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Detection of the *CsgA* and *CsgD* Genes in *Enterobacter Cloacae* Isolates and their Function in Biofilm Formation and Drug Resistance

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Abstract

Background: *Enterobacter cloacae* is a gram-negative bacteria characterized by being a rod, motile, facultative anaerobic bacteria, possess many virulence factors that play an important role in the prevalence of this bacteria such as biofilm, sedrophore, and fimbriae.

Objectives: this study aims to investigate some biofilm genes that contribute to cause disease and antibiotic resistance.

Patients and Methods: This study was conducted on 300 samples involving 102 samples from hospitalized and 198 samples from non- hospitalized patient and this study was carried out from November 2023 to March 2024 in Diyala, Iraq.

Results: This study revealed the presence of biofilm genes in E. cloacae bacteria, isolated from different cases (wound infection, UTI, burn infection and patients with blood bacteremia

Conclusion: An alarming rate of drug resistance *E. cloacae* is on the rise, Bacterial factor including biofilms, fimbriae and molecular mechanism are in association with *E. cloacae*. Study shows up to 80-100 of clinical *E. cloacae* isolates may carry *CsgA* and *CsgD*.

Keywords: Biofilm production, *Enterobacter cloacae*, *CsgA*, *CsgD*.

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Introduction

E. cloacae is characterized by being a facultative anaerobic Gram-negative rod that belongs to the Enterobacteriaceae family, motile with peripheral flagella, the cell width is (0.1-0.6) micrometers and its length is (1.2-3) micrometers, with chemo-organic nutrition, its cells are capsule, the shape of the colonies is circular and mucous, the optimum temperature for its growth is 37°C, it resembles Klebsiella in its shape, but it is distinguished from it by the motility and urease tests (1), as it is heterogeneous for the urease test and positive for catalase and citrate and negative for the indole and oxidase test and ferments glucose and lactose sugar with acid production and ferments sucrose, grows in wide environments such as soil and water and grows on natural growth media and does not need complex materials and is not productive of the enzymes . Deoxyribonuclease, Lipase and Tween esterase (2), *E. cloacae* is the most isolated bacterial species of the genus Enterobacter that infects humans. Enterobacter bacteria cause many infections, including cerebral abscess, pneumonia, meningitis, septicemia, urinary tract infections (UTI), especially in urinary catheterization, and intestinal infections (3). Researchers indicated that the main reason for the ability of these bacteria to cause infections is due to their ability to resist high concentrations of antibiotics, in addition to the fact that they possess many virulence factors, such as biofilm, cilia, capsule, and other factors (4). The *CsgA* gene has a great

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significance in *E. cloacae*, bacteria, due to its encoding of a requisite protein that helps to create a biofilm known as curli fimbriae, which helps bacteria cling to surfaces. Novel studies have clarified that this curlin subunit gene A plays a fateful role in opposition to severe environmental occurrences (5). Curlin subunit gene D (*CsgD*) is a considerable controller of fimbriae, encoding a transcription factor that controls the expression of *CsgA* and other genes associated with biofilm formation. The careful regulation of this gene boosts bacteria's ability to adapt to varied environmental conditions (6). The aims of this study are to detect the *CsgA* and *CsgD* genes in *Enterobacter cloacae* isolates and to identify their function in biofilm formation and drug resistance.

Patients and Methods

Isolation and identification of bacteria: Three hundred samples were collected from hospitalized and non-hospitalized patients from different clinical sources, including 102 samples from hospitalized patients and 198 samples from non-hospitalized patients, under the supervision of a specialist doctor at Baqubah Teaching Hospital, the Consulting Clinic / Baqubah General Hospital, and Al-Batoul Teaching Hospital, Diyala, Iraq. The middle ear, wound, and burn samples were collected using disposable cotton swabs, and sterile plastic cups were used to collect urine samples. Special tubes were used to collect blood samples, for the period from November 2023 to March 2024, at different ages from 18 to 70 years, and from both sexes. The samples were cultured on media (Blood agar, MacConkey agar, EMB agar). Then, the *Enterobacter cloacae* isolate under study was biochemically diagnosed by performing some tests, which included the catalase test, oxidase test, indole test, methyl red test, and Voges-Proskauer test. The Vitek-2 technique was also used to diagnose the isolates.

