

Republic of Iraq

**Ministry of Higher Education and Scientific
Research**

University of AL-Qadisiyah

College of Science

Department of Biology



**Evaluation of a *Staphylococcus Epidermidis* Cell Free
Supernatant Hydrogel For Topical Safety and
Immunomodulatory Effects**

A Thesis Submitted to the Council of the College of Science /
Department of Biology / University of Al-Qadisiyah in a partial
Fulfillment of the Requirements for the Degree of Master of Science in
Biology

By

Rafal Ali Hatef

B.Sc. / Biology/College of Science / University of Kufa

(2018)

Supervised by

Pro. Dr. Maitham Ghaly Yousif

2026 A.D

1448 A.H

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

(وَقُلْ رَبِّ زِدْنِي عِلْمًا)

صَدَقَ اللَّهُ الْعَلِيُّ الْعَظِيمُ

Dedication

To the Master of Creation, sent as a mercy to the worlds, our Holy Prophet Muhammad (Peace Be Upon Him and His Progeny).

I dedicate this work in reverence to his honorable stature and his eternal message in the pursuit of knowledge.

To my Beloved Parents, whose prayers were my provisions and whose unconditional love paved my way. I dedicate this endeavor in profound gratitude for your endless sacrifices that words cannot capture.

To my life partner, my Husband, whose beautiful patience and unwavering encouragement enveloped me throughout this journey. This milestone would not have been possible without you by my side.

To my brother and Sisters, my unbreakable support system. I share the joy of this accomplishment with you, for your imprint is in every step of this success.

To my Friends and Companions, who offered a helping hand and the motivation to persevere whenever the road grew arduous, you have my boundless gratitude.

Rafal

Acknowledgment

First and foremost, I praise and thank Allah for granting me the strength, patience, and determination to complete this thesis.

I would like to express my deepest and most sincere gratitude to my supervisor, **Professor Dr. Maitham Ghaly Yousef**, for his continuous guidance, valuable feedback, and unwavering encouragement throughout this research. His profound knowledge and immense patience have been instrumental in the success of this work.

My sincere thanks and appreciation also go to the Department of Biology, College of Science, at the University of AL-Qadisiyah, for providing the necessary facilities, equipment, and a highly supportive academic environment.

Furthermore, I extend my sincere gratitude to the Deanship of the College of Science, represented by **Professor Dr. Salwan Al-Hamzawi**. My deep appreciation also goes to the Head of the Department of Biology, **Professor Dr. Layth Sareea Mutar**, for his continuous academic support and encouragement.

Rafal

Summary

The cutaneous microbiota encounters persistent environmental and infectious pressures, frequently resulting in dysbiotic conditions. Conventional broad-spectrum antimicrobial interventions may intensify this microbial imbalance, thereby establishing a clinical imperative for efficacious, microbiome-preserving therapeutic modalities. Accordingly, this investigation examines the therapeutic efficacy of postbiotics cell free supernatant (CFS) derived from an indigenous *Staphylococcus epidermidis* strain, prior to formulation, the in vitro bioactivity of the cell free supernatant (CFS) was evaluated using well agar diffusion method, demonstrating notable antibacterial efficacy against *Staphylococcus aureus*. Following this, the powder was incorporated into topical hydrogel formulation to effectively restore dermal homeostasis and regulate innate immunological mechanisms.

This research initiative was executed at the Department of biology, College of Science, University of Al-Qadisiyah, spanning the interval from October, 2025 to August, 2026. The principal aim encompassed the development of a biocompatible, efficacious postbiotic-derived hydrogel and the subsequent assessment of its tissue-regenerative and immunomodulatory properties. In pursuit of these objectives, indigenous *Staphylococcus epidermidis* isolates were procured from dermal swabs obtained from healthy individuals and subsequently cultivated and isolated on Mannitol Salt Agar. The bioactive postbiotic constituents were recovered through extraction and filtration, converted into a lyophilized bioactive formulation, and integrated into a Carbopol matrix utilizing a low-temperature incorporation methodology to maintain hydrogel integrity and maintain pH at 5.5 using triethanolamine.

The in vivo therapeutic efficacy of the formulated hydrogel was initially evaluated utilizing a mouse model system. Histopathological examination confirmed its biocompatibility and concentration-dependent improvements in tissue architecture. Histopathological analysis revealed that the (5%) CFS hydrogel yielded slight structural improvements in the skin tissue while The (10%) CFS hydrogel demonstrated superior capacity for tissue regeneration, facilitated optimal keratinocyte proliferation, enhanced fibroblast function, and exhibited well-organized collagen deposition without evidence of pathological hyperplasia. Subsequently, a clinical investigation was performed with 15 healthy human subjects across a 20-day period to assess the (5%) CFS formulation.

Clinical findings revealed pronounced anti-inflammatory properties, as demonstrated by a statistically significant decrease in localized pro-inflammatory cytokine IL-8 concentrations ($p = 0.026$). Additionally, the formulation effectively potentiated the skin's endogenous immune mechanisms, resulting in a statistically significant 15.9% elevation in the antimicrobial peptide HBD-2 ($p = 0.017$). Although the elevation in LL-37 concentrations was modest ($p = 0.332$), 60% of participants demonstrated a favorable response. In conclusion, the developed *Staphylococcus epidermidis* CFS hydrogel demonstrated safety, efficacy, and non-pathogenic characteristics as a therapeutic modality for promoting dermal regeneration and augmenting intrinsic cutaneous immunity.

List of Content

No.	Contents	Page No.
	Summary	I-II
	List of contents	III-V
	List of tables	VI
	List of figures	VII-VIII
	List of abbreviation	IX
1	Chapter one Introduction	
	Introduction	1-4
2	Chapter two Literatures Review	
2.1	Skin structure and barrier function	6
2.1.1	Structure of Epidermis	6
2.1.2	The skin barrier: physical and chemical components	9
2.1.3	Cutaneous Innate Immunity	10
2.1.3	Skin microbiome: Homeostasis and Dysbiosis	12
2.2.1	Composition and topographical distribution	12
2.2.2	Dysbiosis and it's associated with skin pathologies	15
2.3	Staphylococci	17
2.3.1	Staphylococcus Epidermidis	18
2.3.2	Bioactive metabolites and functional diversity	19
2.4	Microbiome modulation and probiotics	22
2.4.1	Replacement of antibiotics with bacteriotherapy	22
2.4.2	Traditional antibiotic therapy limitation	23
2.4.2.1	Random destruction (collateral damage)	23
2.4.2.2	Antimicrobial resistance (AMR)	23
2.4.2.3	Alteration of immune signaling	24
2.4.3	Postbiotic, Cell-Free Supernatant–Based Approaches	25
3	Chapter Three Material and Methods	
3.1	Materials	27
3.1.1	Laboratory instruments and apparatuses	27
3.1.2	Chemicals	28
3.1.3	Ready-Made culture media	29
3.1.4	Antibiotics	30
3.1.5	Ready kits	30
3.1.6	Primers	32
3.1.7	Study design	33
3.2	Methods	34
3.2.1	Collection of samples	34
3.2.2	Identification of S. epidermidis isolates	34
3.2.2.1	Cultural and microscopic characteristics	35
3.2.2.2	Biochemical tests	35
3.2.2.3	VITEK 2 system	36
3.2.3	Maintenance and conservation of isolates	38
3.2.3.1	Short period conservation	38
3.2.3.2	Long period conservation	38
3.2.4	Sterilization methods	39

3.2.4.1	Wet-Heat sterilization	39
3.2.4.2	Incineration sterilization	39
3.2.4.3	Filter sterilization	39
3.2.5	Characterization of the selected <i>S. epidermidis</i> isolate as source of postbiotic	39
3.2.5.1	Antibiotic susceptibility testing for <i>S. epidermidis</i>	39
3.2.5.2	Polymerase chain reaction	40
3.2.5.2.1	Bacterial DNA extraction	40
3.2.5.2.2	Estimation of extracted total DNA	42
3.2.5.2.3	PCR Master Mix preparation	43
3.2.5.2.4	PCR thermocycler	43
3.2.5.2.5	PCR product analysis	44
3.2.5.3	Antimicrobial activity testing using well-agar diffusion method	45
3.2.5.3.1	Preparation of indicator lawn	45
3.2.5.3.2	Well preparation and sample application	45
3.2.5.3.3	Incubation and zone measurement	46
3.2.6	Preparation of powder from cell-free supernatant	46
3.2.6.1	Pre-lyophilization process	46
3.2.6.2	Lyophilization process	46
3.2.6.3	Powder collection and storage	47
3.2.6.4	Antimicrobial activity of lyophilized CFS	47
3.2.6.5	Hydrogel formulation and processing	48
3.2.7	In vivo evaluation of topical powder on skin of laboratory mice	49
3.2.7.1	Experimental Animals	49
3.2.7.2	Experimental design and Animal grouping	50
3.2.7.3	Histopathological study	51
3.2.8	In vivo Evaluation of gel efficacy on skin microbiome restoration and barrier function	52
3.2.8.1	Application protocol	52
3.2.8.2	Blood sample collection and serum processing	53
3.2.8.3	Measurement of immunological biomarkers using ELISA	53
3.3	Statistical Analysis	56
4	Chapter Four Result and Discussion	
4.1	Isolation and identification of <i>staphylococcus epidermidis</i>	58
4.2	Antibiotic susceptibility testing for <i>S. epidermidis</i>	59
4.3	Molecular detection of <i>mecA</i> gene and phenotypic-genomic variation	63
4.4	Antimicrobial activity of cell-free supernatant of <i>S. epidermidis</i>	65
4.5	Characterization of the Lyophilized Postbiotic Extract Based on Appearance, pH and Mass	68
4.6	Formulation and characterization of the hydrogel base	70
4.7	Antimicrobial activity of lyophilized <i>S. epidermidis</i> CFS against <i>S. aureus</i>	73

4.8	Histological evaluation of the effect of hydrogel containing CFS of <i>S. epidermidis</i> bacteria on mice	77
4.9	Immunological evaluation of the topical formulation	82
4.9.1	In vivo effect of CFS on the Pro-inflammatory cytokine (IL-8)	82
4.9.2	In vivo effect of CFS hydrogel on antimicrobial peptide (HBD-2) levels	87
4.9.3	In vivo effect of CFS hydrogel on antimicrobial peptide (CAMP-LL37) levels	90
	conclusions and recommendations	
	Conclusions	94
	Recommendations	95
	References	97
	Appendices	120
	الخلاصة	أب

List of Tables

No.	Title	Page No.
2-1	Reported bioactive component of <i>Staphylococcus epidermidis</i> strains	21
3-1	The laboratory instruments and apparatuses used in this study.	27
3-2	Chemicals materials that were used in this study with their company and country of origin.	28
3-3	Culture media that were used in this study.	29
3-4	Antibiotic discs that were used in this study.	30
3-5	The kits used in this study with their companies and countries of origin.	30
3-6	The PCR primers for the detection of <i>mecA</i> gene with their sequence and product size.	32
3-7	Identification levels in VITEK2 system.	37
3-8	Standard PCR master mix protocol.	43
3-9	PCR thermocycling program for the molecular detection of <i>S. epidermidis</i> genes.	44
4-1	Distribution and percentage of Coagulase-Negative staphylococci (CoNS) isolate	59
4-2	Antibiotic susceptibility of some antibiotic against the selected isolate <i>S. epidermidis</i> n7.	60
4-3	Statistical analysis of interleukin-8 levels in human volunteers before and after a 20-day application of 5% <i>S. epidermidis</i> cell-free supernatant hydrogel	83
4-4	Statistical analysis of HBD-2 levels before and after 20 days of topical application of <i>S. epidermidis</i> CFS hydrogel	87
4-5	Statistical analysis of CAMP-LL37 marker levels before and after 20 days of topical application of <i>S. epidermidis</i> CFS hydrogel	90

