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Phenotypic and molecular investigation of biofilm formation of Enterobacter cloacae causing urinary tract infections

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Abstract:

Urinary tract infections represent a major expensive, a global common public health issue owing to their high prevalence and the struggling associated with their management. The current investigation aimed to identify the phenotypic and molecular characteristics of biofilm formation of E. cloacae isolates from UTIs by investigated the rate of distribution for two important genes namely: csgA and csgD. The current investigation demonstrated that the studied isolates of E. cloacae isolated from UTIs had a biofilm production rate at 80%. The statistical analysis outcomes indicate significant differences at 0.01 probability level. The outcomes of PCR for (20) E. cloacae isolates showed that the prevalence rates of the three studied genes were 70% and 65% for: csgA and csgD, respectively. The results of statistical analysis indicated that numbers of positive isolates was significantly higher ($p<0.05$) than negative samples for studied genes (csgA and csgD). Conclusion: The results of the current study confirm the clinical challenge posed by biofilm-forming E. cloacae strains, which have the ability to evade host defenses and classical antimicrobial treatments. The ability of E. cloacae to form biofilms was enhanced by 80%, which reinforced its virulence and survival, particularly in hospital settings where biofilms-related infections implicated to chronic disease and treatment failure.

Keywords: UTIs, E. cloacae, csgA, csgD, biofilms

Introduction:

Urinary tract infections (UTIs) are deem from the most prevailing bacterial infections in the world, which are one of most common infections in hospitals and the community (Mach et al., 2020). These infections influencing millions yearly and constituting considerable healthcare burden, particularly in hospitalized and immunocompromised individuals (Li et al., 2025). The most commonly implicated species are the members of Gram-negative bacteria (Rajabi & Dallal, 2015), including Escherichia coli (E. coli), Enterobacter spp., Klebsiella pneumoniae (K.

pneumoniae) (Guermazi-Toumi et al., 2018), Pseudomonas aeruginosa, and Serratia marcescens with increased resistance patterns rates (Rosana et al., 2020). Among these species, E. coli, K. pneumoniae, and Enterobacter cloacae (E. cloacae) are three significant pathogens engaged with UTIs with remarkable resistance phenotypes (Xu & He, 2019). These strains were recognized as the most developed resistance mechanisms in enterobacteria (Jiménez-Guerra et al., 2020).

Recently, attention has focused on E. cloacae, a member of E. cloacae complex (ECC), owin to its altitude incidence in nosocomial UTIs and multidrug resistance profiles (Yassir & Zaid, 2022;Hassan & Motaweq, 2024). The ECC involved various closely related species, including E. cloacae, E. hormaechei, and E. asburiae, which are sharing more than 60% DNA homology and are commonly isolated from clinically relevant samples (Yassir & Zaid, 2022). A decisive virulence feature responsible for persistencing and pathogenicity of E. cloacae in UTIs is the capacity to produce biofilms (Kathi, 2024). The biofilms are assembled microbial communities enclosed by extracellular polymeric substances (EPSs), which have the ability for adhering with biotic and abiotic surfaces (Flemming et al., 2016). These biofilms formation enhance the bacterial survival under antagonistic conditions, inclusive of antibiotics exposure and immune responses of host, thus complicating the therapeutics options and promotion the chronic infections. Studies had revealed that biofilm-producing E. cloacae isolates exhibit significantly higher resistance to antibiotics compared to their planktonic counterparts, underscoring the clinical relevance of this phenotype (Liu et al., 2022; Nohad & Hassan, 2023; Kathi, 2024). The usual methods employed to evaluate the ability to form biofilms is Congo Red Agar (CRA) technique and tissues culture plate procedure (Oleiwis et al., 2021). From the molecular view, different genes were engaging in biofilms regulation alongside the structural integrity, including fimH, fimA, csgD, and rpoS; where these genes are encoding for fimbriae, curli fibers, and stress responses regulators that enable adhesion, EPSs synthesis, and perseverance in presence of environmental stressors (Misra et al., 2022). The expressions of these genes differ among strains and often related with multidrug resistance, which revealing a genetic relationship between biofilms production and antimicrobial resistance mechanism. The current study sought to characterize both phenotypic and molecular characteristics of biofilm formation in E. cloacae isolates from UTIs, focusing on the distribution rates of two principle genes: csgA and csgD.

Materials and methods:

Study design & Samples Collection and processing:

A randomized exploratory investigation was achieved at the period from November 2024 to January 2025. A total of 100 urine specimens from patients with UTIs have been collected from several hospitals in different regions at Babylon province which including: Al-Hashimiya General Hospital, Al-Jumhuri Teaching Hospital and The late Ali Oubis Al-Sultani General Hospital. This investigation focused on isolating E. cloacae isolates from urine samples, which collected and analyzed according to standard laboratory procedures. From all patients, the midstream urine samples were taken and putted in sterile plastic containers, then a disposable loop was employed to streak the specimens streaked on blood agar and MacConkey agar, after that were incubated at

37°C for 24hours. At first, an initial reading was recorded, then specimens were farther incubated for a concluding reading.

Culture procedure & Detection of E. cloacae:

According the guidelines of World Health Organization on routine culturing, all specimens have been cultured employing a semi-quantitative technique (Vandepitte, 2003). The E. cloacae isolates have been isolated by plating 1mL of each urine specimen on nutritional agar, tryptose soya agar, and two differential selective media (Xylose Lysine Deoxycholate agar and Salmonella Shigella agar). These culture plates have been incubated at 37°C for 18-24hours. The detection of E. cloacae isolates has been achieved by identifying the appearance of rough and smooth colonies on tryptose-soya agar. The biochemical assays and cultural characteristics have been used to detect and classify the isolates.

Estimation of Biofilms Formation:

Congo red agar test has been applied to estimate the ability of E. cloacae isolates to produce biofilms, where E. cloacae isolates had been incubated for 24-42h at 37°C in Brain Heart Infusion (BHI) broth supplemented with 5% (w/v) sucrose and 0.08% (w/v) Congo Red. Strains that formed red colonies with a dry, crystalline texture were considered to be producing exopolysaccharides, while white or pink colonies indicated poor exopolysaccharide production.