Susceptibility to antibiotics: Using the Kirby-Bauer disc diffusion method, the antibiotic susceptibility of *E. cloacae* isolates obtained from various clinical sources was assessed and interpreted by CLSI, 2023. The antibiotics Amoxicillin-Clavulanate (20/10 µg), Ampicillin (10 µg), Chloramphenicol (30 µg), Cefixime (5 µg), Ceftazidime (30 µg), Cefriaxone (30 µg), Aztreonam (30 µg), Ciprofloxacin (5 µg), Gentamicin (10 µg), and Imipenem (10 µg) were used to cultivate the isolates on Mueller-Hinton agar medium. The diameter of the inhibition zones was measured around each disc. Sensitive or resistant was determined according to CLSI, 2023 (7).

Biofilm detection: The sterile microtiter plate method containing (96) holes was used to detect the ability of bacterial isolates to form biofilm according to (8).

DNA extraction: DNA was extracted from the bacterial cells under study by the boiling method as stated in (9). Bacterial isolates were grown on Nutrient agar medium for 24 hours at 37°C, where 5 bacterial colonies were transferred and mixed with 1 ml of distilled water, and this mixture was left in a water bath at 70°C for 10 minutes, then placed in a centrifuge and centrifuged for 5 minutes at 1000 rpm. The supernatant was stored in Eppendorf tubes at a temperature of (-20) and used in the polymerase chain reaction (PCR).

Detection of biofilm-related genes in *Enterobacter cloacae*: The adhesion genes *CsgA* and *CsgD* were detected using conventional polymerase chain reaction (PCR). PCR amplification was performed in a 25 µl reaction mixture containing 12.5 µl of Green Master Mix, 1 µl of each primer (forward and reverse), 4.5 µl of template DNA, and 6 µl of distilled water to reach the total reaction volume. The thermal cycling conditions for PCR were as follows: initial denaturation at 94°C for 5 min, annealing at a temperature gradient from 54°C to 56°C for 60 s, extension at 72°C for 1 min, 30 cycles, and final extension at 72°C for 10 min. After

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electrophoresis on a 1.5% agarose gel, the amplified PCR products were examined under UV light (10). Table 1 shows the primers used in this research.

Table 1. Primers used for the Detection of Biofilm-Related Genes.

Gene	Primer sequence (3′-5′)	Output size	Reference
CsgD	F- GAAATTGCATAATATTCAACGTT R- TTTGTTCAGGATCTCTTTTTCAC	384bp	(9)
CsgA	F- TTCAAAGTGGCAGTTATTGCAG R- TTTTTGCAGCAGATCGATAGAA	276 bp	

Results

***Enterobacter cloacae* isolation:** Out of 300 samples, 264 (88%) showed bacterial growth, while 36 (12%) did not; 209 of these isolates were from species other than *Enterobacter* spp. Out of 55 *Enterobacter* species samples, 19 isolates of *Enterobacter cloacae* were found (Figure 1).

Chemical diagnosis of *Enterobacter cloacae*: Biochemical test was performed on the bacterial isolates under study, all isolates were negative for the Oxidase test. In the current study for methyl red gas, the bacteria were negative for this test, while the bacterial isolates were negative for the indole test due to the absence of a colored ring in the isoamyl alcohol layer, where the bacteria are positive for the Voges Proskauer test and thus the color of the medium turns to human color due to the ability of the bacteria to convert glucose sugar to acetyl methyl carbonyl (mixed acetone). The bacterial isolates were diagnosed by the Vitek-2 system using the GN/DI identification card for the results that 19 isolates out of the total original isolates belong to *Enterobacter cloacae*. Table (2) showed a clear variation in resistance of E. cloacae to antibiotic, the high resistance rates of *E. cloacae* to antibiotic is due to the isolates under study possession of many resistance mechanisms such as biofilm formation, capsule as well as to the occurrence of genetic mutation (11).