List of Figures

No.	Title	Page No.
2-1	Skin anatomy.	7
2-2	Skin microbiome distribution.	13
2-3	<i>Staphylococcus epidermidis</i> on mannitol salt agar.	18
3-1	Study design	32
3-2	A standard curve for ELISA data (A) represent standard curve for HBD-2, (B) represent standard curve for IL-8 and (C) represent standard curve for LL-37.	
4-1	Growth of staphylococcus epidermidis and staphylococcus aureus on MSA after incubation of 24h at 37c.	56
4-2	Antibiotic susceptibility of some antibiotics against the selected isolate <i>S. epidermidis</i> n7.	60
4-3	Agarose gel electrophoresis of mecA PCR amplicon (310 bp) from ceftiofur-susceptible <i>S. epidermidis</i> isolates, electrophoresed on [1.5%] agarose gel in [1× TBE] buffer at [80 V] for [60 min] and stained with [ethidium bromide]. Lane L: DNA ladder; Lanes 7, 12, and 20: Positive bands of mecA gene at 310 bp.	62
4-4	Antagonistic effect of <i>staphylococcus epidermidis</i> cell-free extract against <i>staphylococcus aureus</i> .	64
4-5	Lyophilized <i>S. epidermidis</i> cell-free supernatant	67
4-6	Hydrogel containing <i>S. epidermidis</i> cell-free supernatant	70
4-7	Antimicrobial Activity of lyophilized cell-free supernatant of <i>S. epidermidis</i> against <i>S. aureus</i> . (1) represent 0.1g/mL of CFS, (2) represent 0.05g/mL of CFS, (3) represent 0.025g/mL of CFS and (4) represent 0.0125g/mL of CFS.	73
4-8	Microscopic histological sections of the skin in the control group showed the normal skin structure, characterized by a normal-thickness epidermis composed of its four layers. (E) The dermis contained regular collagen fibers (blue arrow) with a normal distribution of blood vessels (3), sebaceous glands, and hair follicles (2). Hematoxylin and eosin staining. Magnification: Figure A: 100X, B: 400X	75
4-9	Histological sections revealed that the epidermal integrity improved (slightly) with more regularly arranged keratinocytes and without pathological hyperplasia of the stratum, slightly thicker than usual (blue arrow), and normal blood vessels, not dilated nor congested (3). Hematoxylin and eosin staining: A: x200; B: x400	76

4-10	This group showed the best histological improvement, with the epidermis becoming more regular (E) and exhibiting a moderate increase in thickness due to activation of keratinocyte proliferation (E). The dermis was characterized by a higher density of organized and parallel collagen fibers (blue arrow) with clear regularity in the structure of hair follicles (2) and blood vessels (3). Hematoxylin and eosin staining: A: x200; B: x400	77
4-11	Comparison of mean Interleukin-8 (IL-8) levels among the healthy control group and volunteers before and after a 20-day treatment with 5% <i>S. epidermidis</i> cell-free supernatant hydrogel. Data are presented as mean $\pm$ SEM	82
4-12	Effect of the <i>S. epidermidis</i> CFS topical gel on HBD-2 levels among the study groups.	86
4-13	Effect of the <i>S. epidermidis</i> CFS topical gel on CAMP-LL 37 levels among the study groups.	89

List of Abbreviations

Abbreviations	Meanings
6-HAP	6-N-hydroxyaminopurine
AD	Atopic dermatitis
AhR signaling	Aryl hydrocarbon Receptor
AMR	Antimicrobial resistance
B16F10	Murine melanoma cell line
CoNs	Coagulase negative staphylococci
CSF	Cell-free supernatant
ESP	Epidermidis serine protease
fnBps	Fibronectin-binding proteins
GLuse	Glutamyl endopeptidase
hBD-2	Human B-defensi-2
hBD-3	Human B-defensi-3
IL-8	Interleukin 8
ITS sequencing	Internal Transcribed Spacer Sequencing
LL-37	Leucine-leucine 37
LTA	Lipoteichoic acid
MAPK	Mitogen-Activated Protein Kinase
MSA	Mannitol salt agar
MSC-secretome	Mesenchymal Stromal Cell secretome
NF-κB	Nuclear Factor kappa-light-chain-enhancer of activated B cells
OS-MRCoNs	Oxacillin susceptible methicillin-resistant coagulase-negative staphylococci
OVOL1	Ovo-Like Transcriptional Repressor 1
PSMs	Phenol-soluble modulins
SCCmec type IV	Staphylococcal Cassette Chromosome <i>mec</i> type IV
Spa	Staphylococcal protein A
TEWL	Transepidermal Water Loss

Chapter one Introduction

1.Introduction

Skin microbiome contains a broad spectrum of microorganisms such as bacteria, fungi, viruses and archaea (Brown and Horswill, 2020). A balanced skin microbiome is critical to the stability of the skin barrier function, local immune response regulation, and the prevention of most microbially-related disorders of the skin, including atopic dermatitis, acne, and certain chronic infections (Severn and Horswill, 2023).

Gram-positive bacteria, especially those of the coagulase-negative staphylococci, are some of the most prevalent bacterial components of the skin microbiome of healthy human skin. *Staphylococcus epidermidis* plays a pivotal role as it is among the most prevalent normal colonizers of the skin (Qi *et al.*, 2025). Conventionally considered as an opportunistic organism that can lead to the occurrence of medical equipment and biofilm-related infections, the latest literature has re-defined it as an active commensal, which is critical to the homeostasis of the skin and helps defend the host against pathogens (Cheung and Otto, 2025).

Recent reports have indicated that some isolates of *S. epidermidis* are able to activate the production of antimicrobial peptides like β -defensin-2, β -defensin-3 as well as synergistically with the antimicrobial peptide LL-37 thereby increasing their host-killing ability against pathogens like *Staphylococcus aureus* and Group A Streptococcus (Simanski *et al.*, 2019).

This behavioral heterogeneity among isolates of *S. epidermidis* also suggests a high level of heterogeneity in strain level. Isolates could also help increase skin barrier strength and inflammatory control, or be linked to barrier breakage or increased inflammatory response, depending on the pathological situation (Landemaine *et al.*, 2023).

Hence, the targeting of local isolates with selective advantageous features is an essential measure towards the creation of selective therapeutic methodologies of skin symbionts, as opposed to conventional antibiotic elimination technologies.

The notion of so-called postbiotics has been developing in recent years. Postbiotics are preparations that contain non-living microorganisms and/or their components or metabolites that are known to produce a health benefit to the host even in the absence of living and reproducing cells (Salminen *et al.*, 2021). A number of studies have indicated that postbiotics prepared using commensal bacteria or probiotic may have antimicrobial, immunomodulatory and anti-inflammatory effects, and are more stable and safer than live-bacteria-based preparations. This qualifies them as a good choice in topical use in personal care and therapeutic products (da Silva Vale *et al.*, 2023).

In this regard, cell-free supernatants, which are the result of culturing of selected isolates of *S. epidermidis*, are of special interest as a type of postbiotic (Shao *et al.*, 2025). They consist of a blend of peptides, small molecules, enzymes, and metabolic products that can impact the skin microbiome and host cell responses without actual transfer of the bacterial cells to the site of application (Qi *et al.*, 2025). Research on supernatant cultures of different commensals and probiotics has suggested that they can promote the integrity of the barrier, decrease the release of inflammatory cytokines by keratinocytes, and promote wound healing in a variety of laboratory and animal models (De Almeida *et al.*, 2023).

Simultaneously, the skin microbiome-based therapies field is moving in the direction of the creation of topical microbial-friendly formulations that can correct microbial imbalances and reestablish microbial community balance with a combination of prebiotics, probiotics,

and postbiotics (Habeebuddin *et al.*, 2022). There is evidence that a more precise approach can be achieved by targeting specific strains or their metabolites to treat conditions like atopic dermatitis (Nakatsuji *et al.*, 2021), acne, and frequent infections by promoting the colonization by beneficial commensal bacteria and limiting the chances of colonization by pathogenic ones (Niedźwiedzka *et al.*, 2024).

1.1 Aim of the study

To evaluate the potential of a cell free supernatant (CFS) derived from a locally isolated *Staphylococcus epidermidis* strain as a postbiotic topical formulation, with emphasis on its antimicrobial activity, physicochemical formulation, dermal safety and modulation of selected innate immune markers.

1.2 The objectives

1. Isolation and Identification of *staphylococcus epidermidis* from healthy human skin using conventional method followed by VITEK-2 system identification.
2. Characterize the selected *S. epidermidis* isolate phenotypically and molecularly, including antimicrobial susceptibility testing and detection of the *mecA* resistance gene.
3. Prepare and lyophilize the cell free supernatant (CFS) obtained from selected *S. epidermidis* isolate and evaluate its antimicrobial activity against *staphylococcus aureus*.
4. Formulate the lyophilized CFS into a Carbopol base topical hydrogel and characterized its basic physical properties and skin compatible pH.
5. Evaluate the dermal tolerability and histopathological effects of the CFS hydrogel at different concentrations in a murine skin model.
6. Conduct a preliminary human tolerability and immunological assessment of the CFS hydrogel by measuring serum IL-8, HBD-2 and LL-37 levels before and after topical application.

Chapter Two

Literature Review

2. Literature Review

2.1 Skin Physiology and Barrier Function

2.1.1 Structure of the Epidermis

The epidermis is the primary anatomical site of the cutaneous microbiome and is the primary barrier the body has against external factors (Yousef *et al.*, 2024). It is a histologically avascular stratified squamous epithelium which is keratinized, and experiences self-renewal in a well-regulated keratinization program (Lee and Kim, 2022). The proliferation, differentiation and programmed cell death of keratinocytes as they move upwards through the basal lamina to the surface maintain epidermal homeostasis, forming four major strata (Lefèvre Utile *et al.*, 2021) as showed in figure (2.1).

The lowest layer is the stratum basale (basal layer), which consists of a single row of proliferative keratinocytes, which renew the layers on top of it and maintain the integrity of the epidermis (Betts *et al.*, 2022). Melanocytes, which produce melanin to shield underlying tissues against ultraviolet radiation and help give the skin its color, are also found in this compartment (Yousef *et al.*, 2024).

The layer directly above it is the stratum spinosum (spinous layer), which contains keratinocytes linked together by numerous desmosomes, which give the tissue mechanical support (Broussard *et al.*, 2020). There are Langerhans cells that are located in this layer that scan microbial antigens and provide a functional interface between the microbiota in the skin and the adaptive immune system (Deckers *et al.*, 2018).

Within the stratum granulosum (granular layer), the keratinocytes store the keratohyalin granules and release lamellar bodies containing lipids and enzymes into the extracellular space which is critical to building barriers (Alonso and Mizuno, 2021; Bouwstra *et al.*, 2023). These exuded lipids not only play a role in water retention and cohesion of the barrier but are important sources of carbon that enhance the growth and activity of commensal skin bacteria (Berdyshev, 2024).

The most superficial layer is the outer stratum corneum (cornified layer), which is composed of 15-30 layers of flattened anucleate corneocytes surrounded by an organized lipid matrix, which forms the major physical barrier of skin (Murphrey *et al.*, 2022). This layer has a slightly acidic surface pH, which ranges between 4.0 and 6.5 and it forms the so-called acid mantle that inhibits the growth of numerous pathogenic microorganisms and promotes the colonization of useful commensals (Fukuda *et al.*, 2024).

The stratum corneum is an ecological niche that is prominent in the case of *Staphylococcus epidermidis*, offering a nutrient pool based on lipids and sweat as well as structured surface that supports long-term colonization (Grice and Segre, 2011; Rahmdel & Götz, 2021). When properly exposed to environmental conditions and found on suitable surfaces, *S. epidermidis* is able to use its adhesins and extracellular matrix components to form biofilms, a capability that underlies its opportunistic behavior in device-associated infections, but also potentially contributes to its persistence on the skin in the long term (Cheung & Otto, 2025; Fernández-Calderón *et al.*, 2025).

