

Prevalence and distribution of *Staphylococcus aureus* virulence enzymes isolated from clinical and environmental sources

Abstract

The goal of this study was to isolate and identify *Staphylococcus aureus* from clinical and environmental sources. This study also looked at the prevalence and distribution of different virulence enzymes among its isolates. For this objective, 65 samples were collected and dispersed among 50 distinct clinical samples and 15 environmental (soil, water, and air) samples. The results revealed that 20 bacterial isolates were *S. aureus*. The isolation rate was 30.76% from all samples, with 13 isolates 20% from a clinical source and 7 isolates 10.76% from an environmental source. The phenotypic detection of virulence enzymes revealed that all isolates, including environmental isolates, produced virulence enzymes (between two and six enzymes), indicating the clinical importance. The isolates produced virulence enzymes at different rates. The results revealed a clear rise in the rate of production of the enzymes: lecithinase and lipase producing at a rate of 95%, protease at 90%, urease at 80%, hemolysin at 60%, and beta-lactamase at 55%.

Keywords: *S. aureus*, Catalase, beta-lactamases, Protease.

<https://www.peerreviewcentral.com/user/inprocessmanuscript>

Introduction

Staphylococcus aureus is a common, ubiquitous bacterium that colonises the skin, nose, and throat. Human ailments caused by it include food poisoning, pharyngitis, meningitis, pneumonia, bacteremia, wounds, and post-operative infections [1]. Furthermore, *S. aureus* can also be detected in the atmosphere, soil, and water [2]. *S. aureus* clinical is owing to the presence of a collection of virulence factors, some of which are naturally present in bacteria and others that are released by them [3].

Extracellular enzymes are secreted by these microorganisms. The most important enzyme is beta-lactamase, which is responsible for beta-lactam antigen resistance. It is encoded by the *blaZ* gene, which is found on a chromosome or plasmid [4]. *S. aureus* also secretes the hemolysin enzyme, which breaks down red blood cells, gives iron to germs, and interferes with host cell membranes. Lecithinase is a protein that degrades phosphorylated lipids, causing tissue deterioration and death. Lipase penetrates fatty skin tissues and subcutaneous fat to break it down [5]. *S. aureus* also produces proteases that destroy host proteins such as human neutrophil protein, antimicrobial platelet protein, and complement protein [6].

Because virulence enzymes are important in the clinical of *S. aureus*, this study was carried out to isolate and diagnose bacteria from clinical and environmental sources, as well as to investigate the prevalence and distribution of various virulence enzymes among its isolates.

Materials and Methods

In this study, 65 samples were collected from different sources in sterilized conditions. 50 from clinical sources (sputum, urine, skin, and blood swabs) and 50 from ambient sources (15 samples that included soil, water and air samples) [7]. The samples were cultured on Mannitol Salt Agar (Neogen / UK) plates for 24 hours at 37°C.

Bacterial isolates emerged on mannitol salt agar as yellow fermented colonies of mannitol sugar. The streak approach was used to select and re-seed bacteria on the purification medium[8].

Microscopic examination

Swabs were obtained from forming colonies and stained with Gram stain on a glass slide. To assess the type and color of cells, the bacteria were viewed under a microscope using an oil lens.

Biochemical test

The tests were conducted according to the conventional method mentioned in[9]. In this test include catalase test, oxidase test, and coagulase test which was conducted in two ways: Slide Coagulase Test and Tube Coagulase Test.

Diagnostics with the Vitek-2 system

The isolates were diagnosed with the Vitek-2 device in Al-Mansour laboratory in Mosul city. This new instrument can easily detect the specific bacteria in any type of isolates.

Phenotypic investigation of virulence enzymes in *S. aureus*

Detection beta-lactamase in vitro

100 µl of penicillin G solution at a concentration of 6 g was poured in test tubes and added to the bacterial suspension before incubating. After mixing, the tubes were kept in the incubation chamber for an hour at 37°C for 18-24 hrs. The solution was then dropped into each tube, followed by a drop of iodine solution, and the tubes were shaken vigorously and allowed for 5 minutes at the laboratory temperature before the results were read [10].

Detection beta-lactamase enzymes using nitrospinedisk

One of the reagents on bacterial growth is the nitrospinedisk. After being moistened with a drop of sterile physiological saline, the nitrospinedisk becomes yellow. The juvenile colony was then moved and placed on the nitrospinedisk. The colour of the disc changed to pink, suggesting the synthesis of beta-lactamase [11].

