhibited serum resistance, while 25% were sensitive, indicating a mixed pattern where proteolytic activity may contribute to serum resistance, but other factors could also be influencing this resistance.

Table 5: Correlation Between Proteolytic Activity FimA gene Presence, , and Serum Resistance in *Enterobacter cloacae* Isolates.

| Isolate | Proteolysis | Resistance | fimA |
|---|---|---|---|
| 1 | Strong | Resistant | + |
| 2 | Moderate | Resistant | – |
| 3 | Moderate | Resistant | + |
| 4 | Moderate | Resistant | – |
| 5 | Strong | Resistant | + |
| 6 | Weak | Sensitive | + |
| 7 | Strong | Resistant | – |
| 8 | Weak | Sensitive | – |
| 9 | Strong | Resistant | + |
| 10 | Moderate | Sensitive | + |
| 11 | Moderate | Sensitive | + |
| 12 | Strong | Resistant | + |
| 13 | Moderate | Resistant | + |
| 14 | Moderate | Resistant | + |
| 15 | Moderate | Resistant | + |
| 16 | Moderate | Resistant | + |
| 17 | Weak | sensitive | + |
| 18 | Moderate | Resistant | – |
| 19 | Moderate | Resistant | – |
| 20 | Strong | Resistant | – |

A chi-square test of independence was performed to examine the relationship between proteolytic activity and serum resistance among E. cloacae isolates. The association was statistically significant, $\chi^2(df=2, N=20) = 9.57$, $p = 0.0082$. This suggests that higher levels of proteolytic activity are significantly associated with increased serum resistance. Notably, all strongly proteolytic isolates were resistant, while all weakly proteolytic isolates were sensitive to serum, indicating a potential role of proteases in mediating resistance to host immune defenses.

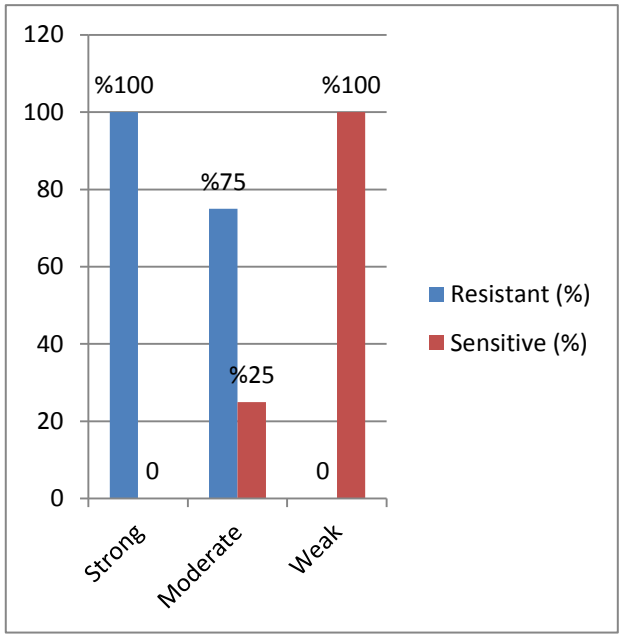


**Fig 4:** Positive Correlation between Serum resistance and Proteolytic ability of Enterobacter cloacae isolates

## Discussion

Among the twenty Enterobacter cloacae clinical isolates investigated in this study, serum resistance was observed in 15 isolates (75%), while the remaining five were sensitive to the bactericidal effect of normal human serum. This resistance is a crucial virulence trait that allows bacteria to survive in the bloodstream by evading the host complement system. The current results suggest a substantial prevalence of this phenotype, in agreement with Zhao and colleagues [18], who demonstrated a correlation between adhesion-related genes and increased resistance to immune clearance mechanisms.

**DOI:** https://doi.org/10.36320/ajb/v17.i3.20336

In parallel, proteolytic activity assessed on skim milk agar revealed that the majority of isolates (30%) exhibited strong casein hydrolysis, with others showing moderate or weak activity. This variability may reflect differential expression of secreted proteases, which play key roles in host tissue degradation and nutrient acquisition. The presence of clear hydrolytic zones in most isolates aligns with findings from Jiang and colleagues [19], who associated high protease activity with increased pathogenicity in E. cloacae strains. Notably, several serum-resistant isolates also exhibited strong proteolytic activity, suggesting a cumulative effect of these traits in enhancing the virulence profile of the organism.

Genotypic analysis revealed the presence of the fimA gene in 65% (13/20) of the isolates. Among them 9 isolates were resist to serum and 4 isolates strong proteolytic activity . This gene, which encodes the major subunit of type I fimbriae, is pivotal in bacterial adhesion and initial colonization of host tissues. Frutos [20] reported that the disruption of fimA significantly reduces bacterial adherence to epithelial surfaces. These findings suggest that the presence of fimA may not only enhance adhesion but may also promote survival and invasiveness through synergistic effects with other virulence determinants, which may reflect its important role in adhesion to host cells, while the other genes were variably distributed and may be associated with different abilities to evade immunity or cause tissue damage. In the current study, the fimA gene was detected in 65% (13 out of 20) of Enterobacter cloacae clinical isolates. This moderate prevalence suggests a potentially significant, yet variable, role of fimA in the pathogenesis of this species.