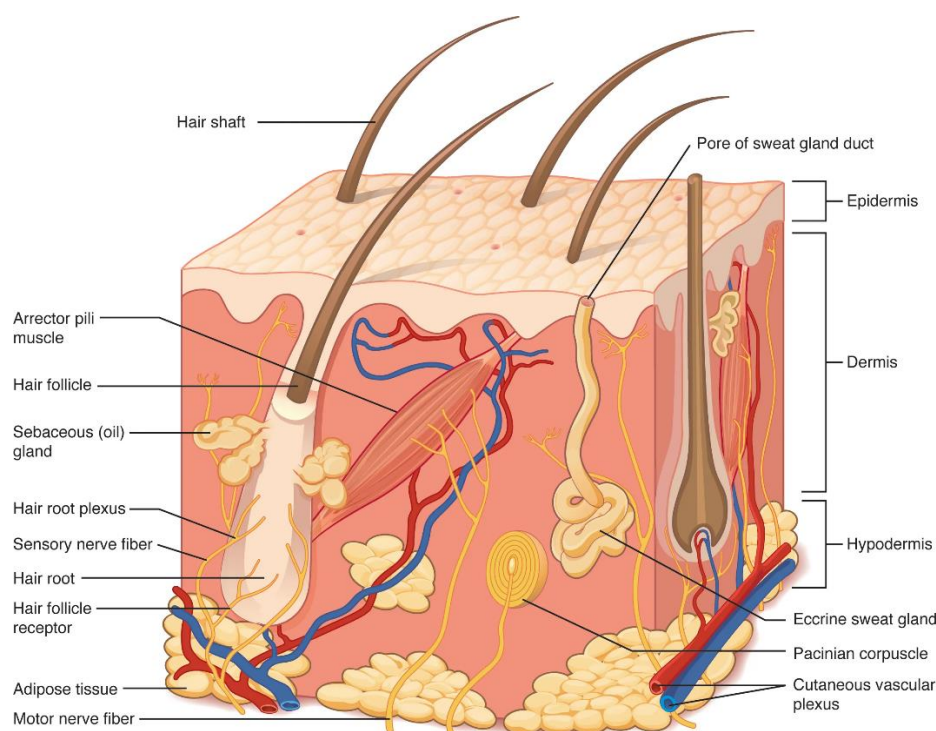


Figure (2.1) Skin anatomy (Betts *et al.*, 2022)

In addition to the stratified structure of the epidermis, there are also diverse adnexal features of the skin such as hair follicles, sebaceous glands and eccrine/apocrine sweat glands, that form discrete microenvironment in the integument (Grice and Segre, 2011; Zhang *et al.*, 2024). Pilosebaceous units constituted by hair follicles and linked sebaceous glands empty on the skin surface and release sebum-rich lipids, which serve as a part of barrier defense, lubrication and the creation of hydrophobic niches, which selectively recruit lipophilic members of the skin microbiota (Harris-Tryon & Grice, 2022).

The eccrine sweat glands, in their turn, release an aqueous liquid, which includes the electrolytes, antimicrobial peptides and organic acids that regulate the surface pH and osmolarity, thus defining microbial composition and affecting the activity of commensal and pathogenic organisms (Baker, 2019; Tricarico *et al.*, 2022).

The stratified epidermis and its extensions together create a non-homogenous environment of physical, chemical and nutritional conditions which dictate the localized settlement of particular microbes, such as *Staphylococcus epidermidis*, and the manner in which their excreted products react with the cutaneous barrier (Li *et al.*, 2023).

2.1.2 The Skin Barrier: Physical and Chemical Components

The cutaneous barrier is conventionally defined as a combination of interrelated physical and chemical elements that, in combination, prevent the body against dehydration and microbial intrusion (Lee and Kim, 2022). Physically, the stratum corneum is organized like the classical brick and mortar where the corneocytes are encased in lamellar lipid bilayers to create a cohesive structure that restricts transepidermal water loss and passive diffusion into the skin (Rajkumar *et al.*, 2023; Rawlings *et al.*, 2024).

On a chemical level, the skin surface is coated with a weakly acidic acid mantle (pH 4.5) of free fatty acids, organic acids, elements of the sweat and antimicrobial peptides that fosters enzymatic activity needed to maintain the skin barrier and selectively supports commensal bacteria like *Staphylococcus epidermidis* and inhibits opportunistic pathogen like *S. aureus* (Iyer *et al.*, 2021; Tan & Lio, 2025).

Such combined physical and chemical defenses are directly applicable to microbiome-based interventions, as commensal-derived postbiotic products have been reported to increase ceramide generation, regulate AMP expression and stabilize surface pH, which strengthens barrier functionality and a more robust, health-related skin microbiome (De Almeida *et al.*, 2023; Hashemi *et al.*, 2025).

2.1.3 Cutaneous Innate Immunity

The cutaneous innate immunity is a non-specific, rapid defense barrier which works in close collaboration with the physical and chemical elements of skin barrier (Lee and Kim, 2022). Keratinocytes have since been identified as active immune regulators, not passive structural cells; they express a large repertoire of pattern-recognition receptors, such as Toll-like receptors and NOD-like receptors, permitting them to recognize microbial- and damage-associated molecular patterns, and to respond to them by generating cytokines, chemokines and antimicrobial peptides (Zhang *et al.*, 2024). In these mediators, keratinocytes coordinate initial containment of invading microbes and determine future adaptive immune responses.

Human β -defensin-2 (hBD-2) and the cathelicidin peptide LL-37 are two of the most relevant of the epithelial antimicrobial mediators. They are both inducible by pro-inflammatory cytokines and microbial stimuli in keratinocytes and have a broad-spectrum activity against Gram-positive and Gram-negative bacteria, viruses and fungi (Lesiak *et al.*, 2024), as well as stimulating chemotaxis, angiogenesis and re-epithelialization during wound repair (Takahashi & Gallo, 2017; Lueschow-Guijosa *et al.*, 2024).

In addition to their individual activity, host AMPs can work in collaboration with commensal secreted factors: the antimicrobial δ -toxin (phenol-soluble modulin - γ) released by *Staphylococcus epidermidis* has been reported to bind both LL-37 and hBD-2, and to interact in a synergistic manner with these peptides to kill Group A Streptococcus. In line with this (Cogen *et al.*, 2010; Severn & Horswill, 2022).

A growing number of probiotic- and commensal-derived cell-free supernatants and postbiotic preparations have been reported to stimulate

the expression of hBD-2 and cathelicidin in keratinocytes, augment AMP deposition in the stratum corneum and microbial killing, and thereby, functionally reinforce the antimicrobial component of the skin barrier (Vieira De Almeida *et al.*, 2023; Hashemi *et al.*, 2025).

Interleukin 8 (IL-8, CXCL8) is a major chemokine that connects epithelial sensing with cellular recruitment in areas of barrier disruption, in that it attracts neutrophils and other immune cells (Hou & Huttenlocher, 2024). Although IL-8 potentiation is critical to the initial clearance of pathogens, prolonged and excessive IL-8 production may also lead to tissue damage and chronic inflammation (Cambier *et al.*, 2023; Rizo-Téllez & Filep, 2024) therefore, an optimal microbiome-regulating intervention would increase the antimicrobial action of AMP without exaggerating IL-8 effects.

In this respect, several studies of cell-free supernatants and postbiotic preparations of commensal or probiotic bacteria demonstrate a typical pattern in which the levels of hBD-2 and cathelicidin are raised or maintained, but those of pro-inflammatory cytokines and chemokines, such as IL-8, are decreased under the inflammatory conditions (Baquer *et al.*, 2023), resulting in enhanced wound healing, diminished inflammation (Zanetta *et al.*, 2023; Hashemi *et al.*, 2025)

These findings form the mechanistic rationale of employing hBD-2, LL-37 and IL-8 as complementary readouts in the current study to determine whether a *Staphylococcus epidermidis* cell-free supernatant is capable of inducing a condition of augmented antimicrobial competence alongside regulated inflammation at the skin surface (Pastar *et al.*, 2020; Qi *et al.*, 2025).

2.2 Skin Microbiome: Homeostasis and Dysbiosis

Rather than serving merely as a physical boundary, the skin maintains a dynamic state of homeostasis through continuous cross-talk with its resident microbiome. This complex microbial community is indispensable for driving immune education, reinforcing barrier integrity, and orchestrating defenses against invading pathogens (Grice & Segre, 2011).

This microbiome structure is not just arbitrary but can be reproducibly structured in a topographical manner and depends mainly on the underlying biophysical characteristics of the site, specifically, whether sebaceous, moist or dry (Pérez-Losada & Crandall, 2023).

In non-pathogenic conditions, commensal communities of coagulase-negative staphylococci such as *Staphylococcus epidermidis*, *Cutibacterium spp.* and other inhabitants of the skin facilitate barrier function and immune homeostasis, and perturbation of this state (dysbiosis) is linked to inflammatory skin diseases including atopic dermatitis and acne (Wojciechowska & Szewczyk, 2025; Kim *et al.*, 2025).

2.2.1 Composition and Topographical Distribution

The microbiota of the cutaneous surface is significantly different on the body surface and depends on a complicated interaction of host-intrinsic aspects (age, sex, genetics), environmental factors (UV radiation, temperature, humidity) and behavioral (hygiene practices, cosmetic and antibiotic use) ones (Byrd *et al.*, 2023; Zhang *et al.*, 2026). In spite of these effects, comparative sequencing studies have consistently demonstrated that the local biophysical niche of a skin site, be it sebaceous, moist or dry as showed in figure (2.2) is the most important determinant of bacterial community structure (Pérez-Losada & Crandall, 2023).

The presence of a high density of sebaceous glands in sebaceous areas, including the forehead, upper back and chest, makes these regions prone to microenvironment with a significant amount of lipids and relatively low oxygen (Grice and Segre, 2011). This niche prefers lipophilic bacteria and communities at sebaceous sites are usually dominated by *Cutibacterium* (formerly *Propionibacterium*) species alongside species of the family *Staphylococcaceae* (Fournière *et al.*, 2020). *Cutibacterium acnes* selectively inhabit the canal of follicles, and *Staphylococcus epidermidis* more often inhabits the follicular opening and the adjoining surface where it feeds on sebum-derived lipids and other follicular constituents (Hamann *et al.*, 2025).

Moist skin sites where humidity and temperature are increased in regions like axillae, inguinal folds and popliteal fossae because of the presence of a significant amount of sweat and the blockage of this moisture (Borrego-Ruiz & Borrego, 2024). Such conditions favor a high bacterial biomass and communities are generally enriched with *Staphylococcus* and *Corynebacterium* species (Severn *et al.*, 2023). The adaptation of *S. epidermidis* to such regions is due to its capacity to withstand high salt levels of the sweat and to metabolize the solutes produced by the sweat, such as urea and amino acids, as a nutrient source, which contributes to its persistence in commensal colonization of the high-humidity niches (Carvalho & Saraiva, 2026).

Dry sites the forearms, legs and dorsal hands are relatively dry regions with relatively few sebaceous and sweat glands and they experience great changes in temperature and moisture, which means that they are relatively desert-like habitats of microbes (Byrd *et al.*, 2018). Ironically, such sites tend to have the highest bacterial diversity and lowest

dominance and usually contain mixed communities containing Proteobacteria, Bacteroidetes and Actinobacteria (Skowron *et al.*, 2021).

S. epidermidis is still a common inhabitant of dry skin despite being less prevalent than in sebaceous and moist environments and plays a role in preserving the barrier and immune regulation by producing antimicrobial and immunomodulatory metabolites (Harris-Tryon & Grice, 2022).