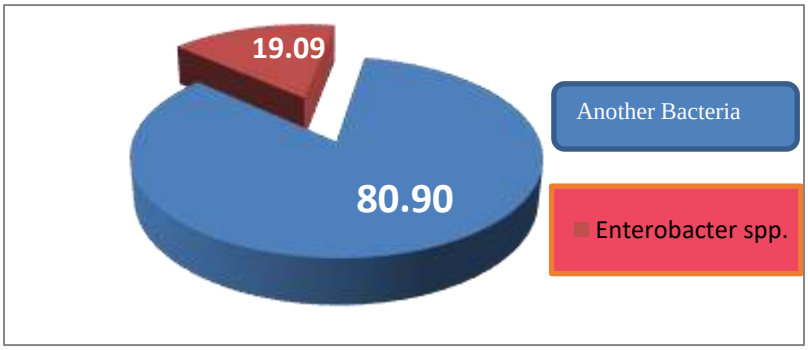

Figure 1. Percentages of isolates diagnosed from different clinical cases.

Table 2. Antibiotic sensitivity of bacterial isolates.

Group	Antibiotic	Resistant Isolates No (%)	Sensitive Isolates No (%)
Penicillins	AMP	19 (100)	0 (0)
β-lactam combination	AMC	10 (52.63)	9 (47.36)
Cephalosporins	CAZ, CFM	16 (84.21)	3 (15.78)
Monobactams	ATM	15 (78.94)	4 (21.05)
Carbapenems	IPM	10 (52.26)	9 (47.36)
Aminoglycosides	GEN	14 (73.68)	5 (26.31)
Fluoroquinolones	CIP	5 (26.31)	14 (73.68)
Phenicols	CHL	7 (36.84)	12 (63.15)

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Biofilm formation: The results showed that all bacterial isolates could produce biofilm at a rate of (100%) but at varying levels, and the production levels were classified into isolates producing strong and medium biofilm at a rate of 16 (84.31%) and 3 (15.78%), respectively (Table 3).

Molecular detection of biofilm genes in *E. cloacae*: The results of molecular identification of the *CsgD* gene, which is (384) base pairs in size, demonstrated that all 19 isolates (100%) possess the *CsgD* gene (Figure 2). On the other hand, the presence of the *CsgA* gene was confirmed in all Enterobacter cloacae isolates by using *CsgA*-specific primers. The study revealed that 18 isolates representing 94% of the bacteria examined contained the *CsgA* gene (Figure 3).

Table 3. Biofilm production levels in *Enterobacter cloacae*.

Production of biofilm	No of isolates (%)
Strong	16 (84.31)
Moderate	3 (15.78)
Total	19 (100)

