



Molecular Identification of Lipase Producing Staphylococcus Epidermidis Isolates from Community and Hospital Samples, Mardan

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Authors' Contribution

All authors equally contributed to the study and approved the final manuscript

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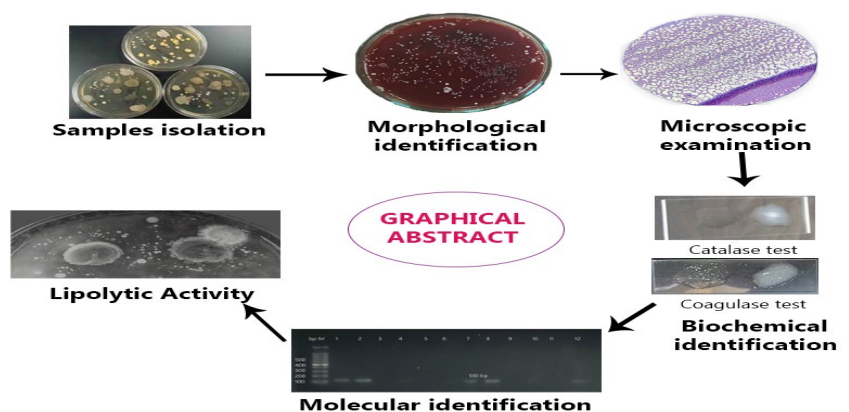
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ABSTRACT

Staphylococcus epidermidis is a facultative anaerobic, non-spore-forming, catalase positive, coagulase negative (CoNS) gram-positive clustering cocci that grow on human skin and mucous membranes hence the commensal flora but can turn pathogenic among the immunocompromised individuals; it's a major cause of nosocomial infections, bacteremia, endocarditis, and wound infections. In the advancement of growth and colonization together with the ability to survive within the microenvironment, lipase has been connected to staphylococci bacteria. The specific objectives of this research are to isolate and categorize bacterial samples from hospital and community facilities. Swabs of the nose and skin were taken. Thus, 15 samples from the hospital and 15 samples from the community were cultured on blood agar. The bacterial species was identified using morphological characteristics, gram staining, and biochemical tests of catalase and coagulase. The conclusion of the study revealed that 29 out of 30 samples had bacterial displacement. Chromatography with gram stain also showed that all the isolates were gram-positive cocci 29 in number. Catalase testing further revealed that 28 of these isolates belonged to staphylococcal species while one of the hospital nasal samples was catalase negative. Regarding the coagulase test result, 27 out of the 29 identified staphylococcal isolates were CoNS. Lipase activity in CoNS was least common and found only in three samples; two hospital isolates from nasal and one weak community isolate. Staphylococcal lipase is described for the purpose of colonizing and subsisting of the existence of microorganism. When performing DNA extraction and PCR amplification to amplify the segment of the *rdr* gene of Staphylococcus epidermidis, sample was identified in 13 of the 27 CoNS isolates. The PCR results revealed that out of all the clinical samples 9 were *S. epidermidis* from hospital sample and 4 from community sample. Therefore, the work highlighted that CoNS especially *S. epidermidis* were prevalent in hospital and community settings.

Graphical Abstract



INTRODUCTION

Staphylococcus epidermidis, belongs to the own family *Staphylococcaceae* (Sinkov et al., 2024). The *Staphylococcaceae* family includes numerous other significant species, each pathogenic and non-pathogenic, consisting of *Staphylococcus aureus* and *Staphylococcus saprophyticus*. *Staphylococcus epidermidis* is a causative agent of bacteremia, endocarditis, wound, nosocomial infections (Aghazadeh et al., 2015). *Staphylococcus epidermidis* forms a part of the normal flora of human skin, predominantly being located on sebaceous areas. Usually not pathogenic when colonizing intact skin, but as an opportunistic pathogen, can cause infection when skin barrier or when it directly enters the blood stream (Otto et al., 2009). *S. epidermidis* is coagulase-negative, and it generally colonizes the skin and mucous membranes, and this organism has the ability to form biofilms on synthetic surfaces such as catheters and prosthetic devices (John et al., 2015). It is an important source of HAIs and is particularly dangerous for immunocompromised patients (Becker et al., 2014). Within health-care related premises, *S. epidermidis* is among the predominant pathogens that cause HAIs, especially bloodstream infections, and endocarditis (Shobo et al., 2021). Through formation of biofilm, *E. Coli* escapes immune response and antibiotic actions, meaning the bacterium becomes a tough adversary on medical devices and hard to eliminate (Foster, 2020). Community-acquired *S. epidermidis* infection is infrequent although it is reported in patients with skin lesions, chronic ulcers or those using medical devices outside health facilities (DeBlieux et al., 2010).

