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Molecular Study of Lysozyme Resistant Gram- negative Bacteria

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Abstract

General Background: Lysozyme, an essential antimicrobial enzyme found in bodily fluids, serves as a vital component of the innate immune system, acting primarily by hydrolyzing bacterial cell walls. Specific Background: While lysozyme effectively targets Gram-positive bacteria, Gram-negative bacteria often display resistance due to their protective outer membrane. Recent studies have suggested that specific genes, such as icaA and OatA, may contribute to lysozyme resistance by enhancing biofilm formation and altering cell wall structure. Knowledge Gap: Limited molecular data exist regarding the prevalence of these resistance genes among Gram-negative pathogens isolated from burn patients, particularly in Iraq. Aims: This study aimed to identify the presence of icaA and OatA genes in Gram-negative bacterial isolates from burn patients and assess their potential roles in lysozyme resistance. Results: Among 36 bacterial isolates, Pseudomonas aeruginosa (72.20%) and Klebsiella pneumoniae (27.80%) were dominant. The icaA gene was detected in 30.60% and the OatA gene in 22.20% of isolates. Novelty: This research provides the first molecular evidence of icaA and OatA gene distribution among lysozyme-resistant Gram-negative bacteria in burn patients in Thi-Qar. Implications: The findings highlight the need for continuous molecular surveillance of resistance determinants to improve antimicrobial strategies and infection control in burn treatment settings.

Highlight :

- Lysozyme is a key natural antibacterial enzyme.
- icaA and OatA genes were found in burn bacterial isolates.
- Monitoring resistance genes is essential.

Keywords : Pseudomonas Aeruginosa, OatA Gene, icaA Gene, Burn Patient, Lysozyme Resistant

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Introduction

In 1921, Sir Alexander Fleming discovered the enzyme lysozyme, which is found in many natural substances, including egg white, nasal mucus, stomach fluids, and tears. One third of the protein in both tear films is lysozyme, which is secreted by the human eye's lacrimal glands [1]. A muramidase called lysozyme facilitates the degradation of peptidoglycan (PG), a polymer made up of sugars and amino acids that creates the cell wall by forming a mesh-like layer outside the plasma membrane [2]. In the lysozyme, the sugar component is made up of N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM), which are connected in alternating fashion. help the hydrolysis of N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) glycosidic β-1,4-linked residues [3]. A strong glycan chain, formed by these molecules, is the structural backbone of the cell wall [4]. The organism lyses because its cell wall is compromised because lysozyme cleaves the connection between N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) [5]. The cationic antimicrobial peptide action of lysozyme, in addition to hydrolyzing peptidoglycan, may damage the bacterial membrane [6]. It has been observed that lysozyme has anti-HIV action in addition to cleaving chito dextrins in fungal cell walls. Many cell types, including monocytes and macrophages, have their lysozyme concentrations elevated after infection [7].
Particularly effective against Gram-positive bacteria, lysozyme is a powerful antibacterial agent. The ability to break the Ẑ-1,4-glycosidic bonds inside the polysaccharide structure of Gram-positive bacterial cell walls is the source of its effectiveness [8]. Lysozyme is able to infect Gram-positive bacteria, while Gram-negative bacteria are less susceptible because lysozyme cannot penetrate their outer membrane, which is decorated with lipopolysaccharides [9]. Beyond their well-known enzymatic function, C-type lysozymes (lysozyme in humans, LysM and LysP in mice) have antimicrobial action [10]. Not only may these cationic proteins hydrolyze peptidoglycan, but they can also interact with bacterial membranes and disrupt them. Their antibacterial actions are a result of a two-pronged approach that includes both enzymatic activity and characteristics that break cell membranes [11].

Methods

A. Sample Collection

The research included fifty burn swabs collected from burn patients at Al-Nasiriyah Teaching Hospital (Thi-Qar). The samples were sent to the microbiological unit at Al-Nasiriyah Teaching Hospital using transport media. They were then grown on regular medium to find the bacteria, and EPI-20 was used to find them again.

