



In Vitro Antagonistic Activity of Cell-Free Supernatant from a Local *mecA*-Positive *Staphylococcus epidermidis* Isolate Against *Staphylococcus aureus*

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Abstract

Background: The human skin microbiome, specifically commensal bacteria such as *Staphylococcus epidermidis*, is important in preventing opportunistic bacteria such as *Staphylococcus aureus* from colonizing the skin. The purpose of this study was to assess the in vitro antagonistic effect of the cell-free supernatant (CFS) obtained from a local *S. epidermidis mecA* positive isolate against *S. aureus*.

Methods: A local isolate of *S. epidermidis* was phenotypically identified by the use of VITEK 2 system and molecularly characterized by Polymerase Chain Reaction (PCR) confirming the presence of the *mecA* gene. The CFS were prepared by centrifuging and filtering them. Later, the antibacterial activity against *S. aureus* was evaluated by the agar-well diffusion method.

Results: Molecular analysis proved to be successful in confirming the local isolate as being *mecA* positive. The in vitro antagonism assay showed that the CFS of *S. epidermidis* isolate had a clear antagonism effect on *S. aureus* with a definite ZOI of 10 mm.

Conclusion: The local *mecA*-positive *S. epidermidis* has significant antagonistic properties against *S. aureus* in the cell free supernatant. These results pave the way for the potential use of postbiotics from local commensals as active ingredients to create topical formulations with the aim of restoring the balance of the human skin microbiome and improving the skin's natural immune barrier functions.

Introduction

Human skin is the largest organ of the body and the main barrier to environmental danger both physically and immunologically (Zhu *et al.*, 2023). A diverse and complex colonization occurs with microorganisms, termed the skin microbiome, that are essential to the maintenance of skin homeostasis and in educating the host's innate immune system (Prajapati *et al.*, 2025). Natural skin defense functions are dependent on a balanced microbiome, while a dysbiosis is often linked to dermatological disease and a risk of infection (Smythe & Wilkinson, 2023).

Staphylococcus epidermidis is one of the most important commensal organisms of human skin (Qi *et al.*, 2025). In normal state, *S. epidermidis* has mutualistic relationship with the host. It uses competitive exclusion to inhibit the growth of some transient opportunistic pathogens, including *Staphylococcus*

aureus (Nurxat *et al.*, 2023), and produces antimicrobial compounds, including various organic acids, phenol-soluble modulins and bacteriocins (Jang *et al.*, 2020). Such oppositional relationship is an elemental part of the natural defense line of the skin.

Prescribed live commensal bacteria (probiotics) have been suggested to restore the balance of the microbiome, but there is a huge concern for biosafety for the clinical use (Roslan *et al.*, 2023). One of the main issues is the potential to develop opportunistic pathogenicity and the development of antimicrobial resistance (Hung *et al.*, 2015). Use of live cells is potentially dangerous, if the *mecA* gene is common in environmental or commensal staphylococcal isolates (Bloemendaal *et al.*, 2010).

In order to avoid use of live resistant cells, recent studies have turned to the use of the cell-free supernatant (CFS), known as "postbiotics" (Gurunathan *et al.*, 2023). Therefore, recent studies have focused on using the cell-free supernatant (CFS) as "postbiotics" to avoid the risks of live resistant cells (Meena *et al.*, 2025). The CFS is a complicated cocktail of soluble bioactive substances and metabolic waste products produced by the bacteria (Pedretti *et al.*, 2024), with therapeutic and antagonistic activities and without the potential of bacterial translocation or the horizontal transfer of resistance genes (Mund *et al.*, 2026).

Hence, the present investigation is designed to assess the in vitro antagonistic activity of the cell-free supernatant (CFS) obtained from an *S. epidermidis* isolate from the local region against *S. aureus*. In this study, the postbiotic potential of this local isolate is targeted to establish a scientific reference for the production, via a cell-free preparation, of a topical formulation that can restore the skin's natural defense functions and its microbiome balance.

Methods

Bacterial Isolation and Phenotypic Identification

A local bacterial isolate was obtained from skin swabs collected from healthy volunteers. Initial culturing and morphological examinations were performed using standard microbiological procedures. The definitive phenotypic identification of the isolate was carried out using the VITEK 2 Compact System (bioMérieux, France) using Gram-positive (GP) identification cards, according to the manufacturer's instructions. A clinical isolate of *Staphylococcus aureus* was obtained from [Insert Hospital/University Name, e.g., University of Al-Qadisiyah] to be used as the target pathogen for the antagonistic assay.