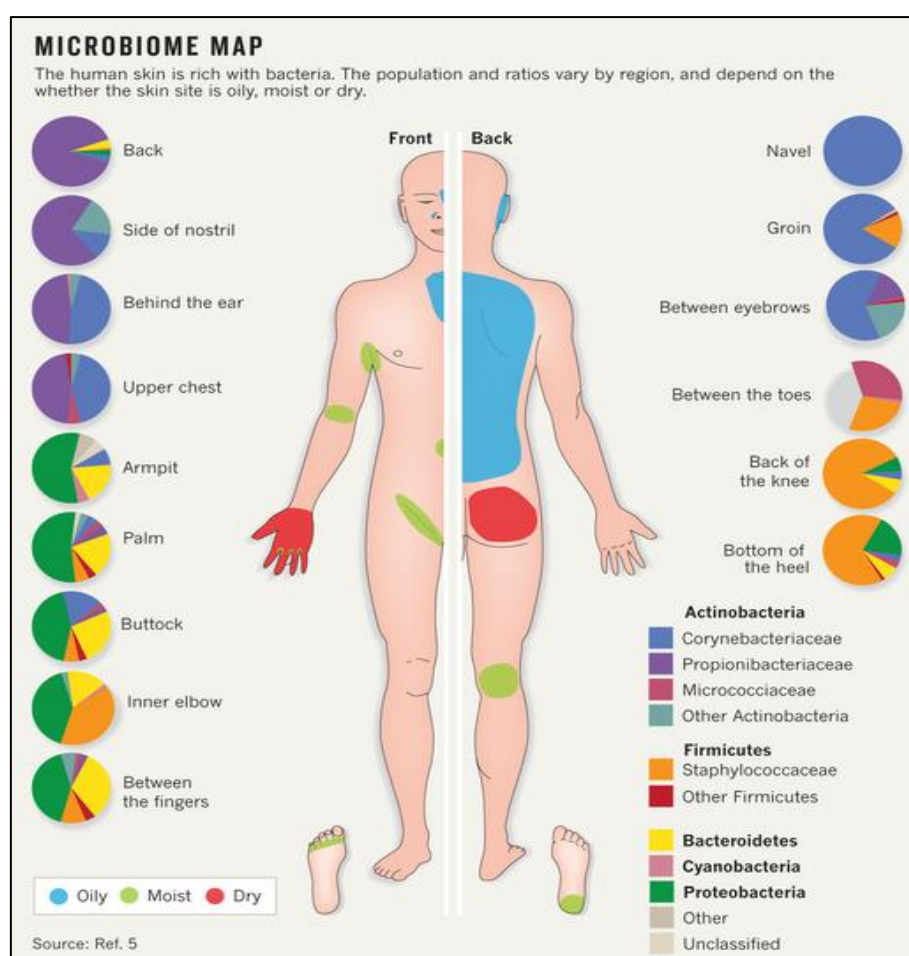


Figure (2.2) Skin microbiome Distribution (Trivedi, 2012)

This diffuse yet focal distribution of *S. epidermidis* to sebaceous, moist and dry locations highlights its pivotal role in cutaneous homeostasis and justifies the reasoning behind using a local commensal isolate and its cell-free supernatant in microbiome-based therapeutic approaches.

2.2.2 Dysbiosis and its associated with skin pathologies

The skin microbiome is maintained in a state of dynamic balance in health, with commensal communities that promote barrier function, regulate immune responses and limit the growth of potentially pathogenic microorganisms (Ye *et al.*, 2026).

Dysbiosis, a qualitative and/or quantitative alteration of these microbial communities, is now known to be not only the result but also a driver of many inflammatory and infectious skin diseases (Li *et al.*, 2025). Microbial sequencing studies of a wide variety of dermatoses consistently reveal that dysbiosis is associated with diminished microbial diversity, loss of protective commensal functions and growth of potentially pathogenic microorganisms, which translates into changes in pattern-recognition receptor signaling, dysregulated cytokine production and barrier dysfunction.

More recently, longitudinal sequencing studies have expanded this picture: De Tomassi *et al.* (2023) provided both a cross-sectional and longitudinal analysis of both atopic dermatitis (AD) and non-AD cohorts, showing that AD is not only associated with a decrease in diversity, but also with a significant increase in total bacterial load due to *S. aureus* overgrowth. The microbiome-level dysbiosis associated with inflammation is reversible with treatment, and tracks clinical healing, consistent with the treatment-restoration finding of Simpson *et al.* (2023) that along with clinical improvement, there was a rapid decrease in *S. aureus* relative abundance and a partial restoration in microbial diversity within lesional skin of people who were treated with dupilumab in a clinical trial.

Akaza *et al.*, (2022) conducted next-generation 16S and ITS sequencing of the microbiome in comedonal contents of inflammatory

acne vulgaris lesions and found that lesions are characterised by the presence of metabolically highly active *Cutibacterium spp.*, as well as *Staphylococcus* and *Malassezia*, but not in the surrounding less active skin surface communities.

Chang *et al.* (2018) conducted 16S rRNA (V1–V3) sequencing on lesional, non-lesional, and healthy skin samples from individuals with psoriasis, revealing that these different states can be differentiated based on their community structure. Notably, psoriatic lesions exhibited significant alterations in the relative abundances of key genera, including a decrease in *S. epidermidis* and *Cutibacterium acnes*, alongside a greater diversity compared to healthy controls.

The most well-studied model of cutaneous dysbiosis is atopic dermatitis (AD). AD lesions and even AD-affected but non-lesional skin are usually characterized by reduced bacterial diversity, overgrowth of *Staphylococcus aureus* and under-representation of health-protective coagulase-negative staphylococci and other commensals (Okamoto & Nakamura, 2026). This change promotes Th2-biased inflammation, tight junction and lipid barrier dysfunction and creates a vicious cycle in which barrier dysfunction promotes *S. aureus* proliferation, and *S. aureus* toxins in turn, promote immune dysfunction and flares (Nakatsuji *et al.*, 2016).

In psoriasis and other psoriasiform conditions, local bacterial community changes include increased *Staphylococcus* and *Corynebacterium* abundance, and altered fungal and viral signatures, and are associated with interferon and Th17-associated immune signaling, which may contribute to the development and persistence of lesion (Celoria *et al.*, 2023; Wu *et al.*, 2025).

2.3 Staphylococci

Staphylococci are Gram-positive, catalase-positive, non-motile, and facultative anaerobic cocci that characteristically arrange in grape-like clusters when observed under a microscope. The genus encompasses numerous species that colonize human skin and mucosal surfaces. Clinically, they are divided into coagulase-positive staphylococci (primarily *Staphylococcus aureus*) and coagulase-negative staphylococci (CoNS), a heterogeneous group that prominently includes *Staphylococcus epidermidis*, alongside *S. saprophyticus* and *S. haemolyticus* (Becker *et al.*, 2014; Paranthaman *et al.*, 2023).

As primary colonizers of human skin, particularly in moist and sebaceous habitats, CoNS are fundamentally considered low-virulence commensals. They play a crucial role in maintaining cutaneous homeostasis by competing with exogenous microbes, interacting with epidermal cells, and modulating local inflammatory tone (Byrd *et al.*, 2018). While *S. aureus* represents the major opportunistic pathogen of this genus—equipped with an extensive arsenal of toxins and virulence factors—CoNS rely more on specific adhesins and biofilm formation for survival (França *et al.*, 2021). Ultimately, the *Staphylococcus* genus represents a broad functional spectrum, ranging from highly virulent pathogens to beneficial commensals that are vital for host defense.

2.3.2 *Staphylococcus epidermidis*

Staphylococcus epidermidis is a gram-positive, facultative anaerobic, coagulase-negative bacterium (Burke *et al.*, 2024) which is a predominant member of the bacterial flora of the integumentary system of the human body, and rarely of the mucosal surfaces (Fournière *et al.*, 2020).

It is a normal skin flora but is a very important opportunistic pathogen, especially in immunocompromised patients and hospitals. The pathogenicity and persistence of this organism is driven mainly by its ability to form robust biofilms on indwelling medical devices, such as catheters and prosthetic implants (Brescó *et al.*, 2017).

Overall, *S. epidermidis* is a non-motile bacterium that forms a characteristic "grape-like" cluster. After 24 hours incubation, it produces small (1-2 mm) compact, raised and white colonies (Aryal, 2024). Biochemically, it is catalase positive, coagulase negative and produces gamma hemolysis (non-hemolytic) on blood agar (Prasad *et al.*, 2012). Colonies are white on MSA (mannitol salt agar) because it does not ferment mannitol and they are small black colonies with no halo on Baird-Parker agar.

2.3.3 Bioactive Metabolites and Functional Diversity

Staphylococcus epidermidis releases a wide range of bioactive metabolites into the extracellular environment such as antimicrobial peptides, bacteriocins, proteases, immunomodulatory lipids and small molecules (Newstead *et al.*, 2020). Such factors are not restricted to mere antimicrobial peptides but also anticancer compounds, immune modulators and anti-biofilm enzymes all of which aid in controlling inflammation, maintaining microbiome balance and stability as well as preventing the domination of the pathogen on the skin surface (Pastar *et al.*, 2020). Notably, a significant number of these activities are mediated by soluble or secreted products that could be isolated in the cell-free supernatant (CFS) and useful functions of these products could be obtained without subjecting the skin to live bacteria.

The bacterium releases Phenol-Soluble Modulins (PSMs) which include PSM γ , PSM δ and δ -toxin, and display a specific cytotoxicity against the major skin pathogens such as *Staphylococcus aureus* and Group A Streptococcus (Cogen *et al.*, 2010).

Notably, *S. epidermidis* itself is comparatively resistant to them, boosting the host antimicrobial peptide (LL-37, hBD-2 and hBD-3) to clear pathogens and trigger neutrophil extracellular traps in skin tissue (Simanski *et al.*, 2019).

In addition to PSMs, some strains also produce lantipeptides and bacteriocins, including Epilancin 15X and Bacteriocin A37, which have a powerful antagonistic effect on microbial competitors in the nasal and skin niches (e.g. *Corynebacterium spp.* and *S. aureus*) by disrupting membranes or penetrating the cytoplasm. Due to their secretion into the extracellular space, these peptides will be found in cell-free supernatants and play a

direct role in the antimicrobial effects of *S. epidermidis*-derived CFS preparations (Newstead *et al.*, 2020; Wu *et al.*, 2023).

To limit the colonization of pathogens, *S. epidermidis* can secrete extracellular proteases, specifically GluSE (glutamyl endopeptidase) and Esp (epidermidis serine protease) (Sugimoto *et al.*, 2013; Abdurrahman *et al.*, 2024). These enzymes selectively cleave essential components of *S. aureus* biofilm matrix: staphylococcal protein A (Spa) and fibronectin-binding proteins (FnBPs) and therefore induce the dissolution of biofilm already established and prevent the colonization of the mucosal and dermal surfaces by biofilms in the first place (Martínez-García *et al.*, 2018)

Consistent with this, *S. epidermidis* cell-free conditioned media containing small molecules and enzymes have been reported to markedly decrease *S. aureus* biofilm formation and to enhance the action of more traditional antibiotics against biofilm-contained bacteria, highlighting the antibiofilm capacity of *S. epidermidis* CFS (Glatthardt *et al.*, 2019).

Anticancer Potential: Recent findings point to possible neoplasia-protective effect. Some strains of *S. epidermidis* synthesize 6-N-hydroxyaminopurine (6-HAP), a purine analog that selectively suppresses the action of DNA polymerase in tumor cells (Li *et al.*, 2025). In experimental models it can be demonstrated that 6-HAP does affect the proliferation of melanoma cells (B16F10) or diminish the occurrence of UV-induced tumors, but has no effect on primary keratinocytes. This gives reason to believe that the skin microbiome can act as a natural system of checking skin cancer (Nakatsuji *et al.*, 2018).