B. Molecular Identification

Table 1: The primers, sequence and size to amplify carbapenem genes

Primers	Primer sequence	Length (bp)	Product size (bp)
OatA F	GGGCATTCGCAGTTATAGGA-3-5	20	1712
OatA R	GCATGTGTTTCCATCGTTTTT-3-5	21	
icaA F	TCTCTTGCAGGAGCAATCAA-3-5	20	188
icaA R	TCAGGCCACTAACATCCAGCA-3-5	21	
R – *OmpA*	ACTCTTGTGGTTGTGGAGCA-3-5	20	

C. DNA Molecular Weight Markers

The molecular weight markers used in agarose gel electrophoresis are listed in the Table 2.

Table 2: DNA molecular weight markers, providers and fragment size

Markers	**Provider/country**	**Fragment sizes (bp)**
bp DNA 100 ladder	Bioneer / Korea	1800,2000 ,1200 , 100,200,300,400,500,600,700,800,900,1000

D. Amplification of *icaA* gene

For each of the 43 bacterial isolates, PCR was used to amplify the IcaA gene, which is involved in biofilm formation. Table 3 contains the primer sequences. In Table 3 you can see the ica A amplification reagents and volumes. It was centrifuged for 2 minutes and vortexed before PCR. The program for the thermal cycler is shown in Table 3.

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Table 3: Reagents (25 µl) for each amplification lysozyme gene

Reagents	Volume (µl)
Go Taq green master mix	12
Template DNA	2
Forward primer	1
Reverse primer	1
Nuclease Free Water	9
Total volume	25

Before being put in the thermal-cycler according to the protocol in Table 3, the mixture was vortexed and centrifuged for 1 minute. Everything you need to know about the icaA gene amplification reagents is in Table 4.

Table 4: Thermal cycler program for *icaA* gene amplification

Stages	Temperature	Time	Cycles
Initial denaturation	C °94	min 5	1
Denaturation	C °94	sec 35	35
Annealing	C °55.5	sec 35	
Extension	C °72	sec 35	
Final extension	C °72	min 5	1

E. Amplification of *OatA* gene

Table 5 lists the reagents and quantities needed for ica A amplification. It was centrifuged for 2 minutes and vortexed before PCR. Table 5 has the details of the thermal cycler system.

Table 5: Reagents (25 µl) for each amplification lysozyme gene

Reagents	Volume (µl)
Go Taq green master mix	12
Template DNA	2
Forward primer	1
Reverse primer	1
Nuclease Free Water	9
Total volume	25

Table 6: Thermal cycler program for *OatA* gene amplification

Stages	Temperature	Time	Cycles
Initial denaturation	C °94	min 5	1
Denaturation	C °94	sec 35	35

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Annealing	C °56	sec 35	
Extension	C °72	sec 35	
Final extension	C °72	min 5	1

F. Statistical analysis

A chi-square was performed to evaluate the differences among the studied tests using SPSS version 17.0, $P \leq 0.05$ were considered as statistically significant [12].

Results

Fifty patients admitted to Al-Nasiryah Teaching Hospital with burns had 36 different bacterial strains identified. At a significance level of $P < 0.05$, there were 26 cases of Pseudomonas aeruginosa (72.20%) and 10 cases of Klebsiella pneumoniae (22.8%). Table seven.

Table 7 : Bacterial Isolates

SamplesNo.	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*
36	*(%72.20) 26	(%27.80) 10

$P \leq 0.05$*

The present study showed the detection of *icaA* gene in 11of 36 (30.60%) bacterial isolates as in Figure (4-1). with no significant differences at $P \leq 0.05$.