Detection Urease

A young developing colony of *S. aureus* was injected into the prepared blood agar medium (Neogen/UK), and the plates were incubated at 37°C for 24 hours. The emergence of a change in the color of the medium from yellow to pink is a favorable result and a sign of urease enzyme secretion [12].

Detection of Hemolysin

The prepared blood agar medium (Neogen/UK) was injected with a young developing colony of *S. aureus*, and the plates were incubated at 37°C for 24 hours. The presence of translucent patches around the colonies implies that the bacteria can examine blood [13].

Detection of Lecithinase & Lipase

Egg Yolk Agar (Neogen/UK) was inoculated with a young developing colony of *S. aureus* and incubated for 24 hours at 37°C. The translucent area around the growth after incubation signifies a positive result for lecithinase synthesis. To examine the lipase enzyme, bacteria were added to egg agar and the plate was incubated for 24 hours. The plate was immersed in a 20% saturated copper sulfate CuSO_4 solution for 20 minutes. The surplus solution was removed, and the plate was dried in the incubator for 30 minutes. Changing the hue of the colonies to a greenish blue yields a good result, indicating that the enzyme is being secreted [14].

Detection of protease

Skim Milk Agar (Microexpress / Spain) medium was inoculated with a young developing colony of the bacteria to be studied and then incubated for 24 hours at 37 °C. The emergence of a translucent region around the bacterial colonies after the incubation period reflects a positive result [14].

Results and Discussion

Because *S. aureus* is resistant to salinity and multiplies in the presence of sodium chloride, the colour of the red phenol index was modified from pink to yellow as a signal of low PH [15].

Gram stain microscopy was used as one of the diagnostic procedures because its cells showed in a spherical shape organized as grapes cluster under a light microscope, figure (1A). *S. aureus* was also replanted in salt and mannitol agar to observe the aforementioned agronomic features, figure (1B). Some biochemical tests were also performed, and all of the *S. aureus* isolates tested positive for the catalase that breaks down hydrogen peroxide H_2O_2 into oxygen gas O_2 and H_2O , which shows as gas bubbles, figure (2A) [10]. A 100% positive result appeared for the coagulase enzyme, which is one of the most important diagnostic tests, figure (B2) [16]. The Vitek-2 was used to confirm the diagnosis of all isolates under examination, figure (3).

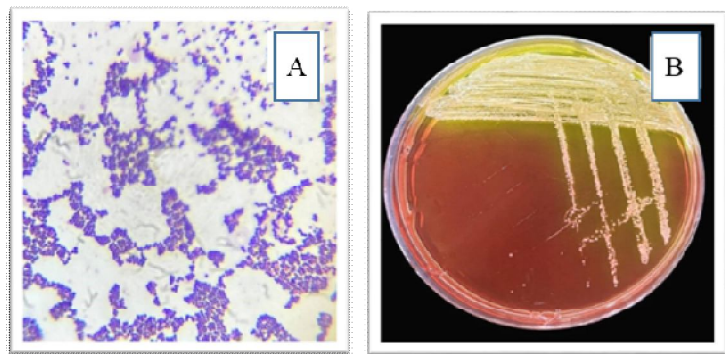


Fig 1: A. *S. aureus* takes Gr⁺ under a light microscope. B: *S. aureus* on a medium of salt and mannitol agar.

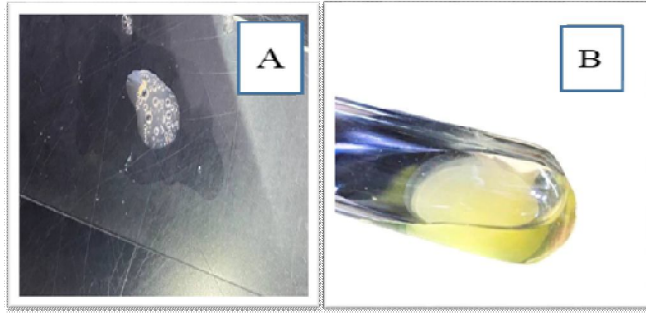


Fig 2:A: A positive result for a catalase test. B:A positive result for a blood clotting test.