Genotypic Analysis:

Genomic DNA of E. cloacae isolates had been was extracted according to the manufacturer's instructions of Genomic DNA Purification Kit (Geneaid/Turkey). The purity of the extracted genomic DNA was determined using a Nano-drop spectrophotometer, which measures DNA concentration (ng/µl) and reads the absorbance at (260/280 nm). The primers specific from (IDT, Canada) for (csgA, csgD and 16Sr RNA) is shown in Table (1). The conventional polymerase chain reaction (PCR) has been applied to amplify studied genes; it had been yielded in 50µl volumes containing 5µl of DNA template, 25µl of PCR Taq Master Mix (Abm/Korea), 3µl from both forward and reverse primers, and 14µl of Nuclease free water (Bioneer/Korea). The conventional PCR thermocycler apparatus (Techne/UK) has been second-hand for genetic detection. Table (2) shows PCR thermocycler setting that was applied for studied genes. The PCR products have been analyzed in (1.5) Agarose that stained using (1%) ethidium bromide (Bio basic/Canada) and photo-documented under UV illumination (UVP/USA).

Table (1): Primers set of biofilms associated genes

Target genes	Primer sequence (5'- 3')		Product size (bp)
csgA	**F**	ATTGCAGCAATCGTAGTTTCTGG	230 bp
	R	ATGAYCTGTCATCAGAGCCCTGG	
csgD	**F**	TGAAARYTGGCCGCATATCAATG	243 bp
	R	ACGCCTGAGGTTATCGTTTGCC	
16SrRNA	**F**	TCC AGA TTA CAA CTT CAC CAG G	**1465bp**
	R	CAA TTC ATA TCT TGT AAC G	

Table (2): PCR Thermocycling settings of biofilms associated genes

Genes name	**csgA**		
PCR steps	**Cycle**	**Temp.**	**Time**
Initial denaturation	1	95C	2min.
Denaturation	35	95C	30 sec.
Annealing		55°C	30 sec.
Extension		72C	45sec.
Final extension	1	72C	7min.
Hold	-	4C	Forever
csgD			
PCR steps	**Cycle**	**Temp.**	**Time**
Initial denaturation	1	95C	2 min.
Denaturation	35	95C	30sec.
Annealing		55°C	30sec.
Extension		72C	45sec.
Final extension	1	72C	7min
Hold	-	4C	Forever
16SrRNA			
PCR steps	Cycler	**Temp.**	**Time**
Initial denaturation	1	95C	5min.
Denaturation	35	95C	30sec.
Annealing		56°C	30sec.
Extension		72C	45sec
Final extension	1	72C	7min
Hold	-	4C	Forever

Results and discussion:

Phenotypic detection of Biofilms formation in E. cloacae isolates:

The current investigation demonstrated that the studied isolates of E. cloacae isolated from UTIs had a biofilm production rate at 80%, as displayed in Figure (1). The statistical analysis outcomes indicate significant differences at 0.01 probability level, as exhibited in Table (3).

Table (3): Phenotypic detection of Biofilms formation in E. cloacae isolates

Total isolated examined	Positive result		Negative result	
	No.	%	No.	%
20	16	80	4	20
X^2	14.4			
P value	<0.0001 HS			

HS: Highly significant difference at P<0.01

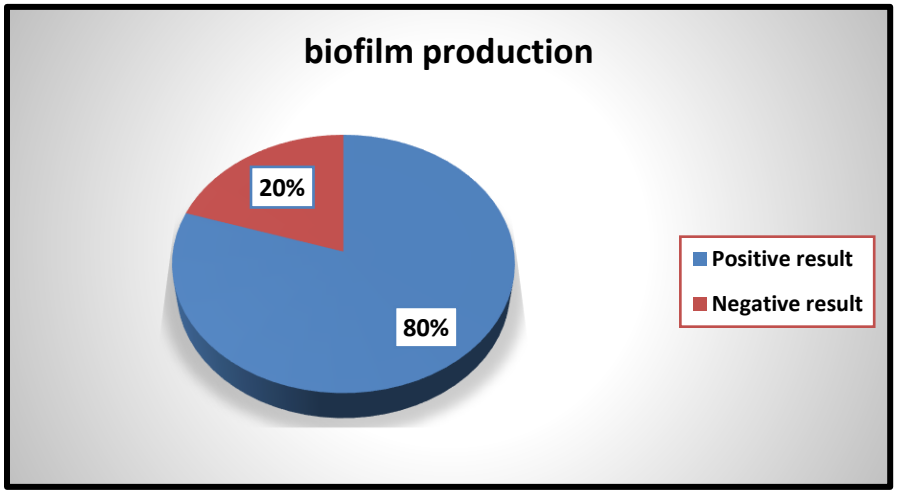

Figure (1): Phenotypic detection of Biofilms formation in E. cloacae isolates

The current investigation demonstrated that detection rate of biofilm formation in E. cloacae isolates from UTIs was 80%, where this high rate emphasizes the clinical relevance of biofilm formation in E. cloacae, exceptionally with respect to permanent and recurrent UTIs, this relevance comes from the ability of biofilm to give reinforced bacterial surviving by shielding against host immunological responses along with antimicrobial agents, so these biofilm complicating the therapeutics outcomes and boosting the chronic infections states. The present finding highlights the pathogenic adaptability of E. cloacae, particularly in hospital setting where biofilm-associated infections represent a major concern. Biofilms are enabling bacterial strains to survive on medical tools and being resistant for various antimicrobial agents.

Biofilm producing deem as a vital virulence factor, which provides a protective environment to allow pathogens to survive and protect from antibiotics (Dincere et al., 2020).

Several Gram-negative bacteria are known for their ability to form biofilms, which are masses of bacterial cells that stick to each other and offer excellent protection against the various challenging conditions of the environment, as well as agents used to kill bacterial organisms. This structure is making these bacteria more resistant to antibiotic than bacteria that do not have biofilm (Garde et al., 2015). Within biofilms, there are bacteria known as sessile bacteria, which undergone a stationary or dormant growth phase and display phenotypic attributes that differ them from planktonic bacteria (Muhammad et al., 2020). N-acyl-l-homo serine lactones (AHLs)-based quorum sensing mechanisms are known to regulate different virulence factors. It is widely assumed that cell-to-cell communication via collective sensing is mainly mediated by AHLs, which act as key signaling molecules (Scoffone et al., 2019; Venkatramanan et al., 2020).

