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Molecular analysis of *Staphylococcus aureus* isolated from clinical samples and natural flora

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ABSTRACT

A total of 100 samples collected from the wound, abscess skin, and normal human flora were investigated for *S. aureus* identification. Overall, in 40 samples, *S. aureus* isolates were present, out of which most strains were isolated from normal human flora (50.0%), followed by wound (37.5%) and burn (12.5%) samples. Moreover, *S. aureus* isolates from all samples could produce extracellular enzymes (catalase, coagulase, urease, and hemolysin–ß) as virulence factors except for some isolates from normal flora samples (unable to produce coagulase enzymes). Therefore, genes encoding the enzymes coagulase and hemolysin were evaluated in 20 *S. aureus* isolates by PCR-specialized primers targeting co-specific genes. The PCR analysis revealed that clinical isolates included both genes. Contrarily, 6 isolates of the normal flora lacked the coa gene, revealing bacterial fingerprints that can be used to distinguish between isolated bacteria and human beings.

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Introduction

Gram-positive, coagulase-positive, catalase-positive *Staphylococcus aureus (S. aureus)* is an anaerobic bacterium that frequently results in nitrate reduction (1). *S.aureus* contributes to the normal flora of human skin and is one of the main sources of pyogenic illnesses (2). *S. aureus* is typically found in the upper respiratory tract and on the skin. They can act as a common pathogen of bloodstream infections, pneumonia, soft tissue and skin infections, postoperative wound infections, or device-related infections (3) by entering deep within the bloodstream, joints, bones, lungs or heart (4). Additionally, *S. aureus* infects a host by secreting dozens of virulence factors that influence the host's immune responses. Such virulence factors include the release of exotoxins which account for around 10% of the entire secretome. Overall, *S. aureus* produces more than 40 recognized exotoxins having similar activities and strong structural similarities, but a deeper look says otherwise (5). Pathogenic *S. aureus* strains often promote infections by producing virulence factors such as potent protein toxins and extracellular enzymes such as lipase, urease, DNase, hemolysin (hly), β-lactamase, coagulase (coa), and lecithinase (6, 7).

As a result, in consideration of the facts previously mentioned, the productivity of some virulence factors of the extracellular enzymes (urease, hemolysin, coagulase, and catalase) from various clinical *S. aureus* isolates is examined. Additionally, the genes for the enzymes coagulase (coa) and hemolysin (hly), which are encoded by PCR using specialized primers that target Co-specific of these genes, were also examined in 20 isolates.

Materials and Methods

Sampling

This study was carried out in the Marjan Teaching Hospital from March 2019 to June 2019. Ethical approval was received at the beginning of the research. Samples were collected from wounds (n=30), abscessed skin (n=35), and healthy people's normal flora (n=35). The clinical specimens have been adequately labelled, transported, and prepared in accordance with the aerobic bacterial culture's requirement.

Isolation and diagnosis

The samples were processed using established microbiological protocols, and suspected *S. aureus* colonies were examined extensively (8). Samples were cultured at 37 C° for 24 h on both blood agar and mannitol salt agar (MSA), and isolates were analyzed consequently for phenotypic features, microscopy and biochemical tests by the reported protocol (9). All strains were grown as per the manufacturer's instructions and sterilized for 15 minutes at 121°C I bar. The plates were examined for staphylococci morphology, and suspect colonies were recognized using standard microbiological procedures (Gram staining, catalase reactions, and coagulase reactions) (10).

Investigation of some virulence factors

The isolate which has the ability to produce coagulase and hemolysin enzyme as virulence factors were selected for further investigations. Using various laboratory culture mediums, the efficiency of virulence factors of the extracellular enzymes (urease, hemolysin, coagulase, and catalase) was examined. For such purpose *S. aureus* isolates,

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Table 1. Primers used in the study.

Primer	Primer sequence (5'---------3')	Expected gene size
Coagulase	5' ATA GAG ATG CTG GTA CAG G3' 5' GCT TCC GAT TGT TCG ATG C 3'	1000-2500 bp
Hemolysin	5' GGTTTAGCCTGGCCTT 3' 5' CATCACGAACTCGTTC 3'	100-1000 bp

including ten normal flora as families sample (coagulase-negative (5) and coagulase positive (5)), and ten clinical samples were selected to evaluate the presence of genes encoding the enzymes coagulase and hemolysin through polymerase chain reaction (PCR) by utilizing specialized primers that target Co-specific genes (*coa* and *hIy*), (Table 1).