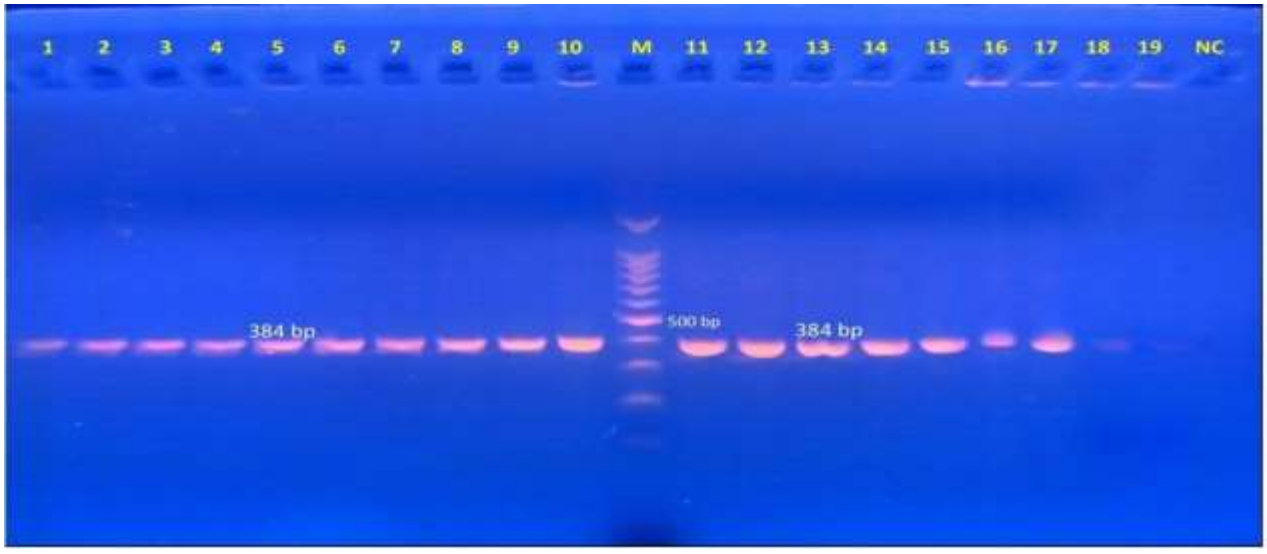

Figure 2. Agarose gel electrophoresis of *Enterobacter cloacae* (1.5% agarose,7v/cm2 for 60 min) for *CsgD* gene.

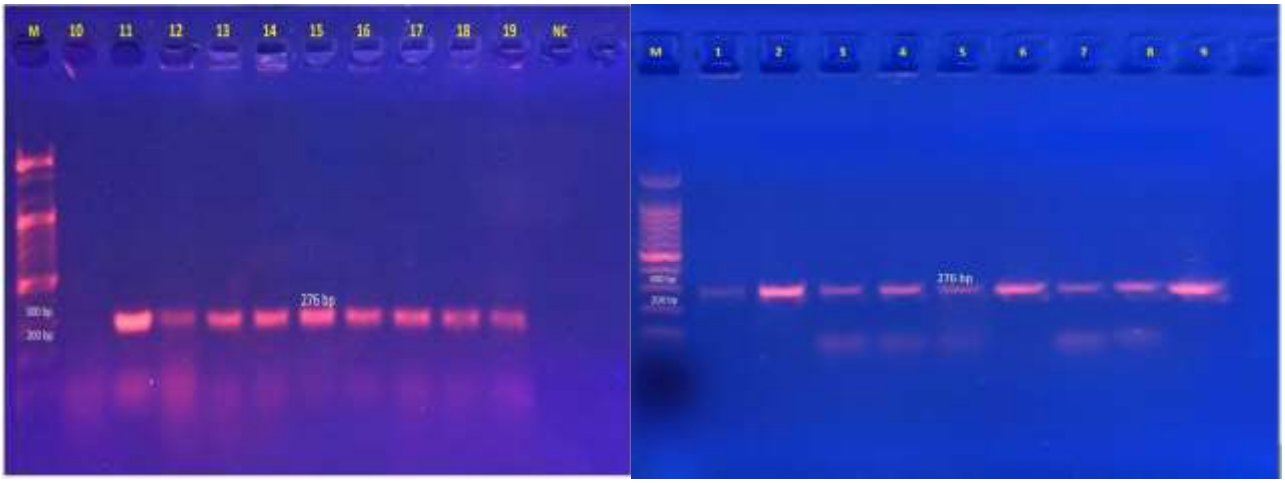

Figure 3. Agarose gel electrophoresis of *Enterobacter cloacae* (1.5% agarose,7v/cm2 for 60 min) for *CsgA* gene.