Molecular Characterization (PCR Detection of *mecA*)

In order to confirm the genetic profile and methicillin resistance of the isolate of *S. epidermidis* found locally, a Polymerase Chain Reaction (PCR) test was performed. The genomic DNA was extracted from bacterial cell using commercially available DNA extraction kit (Presto mini gDNA bacteria kit). The PCR amplification of the *mecA* gene was done using specific primers. The amplified PCR products were separated on agarose gel, stained and looked under UV trans illumination to make sure that the target gene band is there.

Table 1: Primer sequence used in this study

No.	gene	Primer name	Sequence 3-5	Product size	reference
1	<i>mecA</i>	<i>mecA</i> (F)	GATCGGCTGAAACAGTTGAA	290	23
		<i>mecA</i> (R)	GCCTGACCATTACCAGCACT		

Preparation of Cell-Free Supernatant (CFS)

S. epidermidis strain was cultured for 24-48 hours in Tryptic Soy Broth (TSB) under aerobic condition at 37°C and *mecA* positive was obtained. The bacterial culture was then centrifuged for 30 minutes at 4°C at (10,000 rpm) to isolate the bacterial cells. The supernatant was then carefully collected and passed through Millipore membrane filter (0.22 µm) to remove any viable cell present in the supernatant, which was then sterilized.

In Vitro Antagonistic Activity Assay

The antibacterial effect of CFS against *S. aureus* was determined by the agar well diffusion method. In short, *S. aureus* (which corresponds to 0.5 McFarland standard) was swabbed uniformly over the surface of Mueller-Hinton Agar (MHA) plates. The sterile cork borer was used to punch 6 mm diameter wells into the agar. The CFS was added to the wells and equal volumes (50-100 μ L) were added to each well. Plates were then allowed to grow for 24 hours at 37°C. The antagonistic activity was measured by the diameter of the clear zone of inhibition (in mm) around the wells.

Results

Phenotypic Identification via VITEK 2

System The initial morphological and biochemical characterization of the local isolate was definitively confirmed using the VITEK 2 Compact system. The automated analysis utilizing the Gram-positive (GP) identification card positively identified the isolate as *Staphylococcus epidermidis* with an excellent confidence level of probability 99%.

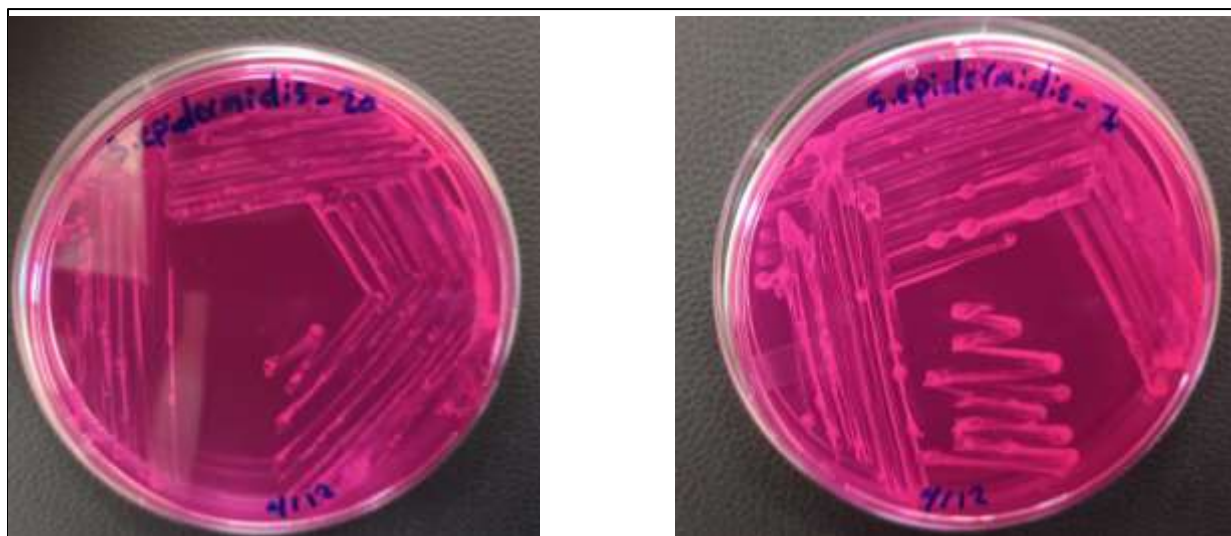


Fig. 1: Isolation and Purification of *S. epidermidis* on Mannitol Salt Agar.

Molecular Confirmation of the *mecA* Gene

After phenotypic identification the molecular profile of the isolate of *S. epidermidis* was assessed. The presence of *mecA* gene was successfully detected by the PCR amplification. The PCR products when subjected to agarose gel electrophoresis, showed a distinct band of 290 base pairs (bps) which corresponds to the *mecA* gene and thus confirmed methicillin resistant genotype of the local isolate.