A study conducted on uropathogenic Escherichia coli (UPEC) isolates in Baghdad reported a much higher prevalence, with fimA detected in 100% (52/52) of the isolates examined [20]. This strong presence reflects the gene's essential role in adhesion and virulence in UPEC, which heavily relies on type I fimbriae-mediated attachment to urinary tract epithelium.

The lower prevalence observed in E. cloacae may indicate species-specific differences in virulence mechanisms, where fimA plays a supportive—but not exclusive—role. It may also reflect genetic diversity among strains or environmental adaptation, particularly in non-urinary sources such as biopsies or semen, which composed a substantial portion of the isolates in study [21].

Isolates with moderate proteolytic activity generally displayed intermediate resistance to serum, supporting the hypothesis of a dose-dependent or regulatory effect of protease expression on serum survival. These findings suggest a positive association between proteolysis strength and serum resistance.

The sequence analysis of the fimA gene revealed minor nucleotide polymorphisms among the positive isolates. Phylogenetic analysis based on these sequences demonstrated that while several isolates clustered closely with known reference strains in GenBank, others formed distinct branches, possibly indicating genetic divergence influenced by clinical source or selective pressures. This diversity within a conserved gene may contribute to functional variation in fimbrial structure or expression, which in turn could modulate interaction with host immunity or environmental resilience.

This study highlights the interdependence of serum resistance, proteolytic capability, and fimA-mediated adhesion as core elements of E. cloacae pathogenicity. The co-occurrence of these factors in the majority of virulent isolates suggests a coordinated expression of virulence mechanisms that enhance survival, tissue invasion, and immune evasion. These findings support the potential use of fimA as both a diagnostic marker and a target for anti-virulence therapies in Enterobacter infections.

Impact of Sample Source on Enterobacter cloacae Characteristics

The source of Enterobacter cloacae isolates significantly influences their genetic diversity, virulence, and resistance profiles. Isolates from intestinal and colonic biopsies often harbor antibiotic resistance genes and may exhibit lower virulence, reflecting their adaptation to the gut microbiota. Urine samples, commonly associated with urinary tract infections (UTIs), show higher virulence and resistance due to recurrent antibiotic exposure. Semen samples provide insight into isolates adapted to the reproductive tract, with unique traits for adherence and immune evasion. These findings highlight the importance of sample source in understanding the pathogenic potential and resistance mechanisms of Enterobacter cloacae

## Conclusion

The *fimA* gene plays a crucial role in bacterial adhesion to host tissues, facilitating colonization and persistence. However, the FimA gene is primarily considered the major structural subunit of Type I fimbriae, its role in pathogenicity goes beyond merely enabling bacterial

**DOI:** https://doi.org/10.36320/ajb/v17.i3.20336

adhesion. FimA is essential for the assembly of Type I fimbriae—a complex structure that includes the proteins FimH and FimF—which contribute to the interaction with host cells. Phylogenetic analysis of the *fimA* gene among *Enterobacter cloacae* complex isolates revealed a high degree of sequence conservation (98%), regardless of geographic origin or isolation source, suggesting that *fimA* is a conserved and functionally important gene within the *Enterobacter* genus. In contrast, serum resistance and proteolytic abilities exhibited greater variability among isolates, indicating that these traits are likely governed by different genetic determinants and may reflect adaptive responses to host-specific or environmental pressures.

## Acknowledgment

The authors gratefully acknowledge the support of **Al-Jumhori Hospital** and the valuable assistance provided by the laboratory staff.

## Funding Information

No external funding was received for this study.

## Author's Contributions

Mays alreem A. Aljebori Responsible for sample collection, genetic studies, and laboratory diagnosis .

Oruba K. AlBermani Genetic analysis and sequencing

Eklas M. Alsharifi Statistical analysis and scientific review

## Ethics

Ethical approval for this study was obtained from the Research Ethics Committee of the College of Science For Women, University of Babylon, Iraq. The study was approved under reference number 61, dated July 8, 2025. Informed consent was obtained from all participants prior to sample collection, and all procedures were conducted in accordance with the ethical standards of the Declaration of Helsinki.

## References

1. Matsuo, A., Matsumura, Y., Mori, K., Noguchi, T., Yamamoto, M., & Nagao, M. (2025). Molecular epidemiology and β-lactam resistance mechanisms of Enterobacter cloacae complex isolates obtained from bloodstream infections, Kyoto, Japan. Microbiology spectrum, 13(4), e02485-24.