The current outcomes align with those of Liu et al. (2022), who revealed that biofilms formation is a common feature across different groups of E. cloacae complex, principally under nutrient-deficient conditions. As well, our finding agree with a previous study demonstrated that all Enterobacter spp. isolates were able of forming biofilms (ALKhalidy, 2024). What more, Oleiwis et al. (2021) reported that biofilms formation rate in E. cloacae isolates was 100% by employing Congo Red Agar and Christensen tube approaches. In addition, Dehkordi et al. (2022) found that 53.85% of E. cloacae isolated from UTIs were vigorous biofilm formers, which more supported the conception that biofilms formation is a significant attribute among clinical isolates.

In contrast, some investigations had documented lower and diversified biofilms formation rates in E. cloacae strains; where the study of Liu et al. (2022) revealed that biofilm formation varied notably across ECC clusters, with only some clusters showed potent biofilms production.

Molecular characterization of E. cloacae using 16SrRNA gene sequencing

The genetic analysis of E. cloacae isolates has been yielded several important findings that can be utilized not only to detect the taxonomic identity of these isolates but also to reveal their evolutionary correspondence. Figure (2) displays the outcomes of PCR for twenty E. cloacae isolates screening for 16SrRNA gene with a product size of 1465bp.

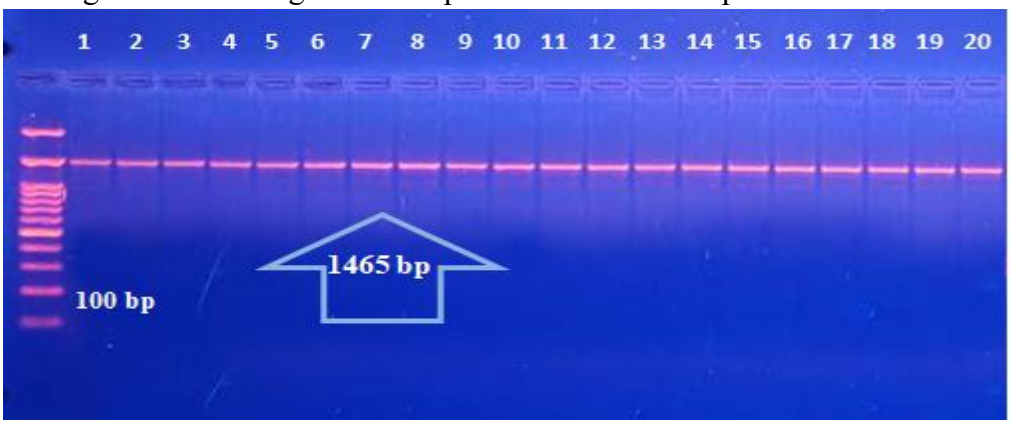

Figure (2): Outcomes of DNA amplification of 16SrRNA gene in E. cloacae isolates using 1.5 agarose gel electrophoresis, product size (1465bp).

These high (>99%) similarity scores from the BLASTn search support the classification of Iraqi clinical isolates as E. cloacae, E. hormaechei, E. asburiae and E. aerogenes thus indicating that 16SrRNA gene sequence is very reliable for bacterial characterization. This characterization is further supported by phylogenetic reconstruction which presented strong monophyletic cluster depended on species and uncover the genetic proximity between Iraqi isolates and reference strains with geographical distance locations as India, China and Brazil except the Egyptian wild type which show divergence upon comparing with four other simian-virus genotypes. This high evolutionary relationship indicated a shared evolution history and also drew attention to the ubiquitous existence of these bacterial species, possibly owing to environmental influence and human activities.

In this regard Figure (3) shows multiple sequence alignment of in DNA sequences of isolates that were involved in the study. This alignment supported the conservation of certain regions and divergence in the number and nature of sequence accession, hence confirming the genetic diversity and evolutionary convergence among isolates. This alignment corresponds to a comparison of nucleotide sequences of 32 isolates. The colored bars display locations of single nucleotide polymorphisms (SNPs), where at least one nucleotide difference at these locations doesn't match the consensus sequence. Nucleotides with the same reference number are marked in same color, while different colors are utilized to indicate variations in variable locations across these sequences.

Concurrently, both transitions and transversions in SNPs between isolates indicated the presence of micro-diversity within bacterial community, as displayed in Table (4). This table exhibited the location of all variants compared with the reference sequence, alongside the nucleotide base in the reference sequence and the correspond base detected in query sequence. The table also displays SNP's type of in each instance of variation, whether it being transition or transversion. The outcomes indicated the presence of variations at several locations, some of which are classified as transitions, which are alterations between bases of same chemical class, and others are classified as transversions, which are alterations between bases of different classes. Some sites, such as positions 22, 28, and 32, showed the presence of more than one type of variations at the same site, indicating a degree of micro-diversity within studied bacterial community. This diversity may be originated from neutral genetic drift or from ongoing local evolution of Iraqi isolates. These subtle genetic differences could be an indication of ongoing local evolution or neutral genetic drift in Iraqi lineages.