Results

Diagnosis of *Staphylococcus* spp.

One hundred samples from the wound, abscess skin and normal human flora were collected to investigate the presence of *S. aureus*. The samples were cultured in nutrient agar containing either blood agar medium, nutrient broth, or MSA medium, respectively. Purification was done on several sub-culturing on corresponding media. Initially, *Staphylococcus* spp were diagnosed based on phenotypic characteristics and biochemical tests. When *Staphylococci* spp were viewed under a microscope, grape-like clusters having large round colonies were observed, as reported previously (11). *Staphylococcus* spp was further identified based on Gram staining, catalase, hemolysin, coagulase, sugar fermentation, and urea test.

Staphylococcal spp colonies obtained from selective media were all Gram-negative and catalase positive. In Figure 1, the growth of bacteria in the blood medium illustrates clear zones of β-hemolysis. Still, the small difference between the zone of β-hemolysis in the pathogenic and the normal flora sample is because of the environment in which bacteria grow.

S. aureus isolates and extracellular enzymes.

Overall, it was found that in 40 samples, *S. aureus* isolates were present. Most *S. aureus* strains were isolated from healthy samples (50.0%) followed by wound (37.5%) and burn samples (12.5%) (Table 2). *S. aureus* can typically be found in humans in the skin, upper respiratory tract, and gut mucosa as a part of the normal microbiota. This explains the variation in the presence of S. aureus isolates in the sample (18). On the other hand, because S. aureus is categorised as a pathobiont, it can spread disease under specific host and environmental conditions (19, 20). Moreover, isolated *S. aureus* strains were tested for the production of extracellular enzymes (a wide variety of exoenzymes) as virulence factors.

The result revealed that *S. aureus* isolates from clinical samples could produce all four tested enzymes i.e., catalase, coagulase, urease and hemolysin–ß. In a similar manner *S. aureus* isolates from families (mean strains) as normal flora samples were also able to produce enzymes, i.e., catalase, urease and hemolysin–ß, except coagulase 70% (14 isolates) (Table 3).

Correlation between bacterial enzymes production

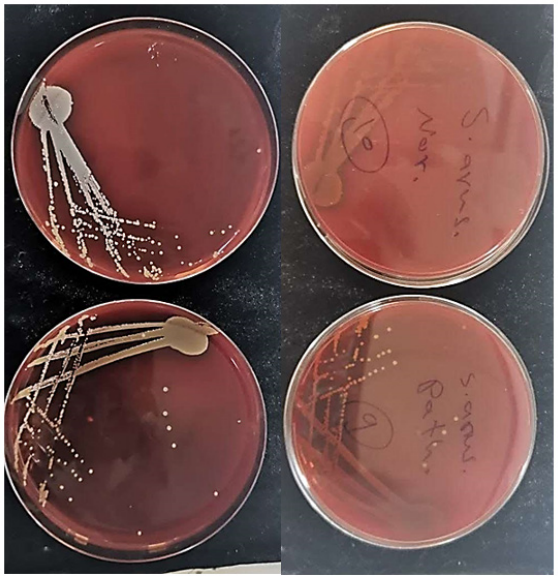

Figure 1. Staphylococcal bacteria (pathogenic and normal flora) on the blood agar.

Figure 2. Bacterial growth on MAS medium.

Table 2. Bacterial distribution throughout the study population.

Type of sample	*S. aureus (n)*	%
healthy people	20	50%
Wound	15	37.5%
Burns	5	12.5%
Total	40	100%

Table 3. The ability of *S. aureus* to produce some extracellular enzymes as virulence factors.