Table 2.1: Reported bioactive component of *Staphylococcus epidermidis* strains

Functional Category	Bioactive Metabolite	Mechanism of Action	Physiological Benefit	Reference
Antimicrobial Defense	PSMs (γ , δ) & δ -toxin	Selectively disrupt membranes of pathogens; δ -toxin synergizes with host AMPs (e.g., LL-37).	Elimination of <i>S. aureus</i> and Group A Streptococcus without harming commensals.	(Cogen <i>et al.</i> , 2010)
	Bacteriocin (Epilancin 15X, A37)	Cause membrane destruction or cytoplasmic infiltration.	Competitive exclusion of nasal and skin rivals like <i>Corynebacterium</i> .	(Newstead <i>et al.</i> , 2020; Wu <i>et al.</i> , 2023).
Anti-Biofilm Activity	Proteases (GluSE, Esp)	Cleave essential biofilm proteins (Spa, FnBPs).	Disassembly of <i>S. aureus</i> biofilms and inhibition of colonization.	(Glatthardt <i>et al.</i> , 2019).
Barrier Reinforcement	Tryptophan Derivatives	Activate Aryl Hydrocarbon Receptor (AhR) in keratinocytes.	Induces expression of barrier genes (CYP1A1) and host defensins (hBD-3).	(Rademacher <i>et al.</i> , 2018)
Anticancer Activity	6-HAP	Acts as a purine analog inhibiting DNA polymerase.	Suppresses tumor cell proliferation (e.g., Melanoma) selectively.	(Nakatsuji <i>et al.</i> 2018)

The use of *S. epidermidis* cell-free supernatant (CFS) is particularly attractive from a translational perspective. By removing viable bacteria through centrifugation and sterile filtration, CFS retains a complex mixture

of secreted peptides, enzymes and small molecules, while minimizing safety concerns related to live bacterial application, antibiotic resistance determinants or uncontrolled colonization (Żółkiewicz *et al.*, 2020).

Experimental studies using *S. epidermidis*–conditioned media have shown that soluble factors alone are sufficient to sensitise keratinocytes towards *S. aureus*, enhance antimicrobial peptide production and amplify clearance of pathogens, as well as to modulate barrier-related gene expression via AhR signaling (Simanski *et al.*, 2019). Moreover, antibiofilm compounds contained in *S. epidermidis* cell-free conditioned media have been demonstrated to inhibit *S. aureus* biofilm formation and to increase the susceptibility of biofilm-embedded bacteria to antibiotics, highlighting their potential as adjunctive topical therapies (Glatthardt *et al.*, 2020).

These observations support the rationale of the present work, which aims to exploit *S. epidermidis* CFS as a postbiotic preparation that concentrates beneficial metabolites while avoiding the risks associated with the direct application of live bacteria.

2.4 Microbiome Modulation and Postbiotics

2.4.1 Replacement of Antibiotics with Bacteriotherapy

Topical and systemic antibiotics have long been the mainstay of management of skin infections and of microbiome-associated dermatoses, such as acne vulgaris and atopic dermatitis. However, rapid advances in skin microbiome research have highlighted important limitations of this “eradication-centered” paradigm and underscored the need to develop ecological restoration strategies collectively referred to as bacteriotherapy or microbiome-targeted therapies. These methods are aimed at restoring a

sustainable, health-related microbial community capable of offering durable colonization resistance and facilitating cutaneous immune homeostasis.

2.4.2 Traditional Antibiotic Therapy Limitations

Although conventional antibiotics have obvious advantages when applied to treat acute skin pathogen-related diseases, they also demonstrate several key disadvantages when used to treat chronic, microbiome-associated skin diseases.

2.4.2.1 Random Destruction (Collateral Damage)

Most of the antibiotics used in dermatology, especially in the treatment of acne and secondary infection in eczema, are broad-spectrum agents that do not discriminate between pathogenic bacteria (e.g. *S. aureus*) and beneficial commensals (e.g. coagulase-negative staphylococci and *Cutibacterium* species). By depleting diverse commensal taxa, such “scorched earth” regimens can reduce overall microbial diversity, weaken colonization resistance and create ecological niches that favor recolonization by antibiotic-resistant strains (Patangia *et al.*, 2022).

Long term human studies have also shown that systemic and topical antibiotic can induce chronic changes in the skin microbiome and resistome, with long term increases in resistant *Staphylococcus* populations and loss of key commensal groups (Andriessen & Lynde, 2014, Jo *et al.*, 2021).

2.4.2.2 Antimicrobial Resistance (AMR)

Decades of extensive and frequently prolonged topical and systemic antibiotic (such as erythromycin, clindamycin and macrolides) use in acne and other inflammatory dermatoses have led to a worldwide resurgence of

antibiotic-resistant *Cutibacterium acnes* and Staphylococcus strains (SanMiguel *et al.*, 2017; Dessinioti & Katsambas, 2022).

Recent surveillance and meta-analyses show that more than half of the clinical *C. acnes* isolates in certain areas exhibit resistance to commonly used agents, especially to macrolides and multidrug-resistant phenotypes are increasingly reported, rendering many standard regimens less effective or obsolete (Alkhawaja *et al.*, 2020; Bhadade *et al.*, 2026).

This tendency is alarming because further use of antibiotics will solely contribute to the further increase of AMR and restrict the range of future treatment possibilities.

2.4.2.3 Alteration of Immune Signaling

Existing microbes play a tonic stimulatory role on cutaneous innate immunity by the action of microbe-associated molecular patterns including lipoteichoic acid (LTA), lipopeptides and other ligands that signal via pattern-recognition receptors (e.g. TLR2) (Biedermann *et al.*, 2015). Such beneficial signals can be blunted by broad antimicrobial regimens that deplete these commensals and result in a hyporesponsive or dysregulated immune state and potentially impair the ability of the skin to respond appropriately to subsequent insults (Jo *et al.*, 2021).

Thus, the impact of antibiotics extends beyond simple pathogen killing to include disruption of microbiome–immune crosstalk that is critical for long-term tissue protection (Francino, 2015). Together, these constraints have driven a conceptual shift from indiscriminate antimicrobial suppression to strategies that restore or engineer health-associated microbial ecosystems.

2.4.3 Postbiotic, Cell-Free Supernatant–Based Approaches

In more general context of bacteriotherapy, there is an increasing interest in postbiotics, which are non-viable cells of microorganisms, cell components or metabolites that confer a health benefit to the host. In practice, postbiotic preparations are often cell-free supernatants (CFS) of probiotic or commensal bacteria, which retain complex mixtures of secreted peptides, organic acids, enzymes and small molecules without the risks that come with the administration of live bacterial samples (Salminen et al., 2021; Vinderola et al., 2022).

Various in vivo and in vitro studies have demonstrated that such CFS can inhibit growth and biofilm formation of skin pathogens, modulate inflammatory signalling and enhance barrier repair, in support of their development into safer, more controllable microbiome-targeted therapies (Drumond et al., 2023; Dinić et al., 2025).

CFS of the selected *S. epidermidis* strains has been reported to inhibit the growth of *Cutibacterium acnes* by fermentation-produced acids, reduce oxidative stress and cytokine production in UVB-injured keratinocytes, and promote barrier-related gene expression, highlighting the inherent anti-inflammatory, antioxidant and barrier-protective capabilities (Nakamura et al., 2020; Shao et al., 2025).

Chapter three

Materials and Methods

3. Materials and Methods

3.1 Materials

3.1.1 Laboratory Instruments and Apparatuses

The laboratory instruments and apparatuses used in this study are listed in table (3-1).

Table (3-1): The laboratory instruments and apparatuses used in this study

No.	Apparatus / Equipment	Company	Origin
1	Autoclave	Express	Germany
2	Burner	Amal	Turkey
3	Deep freezer	Arctiko	Denmark
4	ELISA Incubator	Memmert	Germany
5	ELISA Reader	Bio-Rad	Ireland
6	ELISA Washer	Human	Germany
7	Eppendorf tubes	BioBasic	Canada
8	Gel-Electrophoresis	Bioneer	Korea
9	High Speed centrifuge	Hettich	Germany
10	Hood	Bio Lab	Korea
11	Hot plate with magnetic stirrer	Gallenkamp	England
12	Incubator	GFL	India
13	Light microscope	Olympus	Japan
14	Lyophilizer (AIPHA 2-4LSC Plus)	Christ	Germany
15	Micro Centrifuge	KUBOTA	Japan
16	Micropipettes (1-10 µl, 1-20 µl, 10-100 µl, 100-1000µl)	Gilson	France
17	PCR thermocycler T100	BioRad	USA
18	Petri dishes	China	China
19	Refrigerator	Rexmed	Taiwan
20	Sensitive balance	Sartorius	Germany
21	Shaking incubator	Memmert	Germany
22	Standard wire loop 1µl	Hi- media	India
23	Syringe filters	Millipore	USA
24	Tubes	China	China
25	U.V transilluminator	Wised	Korea
26	VITEK 2- Compact system	Biomerieux	France

27	Vortex mixer	Talboys	USA
28	Water Bath	Poly-science	USA
29	Whatman filter paper (2.5µm)	China	China

3.1.2 Chemicals

Table (3-2) clarifies the various chemical materials that were used in this study.

Table (3-2): Chemical materials that were used in this study with their company and country of origin.

No	Chemicals	Company	Origin
1	Agarose	iNtRON	Korea
2	Carbopol	Himedia	India
3	Crystal violet	CHEM-LAB	Belgium
4	DNA Marker ladder 100-1500bp	BioBasic	Canada
5	Ethanol 99%	BioLabs	UK
6	Ethidium bromide	iNtRON	Korea
7	Glucose	Panreac	Spain
8	Glycerol	Kem	UK
9	Hydrogen peroxide	Panreac	Spain
10	Iodine	CHEM-LAB	Belgium
11	Lysozyme	Geneaid Biotech Ltd	Taiwan
12	Methanol	CHEM-LAB	Belgium
13	Nuclease-free water	BioLabs	UK
14	Proteinase K	Geneaid Biotech Ltd	Taiwan
15	Safranin	CHEM-LAB	Belgium
16	TBE buffer	iNtRON	Korea
17	Triethanolamine	Alpha Chemika	India

3.1.3. Ready-Made Culture Media

Table (3-3) clarifies the culture media that were used in this study.

Table (3-3): Culture Media that were used in this study.

No.	Culture media	Purpose	Company/ Origin
1	Blood agar base	used to investigate the ability of bacteria to hemolyze blood	Labtech / UK
2	Mannitol salt agar	used as a selective and differential media for the growth of <i>Staphylococcaceae</i> bacteria	Hi-media /India
3	Mueller Hinton agar	used to test the susceptibility of isolates to antibiotics	Labtech / UK
4	Nutrient agar	using as a media for preserving and activating the isolates.	BDH / England
5	Tryptic soy broth	using as a media to activate and perpetuate the isolates	
6	Brain heart infusion broth	used to activate and preserve the isolates	Difco/ Germany
7	Brain heart infusion agar	used to activate and preserve the isolates	

All these media were prepared according to company directions in Erlenmeyer flasks sealed with cotton-wool bungs and wrapped with aluminum foil. The flasks were sterilized by autoclaving at 121°C for 15 min. Then distributed into Petri dishes or tubes as needed, conditions of incubation are (37°C for 24 hrs.) to ensure sterility.

3.1.4. Antibiotics

3.1.4.1. Antibiotic Discs

Table (3-4) clarifies the antibiotics discs were used in study for *Staphylococcus epidermidis* isolates.