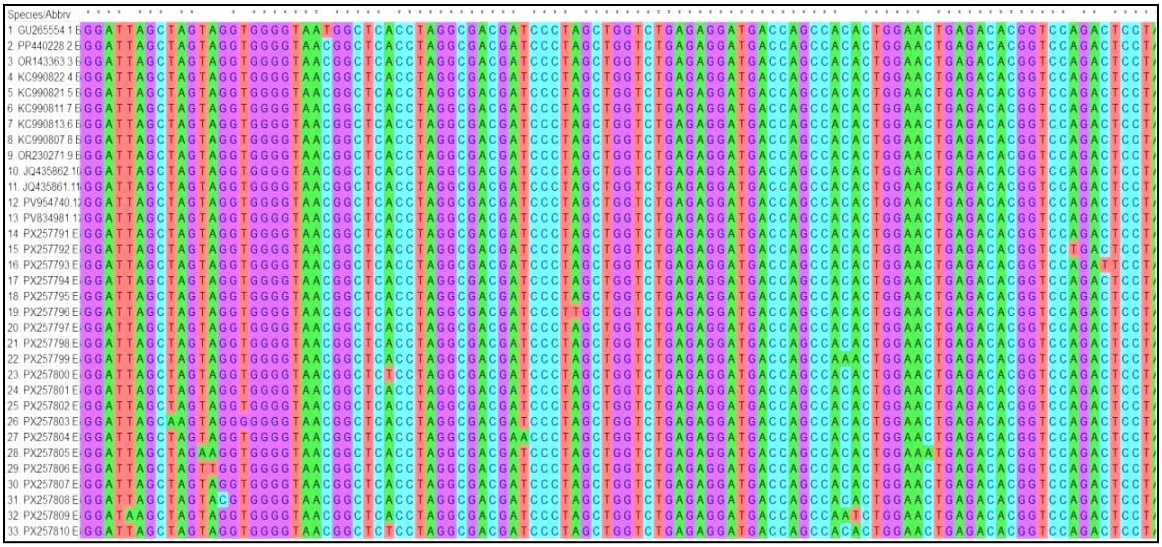

Figure (3): Multiple sequence alignment of 16S rRNA gene sequences of E. cloacae isolates

Table (4): Analysis of SNP variations between the reference sequence and the sequences of Iraqi isolates

Alignment Pos.	Ref._Base	Variant(s)	SNP Type
1	C	T	Transition (C>T)
3	T	C	Transition (T>C)
8	A	G	Transition (A>G)
15	C	T	Transition (C>T)
19	A	T	Transversion (A>T)
22	C	A, T	Transversion (C>A), Transversion (C>T)
28	T,C	A	Transversion (T>A), Transversion (C>A)
31	G	C	Transversion (G>C)
32	T,A	A,T	Transition (T>A), Transversion (A>T)

Table (5) illustrates the outcomes of the BLASTn search, which compared the nucleotide sequences (like 16SrRNA gene) of Iraqi isolates with a database containing known sequences. The table displays the percentage of identity (Per. Ident) between each isolate and reference sequence, in addition to scientific name, database accession number, and country in which reference sequence was documented. These data provide powerful evidence of the taxonomic identity of studied isolates, as the high identity rates (over 98%) indicate that the samples belong to specific

Enterobacter spp. These data also reinforce the findings of phylogenetic analysis and strengthen the reliability of using 16S rRNA gene as a standard factor for bacterial recognition.

Table (5): BLASTn search results for 16S rRNA gene sequences of E. cloacae

Description	Scientific Name	Per. Ident	Accession	Country
Enterobacter sp. strain FMQEB15 16S ribosomal RNA	Enterobacter sp.	99.87%	PX257805	Iraq
Enterobacter sp. strain FMQEB17 16S ribosomal RNA	Enterobacter sp.	99.12%	PX257807	Iraq
E. aerogenes strain T2 16S ribosomal RNA gene	E. aerogenes	98.45%	GU265554.1	India
E. hormaechei strain JCS-3 16S ribosomal RNA gene	E. hormaechei	99.76%	PP440228.2	China
E. hormaechei strain FMQEB12 16S ribosomal RNA	E. hormaechei	100.00%	PX257802	Iraq
E. hormaechei strain FMQEB11 16S ribosomal RNA	E. hormaechei	99.91%	PX257801	Iraq
E. cloacae strain 344 16S ribosomal RNA gene	E. cloacae	99.54%	J0435862.10	Brazil
E. cloacae strain FMQEB7 16S ribosomal RNA	E. cloacae	99.82%	PX257797	Iraq
E. cloacae strain 341 16S ribosomal RNA gene	E. cloacae	99.21%	J0435861.11	Brazil
E. cloacae strain FMQEB8 16S ribosomal RNA	E. cloacae	100.00%	PX257798	Iraq
E. asburiae strain G3 16S ribosomal RNA gene	E. asburiae	98.93%	OR143363.3	China
Enterobacter asburiae strain FMQEB14 16S ribosomal RNA	E. asburiae	99.65%	PX257804	Iraq
E. asburiae strain FMQEB13 16S ribosomal RNA	E. asburiae	99.77%	PX257803	Iraq
E. cloacae strain RJ04 16S ribosomal RNA gene	E. cloacae	98.88%	KC990807.8	India
E. cloacae strain FMQEB5 16S ribosomal RNA	E. cloacae	99.43%	PX257795	Iraq
E. cloacae strain RJ20 16S ribosomal RNA gene	E. cloacae	99.02%	KC990811.7	India

E. cloacae strain FMQEB4 16S ribosomal RNA	E. cloacae	99.91%	PX257794	Iraq
E. cloacae strain 6nak4 16S ribosomal RNA gene	E. cloacae	98.67%	PV954740.12	India
E. cloacae strain FMQEB9 16S ribosomal RNA	E. cloacae	100.00%	PX257799	Iraq
E. cloacae strain RN1 16S ribosomal RNA gene	E. cloacae	99.25%	KC990821.5	India
E. cloacae strain FMQEB6 16S ribosomal RNA	E. cloacae	99.56%	PX257796	Iraq
E. cloacae strain FMQEB3 16S ribosomal RNA	E. cloacae	99.84%	PX257793	Iraq
E. cloacae strain FMQEB2 16S ribosomal RNA	E. cloacae	99.73%	PX257792	Iraq
E. cloacae strain CS8 16S ribosomal RNA gene	E. cloacae	98.79%	OR230271.9	India
E. cloacae strain RJ30 16S ribosomal RNA gene	E. cloacae	99.10%	KC990813.6	India
E. cloacae strain RN2 16S ribosomal RNA gene	E. cloacae	99.33%	KC990822.4	India
E. cloacae strain FMQEB1 16S ribosomal RNA	E. cloacae	100.00%	PX257791	Iraq
E. cloacae strain CIFRLU13 16S ribosomal RNA gene	E. cloacae	98.56%	PV834981.13	India
E. cloacae strain FMQEB10 16S ribosomal RNA	E. cloacae	99.48%	PX257800	Iraq
E. cloacae strain FMQEB20 16S ribosomal RNA	E. cloacae	99.62%	PX257810	Iraq

The outcomes of 16SrRNA gene sequencing analysis of Iraqi E. cloacae isolates demonstrated high accuracy in determining taxonomic identity, by matching rates in BLASTn exceeding 98%, reinforcing significance and reliability of 16SrRNA gene as a standard tool to identify bacterial pathogens. Bartoš et al. (2024) proven that 16SrRNA gene is one of most stable and slowly evolving genes, rendering it suitable to determine taxonomic identity at genus level, despite its potency to differentiate between subspecies is restricted in some cases.