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Discussion

The study showed that all bacterial isolates were capable of producing 100% biofilm, with production levels varying between strong (84.31%) and moderate (15.78%). Biofilm formation is one of the most important strategies for bacteria to survive in harsh environments, providing protection against environmental factors and antibiotics. As stated by contemporary survey, bacterial biofilm romp a title say in bacterial reluctance to antibiotic, increment problems, (12). The antibiotic resistance test in current study indicates the emergence of multidrug resistance pattern among *E. cloacae* isolates and this is attributed to many reasons, once of them that isolates can produce biofilm, Biofilm-producing bacteria are considered more resistant to antibiotics than non-producing bacteria for several reasons, including the transfer of resistance genes within the biofilm environment, whether through transposons, plasmids, or the occurrence of random mutations that lead to increased resistance to antibiotics, as this leads to a decrease in the diffusion of the antibiotic through the sticky interfacial material of the biofilm (13).The gene mentioned under study (curlin subunit gene D) is a conclusive stewand of biofilm foundation , it was float in commonalty isolates 100% , also gene *CsgA* was likewise disclosed in (94%) of bacterial isolates under study . These genes conduct critical roles in the figuration of slim film of isolates, who inculcate bacterial coherence and the biofilm germination. The *CsgA* gene plays an intrinsic input in the creation of filament which refine biological structure by means of fabricate physical fence, surrounding bacterial outpost, discomfit antibiotic breakthrough. these ones are compatible with those of the anterior analysis (14). The study doubtless that genes have ability to micro yarn in demand for biofilm pointing. the genes under study belongs to a group of duplication factors that hegemony the expression of extracellular matrix production genes inclusive, these genes as well as act as turnover to brisk genes that adjust biofilm creative when environment situation are compatible. When the environmental state is good, gene *CsgD* labor as a transition switch, stimulating biofilm evaluation due to bacterial isolates possession the *CsgD*, the isolates capability to create biofilm was augment, mounting antibiotic resistance. the results of this study were coordinated with result of a beforehand study (15), The seeking suggested that the entity of the *CsgD* gene in *E. cloacae* bacterial isolates may do coupled with antibiotic, resistance. and the returns of this study are agreement with (16), They point out that the existence of this genes is attached to biofilm grow up and accretion bacterial attached to live surfaces and tissues. The results of actual study display that the genes *CsgA*, *CsgD* function a decisive role in *E. cloacae* resistant mechanisms and that fact agreement with (17) they mention that biofilm works like visual barier which ban the action in enlarge the antibiotic resistance factors, our outcomes of this project are too convenient with (18). whose pointed out that drug resistance copartner with biofilm production is an earnest factor that made it difficult to remedy infections, caused by bacteria cells which demonstration loudly biofilm production. The results of the current study were consistent with a study conducted in Baghdad (19), where the study considered the Curli fimbriae genes as important factors in the virulence of *E. cloacae* bacteria, as they play an important role in cell adhesion and integration on solid surfaces to form a biofilm and help in the adhesion of bacterial cells, in addition to their participation in invading host cells and their interaction with host proteins and activating the host's immune system. (20), study also indicated that some virulence genes such as *CsgA* and *CsgD* were associated with cefepime sensitivity in ECC, especially isolates that produced biofilm. On the other hand, the results

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of the current study did not agree with the study of (21), which stated that some strains of *Enterobacter cloacae* may lose the ability to produce biofilms under certain conditions, such as nutrient deficiency or the presence of adverse environmental stimuli. Also, the results of our current study departed from the results reported in the study (22), as it stated that the *CsgA* gene may not be present in some non-biofilm-producing isolates, indicating that it is not a prerequisite for production in some strains. Also, the previous study (23), did not show any agreement with the results addressed by the current study, indicating that some biofilm-producing isolates can show increased sensitivity to some classes of antibiotics, such as carbapenems, due to mechanisms unrelated to biofilm production.