Type of samples	No.	Urease test	Coagulase test	Catalase test	Hemolysis test
Healthy people	20	100%	70%	100%	100%
Wounds	15	100%	100%	100%	100%
Burns	5	100%	100%	100%	100%

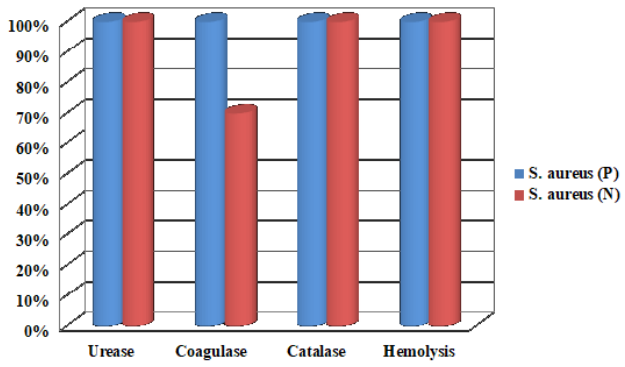

Figure 3. Enzyme production by *S. aureus* (N) and *S. aureus* (P). *P= Pathogenic. *N= Normal flora.

In Table 3, it was noticed that 30% of the *S. aureus* isolated from the normal flora (from the hosts) did not produce coagulase, while all pathogenic samples produced selected enzymes at 100%. These findings remain consistent when repeating the examination after two weeks in the same samples and from the same people, which means that these bacteria are the bacterial fingerprint of these people and their inability to produce this enzyme can be relied upon as a distinguishing mark (Figure 3).

Molecular detection of coa and hly gene

A total of 20 *S. aureus* isolates, including normal flora as family's sample (10) (coagulase-negative (5) and coagulase positive (5)), and clinical samples (10) were selected based on their ability to produce *coa* and *hly* enzyme as virulence factors. To evaluate the presence of genes encoding the enzymes *coa* and *hIy*, a PCR test was performed. The result showed the presence of the *hIy* gene in all studied isolates, while the *coa* gene was present in all clinical samples (10) but not present in 6 families isolation, as shown in Figures 4-6.

Correlation between bacterial production of enzymes and gene analysis

The obtained results signified the *S. aureus* ability to produce extracellular enzymes that express virulence factors. Still, there are some bacterial isolates from normal flora samples which did not produce coagulase enzymes even after repeating the examination more than once. Therefore, genes responsible for the production of these enzymes were examined, and it was found that only those isolates which were negative for the coagulase assay do not possess *coa* gene (Table 4).

Discussion

Table 4. The presence of *coa* and *hly* genes.

***S. aureus* isolates**				
Coagulase test	**Positive**			**Negative**
Total	15			5
	P(10)	N(5)		N(5)
coa gene	+	+ (4)	-(1)	-
hly gene	+	+		+

*P= Pathogenic. *N= Normal flora.

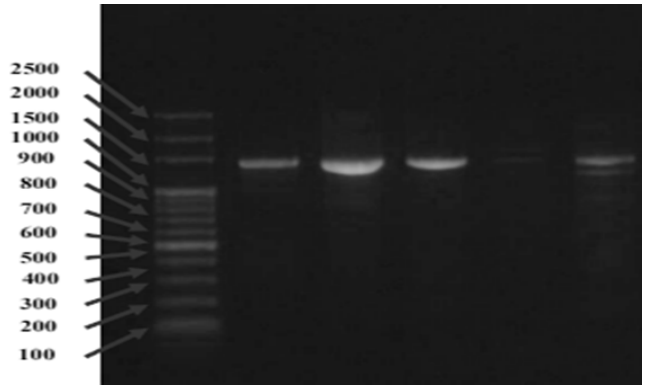

Figure 4. PCR results of *coa* gene of *S. aureus* bacteria isolated from healthy people using specific primer on agarose gel (5.1%) and 60 volts for two hours.

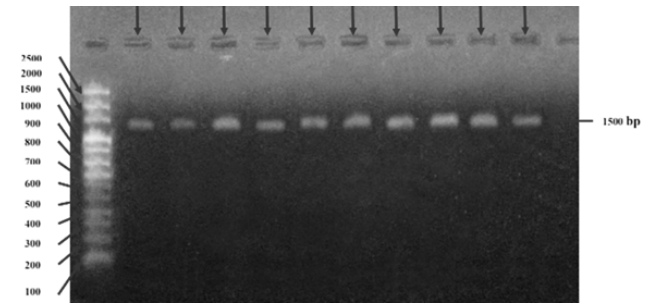

Figure 5. PCR results of DNA isolates of *S, aureus*. bacteria isolated from healthy people, wound and burn infection using specific primers of *coa* gene on agarose gel (5.1%) and 60 volts for two hours.