Nevertheless, emergence of subtle differences in SNP sites between isolates, as shown in sequence alignment, points out the presence of micro-diversity within local bacterial populations. This diversity may originate from neutral genetic drift or growing local evolution. On the other hand, phylogenetic reconstruction demonstrates monophyletic clusters between Iraqi isolates and

strains from faraway regions like India and China and Brazil, revealing a common evolutionary history and common distribution for these species. It revealed that the similarity in this genetic pattern could be returned to presence of horizontal gene transferring, exceptionally in 16SrRNA gene, which justifies the evolutionary interfering between isolates from nonidentical environments (Sato and Miyazaki, 2017).

The findings of Abdul-Razzaq et al. (2013), conducted in Babylon province on clinical isolates of both E. cloacae and E. aerogenes, reinforces our findings; where this study demonstrated that (70.84%) of isolates return to phylogenetic group B2, with phylogenetic group B23 being the most common (12/17 isolates), suggested a locally dominant evolutionary pattern.

The current study also aligns with another local study in Basrah province that emphasized the accuracy of 16SrRNA gene as a standard gene for genetic characterization; this study also showed diversity among species isolated from different sources, including E. cloacae, E. hormaechei, and E. asburiae, revealing presence of a diverse bacterial population in Iraqi environment. The outcomes of phylogenetic analysis showed clear genetic clusters between local isolates and strains from other geographic regions. This genetic overlapping increased the importance of combining the molecular analysis with environmental data to understand more premises about bacterial development locally and contributes toward establishing a genomic database that might be employed in genetic tracking researches and epidemiological surveillance (Abbas & Radhi, 2016).

Phylogenetic analysis:

Figure (4) shows the phylogenetic tree constructed according to 16SrRNA gene sequence analysis, which explicates the pattern of evolution and genetic diversity among different strains of Enterobacter strain including Iraqi isolate. This tree presented clear clustering of isolates based on some taxa such as E. cloacae, E. hormaechei, E. asburiae, and E. aerogenes. The close tacking between Iraqi isolates and the reference sequences from other countries (India, China and Brazil) indicate that these bacterial species have a common origin of evolution with worldwide distribution. The small genetic differences within certain groups also indicate micro-diversity of the isolates, which is due either to local adaptation or neutral drift. Furthermore, the low genetic differences within certain clusters indicate that the isolates constitute micro-diversity and they may be resulted from local adaptation or neutral genetic drift. Reconstructing this phylogenetic tree is an important approach to knowledge the population construction and genetic association of Enterobacter spp. isolates. According to complete phylogenetic analysis, the strains studied in Iraq were well separated and allocated to distinct species; E. cloacae, E. hormaechei, E. asburiae, and E. aerogenes. The high rates of matching obtained in the BLASTn comparisons would strengthen this classification at species level, 16SrRNA gene used as an standard gene. These results were also approved by phylogenetic analysis with clear evolutional relationships between Iraqi isolates and worldwide reference strains indicating the cosmopolitan nature of these bacteria.

Figure (4): The phylogenetic tree based on the 16SrRNA gene sequence shows the relationships between the Enterobacter isolates isolated in Iraq and the reference strains found in global databases. The maximum likelihood method with the Tamura-Nei model was used to infer the evolutionary history of these isolates.

The close relationship between Iraqi isolates and reference strains from other countries suggested a common evolutionary origin and global distribution of these bacterial species. Sato and Miyazaki (2017) described and justified this pattern of genetic similarity depending on horizontal gene transferring, particularly in 16SrRNA gene. However, fine branching across species reflects micro-diversity among these isolates. The present investigation bolsters up the significance of integration the molecular analysis with environment and clinical information within a public health framework, participating to develop of successful strategies for epidemiological inspection and infections management.

Molecular detection of biofilm genes in E. cloacae:

The present study detects the presence of three genes that were associated with biofilm formation in Enterobacter spp., which included: csgA, and csgD genes. The outcomes of PCR for (20) E. cloacae isolates demonstrated that csgA gene with 230bp product size was identified in 14 put of 20 isolates (70%), as shown in (Figure 5). The csgD gene was found in 13 out of 20 isolates at rate of 65%, with a 243bp product size, as shown in (Figure 6). The results of statistical analysis exhibited in Table (6), which indicated that numbers of positive isolates was significantly higher ($p<0.05$) than negative samples for studied genes (csgA and csgD).