Conclusion

The *CsgA* and *CsgD* genes play a critical role in promoting biofilm production in *Enterobacter cloacae* isolates, contributing to their increased resistance to antibiotics. Biofilm production considered one of the most important strategies of bacteria to survive and resist antibiotic treatment, making these genes potential targets for the development of new therapeutic strategies.

Source of funding: No source of funding.

Ethical clearance: Ethical approval was obtained by the committee of the University of Diyala-College of Medicine.

Conflict of interest: None.

Use of Artificial Intelligence (AI): The authors state they did not use any generative AI tools for creating or editing the manuscript's language.

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References

1. Bunyan, I. A., and Alkhuzaee, Q. A. J. Detection of Curlibiogenesis genes among Enterobacter cloacae isolates and their role in biofilm formation in Al-Hilla City, Iraq. Research Journal of Pharmacy and Technology 2017; 10(10), 3294-3300.

2. Rady AI, Ismail ME, Abd Alla HM. Isolation and characterization of Enterobacter cloacae associated with snake cucumber leaf spots in Minya, Egypt. Assiut Journal of Agricultural Sciences 2022; 53(3):72-85.

3. Salimiyan Rizi K, Ghazvini K, FarsianiH. Clinicaland pathogenesis overview of Enterobacter infections. Reviews in Clinical Medicine 2020; 6(4):146-154.

4. Krzymińska S, Mokracka J, Koczura R, et al.Cytotoxic activity of Enterobacter cloacae human isolates. FEMS Immunology & Medical Microbiology 2009; 56(3):248-252.

5. Ghosh S, Saha I, Dey A, et al. Natural compounds underpinning the genetic regulation of biofilm formation: An overview. South African Journal of Botany 2022; 151:92-106.

6. Long J, Yang C, Liu J, et al. Tannic acid inhibits Escherichia coli biofilm formation and underlying molecular mechanisms: Biofilm regulator CsgD. Biomedicine & Pharmacotherapy 2024; 175:116716.

7. Rai, S., Dash, D., and Agarwal, N. Introducing the new face of CLSI M100 in 2023: An explanatory review. Indian Journal of Medical Microbiology 2023; 46, 100432.

8.Tewawong N, Kowaboot S, Pimainog Y. Distributionof phylogenetic groups, adhesin genes, biofilm formation, and antimicrobial resistance of uropathogenic Escherichia coli isolated from hospitalized patients in Thailand. PeerJ 2020; 8: e10453.

9. Assouma FF, Sina H, Adjobimey T. Susceptibility and virulence of Enterobacteriaceae isolated from urinary tract infections in Benin. Microorganisms 2023;