Since lipase enzymes are relevant to bacterial pathogenicity and metabolism because they are involved in the acquisition of nutrients, evasion from the host immune system, and adaptation to the host (Suleiman et al., 2024). Triacylglycerolases or lipases are enzymes that split triglycerides into fatty acids and glycerol, basic constituents of bacterial exponent and growth. *Staphylococcus aureus* secrete lipases help in the breakdown of host lipids which allows the bacteria to infect the tissues and form pus (Al-Sa'ady). Besides facilitating the penetration and evasion of host immune responses, bacterial lipases also help in destruction of lipid barriers to host colonization and invasion of host cells (Chen & Alonzo et al., 2019). Concerning immune evasion ability, bacterial lipases play a role by degrading immune activating lipid molecules which otherwise would trigger immune response (Chen, X. and Alonzo et al., 2019). Also, lipase production provides performance and functional FRE flexibility for bacteria in various circumstances that benefit bacterial survival in environments where nutrients are scarce and in environments where lipids are plentiful (Rameshwaram et al., 2018).

Usually, *S. epidermidis* is a harmless inhabitant of human skin that rarely is pathogenic to healthy humans, but can become a menace to compromised hosts with underlying diseases or people using medical prostheses (Omar et al., 2010). The studies have also shown that the lipase activity in *S. epidermidis* also increases the bacteria's ability to develop biofilms that are in effect a shield making the organisms hard to eliminate through antibiotics or the immune system (Stehr et al., 2003). Local conditions of the

environment and the healthcare system might play a role in the alteration of *S. epidermidis* and reduce lipase activity as well as pathogenicity (Claudel et al., 2019). Such knowledge of local changes comprehensively can contribute to defining infection control measures in the examined region (Chen & Alonzo, 2019). Furthermore, the study on the lipase producing *S. epidermidis* finds direction with the general attempts to combat antibiotic resistance, as the bacteria forming biofilms targets for therapy because of their increased resistance mechanisms (Gupta et al., 2004). It may therefore be critical to identify the nature and behavior of local strains in order to develop better treatment strategies in places such as Mardan. The goal of this research is to systematically isolate and describe lipase producing strains of *Staphylococcus epidermidis* by prevalence of sample types from different sources in Mardan to screen lipase production. The study aims at comparing the lipase activity levels of these isolates to identify biochemical characteristic related to pathogenic tendencies that will help in understanding a relationship between lipase synthesis and pathogenicity of local *S. epidermidis* isolates. Furthermore, the study will further compare lipase activity and pathogenic characteristics of isolates at geographical and source level and determine whether source variation exists in lipase levels of isolates in Mardan.

METHODOLOGY

Sample collection

Sample of skin and nose were taken from Mardan Medical Complex. A total of 15 samples were taken from the hospital, including 7 from the skin surface and 8 from the nasal cavity were collected from the community.

Isolation and Identification

Laboratory cultivation of the samples was done using blood agar (Koksal et al., 2009) at 37°C for 24 hours (Sheikh et al, 2019). The isolates were further identified through gram staining, catalase and coagulase tests.

Lipolytic Activity and DNA Extraction

Bacterial isolates in this study were streaked on the agar medium 10 gm peptone, 5 gm NaCl, 0.1 gm CaCl₂, 20 gm agar and 1 mL Tween 20 per litre. The plates were then incubated at 37°C for 24 hours. DNA extraction was done by suspending 3-4 colonies of pure bacterial culture in 5% of Chelex reagent. The obtained mixture was shaken at 450 rpm and 37°C.

Identification of *rdr*

PCR was used to amplify the (*rdr*) gene.

The *rdr* gene primer sequence was utilized according to (Shome et al., 2011).

Table 3.1

Primer sequences for rdr.

Gene	Primer Sequence	Amplification	References
<i>rdr-f</i>	AAGAGCGTGGAGAAAA CAAG	130	(Shome et al, 2011)
<i>rdr-f</i>	TCGATACCATCAAAG TTGG		

PCR reactions were performed in a BioRad thermal cycler, for target gene copy number amplification of the samples, PCR cocktail: 1µL DNA sample, 1µL forward primer, 1µL

reverse primer, 9.5µL DNAase-free H₂O, 12.5µL master mix.

Agarose Gel Electrophoresis

A 1% agarose gel was prepared containing 2 ml of TAE buffer with which 7 µL solution of ethidium bromide was added. A 100 bp ladder was loaded in the first well of the gel, with which 10 µL of the PCR product were loaded in each well accordingly. Under 120 V, the gel electrophoresis procedure was carried out for 30 minutes. Following the procedure, an ultraviolet (UV) beam was used to evaluate the gel.