bioMérieux Customer:		Al-Mansur Laboratory															
Microbiology Chart Report																	
Patient Name: OM, 29	Location: Lab ID: 225	Patient ID: 225	Physician: Isolate Number: 1														
Organism Quantity:																	
Selected Organism : <i>Staphylococcus aureus</i>																	
Source:	Collected:																
Comments:																	
Identification Information		Analysis Time: 4.87 hours	Status: Final														
Selected Organism		99% Probability <i>Staphylococcus aureus</i>															
ID Analysis Messages		Bionumber: 010402022363231															
Biochemical Details																	
2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	-	11	AGLU	(-)
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	+
20	LeuA	-	23	ProA	-	24	BGUPr	-	25	AGAL	-	26	PyrA	+	27	BGUR	-
28	AlaA	-	29	TyrA	-	30	dSOR	-	31	URE	-	32	POLYB	+	37	dGAL	-
38	dRIB	-	39	ILATK	+	42	LAC	-	44	NAG	+	45	dMAL	+	46	BACI	-
47	NOVO	-	50	NC6.5	+	52	dMAN	+	53	dMNE	+	54	MB4G	+	56	PUL	-
57	dRAF	-	58	O129R	+	59	SAL	-	60	SAC	+	62	dTRE	+	63	ADH2s	-
64	OPTO	+															

Fig 3: Diagnostic Result from using the Vitek-2.

Based on the aforementioned diagnostic processes, 20 isolates of *S.aureus* were obtained, with an isolation rate of 30.76% of all samples analyzed, Table (1). The isolates were divided into 13 isolates 20% from a clinical source and 7 isolates 10.76% from an environmental source. Isolates were recovered from all clinical tissues examined. The same is true for many environmental samples such as soil, water, and air, Table (2)

Table 1: Number and percentages of *S. aureus* isolated from clinical and environmental sources.

Sample source	No.of Sample	<i>S. aureus</i> isolates	%
clinical	50	13	20
Environmental	15	7	10.76
Total	65	20	30.76

Table 2: The percentage of *S. aureus*isolates from different clinical and environmental sources.

Source of isolates	No.	%
clinical isolates		
Airways	1	7.7
Pus	2	15.4
Dialysis	2	15.4
Blood	1	7.7
Urea	2	15.4
Dermatitis	4	30.7
Eye inflammation	1	7.7
Total	13	100
Environmental isolates		
Water	3	42.8
Soil	3	42.8
Air	1	14.4
Total	7	100

Toxic shock syndrome is caused by the release of toxins into the body [17, 18]. Other compounds, in addition to enzymes, toxins, and surface proteins, can assist bacteria invade the body's tissues [19]. Toxins A, B, and D are the most deadly, causing food poisoning [20]. Furthermore, *S. aureus* has the ability to spread through water, causing health issues in humans as well as economically significant aquatic species like fish [21].

Prevalence and distribution of virulence enzymes

S. aureus has a set of virulence factors that are either contained in the cell structure or produced as enzymes, allowing the bacterium to avoid the immune system and infiltrate human tissues [22]. According to the data indicated in Table (3), all of the isolates investigated with diverse sources of isolation produced virulence enzymes (2-6), including the environmental isolates. This confirms the relevance of this bacteria from a clinical standpoint, regardless of the source of its isolation [23].

Table 3: Production and distribution of virulence enzymes of *S. aureus* isolates from different sources.

Isolates	Source	Virulence enzymes					
		Beta-lactamase	Urease	Hemolysin	Protease	Lipase	Lecithinase
1	Airways	+	+	γ_-	-	+	+
2	Pus	+	+	$\alpha+$	+	+	+
3	Pus	+	-	γ_-	+	+	+
4	Dialysis	+	-	$\alpha+$	-	-	-
5	Dialysis	-	+	$\alpha+$	+	+	+

6	Blood	+	+	α +	+	+	+
7	Urine	-	+	\square +	+	+	+
8	Urine	-	+	\square +	+	+	+
9	Dermatitis	-	+	α +	+	+	+
10	Dermatitis	+	+	γ -	+	+	+
11	Dermatitis	+	+	α +	+	+	+
12	Dermatitis	-	-	γ -	+	+	+
13	Eye inflammation	+	-	\square +	+	+	+
14	Water	-	+	\square +	+	+	+
15	Water	+	+	γ -	+	+	+
16	Water	+	+	γ -	+	+	+
17	Soil	-	+	\square +	+	+	+
18	Soil	+	+	γ -	+	+	+
19	Soil	-	+	γ -	+	+	+
20	Air	-	+	\square +	+	+	+

The rate of creation of each type of virulence enzyme varied depending on the isolate, however the current investigation found a clear rise in the rate of production of these enzymes. As indicated in figure 4, the isolates produced the most lecithinase and lipase 95%, whereas protease was 90%, urease 80%, hemolysin 60%, and beta-lactamase 55%.