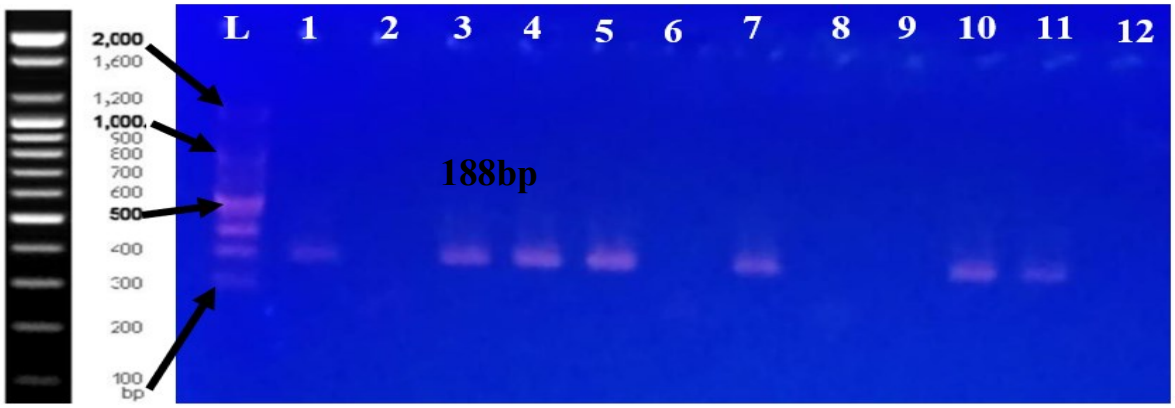

Figure 1: There was an amplified icaA gene (188 kb) in an agarose gel electrophoresis model (1.5% of the time). Direction L: one hundred beats per minute Permanent marker. A, B, C, 7, 10, and 11 lanes: Ica is a Specific bacterial isolates' A gene bands.

According to Figure (4-2), 8 out of 36 bacterial isolates (22.20%) had the OatA gene, and there were no significant differences at $P < 0.05$.

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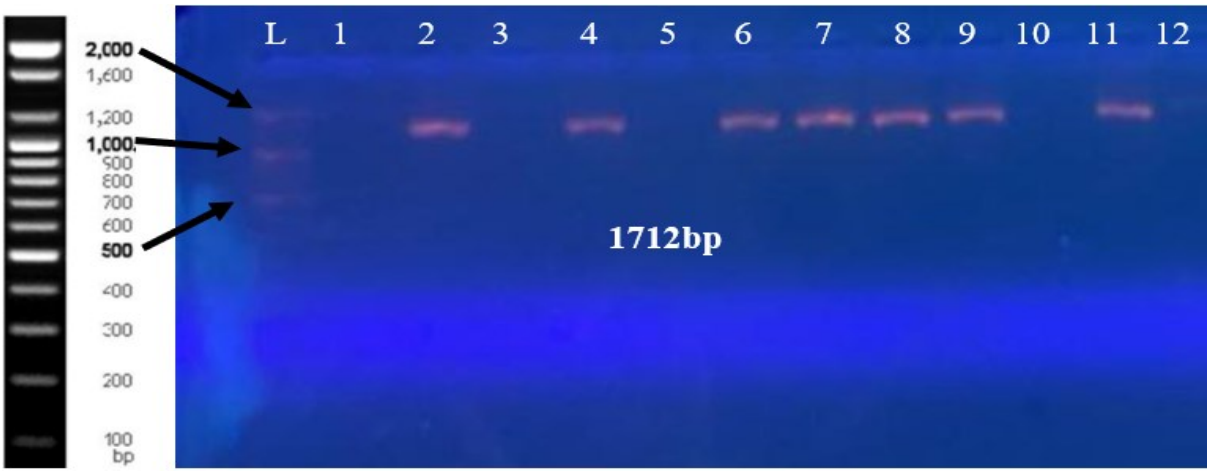

Figure 2: The OatA gene was found to be amplified (1712 bp) in an agarose gel electrophoresis model with a 1.5% success rate. Direction L: one hundred beats per minute Permanent marker. Bacterial isolates' OatA gene bands are located on Lanes2,4,6,7,8,9, and 11.