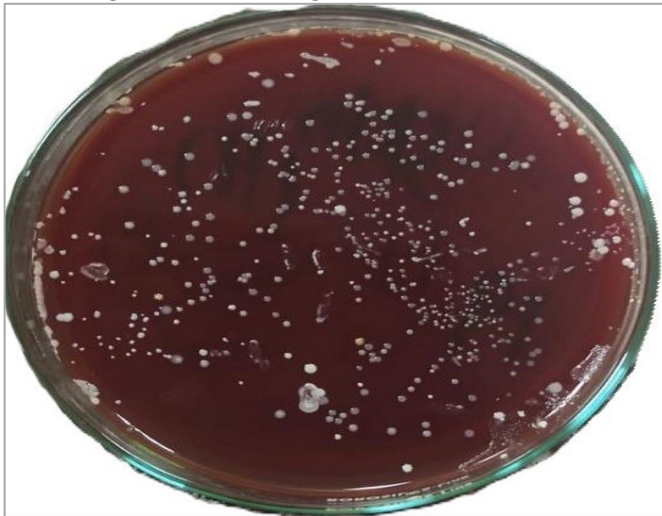
RESULTS

Culturing

Twenty-nine out of thirty samples that were collected and processed for culture showed Bacterial growth. All fifteen hospital samples, community eight skin samples and Six (6) out of seven (7) samples of community nasal showed growth. While one (1) sample got contaminated.

Figure 1

Bacterial growth on blood agar

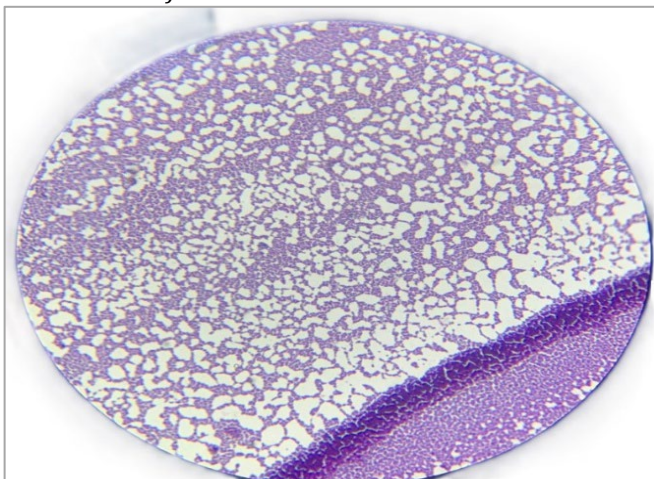


Microscopic Examination

Under a microscope, clusters of gram-positive cocci have been identified. All the Twenty-nine (29) isolates from Twenty-nine (29) culture plates showed Gram-positive results.

Figure 2

Gram stain confirmation



Biochemical Identification

The catalase activity in twenty nine bacterial isolates yielded positive reaction in all the twenty nine samples except one and it's from the nasal swab sample collected from the hospital. The coagulase test was negative in twenty-seven of the twenty-nine isolates, and the only positive test was given by the formation of the clot or the fibrin on the slide covering the bacterial mass.

Molecular Identification of *S. epidermidis*

Thirteen (13) isolates out of Twenty-seven (27), CoNS showed PCR-positive results. There were 13 bands seen parallel to the standard or ladder indicated that all the all the 13 samples were of *S. epidermidis* due to the presence of its *rdr* gene which is 130bp.

Figure 3

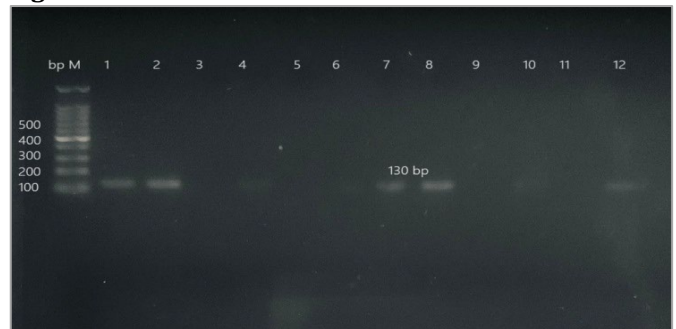
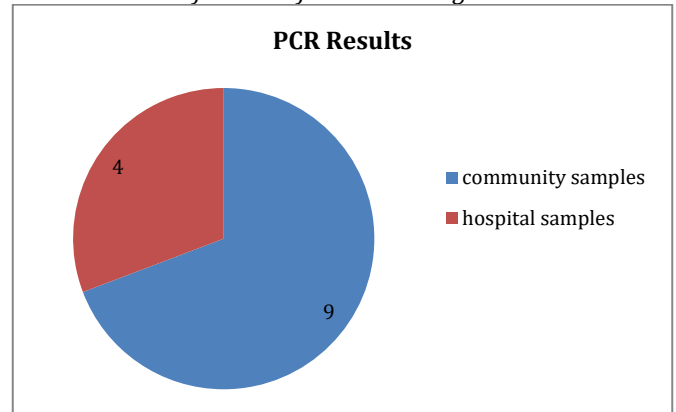