PHENOTYPIC DETECTION OF VIRULENCE ENZYMES

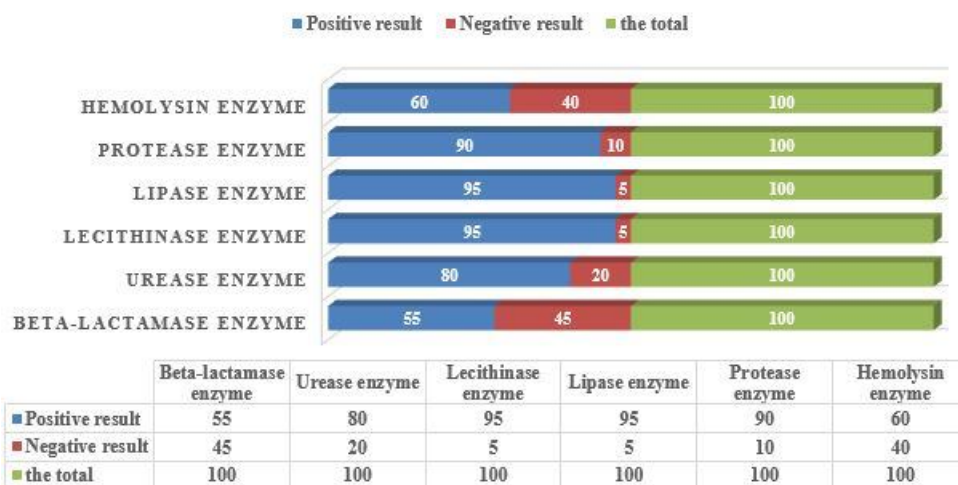


Fig 4: Production rates of virulence enzymes of *S. aureus* isolates.

The results showed that the isolates produced 95% of the lecithinase as shown in figure (5). *S. aureus* secretes an enzyme called lecithinase, which disturbs host tissues by dissolving phospholipids in eukaryotic cell membranes, allowing germs to proliferate and access nutrition [24]. The current study's findings also revealed that the rate of production lipase was high 95% as shown in figure (6). The lipase enzyme breaks down fats and allows them to linger in fatty tissues, damaging the host tissues. The enzyme also plays a crucial role in the production of biofilms and cysts [25].

The activity of the protease production was studied, as the rate of its production approached 90% as shown in figure (7). It plays a significant role in bacterial clinical because it breaks the peptide bonds that bind amino acids, destroying proteins and regulating the immune response. It causing necrosis and damage to host tissues. In addition to obtaining nutrients essential for bacterial development from hydrolyzed proteins derived from host tissues such as collagen [26].

The recent investigation found that 80 percent of the isolates producing urease, figure (8). The urease enzyme is important in the clinical of *S. aureus* because it decomposes urea into carbon dioxide and ammonia during the colonization stage. *S. aureus* is at the forefront of pathogens that cause urinary tract infections and kidney stones, hence the urease enzyme protects bacteria from excessive urea concentrations [27].

The results revealed that the concentration of hemolysin enzyme was 60%, figure (9). One of the key virulence factors in *S. aureus* is the development of hemolysin enzyme, which destroys erythrocytes by dissolving the cell membrane enclosing the globule, resulting in anemia and a weakening of the immune response. This also aids in the provision of iron, which is required for bacterial metabolic processes. Hemolysin also damages leukocytes and macrophages, as well as attacking sphingomyelin in animal cell membranes, allowing bacteria to infiltrate and spread within the host [28].

Two approaches were used to investigate beta-lactamase synthesis. The in-tube approach was utilized, with penicillin G as the foundation of the examination and nitrosphe disk as illustrated in the figure (10). According to the current study's findings, the percentage of isolates producing beta-lactamase by both methods was 55%. *S. aureus* has numerous antibiotic resistance mechanisms, particularly against penicillin and cephalosporin. One of the most prevalent is the creation of beta-lactamase, which breaks the beta-lactam ring in antibiotic manufacture, resulting in resistance [29].