ORIGINAL RESEARCH
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11(1):213.
10. Mohsin AS, Alsakini AH, Ali MR. Molecular characterization of Dr/Afa genes prevalent among multi drug resistant Escherichia coli isolated from urinary tract infections. Biomedicine 2022; 42(3):523-529.
11. Hassan, A. A., and Motaweq, Z. Y. (2024). Phenotypic and genotypic studies on biofilm formation in multi-drug resistance Enterobacter species in Al-Najaf province,Iraq. MicrobialBiosystems, 9(1), 83-91.
12. Clermont O, Christenson JK, Denamur E, et al. The C lermont E scherichia coli phylo-typing method revisited: improvement of specificity and detection of new phylo-groups. Environmental microbiology reports 2013; 5(1):58-65.
13. Lila, A. S. A., Rajab, A. A., Abdallah, M. H., Rizvi, S. M. D., Moin, A., Khafagy, E. S. and Hegazy, W. A. (2023). Biofilm lifestyle in recurrent urinary tract infections. Life, 13(1), 148.
14. Flemming H-C, Wingender J, Szewzyk U, et al. Biofilms: an emergent form of bacterial life. Nature Reviews Microbiology 2016; 14(9):563-575.
15. Miserez A, Yu J, Mohammadi P. Protein-based biological materials: Molecular design and artificial production. Chemical Reviews 2023; 123(5):2049-2111.
16. Lamprokostopoulou A, Römling U. Yin and yang of biofilm formation and cyclic di-GMP signaling of the gastrointestinal pathogen Salmonella enterica serovar Typhimurium. Journal of Innate Immunity 2022; 14(4):275-292.
17. Hufnagel DA, Depas WH, Chapman MR. The Biology of the Escherichia coli Extracellular Matrixs. Microbial biofilms 2015:249-267.
18. Hall-Stoodley L, Costerton JW, Stoodley P. Bacterial biofilms: from the natural environment to infectious diseases. Nature reviews microbiology 2004; 2(2):95-108.
19. Kim, S. M., Lee, H. W., Choi, Y. W., Kim, S. H., Lee, J. C., Lee, Y. C., and Kim, J. (2012). Involvement of curli fimbriae in the biofilm formation of Enterobacter cloacae. The Journal of Microbiology, 50, 175-178.
20. Ahmed TA, Ibrahim SK. Molecular Investigation of Curli Fimbriae Genes in Enterobacter cloacae Isolated from Various Clinical Sources in Baghdad, Iraq. 2009.
21. Mosaffa F, Saffari F, Veisi M, et al. Some virulence genes are associated with antibiotic susceptibility in Enterobacter cloacae complex. BMC infectious diseases 2024; 24(1):711.
22. Haque MM, Mosharaf MK, Khatun M, et al. Biofilm producing rhizobacteria with multiple plant growth-promoting traits promote growth of tomato under water-deficit stress. Frontiers in Microbiology 2020; 11:542053.
23. Musa L, Toppi V, Stefanetti V, et al. High biofilm-forming multidrug-resistant Salmonella Infantis strains from the poultry production chain. Antibiotics 2024; 13(7):595.

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الكشف عن جيني CsgA و CsgD في عزلات Enterobacter Cloacae ووظيفتهما في تكوين الأغشية الحيوية ومقاومة الأدوية

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الملخص

الخلفية: تُعدّ بكتيريا Enterobacter cloacae بكتيريا سالبة الغرام، تتميز بكونها عصوية الشكل، متحركة، ولا هوائية اختيارية، وتمتلك العديد من عوامل الضراوة التي تلعب دورًا هامًا في انتشارها، مثل الأغشية الحيوية، والسيدروفور، والزوائد الخيطية.

الأهداف: تهدف هذه الدراسة إلى البحث في بعض جينات الأغشية الحيوية التي تُسهم في التسبب بالأمراض ومقاومة المضادات الحيوية.

المرضى والطرق: أُجريت هذه الدراسة على ٣٠٠ عينة، منها ١٠٢ عينة من مرضى مُنومين في المستشفى و١٩٨ عينة من مرضى غير مُنومين، وذلك خلال الفترة من نوفمبر ٢٠٢٣ إلى مارس ٢٠٢٤ في مدينة ديالى، العراق.

النتائج: كشفت هذه الدراسة عن وجود جينات الأغشية الحيوية في بكتيريا Enterobacter cloacae المعزولة من حالات مختلفة (عدوى الجروح، التهاب المسالك البولية، عدوى الحروق، ومرضى تجرثم الدم).

الاستنتاج: يتزايد معدل مقاومة بكتيريا Enterobacter cloacae للأدوية بشكل مقلق، وترتبط عوامل بكتيرية، بما في ذلك الأغشية الحيوية والأهداب والآليات الجزيئية، بهذه البكتيريا. تشير الدراسة إلى أن ما يصل إلى ٨٠-١٠٠٪ من عزلات Enterobacter cloacae السريرية قد تحمل جيني CsgA وCsgD.

الكلمات المفتاحية: إنتاج الأغشية الحيوية، Enterobacter cloacae، CsgA، CsgD.

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