Table (3-4): Antibiotic Discs that were used in this study.

No .	Antibiotic	Sym bol	Class	Con. (µg)	Company / Origin
1	Cefoxitin	FOX	β-Lactams	30	Liofilchem /Italy
2	Vancomycin	VA	Glycopeptides	30	HIMEDIA/ India
3	Linezolid	LN	Oxazolidinones	30	
4	Rifampin	RA	Ansamycins	5	Bioanalysis/ Turkey
5	Ciprofloxacin	CIP	Fluoroquinolones	10	
6	Clindamycin	DA	Lincosamides	10	
7	Doxycycline	DO	Tetracyclines	10	
8	Tetracyclines	TE	Tetracyclines	10	
9	Gentamicin	CN	Aminoglycosides	10	TMMEDIA/ India
10	Trimethoprim	TR	Folate Pathway Antagonists	5	
11	Levofloxacin	LE	Fluoroquinolones	5	
12	Erythromycin	E	Macrolides	15	

3.1.5. Ready Kits

The commercial kits used in the present study of immunological and molecular methods are shown in Table (3-5).

Table (3-5): The kits used in this study with their companies and countries of origin.

No.	Kit	Company	Country
1	Human Cathelicidin antimicrobial peptide ELISA Kit	BT LAB	China
	Standard Solution		
	Pre-coated ELISA Plate		

	Standard Diluent		
	Streptavidin-HRP		
	Stop Solution		
	Substrate Solution A		
	Substrate Solution B		
	Wash Buffer Concentrate		
	Biotinylated Human CAMP; LL-37 Antibody		
2	Human Beta-Defensin 2 ELISA Kit	BT LAB	China
	Standard Solution		
	Pre-coated ELISA Plate		
	Standard Diluent		
	Streptavidin-HRP		
	Stop Solution		
	Substrate Solution A		
	Substrate Solution B		
	Wash Buffer Concentrate		
	Biotinylated Human DEFB2 Antibody		
3	Human Interleukin 8 ELISA Kit	BT LAB	China
	Standard Solution		
	Pre-coated ELISA Plate		
	Standard Diluent		
	Streptavidin-HRP		
	Stop Solution		
	Substrate Solution A		
	Substrate Solution B		
	Wash Buffer Concentrate		
	Biotinylated Human IL-8 Antibody		
4	Presto™ Mini gDNA Bacteria Kit	Geneaid	Taiwan
	Gram+ Buffer		
	GT buffer lysis buffer		
	GB buffer binding buffer		
	Proteinase K		
	W1 buffer		
	Wash buffer		
	Elution buffer		
	GD column		
	Collection tube 2ml		
	Lysozyme		
5	GoTaq® Green PCR master Mix	Promega	USA
	Taq DNA polymerase		

	dNTPs (dATP, dCTP, dGTP, dTTP)	
	Tris-HCl pH 9.0, KCl, & MgCl ₂ Stabilizer and loading dye	
6	Vitek 2 compact kit	France

3.1.6. Primer

The PCR primers used for the molecular detection of the *mecA* gene were adopted from a previously published study (Shrestha *et al.*, 2020). These primers were synthesized by Macrogen (South Korea) and procured as a lyophilized powder through the Al-Amin Center for Advanced Biotechnology Research, Iraq. Upon arrival, they were reconstituted by adding 290 µl of nuclease-free water to each vial to prepare a stock solution of 100 pmol/µl, according to the manufacturer's instructions. Subsequently, a working solution of 10 pmol/µl was prepared for the PCR assays. The primer sequences and details are listed in Table (3-6).

Table (3-6): The PCR primer for detection of *mecA* gene with their sequence and product size.

Primer	Sequence (5'-3')		Product Size	Reference
<i>mecA</i> gene	F	GTAGAAATGACTGAACGTCC GATGA	310bp	(Shrestha <i>et al.</i> , 2020)
	R	CCAATTCCACATTGTTTCGGT CTAA		

3.1.8 Study Design

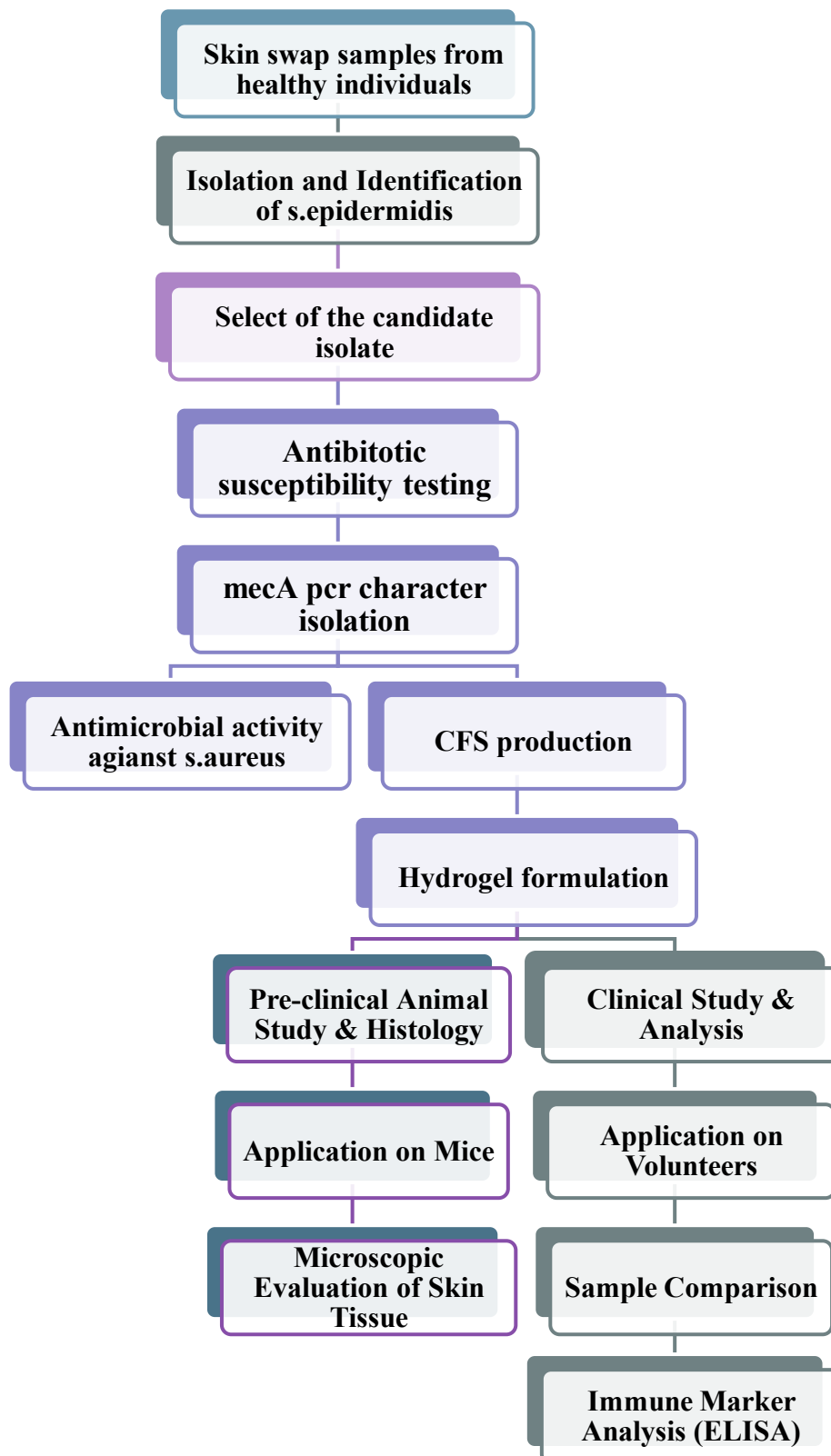


Figure (3-1): Study Design

3.2 Methods

3.2.1 Collection of Samples

To minimize the influence by external factors on normal skin microbiota, 110 skin swab samples were collected from healthy individuals (not hospitalized within the three months preceding swab collection, and not exposed to antibiotics during the same time frame) between 15th of October 2025 and 20th of March 2026.

The swabs were collected and immediately cultured onto Mannitol Salt Agar (MSA), a selective media with high salt concentration that selects for growth of *Staphylococcus* species. Plates were then incubated at 37°C for 24 hours. After incubation, colonies with morphology similar to *Staphylococcus epidermidis* (non-mannitol fermenting and pink to red) were selected. All these colonies were then re-streaked on new MSA plates and subcultured to obtain pure isolates for subsequent identification and analysis.

3.2.2 Identification of *S. epidermidis* isolates

Isolates obtained were subjected to preliminary identification by morphological and standard biochemical characteristics specifically the isolates with normal skin flora characters. Non-hemolytic, mannitol non-fermenting and typical commensal isolates were selected. Selected isolates were confirmed to the species level by Vitek 2 system for accurate species-level identification.

3.2.2.1. Cultural and Microscopic Characteristics

Isolates were inoculated onto Mannitol Salt Agar (MSA) plates and incubated at 37°C for 18 to 24 hours. Following incubation, the plates were examined for cultural characteristics typical of *Staphylococcus epidermidis*, specifically focusing on colony morphology and non-mannitol fermentation. Selected isolates were subsequently subjected to Gram staining and evaluated under a light microscope to determine their Gram reaction and cellular arrangement.

3.2.2.2. Biochemical Tests

A. Catalase Test

A drop of 3% hydrogen peroxide (H₂O₂) was placed on a clean glass slide. A wooden stick full of bacterial culture was then introduced into the drop. If the bubbles appeared immediately, the result was considered a positive one, thus confirming the isolate to be of the genus *Staphylococcus* (Benson, 2002).

B. Oxidase Test

An oxidase reagent-soaked filter paper was used. Using a sterile wooden stick, a part of the tested isolate was transferred and spread on the paper. No colour change (no development of dark blue or purple colour) was regarded as negative result and is typical of *Staphylococcus epidermidis* (Benson, 2002).

C. Blood hemolysis test

The test was employed in order to study the ability of bacteria to produce hemolysin. All of the bacteria were tested by streaking onto a blood agar plate and checked for their ability to grow 5% of Human blood and incubated for 24 hrs. at 37 °C. Then the type of hemolysis produced by growing colonies was observed on the blood agar (Collee *et al.*,1996).

3.2.2.3 VITEK2 System

This device comprised of a pc, a scanner / incubator composed of many internal components including a card recorder, a card filling system, a recorder loading system, a card sealer, a code scanner, and an incubator, as well as transmission optical, waste disposal, electronic control instruments and firmware. The device has been fitted with an expanded diagnostic Data-base for all the diagnostic tests to include an enhanced microbial diagnostic efficiency to eliminate the need for additional testing to increase protection for both test and user. For this analysis the GP card was used for Gram Positive bacterial species comprising 47 substrates (biochemical tests). The following phases were set based to the manufacturer's instructions. The procedure included:

1- Suspension Preparation

Normal saline (3 ml) was placed in a test tube and inoculated with the isolated colony. The test tube was placed into a colony standardization turbidity testing system to standard 0.5 McFarland solution.

2- Inoculation

The standard inoculum was inserted into the cassette, and a sample ID number was inserted into the PC program via bar code. The VITEK 2

card model was then scanned from the bar code placed onto the card during fabrication, and the card was linked to the sample Identification number.

3- Card Sealing and Incubation

The cassette was then put inside the filler box. The cassette was moved to the reader / incubator board, when the cards were loaded in. The instrument handled all subsequent steps: regulates the incubation temperature, optical card readings and continuously tracks and transmissions the examination data to the device for analysis.

4- Test Reactions

Calculations to assess reactions for each test were performed on raw data and compared to thresholds. The test results of the reaction appear on the VITEK 2 as +, -, (-) or (+).