Figure 4

molecular identification of isolate through PCR

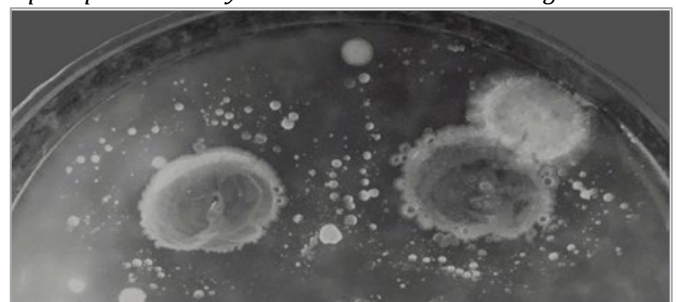


Lipolytic Activity

Lipase was produced by Three (3) isolates out of Twenty-seven (27) CoNS isolates. Among the Fifteen (15) hospital CoNS isolates Two (2) of hospital nasal isolates strongly showed positive lipase activity. While only one (1) community nasal isolate showed weak positive lipase activity.

Figure 5

Lipase production by CoNS isolates on nutrient agar



DISCUSSION

S. epidermidis is especially implicated in wound infection, bacteremia, endocarditis and prosthetic-associated infection and among all the properties that contribute to its pathogenicity, ability to form biofilm is especially important (Cheirifi et al., 2013; Yao et al., 2005). Adhesion is a crucial stage since it selects the bacteria that will colonise, form biofilms and later be resistant to antibiotics and the body's defences when they use medical instruments such as catheters (Gotz, 2002; Knobloch et al., 2008). Moreover, *S. epidermidis* has an edge in changing environments and metabolic status which facilitate its colonization in clinical setting (Vincent, 2003; Zarb et al., 2012). Lipase activity was of particular interest to this study because lipids are common on human skin and may provide bacterial nourishment (Thomson et al., 1999). Catalase and coagulase tests were performed in the first instance to confirm *S. epidermidis* because it is coagulase negative while *S. aureus* is coagulase positive (Kloos & Bannerman, 1994). After identification, biochemical tests on the newly developed low nutrient media containing lipid substrates in order to detect lipase production and activity as evidenced by clear zones surrounding the colonies where lipids were broken down (Sampaio & Rozental, 2010). PCR analysis supported the findings regarding lipase genes presence, as molecular approaches were used. Among the thirty-two sampled, three demonstrated lipase enzyme activity with two samples being strongly positive and one sample being weakly positive. It is important to note that nine of the positive samples were obtained from community samples as against four from other settings. This suggest that the

lipase activity observed may not be attributed to *S. epidermidis* alone, they could be contaminated with lipase producing organism such as *Bacillus* and *Pseudomonas aeruginosa* (Yassein et al., 2021). While 8 of the samples were newly identified as catalase-negative, it is possible that contamination may have led to the misidentification of some of these samples as *S. epidermidis*. In conclusion, it is possible to confirm the lipase activity of *S. epidermidis* Nevertheless, with the data on the constant dilemma of contamination and misidentification of similar characteristics of microorganisms (Lee & Iandolo, 1986; Nicolaides, 1974).

CONCLUSION

According to the Gram staining, cocci were Gram-positive and clustered; 28 of the 29 isolates were positive for catalase. Of these, 27 were found to be coagulase negative, thus placing them as coagulase negative staphylococci or CoNS. Of these 27 CoNS isolates, three isolates demonstrated lipase activity and both hospital and community-acquired strains were positive for this enzyme. After confirmation of the catalase positive and coagulase negative results DNA extraction and PCR were done in which 13 out of 27 isolates tested positive for *S. epidermidis* through detection of 130 bp *rdr* gene fragment.

Future Recommendation

It is especially important to gain a detailed understanding of the mechanisms of *S. epidermidis* infection. There is need to link the virulent factors of *S. epidermidis* with disease outcome.

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