2. Brust, F. R., Boff, L., da Silva Trentin, D., Pedrotti Rozales, F., Barth, A. L., and Macedo, A. J. (2019). Macrocolony of NDM-1 producing Enterobacter hormaechei subsp. oharae generates subpopulations with different features regarding the response of antimicrobial agents and biofilm formation. Pathogens 8:49. doi: 10.3390/pathogens8020049

3. MangeJP,StephanR,BorelN,WildP,Kim KS,PospischilA,etal.Adhesiveproperties of Enterobacter sakazakii to human epithelial and brain microvascular endothelial cells. BMC microbiology. 2006; 6:58. https://doi.org/10.1186/1471-2180-6-58

4. StonesDH,KrachlerAM.Againstthetide: the role of bacterial adhesion in host colonization. Biochem SocTrans2016;44:1571–80. https://doi.org/10.1042/BST20160186 PMID: 27913666

5. Laarman AJ, et al. Staphylococcus aureus metalloprotease aureolysin cleaves complement C3 to mediate immune evasion. J Immunol. 2011;186(11):6445–53.

6. Pokharel, P., Habouria, H., Bessaiah,

H., & Dozois, C. M. (2019). Serine Protease Autotransporters of the Enterobacteriaceae (SPATEs): Out and About and Chopping It Up. Microorganisms, 7(12), 594

7. Salarizadeh N, Mokhtari S, Ranjbar R, et al. Detection of proteolytic activity using skim milk agar method. ResearchGate. 2014. Available from: https://www.researchgate.net/figure/Proteolytic-activity-as-detected-by-the-skim-milk-agar-plate-method_fig1_282253844

8. Paudel, A., Panthee, S., Hamamoto, H., Grunert, T., and Sekimizu, K. (2021). YjbH regulates virulence genes expression and oxidative stress resistance in Staphylococcus aureus. Virulence 12, 470–480. doi: 10.1080/21505594.2021. 1875683

9. N. I. K, Al-Barhawee & J.M. Ahmed, "Using Sequencing Technique for Diagnostic Different Species of Genus Rhizobium Which Isolated from Legume Plants. Iraqi Journal of Science, vol.63, no. 10, pp.4213-4224, 2022.

10. N. I. K, Al-Barhawee & J.M. Ahmed, "Using Sequencing Technique for Diagnostic Different Species of Genus Rhizobium Which Isolated from Legume Plants. Iraqi Journal of Science, vol.63, no. 10, pp.4213-4224, 2022

11. Lee, J., Park, Y. K., Kim, S. Y., Kim, D. H., Kim, B., Lee, H., & Lee, J. Y. (2023). Serum resistance and virulence of Enterobacter cloacae complex species in Galleria mellonella infection model. Journal of Microbiology, 61(1), 47–56. https://doi.org/10.1007/s12275-023-2105-3

12. Masiello, S. N., N. H. Martin, A. Trmčić, M. Wiedmann, and K. J. Boor. "Identification and characterization of psychrotolerant coliform bacteria isolated from pasteurized fluid milk." Journal of Dairy Science 99, no. 1 (2016): 130-140.

13. Saran, S., Isar, J., & Saxena, R. K. (2007). A modified plate assay for detection of microbial protease activity. Journal of Basic Microbiology, 47(6), 444–446. https://doi.org/10.1002/jobm.200700052

14. Sneath P.H.A. and Sokal R.R. (1973). Numerical Taxonomy. Freeman, San Francisco.

15. Tamura K., Nei M., and Kumar S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. Proceedings of the National Academy of Sciences (USA) 101:11030-11035.

16. Tamura K., Stecher G., Peterson D., Filipski A., and Kumar S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution30: 2725-2729.

17. Zhao, Y., Sun, J., & Li, X. (2022). Serum resistance in Enterobacter species: Association with fimbrial gene expression. Infection and Immunity, 90(6), e00013-22. https://doi.org/10.1128/iai.00013-22

18. Jiang, Y., Liu, C., Zhang, J., & Zhou, Y. (2021). Protease production and virulence potential in clinical Enterobacter cloacae strains. Journal of

Clinical Microbiology, 59(7), e01234-21. https://doi.org/10.1128/JCM.01234-21

19. Frutos-Grilo, A., González-Bardanca, M., Pérez-Tanoira, R., & Gómez-Garcés, J. L. (2023). Enterobacter cloacae exerts different adhesion and invasion capacities against different host cell types. PLOS ONE, 18(10), e0289334. https://doi.org/10.1371/journal.pone.0289334
20. Alwan, S. A., & Jassim, H. A. (2024). Antibiotic Resistance, Biofilm Formation, and Identification of FimH and FimA Adhesion Genes in Uropathogenic Escherichia coli Isolated from Patients in Baghdad Province. Iraqi Journal of Science, 65(2), 444–451. https://ijs.uobaghdad.edu.iq/index.php/eijs/article/view/10854