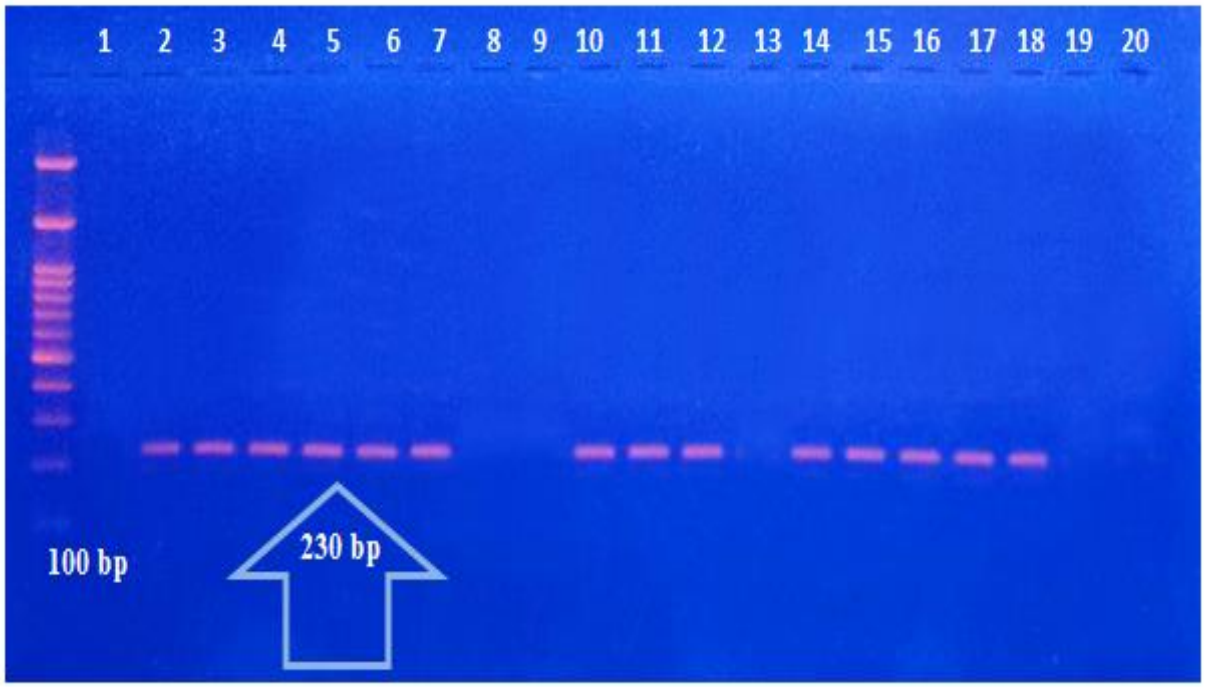

Figure (5): Results of DNA amplification of csgA gene in E. cloacae isolates using 1.5 agarose gel electrophoresis, product size (230bp).

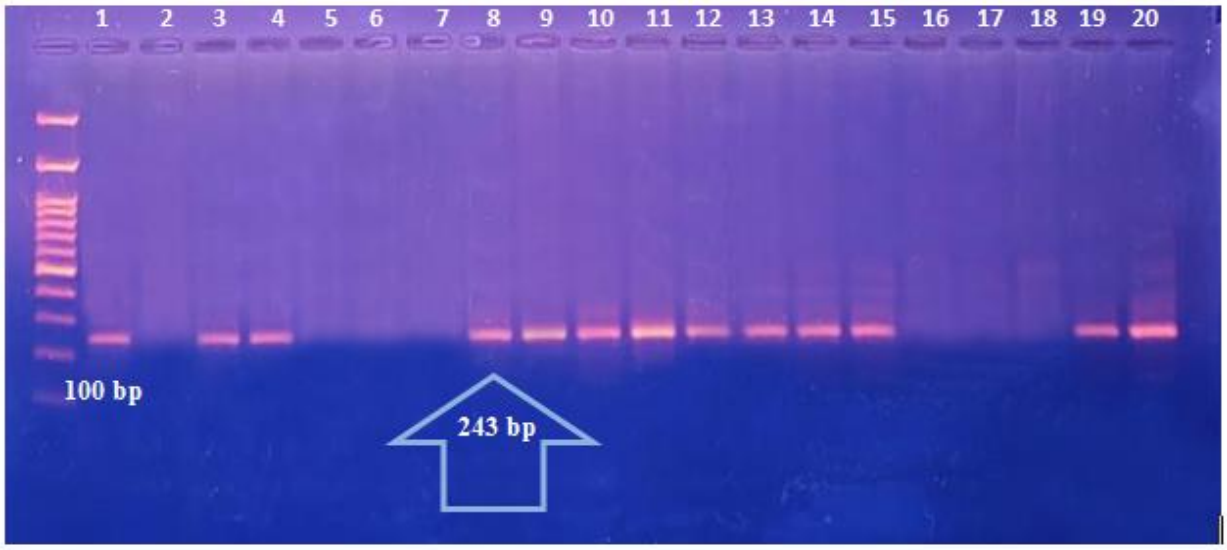

Figure (6): Results of DNA amplification of csgD gene in E. cloacae isolates using 1.5 agarose gel electrophoresis, product size (243bp).

Table (6): Distribution of biofilm genes among E. cloacae

Virulence genes	No. (%) of positive isolates	No. (%) of negative isolates	Total No. (%)	P value
csgA	14(%70)	6(%30)	20(%100)	0.0001*
csgD	13(%65)	7(%35)	20(%100)	0.0027*
P value	**0.330 NS**			

*Significant differences p< 0.05 are indicated by chi-square test; NS indicates non-significant differences p> 0.05.

Molecular inspection of biofilms associated genes showed that 70% of E. cloacae isolates had csgA gene, 65% harbored the csgD gene. These results accentuated that the high frequency of curli fimbriae subunit in UTI isolates of E. cloacae means a strong ability to establish biofilm. These genes encodes the major subunits of curli fimbriae system, which play a critical role in biofilms formation, surface adhesions and auto-aggregation. Self-aggregation increases with curli fimbriae synthesis at low temperatures (Goulter et al., 2010). CsgA subunit is secreted outside the cell, and then convened into fibers by CsgB, which is binding to bacterial outer membrane. There are two different operons are regulating curli fimbriae: first one the csgBAC operon that encodes fundamental curli subunits CsgA and its homologue CsgB, the second is csgDEFG operon that encodes csgD, csgE and csgF, which serve as chaperones mandatory for effective curli fabrication (Dueholm et al., 2012). csgA gene is coding the prime structural unit of curli fibers, whilst csgD

gene serves as a transcriptional regulator that stimulates csgBAC operon, so participating with starting of curli biogenesis (Dueholm et al., 2012).

Numerous bacterial surface structures like curli, flagella, pili, and exopolysaccharides, are implicated in several aspects of biofilms formation (Kalantar et al., 2008). A remarkable correlation was discern between biofilms formation and mRNA expressions rates of csgA as well as csgD. Curli protein fimbriae seem as intertwist fibers, and curli-proficient strains pose mature biofilms (Kim et al., 2012).

Rasheed et al. (2021) demonstrated that curli expressions in E. cloacae is a decisive factor in biofilms formation and represents initial stage of its pathogenicity, this study supports our findings that found a high percentage of E. cloacae isolates form biofilms, at 80%, and also a high percentage of expression of gene responsible for curli production. The current findings consistent with those reached by Ahmad et al. (2020), they revealed that more than 80% of isolates belong to Enterobacteriaceae possessed csgA gene. The current study also aligns with a study by Al-Radhwany and Al-Taee (2024), which confirmed the presence of both csgA and csgD genes in all E. cloacae isolates, indicating 100% prevalence in studied isolates. Likewise, Oleiwis et al. (2021) revealed that E. cloacae isolated from both clinical and environmental origins expressed csgA and csgD genes at rate of 100%, strengthening the functions of these genes in biofilms formation.