The findings of our current study on the production rates of virulence enzymes investigated by *S. aureus* accord with the findings of many other studies, including [5, 30, 31 and 32]. On the other hand, the production rates of virulence enzymes in the current study differed with the results of other studies [14, 24, and 33]. This disparity in results could be attributed to the different isolates, their sources of isolation, the kind of assays used, and the conditions under which these tests were performed.

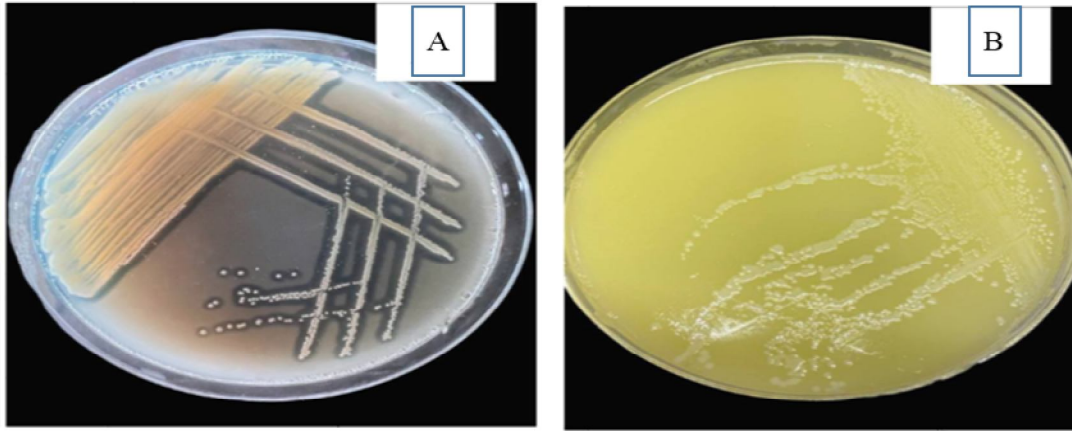


Fig 5: Lecithinase production A: Positive result. B: Negative result.

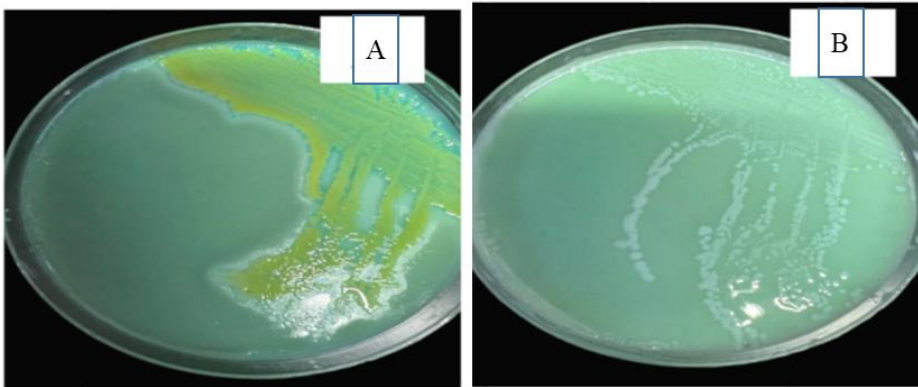


Fig 6: Production of lipase. A: Positive result. B: Negative result.

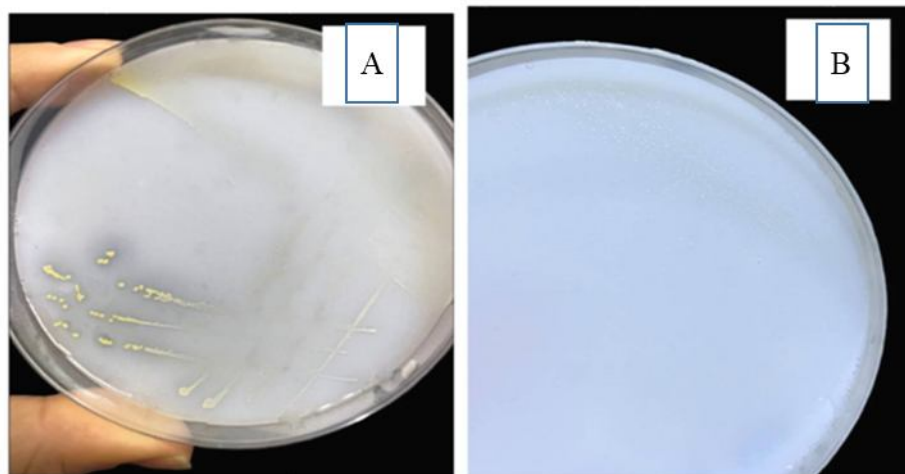


Fig 7: Production of protease. A: Positive result. B: Negative result.