According to the data, Pseudomonas aeruginosa has 8 copies of the icaA gene (30.80%) and 6 copies of the OatA gene (23.10%). As shown in Table 8, the prevalence of Klebsiella pneumoniae was 3% for the icaA gene and 2% for the OatA gene.

Bacterial isolates	No. of isolate	*icaA* gene	*OatA* gene
Pseudomonas aeruginosa	26	(%30.80) 8	(%23.10) 6
Klebsiella pneumoniae	10	(% 30) 3	(% 20) 2

Discussion

Lysozyme serves as a crucial antimicrobial enzyme within the body's initial defense mechanisms and is present in various bodily fluids and tissues, including the skin [13]. The findings indicate that Pseudomonas aeruginosa was the predominant bacterial isolate from burn patients, succeeded by Klebsiella pneumoniae. This aligns with prior research demonstrating that P. aeruginosa is among the most prevalent pathogens in burn environments, attributable to its significant resistance to extreme conditions and antibiotics. Elfadadny et al. [14]. The icaA gene was identified in approximately 30.60% of the total isolates, indicating its potential role in augmenting bacterial resistance to host defense mechanisms, including lysozyme. The occurrence of this gene in both P. aeruginosa and K. pneumoniae reinforces the hypothesis that lysozyme resistance is not confined to a single bacterial species, but may be a widespread characteristic among various pathogens. Nuryastuti et al. [15]. The OatA gene was identified at a significantly lower frequency (22.20%) and was expressed in a limited number of isolates from both species. This gene is implicated in the modification of the bacterial cell wall, thereby diminishing the efficacy of lysozyme. The low frequency observed in this study suggests a limited role in resistance to the isolated bacteria, or the possibility that alternative resistance mechanisms may be at play [16]. In comparison to prior studies, the frequency of icaA gene expression aligns with certain reports; however, the distribution varies among bacterial species, which may indicate differences in isolation sources, environmental conditions, or acquired genes.

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Conclusion

The findings underscore the necessity of monitoring resistance genes in burn bacteria, both for therapeutic purposes and to comprehend the evolution of their defense mechanisms. The authors highlight the necessity for broader studies that incorporate larger sample sizes and various bacterial species, concentrating on the correlation between the presence of these genes and the severity of infection or treatment response.