On the other hand, other studies had demonstrated lower prevalence rates, where in the study of Saadi et al. (2024) the prevalent rate of csgA and csgD genes were only 13% of Enterobacter spp. isolates from clinically cases in Najaf city. This variation can be attributed to various origins of samples, geographical localization or genetic diversity of strains. Similarly, csgD gene was detected from multidrug-resistant Enterobacter (42.9%) by Hassan and Motaweq (2024) showed correlation between the biofilm genes with resistance ability.

The considerably high occurrence of csgA and csgD in our presented research also support the declaration that biofilm creation is a common strategy may be conserved amongst Enterobacter spp. This observation is of special interest to the clinic, where biofilms contribute to persistance of infections and fortify resistance against antibiotics.

Conclusion:

The findings of the present work validate that biofilm-forming E. cloacae strains are a clinical challenge, as they can circumvent classical host defense mechanisms and antimicrobial therapy. The victory and survival of E. cloacae were based on the ability to form biofilms, which was increased by almost 80% in this bacterium that strengthens the virulence of this pathogen, especially in hospitals as biofilm-related infections are linked to chronic disease and treatment failure. The main trait that provides these isolates with the capacity to form biofilm is identified with respect to the genes encoding those, including csgA and csgD, which are greatly present in current research.

Declaration of Competing Interest:

The researcher declares that there are no known financial interests or personal relationships that could have influenced the work reported in this paper.

References:

Abbood, H. K.& Hateet, R. R. (2025). Green synthesis of gold nanoparticles (AuNPs) using pathogenic bacteria Acinentobacter baumanii with evulation their antibacterial activity. Misan Journal for Academic studies, 24(53), 62-72. http://www.misan-jas.com/index.php/ojs/article/view/852

Abdulrazzaq , Y. A., Ali, O. A. (2025). Evaluation of galectin-3 and peptidyl arginine deiminase-4 levels in saliva for periodontal health, gingivitis and periodontitis. Misan Journal for Academic studies, 24(53), 15-26. http://misan-jas.com/index.php/ojs/article/view/848

ALKhalidy, A. A. A. H. (2024). Molecular characterization of virulence factors genes among Enterobacter species isolated from different clinical sources (Master's thesis, University of Kufa, Faculty of Science, Department of Biology–Microbiology).

Al-Radhwany, S. D., & Al-Taee, H. A. (2024). Molecular detection and presence of csgA and csgD genes that share in biofilm production in Enterobacter cloacae by PCR sequence technique. Malaysian Journal of Microbiology, 20(6). https://mjm.usm.my/uploads/issues/2027/SBM-61-formatted-0049.pdf

Al-Saadi, T., Yassir, S. M., Sead, F. F., Ismail, M. A., & Katab, F. K. (2024). Detection of adhesion genes in Enterobacter spp. Isolated from clinical cases in Al-najaf city. Ain Shams Medical Journal, 75(2), 331-336. https://doi.org/10.21608/asmj.2024.273200.1232

Dehkordi, E. B., Tajbakhsh, E., & Momtaz, H. (2022). Molecular characterization of Enterobacter cloacae isolated from urinary tract infections. Jundishapur Journal of Microbiology, 15(5). doi: 10.5812/jjm-122718

Dincer, S., Uslu, F. M., & Delik, A. (2020). Antibiotic resistance in biofilm. Bacterial biofilms, 10, 9.

Dueholm, M. S., Albertsen, M., Otzen, D., & Nielsen, P. H. (2012). Curli functional amyloid systems are phylogenetically widespread and display large diversity in operon and protein structure. PloS one, 7(12), e51274. https://doi.org/10.1371/journal.pone.0051274

Flemming, H. C., Wingender, J., Szewzyk, U., Steinberg, P., Rice, S. A., & Kjelleberg, S. (2016). Biofilms: an emergent form of bacterial life. Nature Reviews Microbiology, 14(9), 563-575. https://doi.org/10.1038/nrmicro.2016.94

Frutos-Grilo E, Kreling V, Hensel A, Campoy S (2023). Host-pathogen interaction: 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

Garde, C., Welch, M., Ferkinghoff-Borg, J., & Sams, T. (2015). Microbial biofilm as a smart material. Sensors, 15(2), 4229-4241. https://doi.org/10.3390/s150204229

Ghonaim, M. M., Elkhyat, A. H., El-Hefnawy, S. M., & Hossam Eldeen, E. A. (2018). FimH Adhesin among Enterobacter spp. Isolates and its Relation to Biofilm Formation and Antimicrobial Resistance Pattern. Egyptian Journal of Medical Microbiology, 27(4), 45-54. https://doi.org/10.21608/ejmm.2018.285640

Goulter, R. M., Gentle, I. R. and Dykes, G. A. (2010) 'Characterisation of curli production, cell surface hydrophobicity, autoaggregation and attachment behaviour of Escherichia coli O157', Current Microbiology, 61(3), pp. 157–162. https://doi.org/10.1007/s00284-010-9589-2

Guermazi-Toumi, S., Boujlel, S., Assoudi, M., Issaoui, R., Tlili, S., & Hlaiem, M. E. (2018). Susceptibility profiles of bacteria causing urinary tract infections in Southern Tunisia. Journal of global antimicrobial resistance, 12, 48-52. https://doi.org/10.1016/j.jgar.2017.09.004

Hassan, A. A., & Motaweq, Z. Y. (2024). Phenotypic and genotypic study of biofilm formation in multi-drug resistance Enterobacter species in Al-Najaf province, Iraq. Microbial Biosystems, 9(1), 83-91. https://doi.org/10.21608/mb.2024.360258

Jiménez-Guerra, G., Borrego-Jiménez, J., Gutiérrez-Soto, B., Expósito-Ruiz, M., Navarro-Marí, J. M., & Gutiérrez-Fernández, J. (2020). Susceptibility evolution to antibiotics of Enterobacter cloacae, Morganella morganii, Klebsiella aerogenes and Citrobacter freundii involved in urinary tract infections: an 11-year epidemiological surveillance study. Enfermedades infecciosas y microbiologia clinica (English ed.), 38(4), 166-169. https://doi.org/10.1016/j.eimce.2019.07.003

Kalantar E, Motlagh M, Lordnejad H, Beiranvand S. The prevalence of bacteria isolated from blood cultures of iranian children and study of their antimicrobial susceptibilities. Jundishapur J Nat Pharm Prod 2008;3(1):1–7.