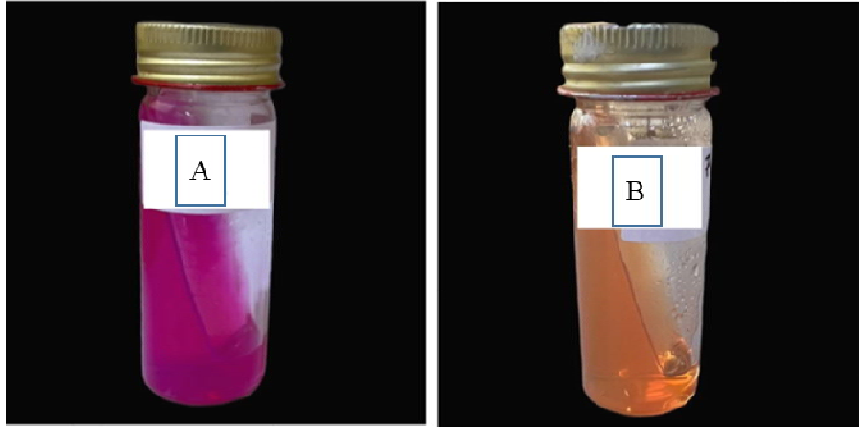


Fig 8: Production of urease. A: Positive result. B: Negative result.

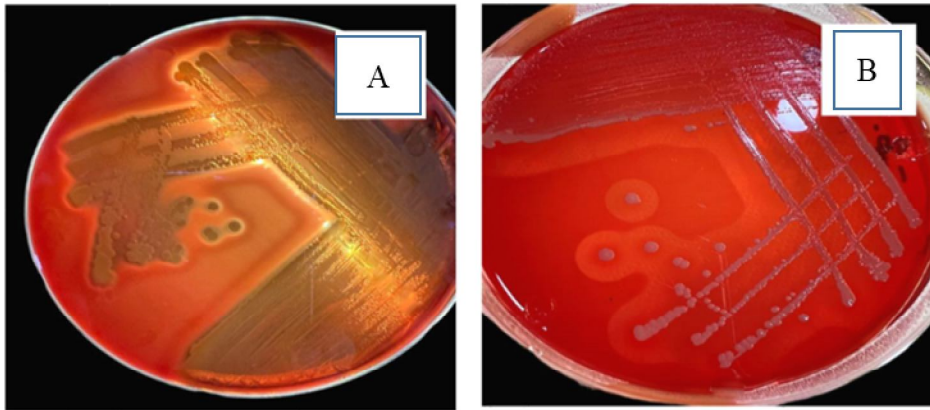


Fig 9: Production of hemolysin. A: Beta result result. B: Alpha result.

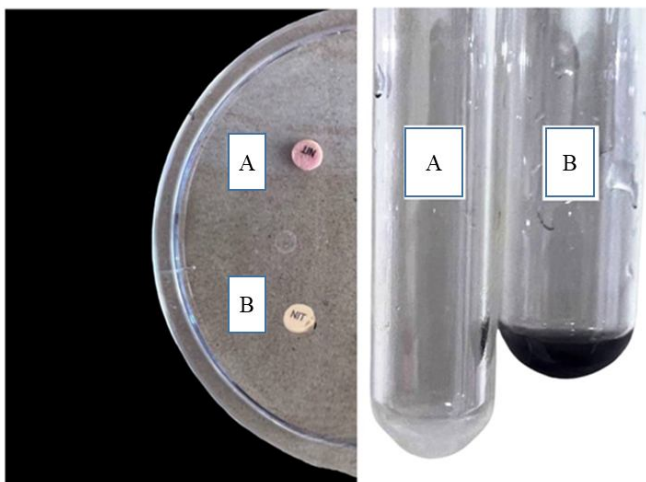


Fig 10: Production of beta-lactamase by two methods: tubes and nitrospirin tablets. A: Positive result. B: Negative result.