5- Identification Levels

An enigmatic bio-pattern for each taxon was matched to the reaction data memory, and a numerical likelihood calculation was conducted. The numerical probability estimation given different qualitative degrees of recognition. The various rates and related details are shown in the table (3-8).

Table (3-7): Identification Levels in VITEK 2 System

ID Message Confidence Level	Choices	% Probability	Comments
Excellent	1	96 to 99	N/A
Very Good	1	93 to 95	N/A
Good	1	85 to 88	N/A
Low Discrimination	2 to 3	Sum of choices – 100; after resolution to one choice, percent probability reflects the number associated with the selected choice.	2 to 3 taxa exhibit the same biopattern. Separate by supplemental testing.
Unidentified Organism	> 3 or 0	N/A	Either > 3 taxa exhibit the same biopattern or very atypical biopattern. Does not correspond to any taxon in the database. Check Gram stain and purity.

3.2.3 Maintenance and Conservation of isolates

3.2.3.1 Short Period Conservation

Pure bacterial isolates were grown on a slant of BHI agar in universal tubes and then kept for a maximum of 1 month at 4°C (Smith and Taubman, 1996).

3.2.3.2 Long Period Conservation

Pure bacterial isolates were cultivated in sterile BHI broth at 37 °C for 18 to 24 hours. Subsequently, the bacterial culture and sterile glycerol were transferred into sterile tubes at a 3:1 volumetric ratio. The suspension was thoroughly mixed using a vortex mixer, sealed with Parafilm, and stored at -20 °C for a period of 1 to 2 years (Karch et al., 1995).

3.2.4. Sterilization Methods

3.2.4.1 Wet-Heat Sterilization

Sterilization of culture media and some solution by Autoclaving at 121°C and 15 psi (pounds per square inch) for 15 min.

3.2.4.2 Incineration Sterilization

It is used to sterilize the loop and forceps by Bunsen burner.

3.2.4.3 Filter Sterilization

Using Millipore filter at 0.22 and/or 0.45µm to sterilize solution that affected with high temperature, or the target being cell free solution

3.2.5 Characterization of the selected *S. epidermidis* isolate as source of postbiotic

3.2.5.1 Antibiotic Susceptibility Testing for *S. epidermidis*

The modified Kirby-Bauer method (Vandepitte et al., 2003) was used as follows: Mueller-Hinton agar was prepared from a dehydrated base as the company instructed (labtech/ UK). The media was chilled to (45-50°C) and poured into Petri dishes. When the agar was solidified and the plates were dried for immediate use. Inoculums were prepared, by transferring 3-5 colonies of the selected *S. epidermidis* into a tube of 5ml of normal saline. The suspension was mixed well and compared with McFarland tube to get a culture of 1.5×10^8 CFU/ml by modifying to turbidity standard of McFarland 0.5. This suspension was used within 30 min of preparation. Next, the plates were seeded by a sterilized swab. After each application, the inoculum was permitted to dry at ambient temperature for a brief period. By using sterile forceps, the selected antibiotic disc

previously mentioned in (table 3-4) was positioned on the inoculated plate. The plates were incubated for 18-24 hours at 37 °C in an inverted position. Upon incubation, using a metric scale, the diameter of the inhibition-zone was measured in millimeters. The diameter of the inhibition region was translated for the individual antibiotic discs into sensitive, intermediate and resistant groups compared to the standard inhibition proposed by the criteria defined by the clinical and laboratory standards (CLSI, 2026).

3.2.5.2 Polymerase Chain Reaction

Using *mecA* as a molecular marker for MRSE, molecular identification was performed on *Staphylococcus epidermidis* using the polymerase chain reaction (PCR) methodology according to the following steps:

3.2.5.2.1 Bacterial DNA extraction

The Presto™ Mini gDNA Bacteria Kit was used to isolate bacterial genomic DNA according to the manufacturer's procedure which includes the following steps:

1-Sample Preparation

A volume of cultured bacterial cells (up to 1×10^9 cells) was transferred into a 1.5 ml microcentrifuge tube and then centrifuged for 1 minute at 10,000 rpm after which the supernatant was removed.

2-Cell Lysis Steps

- a. The cell pellet was re-suspended in 180 µl of GT buffer, followed by the addition of 100 µl of lysozyme to facilitate the lysis of Gram-

positive bacteria. The resulting mixtures were incubated at 60°C for a duration of 10 minutes.

- b. In the incubation phase, the mixture tubes were shaken every 3 minutes. Each tube received 200 µl of GB buffer, which was thoroughly mixed using a vortex for 10 seconds. Subsequently, the tubes were incubated at 60°C for an additional 10 minutes, with inversion performed every 3 minutes.

3-DNA binding steps

- a. Two hundred microliters of absolute ethanol were introduced and subsequently vortexed, while any precipitates were dislodged through pipetting.
- b. A GD column was set up in a 2 ml collection tube and all the mixtures including any precipitates were placed in the GD column. The solution was subsequently subjected to centrifugation at a speed of 10,000 revolutions per minute for a duration of one minute. The 2 ml collection tubes with the flow-through were discarded and the GD column was then placed in a new 2 ml collection tube.

4-Washing Steps

- a. A volume of 400 µl of W1 buffer was introduced to the GD column, followed by centrifugation at 10,000 rpm for a duration of 1 minute. The resulting flow-through was discarded, and the GD column was subsequently returned to the 2 ml collection tube.
- b. A volume of 600 µl of Wash Buffer was introduced to the GD column, followed by centrifugation at 10,000 rpm for a duration of one minute. The flow-through was discarded and the GD column was again inserted into the 2 ml collection tube, and the tubes were

centrifuged once again for 2 minutes at 12,000 rpm to ensure that the column matrix was enough to be dried.

5-Elution Steps

- a. A 100 μ l of pre-heated elution buffer was placed in the middle of the dried GD column in a sterile 1.5 ml microcentrifuge tube
- b. The elution buffer was left to stand for at least 3 minutes to ensure its complete absorption into the matrix. Subsequently, the tubes underwent centrifugation at 10,000 rpm for a duration of 1 minute to facilitate the elution of the purified DNA.

3.2.5.2.2 Estimation of extracted total DNA

The extracted total DNA was checked by using Nanodrop (Thermo Scientific NanoDrop Lite UV Visible Spectrophotometer, USA) that measured DNA concentration (ng/ μ L) and checked the DNA purity at absorbance (260 /280 nm) as following steps:

- 1- After opening up the Nanodrop software, chose the appropriate application (Nucleic acid, DNA).
- 2- A dry wipe was taken and cleaned the measurement pedestals several times. Subsequently, 2 μ l of nuclease-free water was meticulously pipetted and applied to the surface of the lower measurement pedestals to calibrate the system
- 3- The Nanodrop sampling arm was lowered and 1 μ l DNA sample was measured.

3.2.5.2.3 PCR master mix preparation

Master mix reactions for *mecA* gene were prepared by using (GoTaq®Green PCR master kit) and this master mix was done according to company instructions as following table (3-9).

Table (3-8): Standard PCR master mix protocol

PCR Master mix	Volume
DNA template 5-100ng	5µL
Forward primer (10pmol)	2µL
Reverse primer (10pmol)	2µL
GoTaq®Green PCR master	12.5µL
Nuclease-free water	3.5µL
Total volume	25µL

The PCR reaction mixtures were formulated utilizing the GoTaq®Green Master Mix (Promega) in accordance with the guidelines provided by the manufacturer. The components of the reaction, as outlined in Table (3-9), were combined in sterile PCR tubes. Subsequently, the tubes underwent a brief centrifugation using an Exispin vortex centrifuge at a speed of 3000 rpm for 3 minutes to ensure that the contents settled at the bottom, before being transferred to the T100 PCR Thermocycler (Bio-Rad, USA).

3.2.5.2.4 PCR Thermocycler

The PCR thermocycler conditions for *mecA* gene were determined using the Optimise Protocol Writer™ online application and were executed on a conventional PCR thermocycler, as shown in Table (3-10).

Table (3-9): PCR thermocycling program for the molecular detection of *S. epidermidis mecA* gene.

PCR step	Temp.	Time	Repeat
Initial Denaturation	95°C	5min	1
Denaturation	95°C	30sec.	35 cycles
Annealing	58°C	30sec.	
Extension	72°C	1 min	
Final extension	72°C	5 min	1
Hold	4°C	Stop	-

3.2.5.2.5 PCR product analysis

The PCR products were analyzed by agarose gel electrophoresis method as following steps according to (Sambrook & Russell, 2006):

- 1- Agarose gel was prepared by using 0.5X TBE and dissolving the agarose in a microwave with frequent swirling until completely melted for 5 minutes and left to cool to approximately 50°C.
- 2- Then 3µl ethidium bromide stain was added into the agarose gel solution.
- 3- The agarose gel solution was poured into the tray after fixing the comb in the proper position and left to solidify for 15 minutes at room temperature, then the comb was removed gently from the tray.
- 4- The gel tray was fixed in the electrophoresis chamber and filled with 0.5X TBE buffer.
- 5- PCR products (10µl) were loaded to each well with added 5µl (DNA ladder marker) in the first well. Electrophoresis was run at 100 volts and 80 mA for 1 hour.
- 6- PCR products were visualized by using UV Transilluminator.

3.2.5.3 Antimicrobial Activity Testing Using Well-Agar Diffusion

Method

The antimicrobial activity of *S. epidermidis* cell free supernatant (CFS) was determined by the well-agar diffusion method against indicator bacteria (*S. aureus*). It is a quantitative assay that is based on the measurement of the diameter of inhibition zones to assess the production of bioactive compounds (Balouiri *et al.*, 2016).

3.2.5.3.1 Preparation of Indicator Lawn

At first the isolates of *Staphylococcus aureus* were cultured on Mannitol Salt Agar (MSA) at 37°C overnight and several colonies were collected and suspended in normal saline to reach a turbidity of 0.5 McFarland (1.5×10^8 CFU/ml) standard. One hundred microliters (100 µl) of every standardized suspension was spread evenly with a sterile cotton swab on the surface of Mueller-Hinton Agar (MHA) plates, to form a uniform lawn. The excess fluid was drawn off by swirling the swab around the plate edge. The plates were dried at room temperature for 15 minutes prior to well punching.

3.2.5.3.2 Well Preparation and Sample Application

Wells were made in the agar surface by using a sterile cork borer with a diameter of 6 mm. A volume of 100 µl of filter-sterilized *S. epidermidis* cell-free supernatant was added to each well.

3.2.5.3.3 Incubation and Zone Measurement

The plates were maintained at an incubation temperature of 37°C for a duration of 24 hours. The diameter of inhibition zones (mm) around each well was measured using a metric ruler to determine the antimicrobial activity. The positive zones for the bioactive metabolites activity were obtained only with clear zones of ≥ 10 mm.

3.2.6 Preparation of Powder from Cell-Free Supernatant

Cell-free supernatants obtained from *S. epidermidis* cultures were converted to powder form using lyophilization (freeze-drying) technique (Martin Christ Alpha 2-4 LSC plus) to obtain stable postbiotic powder for long-term storage and further applications.

3.2.6.1 Pre-Lyophilization Preparation

Selected *S. epidermidis* isolates were cultivated in TSB medium in shaking incubator at 150 rpm, 37°C for 24 hours. The bacterial cell-free supernatant was obtained by centrifugation using an Optima XPN-100 ultracentrifuge at 10,000 rpm and 4°C for 30 minutes. The supernatant was retrieved and then passed through syringe filters with a pore size of 0.22 μm to ensure complete sterility. The cell-free filtered supernatant was then moved to sterile glass petri dishes, frozen at -80°C and then lyophilized.