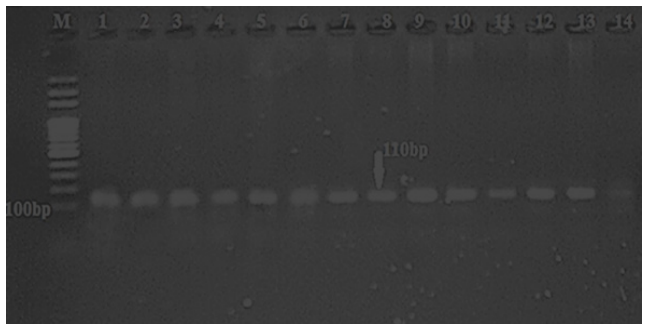

Figure 6. PCR results of DNA isolates of *S, aureus* bacteria isolated from healthy people, wound and burn infection using specific primers of *hIy* genes on agarose gel at a concentration (5.1%) and a good difference (60) volts for two hours.

On MSA, medium pathogenic *S. aureus* produces small colonies surrounded by yellow zones due to mannitol fermentation which clearly distinguishes *S. aureus* from *S. epidermidis* (Figure 2) (12, 13). 90% of *S. aureus* strains are known for urease production (14) because it is important for bacteria in environmental adaptation, pathogenicity, and protection against human immunity. As a result, urease synthesis was indicated by a vivid pink colour on the slant that extended into the butt after a few hours of incubation. A vivid pink colour showed urease production in Stuart's urea broth throughout the broth (15).

Fibrinogen can be turned to fibrin by the protein enzyme known as coagulase, which is produced by several bacteria. It is used in the lab to differentiate between different Staphylococcus isolates. *S. aureus* is coagulase-positive generally, which is significant since it signifies that

a positive coagulase test indicated that *S. aureus* was present in the determined sample (16, 17). A negative coagulase test reveals the presence of organisms like S. epidermidis or S. saprophyticus that are coagulase negative. More typically, opportunistic infection is associated with coagulation-negative Staphylococci species. (1). In most cases, isolated cultures were purified by serial dilution on reputable media, and the acquired pure cultures were maintained at 37°C for 24-48 h, then stored at 4°C in a refrigerator for further investigation. The find results are in accordance with the Abdul-Kareem et al., findings who reports the presence of *hIy* gene (virulence factor of haemolysine enzyme) in *S. aureus* isolate from burns, wounds and skin flora (21). Similarly, it is also reported that most strains of *S. aureus* are the producer of the *coa* enzyme as a significant virulence factor of pathogenicity (22-24). This result helps to link the existence of a relationship between people and isolated bacteria, which helps in the diagnosis and identification of bacterial fingerprints for families.

The *S. aureus* isolated from pathological conditions and a healthy person can produce some virulence factors extracellular enzymes such as coagulase enzyme, catalase, hemolysin, and urease. While not all *S. aureus* isolated from normal persons produced coagulase enzymes. From PCR analyses, it was revealed that this difference is due to the absence of the *cos* gene in *S. aureus* isolates from normal flora in comparison to clinical isolates where *cos* genes are detected in all selected samples.

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Authorship
Every writer is recognized as an author because they have all completed the requirements for authorship and approved the final product.

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Normative Consent
From March to June 2019, this study was conducted at the Marjan Teaching Hospital. The research was given the go-ahead after receiving ethical permission.

Competing interests
There are no conflicts of interest for the authors.

Data accessibility declaration
The manuscript that has been submitted contains data.

