

Original Research Article

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 pathogenic 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 pathogenic 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 pathogenicity 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: *Staphylococcus aureus*, virulence enzymes, beta-lactamases, Protease.

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]. *S. aureus* can also be found in the atmosphere, soil, and water [2]. *S. aureus*' pathogenicity 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 pathogenesis 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.

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Materials and Methods

In this investigation, 65 samples were collected, 50 from pathological 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.

Comment [K3]: Sample collection method not clearly stated. Clarify figures stated. How was the collection done??

Bacterial isolates that showed as fermented yellow colonies of mannitol sugar on agar-salt. The planning method was used to choose and replant mannitol medium on nutritional agar medium for purification [8].

Comment [K4]: Not clear. Rewrite

Microscopic examination

Swabs from the developing colonies were prepared on a glass slide and stained with Gram stain and examined under the microscope with an oil lens to identify the type and color of the cells.

Comment [K5]: State how it was done.

Biochemical test

The tests were conducted according to what was mentioned in the sources (MacFaddin, 2000). In addition to: Catalase test, oxidase test, and coagulase test which was conducted in two ways: Slide Coagulase Test and Tube Coagulase Test.

Comment [K6]: How was this done??

Diagnostics with the Vitek-2 system

The isolates were diagnosed with the Vitek-2 device in Al-Mansour laboratory in Mosul city.

Comment [K7]: What is this?? And how was it done?

Phenotypic investigation of virulence enzymes in *S. aureus*

Detection beta-lactamase in vitro

100 µl of the prepared Penicillin G solution at a concentration of 6 g was placed into test tubes containing a bacterial suspension at the age of 18-24 hours. After mixing, the tubes were kept in the incubation chamber for an hour at 37 degrees Celsius. The starch 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 [9].

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Detection beta-lactamase enzymes using nitrospine tablets

Comment [K13]: What is this?

The hue of the nitrospine pill changed to yellow after being moistened with a drop of sterile physiological saline. The juvenile colony was then moved and placed on the nitrospine tablet. The colour of the disc changed to pink, suggesting the synthesis of beta-lactamase [10].

Detection Urease

The prepared solid urea medium (Neogen/UK) was inoculated with a young developing colony of *S. aureus* and incubated at 37°C for (24-48) 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 [11].

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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 [12].

Comment [K15]: Into what??

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 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 [13].

Comment [K16]: Define this. First mention

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 [13].

Results and Discussion

65 samples were gathered from various pathological and environmental sources for this study. In the initial isolation process, mannitol medium was based on salt agar and as an electoral source for *S. aureus*. Mannitol sugar, salt, and phenol red index are the key components of the medium. Because the bacteria ferment the mannitol sugar, the colonies appear golden yellow in it. Because these bacteria are resistant of salinity and proliferate in the presence of sodium chloride, the color of the red phenol index was changed from pink to yellow as a signal of low pH [14].

Comment [K17]: Recast. Provide a brief introduction.

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) [15]. The Vitek-2 was used to confirm the diagnosis of all isolates under examination, Figure (3).

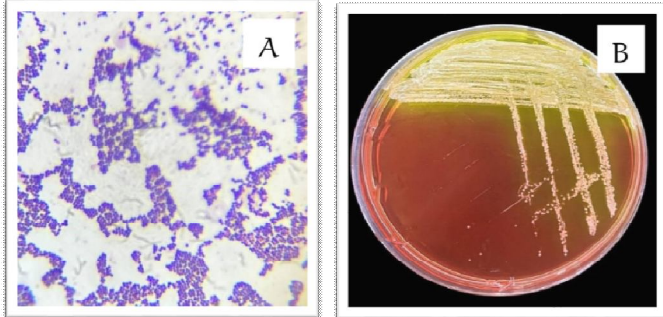


Fig 1: A. *S.aureutakes* Gr+ under a light microscope. B: *S. aureus* on a medium of salt and mannitol agr.

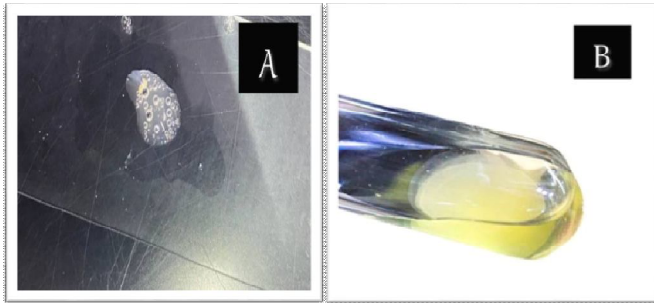


Fig 2:A: A positive result for a catalase test. B:A positive result for a blood clotting test.