Conclusions

The phenotypic investigation of virulence enzymes revealed that all investigated isolates from various sources of isolation, including environmental isolates, produced virulence enzymes. This indicates the relevance of *S. aureus* in clinical, independent of the source of isolation. The isolates' production rates of each type of virulence enzyme varied, and the results demonstrated a definite increase in the production rate of these enzymes.

Acknowledgments

The authors send thankful letter to the college of Science for documenting this work.

References

1. Bitrus, A. A., Olabode, M. P., Abbas, M. A., &Goni, M. D. (2018). *Staphylococcus aureus*: A Review of Antimicrobial Resistance Mechanisms. *Veterinary Sciences: Research and Reviews*, 4(2).
2. Natheer, F. (2019). Molecular Characterization of SEB Gene of *Staphylococcus aureus* Isolated from Raw Milk and Locally-produced Cheese. Master candidate.
3. Zainulabdeen, S. M. S., &Dakl, A. A. (2021). "Review Article Pathogenicity and virulence factors in *Staphylococcus aureus*. *Muthanna Journal of Pure Science*, 8(1): 109-119.
4. Khudher, K.K., &Jabur, S.G. (2020).The Prevalence of some virulence plasmid genes in *Staphylococcus aureus* infection.
5. Zaiean, A. M. (2020). Phenotype and Genotype Detection About the most Important Virulence Factors for Some Bacteria Species which isolated from Children's Nails infected with Diarrhea.*Journal of Education and Scientific Studies*. 6(16):37-48.
6. Lehman, M. K., Nuxoll, A. S., Yamada, K. J., Kielian, T., Carson, S. D., & Fey, P. D. (2019). Protease-mediated growth of *Staphylococcus aureus* on host proteins is opp3 dependent. *MBio*, 10(2): e02553-18.<https://doi.org/10.1128/mBio.02553-18>.
7. Khadem, A. Y., Farhan, A. A., &Ateea, K. E. (2018). Genetic study of *Staphylococcus aureus* Isolates from Different Environmental Source. *Diyala Journal of Medicine*, 15(1): 41-49.
8. Procop, G. W., Church, D. L., Hall, G. S., Janda W. M., Koneman, E. W, Scherckenberger, P.C., Woods, G.L. (2017). Koneman's Color Atlas and Textbook of Diagnostic Microbiology Color. 7th Ed. Philadelphia Wolters Kluwer Health.
9. MacFaddin, J. F. (2000). Biochemical tests for identification of medical bacteria, Williams and Wilkins. Philadelphia, PA, 113.

10. Alabdeen, Z., & Ahmed, B. H. (2021). Effect of Pomegranate Peel Extract on the Production of Some Enzymes in *Proteus* spp Isolated from Different Clinical Samples in Kirkuk city. *Kirkuk University Journal-Scientific Studies*, 16(3): 1-11.
11. Leboffe, M. J., & Pierce, B. E. (2011). A photographic atlas for the microbiology laboratory. 4thed. Morton Publishing Company, USA.
12. Brown, A., & Smith, H. (2014). Benson's Microbiological Applications, Laboratory Manual in General Microbiology, Short Version. McGraw-Hill Education.
13. AL-taai, H.R.R., Farhan, A.A. & Chiad, A.A. (2017). Combination effect of Erythromycin and Clindamycin on *Staphylococcus aureus*, Isolated from Different Clinical Sources. *Diyala journal For Pure Science.*, 13(3):114-130.
14. Nawfal, O., Bahjat, N., & Altaii³, H. (2021). Molecular detection of VRSA and MRSA strains in some infection in nineveh hospitals, Iraq. 21, 1785.
15. Mohammed, Noor Thaeer Saleh. (2022). Variation in the virulence genes of methicillin-resistant *Staphylococcus aureus*, Master's thesis, College of Science, University of Mosul.
16. Khwen, N. N. (2021). Some biological applications of locally purified DNase from *Staphylococcus aureus* and *Staphylococcus gallinarum*. Doctoral dissertation, Mustansiriyah University, Iraq.
17. Daoudi, Hanin Fadel Abbas. (2016). Study of the effect of some plant extracts and antibiotics on some species of the genus *Staphylococcus spp.* isolated from the upper respiratory tract, Master's thesis, College of Education for Girls, Tikrit University.
18. Al-Akedi, Ahmed. (2014). Study of the prevalence of *Staphylococcus aureus* resistant to MARS (methicillin) and its resistance to Glycopeptides in Damascus Children's Hospital, PhD thesis, Faculty of Human Medicine, Damascus University.
19. Mater, A. D., Jasim, M., & Shallal, M. (2019). Detection of intracellular adhesion (ica) B gene in *Staphylococcus aureus* isolated from wound infections. *University of Thi-Qar Journal of Medicine*.
20. Jassim, S. A., & Kandala, N. J. (2021). Molecular detection of enterotoxin genes of multiresistant *Staphylococcus aureus* isolates from different sources of food. *Iraqi Journal of Science*, 62(1): 61-74.
21. Al-Salman, M. A. (2012). Use The Crustacean Cycloypria as Bioclaner for Bacteria *Escherichia coli* & *Staphylococcus aureus* in Contaminated Water. *Ibn Al-Haitham Journal for Pure and Applied Sciences* 25 (1): 55-64.
22. González-Martín, M., Corbera, J. A., Suárez-Bonnet, A., & Tejedor-Junco, M. T. (2020). Virulence factors in coagulase-positive staphylococci of veterinary interest other than *Staphylococcus aureus*. *Veterinary Quarterly*, 40(1): 118-131.