References

1. Otarigho B, Falade MOJB. Analysis of antibiotics resistant genes in different strains of Staphylococcus aureus. Bioinformation 2018;14(3):113.
2. Bennett JE, Dolin R, Blaser MJ. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases E-Book: Elsevier Health Sciences; 2019.
3. Tong SJS, Eichenberger, E., Holland, TL, Fowler, VG. C, Davis, J. Clin Microbiol Rev2015:603-61
4. Bitrus A, Peter O, Abbas M, Goni MJVSR, Reviews. Staphylococcus aureus: a review of antimicrobial resistance mechanisms. Future Microbiology2018;4(2):43-54.
5. Cong Y, Yang S, Rao XJJoAR. Vancomycin resistant Staphylococcus aureus infections: A review of case updating and clinical features. A review of case updating and clinical features. 2020;21:169-76.
6. Bamigboye BT, Olowe OA, Taiwo SSJEJoM, Immunology. Phenotypic and molecular identification of vancomycin resistance in clinical Staphylococcus aureus isolates in Osogbo, Nigeria. .European Journal of Microbiology and Immunology 2018;8(1):25-30
7. Cheung GY, Bae JS, Otto M. Pathogenicity and virulence of Staphylococcus aureus. Virulence. Virulence. 2021;12(1):547-69.
8. Isenberg HJW, DC. Clinical microbiology procedures handbook, p 5.1. 2 ASM Press. 2004.
9. Ferens WA, Davis WC, Hamilton MJ, Park YH, Deobald CF, Fox L, et al. Activation of bovine lymphocyte subpopulations by staphylococcal enterotoxin C. Infection and Immunity, 01 Feb 1998, 66(2):573--80.
10. Karmakar A, Dua P, Ghosh C. Biochemical and molecular analysis of Staphylococcus aureus clinical isolates from hospitalized patients. Canadian journal of infectious diseases and medical microbiology. 2016;2016.
11. Ray CG, Ryan KJ. Sherris medical microbiology: McGraw-Hill; 2010.
12. Harrigan WF. Laboratory methods in food microbiology: Gulf professional publishing; 1998.
13. Cowan ST. Cowan and Steel's manual for the identification of medical bacteria: Cambridge university press; 2003.
14. Murchan S, Aucken H, O'neill G, Ganner M, Cookson B. Emergence, spread, and characterization of phage variants of epidemic methicillin-resistant Staphylococcus aureus 16 in England and Wales. Journal of clinical microbiology. 2004;42(11):5154-60.
15. Dahlén G, Hassan H, Blomqvist S, Carlén A. Rapid urease test (RUT) for evaluation of urease activity in oral bacteria in vitro and in supragingival dental plaque ex vivo. BMC Oral Health. 2018;18(1):1-7.
16. Varrone JJ, de Mesy Bentley KL, Bello-Irizarry SN, Nishitani K, Mack S, Hunter JG, et al. Passive immunization with anti-glucosaminidase monoclonal antibodies protects mice from implant-associated osteomyelitis by mediating opsonophagocytosis of Staphylococcus aureus megaclusters. . J Orthop Res. 2014 October ; 2014;32(10):1389-96.
17. Matthews K, Roberson J, Gillespie B, Luther D, Oliver SJJoFP. Identification and differentiation of coagulase-negative Staphylococcus aureus by polymerase chain reaction. journal of food protection 1997;60(6):686-8.
18. Wollina UJC, cosmetic, dermatology i. Microbiome in atopic dermatitis. Clinical, Cosmetic and Investigational Dermatology 2017;10:51..
19. Otto MJErod. Staphylococcus colonization of the skin and antimicrobial peptides. Expert Review of Dermatology 2010;5(2):183-95.
20. Jassim YA, Al-Amery SMHJDIT. Purification and characterization of protease and lipase from Pseudomonas aeruginosa isolated from some wound and burn infection. Drug Invention Today 2019;11(10).
21. Abdul-Kareem HS, Husain AS. Genetic Comparative Study of Staphyolococcus aureus & Staphylococcus epidermidis Isolated from Wounds, Burns and Skin Flora. Iraqi Journal of Science. 2015;56(1):708-12.
22. Cuny C, Wieler LH, Witte W. Livestock-associated MRSA: the impact on humans. Antibiotics. Antibiotics journal 2015;4(4):521-

43.
23. Javid F, Taku A, Bhat MA, Badroo GA, Mudasir M, Sofi TA. Molecular typing of Staphylococcus aureus based on coagulase gene. Veterinary world. 2018;11(4):423.
24. de Freitas Guimarães F, Nóbrega DB, Richini-Pereira VB, Marson PM, de Figueiredo Pantoja JC, Langoni H. Enterotoxin genes in coagulase-negative and coagulase-positive staphylococci isolated from bovine milk. Journal of dairy science. 2013;96(5):2866-72.
25. Little, S.V.; Hillhouse, A.E.; Lawhon, S.D.; Bryan, L.K. Analysis of Virulence and Antimicrobial Resistance Gene Carriage in Staphylococcus aureus Infections in Equids Using Whole-Genome Sequencing. mSphere Clinical Science and Epidemiology2022, 6, e00196-e20.