bioMérieux Customer:		Al-Mansur Laboratory		Patient ID: 228													
Microbiology Chart Report				Physician:													
Patient Name: LAB_56				Isolate Number: 4													
Location:																	
Lab ID: 228																	
Organism Quantity:																	
Selected Organism : <i>Staphylococcus aureus</i>																	
Source:				Collected:													
Comments:																	
Identification Information		Analysis Time: 4.35 hours		Status: Final													
Selected Organism		99% Probability		<i>Staphylococcus aureus</i>													
ID Analysis Messages		Bionumber:		015402062763231													
Biochemical Details																	
2	AMY	-	4	PIPLC	-	5	DKYL	-	9	ADH1	+	9	GGAL	-	11	AGLU	-
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOB	+
20	LsuA	-	23	ProA	-	24	BGUFr	-	25	ACLAL	-	26	PyrA	-	27	GGUR	-
28	AlaA	-	29	TyrA	-	30	CSOR	-	31	LFRE	-	32	POLYB	+	37	dGAL	+
38	SPB3	-	39	ILATk	+	42	LAC	-	44	PNAG	+	45	dMAL	+	46	BACI	+
47	NCVO	-	50	NCOS	+	52	OMAN	+	53	GMNE	+	54	MBG3	-	56	PUL	-
57	dRAF	-	58	O12PR	+	59	SAL	-	60	SAC	+	62	dTRE	-	63	ADH2a	-
64	OPTG	-															

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

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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 pathogenic source and 7 isolates 10.76% from an environmental source. Isolates were recovered from all pathological tissues examined. [The same is true for many environmental samples such as soil, water, and air, Table (2)]

Comment [K19]: Any citation to confirm??

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

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

Comment [K20]: Use scientific tables

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

Source of isolates	No.	%
Pathological 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

Comment [K21]: Use scientific tables.

The isolation of *S. aureus* from all analyzed sources indicates their dissemination in various contexts and the associated hazard of their existence as one of the most important types of pathogenic bacteria that cause a variety of diseases such as pneumonia, skin infections, and bloodstream inflammation. Toxic shock syndrome is also induced by systemic toxin release, in which virulence factors, including cell wall structure, contribute to the host's immune system's

resistance, and the capsule strives to resist the process of phagocytosis [16, 17]. In addition to enzymes, poisons, surface proteins, and other substances that help bacteria infiltrate the body's tissues and cause infection [18]. Because of *S. aureus*'s propensity to secrete enterotoxins, the incidence of foodborne illnesses has also increased (SEs). The most dangerous toxins are kinds A, B, and D, which cause food poisoning [19]. *S. aureus* can also spread through water, which may contain its toxins, producing health problems in humans and even economically important aquatic creatures such as fish [20].

Comment [K22]: Recast. Not succinct

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 [21]. 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 pathogenic standpoint, regardless of the source of its isolation [22].

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

Isolates	Source	Virulence enzymes					
		Beta-lactamase	Urease	Himolysin	Protease	Lipase	Lecithinase
1	Airways	+	+	γ_-	-	+	+
2	Pus	+	+	$\alpha+$	+	+	+
3	Pus	+	-	γ_-	+	+	+
4	Dialysis	+	-	$\alpha+$	-	-	-
5	Dialysis	-	+	$\alpha+$	+	+	+
6	Blood	+	+	$\alpha+$	+	+	+
7	Urine	-	+	$\square+$	+	+	+
8	Urine	-	+	$\square+$	+	+	+
9	Dermatitis	-	+	$\alpha+$	+	+	+
10	Dermatitis	+	+	γ_-	+	+	+
11	Dermatitis	+	+	$\alpha+$	+	+	+
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	-	+	□+	+	+	+

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%.

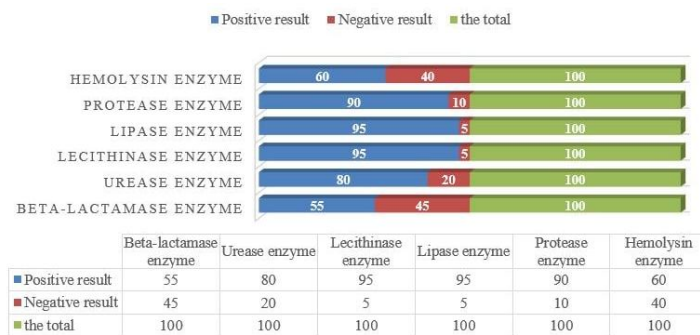


Fig 4: Production rates of virulence enzymes of *S. aureus* isolates.

The results showed that the isolates produced 95% of the lecithinase, 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 [23]. The current study's findings also revealed that the rate of production lipase was high 95%, 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 [24].

The activity of the protease production was studied, as the rate of its production approached 90%, Figure (7). It plays a significant role in bacterial pathogenicity 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 [25].

The recent investigation found that 80 percent of the isolates producing urease, Figure (8). The urease enzyme is important in the pathogenesis 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 [26].

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 [27].

Two approaches were used to investigate beta-lactamase synthesis. The in-tube approach was utilised, with penicillin G as the foundation of the examination and nitrosphe tablets 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 [28].

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, 29, 30 and 31]. On the other hand, the production rates of virulence enzymes in the current study differed with the results of other studies [23, 32 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.