References

1. T. V. Tullio, R. Spaccapelo, and M. Polimeni, "Lysozymes in the Animal Kingdom," in Human and Mosquito Lysozymes: Old Molecules for New Approaches Against Malaria, Cham, Switzerland: Springer International Publishing, 2014, pp. 45–57.
2. I. S. Vavilina, A. A. Shpak, T. A. Druzhkova, A. B. Guekht, and N. V. Gulyaeva, "Shedding Valuable Tears: Tear Fluid as a Promising Source of Disease Biomarkers," Neurochemical Journal, vol. 17, no. 4, pp. 702–714, Dec. 2023, doi: 10.1134/S1819712423040121.
3. N. Nawaz, S. Wen, F. Wang, S. Nawaz, J. Raza, M. Iftikhar, and M. Usman, "Lysozyme and Its Application as Antibacterial Agent in Food Industry," Molecules, vol. 27, no. 19, p. 6305, Sep. 2022, doi: 10.3390/molecules27196305.
4. K. H. Nam, "Crystal Structure of Human Lysozyme Complexed with N-Acetyl-α-D-Glucosamine," Applied Sciences, vol. 12, no. 9, p. 4363, Apr. 2022, doi: 10.3390/app12094363.
5. T. Yang and W. Yan, "Strategies for Enhancing the Antibacterial Efficacy of Lysozyme and the Resulting Outcome," International Journal of Biological Macromolecules, vol. 143, pp. 137–146, Apr. 2025, doi: 10.1016/j.ijbiomac.2025.04.137.
6. K. Skrzyniarz, J. Sanchez-Nieves, F. J. de la Mata, M. Lysek-Gladysinska, K. Lach, and K. Ciepluch, "Mechanistic Insight of Lysozyme Transport Through the Outer Bacteria Membrane with Dendronized Silver Nanoparticles for Peptidoglycan Degradation," International Journal of Biological Macromolecules, vol. 237, p. 124239, May 2023, doi: 10.1016/j.ijbiomac.2023.124239.
7. P. Biswas, G. Mukherjee, J. Singh, A. Rastogi, and R. Banerjee, "Enzymes in Health Care: Cost-Effective Production and Applications of Therapeutic Enzymes in Health Care Sector," in Bioprospecting of Enzymes in Industry, Healthcare and Sustainable Environment, Singapore: Springer Singapore, 2021, pp. 291–314.
8. A. Rkhaila, T. Chtouki, H. Erguig, N. El Haloui, and K. Ounine, "Chemical Properties of Biopolymers (Chitin/Chitosan) and Their Synergic Effects with Endophytic Bacillus Species: Unlimited Applications in Agriculture," Molecules, vol. 26, no. 4, p. 1117, Feb. 2021, doi: 10.3390/molecules26041117.
9. D. A. Heesterbeek et al., "Outer Membrane Permeabilization by the Membrane Attack Complex Sensitizes Gram-Negative Bacteria to Antimicrobial Proteins in Serum and Phagocytes," PLoS Pathogens, vol. 17, no. 1, p. e1009227, Jan. 2021, doi: 10.1371/journal.ppat.1009227.
10. P. Ferraboschi, S. Ciceri, and P. Grisenti, "Applications of Lysozyme, an Innate Immune Defense Factor, as an Alternative Antibiotic," Antibiotics, vol. 10, no. 12, p. 1534, Dec. 2021, doi: 10.3390/antibiotics10121534.
11. V. C. Oliveira et al., "Characterization of a Peptidoglycan-Degrading Protein: Biochemical and Antimicrobial Characteristics, Antibiotic Synergism, and Delivery System Innovation," Probiotics and Antimicrobial Proteins, vol. 1, no. 2, Oct. 2024, doi: 10.1007/s12602-024-01345-8.
12. H. Fadhil, "Association Between the Demographic Characteristics of Patients and the Severity of COVID-19," University of Thi-Qar Journal of Science, vol. 10, no. 2, pp. 92–97, Dec. 2023.
13. P. Ferraboschi, S. Ciceri, and P. Grisenti, "Applications of Lysozyme, an Innate Immune Defense Factor, as an Alternative Antibiotic," Antibiotics, vol. 10, no. 12, p. 1534, Dec. 2021, doi: 10.3390/antibiotics10121534.
14. A. Elfadadny et al., "Antimicrobial Resistance of Pseudomonas Aeruginosa: Navigating Clinical Impacts, Current Resistance Trends, and Innovations in Breaking Therapies," Frontiers in Microbiology, vol. 15, p. 1374466, Apr. 2024, doi: 10.3389/fmicb.2024.1374466.
15. T. Nuryastuti, H. C. van der Mei, H. J. Busscher, S. Iravati, A. T. Aman, and B. P. Krom, "Effect of Cinnamon Oil on icaA Expression and Biofilm Formation by Staphylococcus Epidermidis," Applied and Environmental Microbiology, vol. 75, no. 21, pp. 6850–6855, Nov. 2009, doi: 10.1128/AEM.00875-09.
16. A. Bera, S. Herbert, A. Jakob, W. Vollmer, and F. Gotz, "Why Are Pathogenic Staphylococci So Lysozyme Resistant? The Peptidoglycan O-Acetyltransferase OatA Is the Major Determinant for Lysozyme Resistance of Staphylococcus Aureus," Molecular Microbiology, vol. 55, no. 3, pp. 778–787, Feb. 2005, doi: 10.1111/j.1365-2958.2004.04429.x.