23. Kot, B., Wierzchowska, K., Piechota, M., &Gruzevska, A. (2020). Antimicrobial Resistance Patterns in Methicillin-Resistant *Staphylococcus aureus* from Patients Hospitalized during 2015-2017 in Hospitals in Poland. *Medical Principles and Practice*, 29(1): 61–68.
24. Jameel, Z. J., Nayef, S. H., & Al-mahdawi, M. A. (2015). Study Some Virulence Factors of CO-agulase Positive *Staphylococci* and CO-agulase Negative *Staphylococci* Isolated from Different Parts of Human Body. *Tikrit Journal of Pure Science*, 20(4): 47-54.
25. Jahantigh, H. R., Faezi, S., Habibi, M., Mahdavi, M., Stufano, A., Lovreglio, P., &Ahmadi, K. (2022). The Candidate Antigens to Achieving an Effective Vaccine against *Staphylococcus aureus*. *Vaccines*, 10(2): 1–18.
26. Muter, O. S. (2018). Detiction of some Virulence Factors for *Staphylococcus aureus*Isolated from Conjunctivitis in Numan Teaching Hospital. *Journal of university of Anbar for Pure science*. *Journal of university of Anbar for Pure science*, 12(1):1-9.
27. Gazar, Zina Hassan and Kholoud, Raghad Abdel Latif. (2010). Extraction and purification of urease enzyme from *Staphylococcus aureus* isolated from patients with urinary tract infection, *Journal of the College of Education*, no. 1, p. 231-242.
28. Al-Moussawi, Alia Hussein Jamil. (2017). Study of the effect of some plant extracts on the biofilm formation of methicillin-resistant *Staphylococcus aureus*, Master's thesis, College of Education for Girls, University of Kufa.
29. Efuntoye, M. O., &Amuzat, M. A. (2007). Beta Lactamase production by *Staphylococcus aureus* from children with sporadic Diarrhoea in Ibadan and Ago-Iwoye, Nigeria. *African Journal of Biomedical Research*, 10(1).
30. AL-Samaraey, A. A. A. and Jaafar, N. A. (2021). Study of some virulence factors of *Staphylococcus aureus* isolated from wound and burn infections in Samarra city and evaluate their sensitivity to antibiotics. *Samarra Journal of Pure and Applied Science*, 3(3): 72-81.
31. Dawod Salman, A., &Abass Ali, E. (2017). Identification of *Staphylococcus aureus* Isolated from Different Infections and Study the Ability of Nuclease Production. *Diyala Journal for Pure Science*, 13(2), 215–225.
32. Rasheed, H., Farhan, A., & Ahmed, A. (2017). Combination effect of Erythromycin and Clindamycin on *Staphylococcus aureus*, Isolated from Different Clinical Sources. *Diyala Journal for Pure Science*, 13(3): 114–130.
33. Ibrahim, Mustafa RaadJawad. (2013). Detection of some genes responsible for virulence and antibiotic resistance factors in *Staphylococcus aureus* isolated from different clinical cases in Al-Diwaniyah city, Master's thesis, College of Science, University of Al-Qadisiyah.