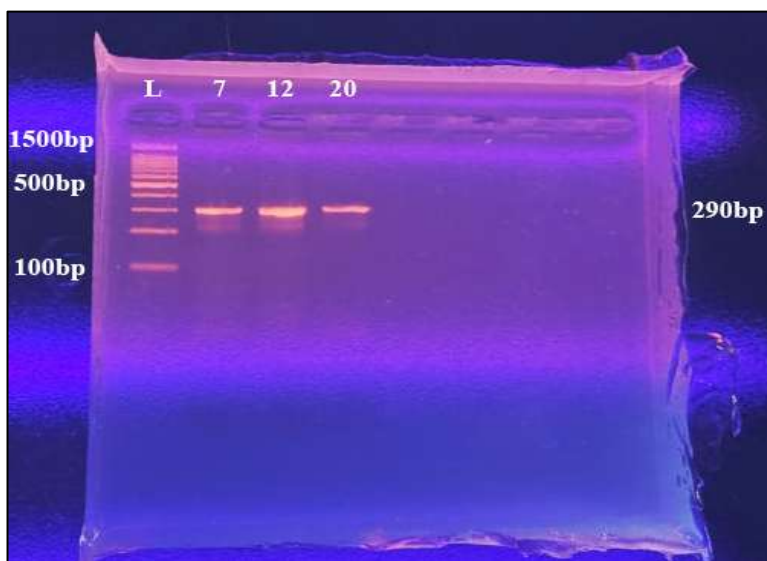


Fig. 2: Agarose Gel Electrophoresis of *mecA* PCR Products from *S. epidermidis* Isolates.

In Vitro Antagonistic Activity

The antagonistic potential of Cell-free supernatant from the *mecA*-positive *S. epidermidis*. The clinical *S. aureus* strain was used to test the isolate. The agar well diffusion method Proved to be very effective in exhibiting antibacterial activity. The regenerated CFS was an effective inhibitor of the growth of *S. aureus* which resulted in a zone of inhibition 10 mm in diameter.



Fig. 3: Antimicrobial activity of Cell-free Supernatant of *Staphylococcus epidermidis* extract against *Staphylococcus aureus* evaluated by the agar well diffusion method.

Discussion

The skin microbiome is a dynamic ecosystem, in which commensal bacteria, such as *Staphylococcus epidermidis*, are critical for preventing colonization of skin by pathogens (Sharma *et al.*, 2025), such as *Staphylococcus aureus* (Mao *et al.*, 2022). The purpose of this study was to take advantage of this natural antagonism and assess the postbiotic potential of a local isolate of *S. epidermidis*.

The first important step was to definitively identify the local isolate as *S. epidermidis* using the VITEK 2 system (Landemaine *et al.*, 2023). The genetic identification of *mecA* however, raised another important biosafety issue. Resistance to methicillin is due to the *mecA* gene, which encodes an altered penicillin-

binding protein (PBP2a) (Myo *et al.*, 2025). The presence of these resistance genes in human commensal skin flora indicates potential dangers of applying live bacteria (probiotics) to topical products as these organisms may be a source of horizontal gene transfer to pathogenic organisms (Phan *et al.*, 2025).

In order to avoid this hazard, our study focused on the cell-free supernatant (CFS). The successful preparation and lyophilization of the CFS guaranteed that secreted bioactive metabolites were retained, while there was no risk of any live resistant cells.

The antagonistic activity of the plant was shown by a clear zone of inhibition of 10 mm against *S. aureus* in in vitro antagonistic assay. This finding is of great importance as it validates that the antagonistic effect of this local *S. epidermidis* strain is not just due to competitive exclusion from live cells, but is also secreted extracellularly by the cells, playing the bulk of the antagonistic activity. These are probably a cocktail of bacteriocins, antimicrobial peptides (AMPs) (Aggarwal *et al.*, 2022), phenol soluble modulins (PSMs) and organic acids (Liang & Xing, 2023). These stable, cell-free metabolites have the capacity to inhibit *S. aureus* in the in vitro setting of the CFS, suggesting a therapeutic potential. In addition, using a local isolate will be beneficial because it can be phenotypically adapted to the environmental conditions of the area (Nurxat *et al.*, 2023), as well as the microbiome profile of the regional population (Traisaeng *et al.*, 2019).

Conclusion

The results of this study indicated that the cell-free supernatant of a local *mecA*-positive *S. epidermidis* isolate showed considerable in vitro antagonistic activity against *S. aureus* with a clear zone of inhibition of 10 mm. The use of the lyophilized postbiotic fraction can take advantage of the antimicrobial effect of the commensal strain without the biosafety concerns of using the *mecA* resistance gene. This gives a strong scientific basis for safe, cell-free topical formulations designed to restore skin microbiome balance and strengthen the natural barrier of skin against opportunistic pathogens in the future.

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