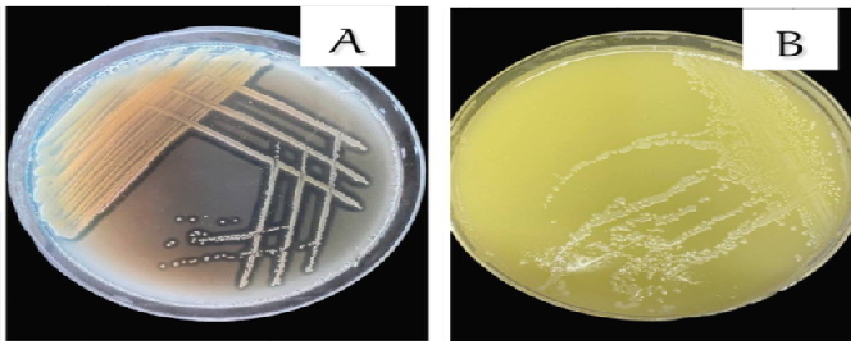


Fig 5: Lecithinase production A: Positive result. B: Negative result.

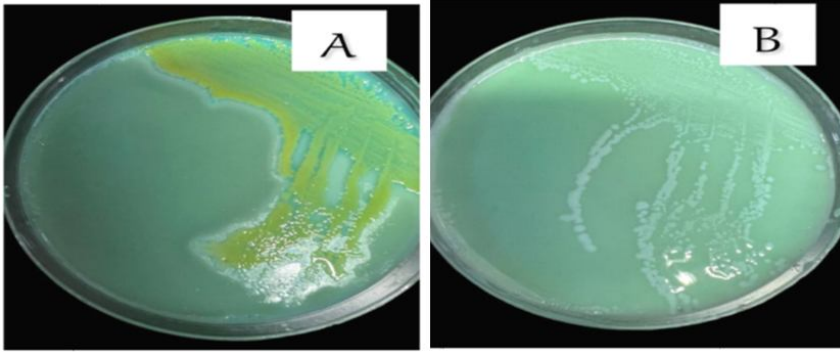


Fig 6: Production of lipase. A: Positive result. B: Negative result.

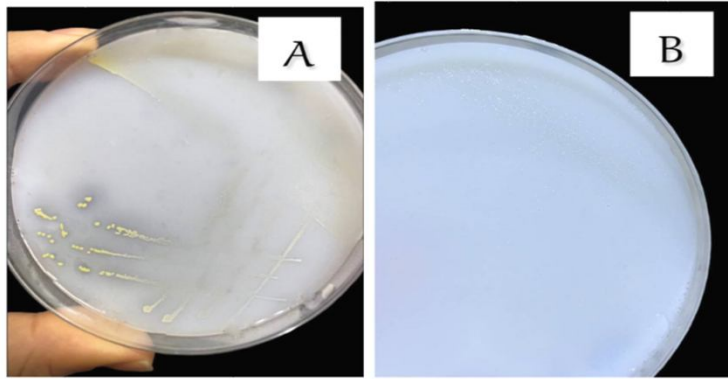


Fig 7: Production of protease. A: Positive result. B: Negative result.



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

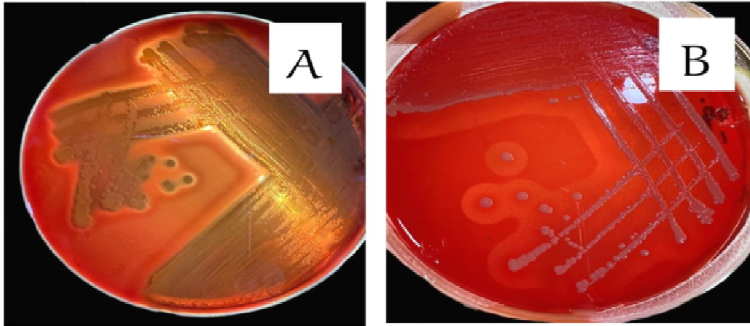


Fig 9: Production of hemolysin. A: Positive result. B: Negative result.

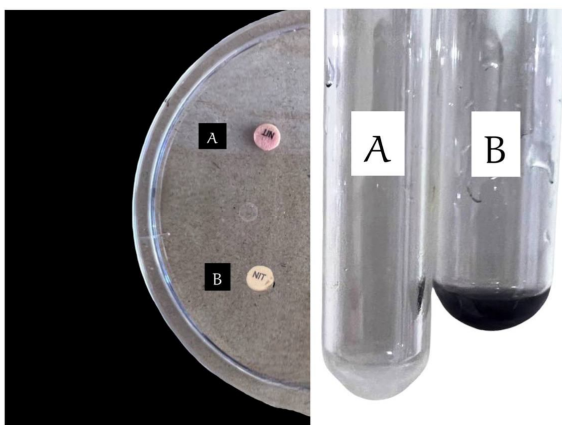


Fig 10: Production of beta-lactamase by two methods: tubes and nitrospirin tablets. A: Positive result. B: Negative result.

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 pathogenesis, 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.

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