Kathi, S. (2024). Enterobacter spp. Virulence Factors and Biofilm Components: Synthesis, Structure, Function, and Inhibitors. In ESKAPE Pathogens: Detection, Mechanisms and Treatment Strategies (pp. 349-365). Singapore: Springer Nature Singapore. https://doi.org/10.1007/978-981-99-8799-3_12

Kim, S. M., Lee, H. W., Choi, Y. W., Kim, S. H., Lee, J. C., Lee, Y. C., ... & Kim, J. (2012). Involvement of curli fimbriae in the biofilm formation of Enterobacter cloacae. The Journal of Microbiology, 50(1), 175-178. https://doi.org/10.1007/s12275-012-2044-2

Li, L., Li, Y., Chen, Y., Hou, H., Wang, J., Liu, M., ... & Wang, S. (2025). Global, regional, and national lifetime probabilities of urinary tract infections and interstitial nephritis from 1990 to 2021. Journal of Health, Population, and Nutrition, 44, 231. https://doi.org/10.1186/s41043-025-00950-y

Liu, S., Chen, L., Wang, L., Zhou, B., Ye, D., Zheng, X., ... & Ye, J. (2022). Cluster differences in antibiotic resistance, biofilm formation, mobility, and virulence of clinical Enterobacter cloacae complex. Frontiers in microbiology, 13, 814831. https://doi.org/10.3389/fmicb.2022.814831

Liu, S., Chen, L., Wang, L., Zhou, B., Ye, D., Zheng, X., ... & Ye, J. (2022). Cluster differences in antibiotic resistance, biofilm formation, mobility, and virulence of clinical Enterobacter cloacae complex. Frontiers in microbiology, 13, 814831. https://doi.org/10.3389/fmicb.2022.814831

Mach, F., Marchandin, H., & Bichon, F. (2020). Traitement et prévention des infections urinaires. Actualités pharmaceutiques, 59(598), 48-52. https://doi.org/10.1016/j.actpha.2020.06.023

Mahshouri, P., Alikhani, M. Y., Momtaz, H. E., Doosti-Irani, A., & Shokoohizadeh, L. (2025). Analysis of phylogroups, biofilm formation, virulence factors, antibiotic resistance and molecular typing of uropathogenic Escherichia coli strains isolated from patients with recurrent and non-recurrent urinary tract infections. BMC Infectious Diseases, 25(1), 267. https://doi.org/10.1186/s12879-025-10635-w

Misra, T., Tare, M., & Jha, P. N. (2022). Insights into the dynamics and composition of biofilm formed by environmental isolate of Enterobacter cloacae. Frontiers in microbiology, 13, 877060. https://doi.org/10.3389/fmicb.2022.877060

Muhammad, M. H., Idris, A. L., Fan, X., Guo, Y., Yu, Y., Jin, X., ... & Huang, T. (2020). Beyond risk: bacterial biofilms and their regulating approaches. Frontiers in microbiology, 11, 928. https://doi.org/10.3389/fmicb.2020.00928

Nohad, E. S., & Hassan, M. H. (2023). Antibiotic resistance in Enterobacter cloacae from Anbar hospitals. Journal of Wildlife and Biodiversity, 7(Special Issue), 701-712. https://doi.org/10.5281/zenodo.10367341

Oleiwis, S. R., Najim, S. S., & Radif, H. M. (2021). Morphological and Molecular Study of Biofilm Formation by Enterobacter cloacae. Ann Trop Med Public Health, 24(4), 176-186. file:///Users/charmed/Downloads/Telegram%20Desktop/about:blank

Rajabi, Z., & Dallal, M. M. S. (2015). Study on bacterial strains causing blood and urinary tract infections in the neonatal intensive care unit and determination of their antibiotic resistance pattern. Jundishapur Journal of Microbiology, 8(8), e19654. https://doi.org/10.5812/jjm.19654v2

Rasheed, M. N., Al-Saadi, B. Q. H., Hasan, O. M., Salman, N. Y., & Faisal, S. (2021). Study the role of Ph in curli biogenesis gene expression in Enterobacter Cloacae local isolates. Indian Journal of Forensic Medicine & Toxicology, 15(1), 1260-1264.

Rosana, Y., Ocviyanti, D., Halim, M., Harlinda, F. Y., Amran, R., Akbar, W., ... & Akhmad, S. R. P. (2020). Urinary tract infections among Indonesian pregnant women and its susceptibility pattern. Infectious diseases in obstetrics and gynecology, 2020(1), 9681632. https://doi.org/10.1155/2020/9681632

Scoffone, V. C., Trespidi, G., Chiarelli, L. R., Barbieri, G., & Buroni, S. (2019). Quorum sensing as antivirulence target in cystic fibrosis pathogens. International journal of molecular sciences, 20(8), 1838. https://doi.org/10.3390/ijms20081838

Vandepitte J. (2003). Basic laboratory procedures in clinical bacteriology. World Health Organization, Geneva.

Venkatramanan, M., Sankar Ganesh, P., Senthil, R., Akshay, J., Veera Ravi, A., Langeswaran, K., ... & Shankar, E. M. (2020). Inhibition of quorum sensing and biofilm formation in Chromobacterium violaceum by fruit extracts of Passiflora edulis. ACS omega, 5(40), 25605-25616. https://doi.org/10.1021/acsomega.0c02483

Xu, J., & He, F. (2019). Genomic analysis of two bacterial strains co-isolated from a urinary tract infection: NDM-1-producing Enterobacter cloacae accompanied by extended-spectrum β-lactamase-producing Escherichia coli. Journal of Global Antimicrobial Resistance, 17, 198-200. https://doi.org/10.1016/j.jgar.2019.04.007

Yassir, S. M., & Zaid, B. A. (2022). Phenotypic and genotypic detection of Enterobacter spp isolated from food. International Journal of Health Sciences, 6(S5), 9727–9736. https://doi.org/10.53730/ijhs.v6nS5.12071