3.2.6.2 Lyophilization Process

The supernatants were then frozen and freeze-dried by using Christ Freeze-dryer (Martin Christ Alpha 2-4 LSCplus) Lyophilizer. The following lyophilization conditions were used:

- Primary drying: Vacuum (0.1 mbar) at -50°C for 24-36 hours

- Secondary drying: Vacuum-dried by gradually increasing the temperature to 25°C for 12 hours.

The lyophilization process continued until there was no more ice sublimation (when the weight remained constant).

3.2.6.3 Powder Collection and Storage

The lyophilized powder obtained was collected under aseptic conditions and weighed on an analytical balance, which resulted in a white to off-white lyophilized powder. Powder was placed in sterile Eppendorf tubes or amber glass vials and then parafilm-sealed and stored at -20°C.

3.2.6.4 Antimicrobial Activity of Lyophilized Cell-Free Supernatant

The antimicrobial activity of the lyophilized cell-free supernatant (CFS) was evaluated using the agar well diffusion method (Balouiri *et al.*, 2016).

The *S. aureus* pathogenic indicator microorganism was cultured on Mannitol Salt Agar (MSA) at 37°C overnight and several colonies were collected and suspended in normal saline to reach a turbidity of 0.5 McFarland (1.5×10^8 CFU/ml) standard. A standardized bacterial suspension was taken with a sterile cotton swab and evenly spread over the surface of the Mueller-Hinton Agar plates to provide a confluent lawn of the bacteria.

A sterile cork borer was used to aseptically bore holes into the inoculated agar, each 6 mm in diameter and spaced apart by equal distances.

The CFS lyophilized powder was serially diluted two-fold in sterile distilled water to provide 0.1, 0.05, 0.025 and 0.0125 g/mL of CFS. Subsequently, the precise volume of 50µL was carefully added to each

prepared concentration and then carefully added to the appropriate well. The plates were then incubated aerobically at 37°C for 24 hours. The plates were then observed for the presence of a clear zone that indicated bacterial growth inhibition after the incubation period. The diameters of these inhibition zones were measured in millimeters (mm) to quantitatively evaluate the antimicrobial effectiveness.

3.2.6.5 Hydrogel formulation and processing

Hydrogel was processed with Carbopol as a gelling base. One gram of Carbopol powder was weighed and slowly added to 100mL of sterile distilled water with constant magnetic stirring to inhibit any aggregations. The solution was allowed to stand overnight at 4 °C to allow complete swelling of the polymer network. Then (5%) Glycerin was mixed as humectant and plasticizer and further gentle stirring was done to get a homogeneous base.

The cell-free extract of *Staphylococcus epidermidis* was freeze dried and then redissolved in a specific volume of chilled sterile distilled water to maintain the bioavailability of the compounds, in a separate step. The dissolved bacterial extract was very slowly and carefully added to the liquid Carbopol base to minimize mechanical stress, to prevent any air bubble formation and to ensure homogenous distribution of the bacterial extract throughout the polymer base.

Finally, the pH was adjusted by slowly adding drops of triethanolamine (TEA) and gently and continuously stirring with a hand-held stirrer until a clear, viscous, aqueous gel was obtained. The final pH of the gel was adjusted to approximately 5.5, which corresponds to the

physiological pH of the skin. The final gel was then kept in sterile, airtight containers at 4°C until used in further biological assays.

3.2.7 In Vivo Evaluation of the Topical Product on Laboratory Mice

Animal models are used to mimic the complex topology of the human body. The following guidelines were the backbone of the procedure conducted in this study (Malachowa *et al.*, 2019).

The efficacy and safety of *S. epidermidis* cell-free supernatant powder as a topical skin treatment were assessed in a mouse skin model. The effectiveness of the formulated product on skin irritation potential was tested in this experiment.

3.2.7.1 Experimental Animals

Healthy adult male (6-8 weeks old, 20-25 g) BALB/c mice were acquired from the animal house at the College of veterinary Medicine, University of Al-Qadisyiah. Mice were adapted to the laboratory environment for seven days before experimentation, and housed under standard laboratory conditions (12 h light/dark cycle, 22±2°C, 50-60% humidity) with unlimited access to standard mouse chow and water. The animal ethics committee at the institution gave approval for all the procedures.

3.2.7.2 Experimental Design and Animal Grouping

The vivo experiment was used to assess the dermal tolerability of the postbiotic extract. The number of healthy mice used was (10). Before topical application, the dorsal area (back) of each mouse was gently shaved with an electric clipper (about 2x2 cm), being careful not to cause physical abrasions or damage to the epidermal layer.

Following a 24-hour rest period post-shaving to rule out mechanical irritation, all the mice were randomly divided into three groups as follows:

- Group I (Control Group): Three mice were used as a control, where a hydrogel free of CFS was topically applied on their shaved dorsal skin.
- Group II (5% Treatment Group): Hydrogel containing (5%) concentration of the CFS was topically applied on the shaved dorsal skin of three mice.
- Group III (10% Treatment Group): Hydrogel containing (10%) concentration of the CFS was topically applied on the shaved dorsal skin of three mice.

The hydrogel was evenly applied to the shaved areas with sterile cotton swabs. Application was done once a day for a continuous 14 days. In the experimental period, the skin treated areas were observed each day for macroscopic changes such as erythema (redness) and edema (swelling) in the skin. After the experimental period, animals were sacrificed and skin tissue biopsies were carefully removed from the treated areas and fixed in 10% neutral buffered formalin for further processing and histopathology analysis.

3.2.7.3 Histopathological study

The researchers in this study adopted a procedure similar to that described by Scheuer & Chalk, (1986) was:

A- Presentation of tissues: Until they were ready to be used for sectioning, skin tissue biopsies from the treated mice.

B- Sectioning: Tissue samples were sectioned according to standard histological protocols as follows:

- Fixation: The specimens were fixed in 10% formalin and then washed in running water.
- Dehydration: The specimens were dehydrated by soaking them in ethyl alcohol at a concentration of 50, 70, 90, and 100 % for one hours at each level.
- Clearing treatment with a mixed solution of alcohol and xylene following dehydration and then followed by three minte in pure xylene.
- Infiltration: Mixture the tissues in xylene and parafin (at 60) infiltrated for two hours. It was then transferred to 60°C parafin bath for (4) hours.
- Embedding: Specimens were infiltrated with parafine, then placed in plastic blocks with soluble parafine heated to (60 c°) and left to harden at room temperature.
- Sectioning: Specimens were sectioned using a rotary microtome (5 µm thickness), and the sections were then placed in a water bath at (60 c°) before being transferred on slides of glass.
- Harris hematoxylin-eosin (H&E) stain was used to colorize the sections in this study.

- Mounting: fixed the tissue on slide by using D.P.X materials. Histological alterations were looked for by examining tissue sections under a microscope.

3.2.8 In vivo Evaluation of Gel Efficacy on Skin Microbiome

Restoration and Barrier Function

Healthy human volunteers, aged between (25-45) years, participated in this evaluation. volunteers who had topical or systemic antibiotics in the previous 4 weeks, any skin infections or a history of skin diseases (such as eczema or psoriasis) were not included.

3.2.8.1 Application Protocol

On the volar forearm of each volunteer two symmetrical areas (2×2 cm) were marked. The area to be treated was marked for the application of the prepared gel containing *Staphylococcus epidermidis* cell-free supernatant, whereas the other contralateral area was marked for local control (application of the gel base alone).

The main aim of the contralateral control area was to assess the localized skin compatibility, physical tolerance and to visually compare any topical reactions (erythema/irritation). A pre-determined volume of 0.1 mL of the corresponding gel was given once a day for 20 days. Participants were asked to follow their usual hygiene practices except that they were not to use any other cosmetic or therapeutic product on the test areas.

3.2.8.2 Blood Sample Collection and Serum Processing

Blood samples (approximately 5 mL) were collected from the volunteers via venipuncture using sterile disposable syringes. To evaluate the systemic immunological response to the formulated topical CFS hydrogel, the sampling was performed at two distinct time intervals: at baseline day 0, prior to the application of the product and after 20 days of continuous topical treatment. The drawn blood was transferred into sterile plain tubes and allowed to coagulate at room temperature for 20-30 minutes. Following coagulation, the samples were centrifuged at 3000 rpm for 15 minutes. The obtained clear serum was carefully aspirated, divided into aliquots in sterile Eppendorf tubes to prevent repeated freeze-thaw degradation, and stored at -20 °C until further immunological analysis.

3.2.8.3 Measurement of Immunological Biomarkers using ELISA

The serum levels of three key immunological markers: Interleukin-8 (IL-8), Human Beta-Defensin 2 (HBD-2), and Cathelicidin (LL-37), were quantitatively evaluated to assess the efficacy of the topical CFS hydrogel. The measurements were performed using commercially available sandwich Enzyme-Linked Immunosorbent Assay (ELISA) kits (BT LAB, China) strictly adhering to the respective manufacturer's protocol.

A. Assay procedure:

1. Standard solutions and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature.
2. Determined the number of strips required for the assay. Insert the strips in the frames for use. The unused strips should be stored at 2-8°C.

3. Added 50µl standard to standard well. Don't add biotinylated antibody to standard well because the standard solution contains biotinylated antibody.
4. Add 40µl sample to sample wells and then add 10µl (anti-DEFB2, anti-IL-8 or anti-CAMP; LL-37) antibody to sample wells, then add 50µl streptavidin-HRP to sample wells and standard wells (Not blank control well). Mix well. Cover the plate with a sealer. Incubate 60 minutes at 37°C.
5. Remove the sealer and wash the plate 5 times with wash buffer. Soak wells with 300µl wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirate or decant each well and wash 5 times with wash buffer. Blot the plate onto paper towels or other absorbent material.
6. Added 50µl substrate solution A to each well and then add 50µl substrate solution B to each well. Incubate plate covered with a new sealer for 10 minutes at 37°C in the dark.
7. Added 50µl Stop Solution to each well, the blue color will change into yellow immediately.
8. Determined the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

B. Calculation of Result:

The ELISA results were calculated depend on the optical density reading for each standard and samples optical density. Then the standard curve was plotted by the mean OD value for each standard on the X-axis against the concentration on the Y-axis and draw a best fit curve through the points on the graph.

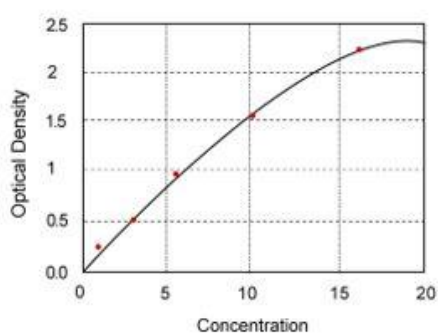
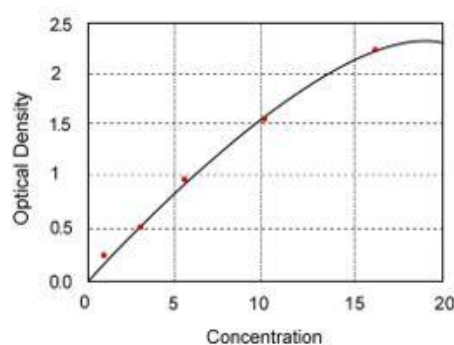
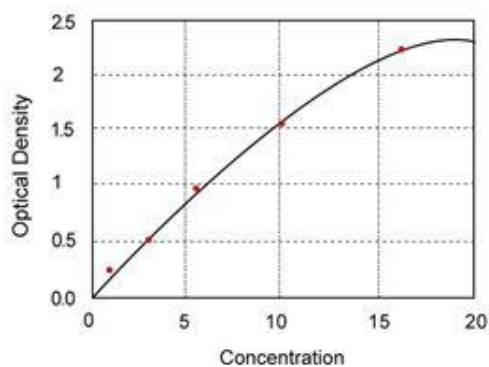
**A****B****C**

Figure (3-2): A standard curve for ELISA data (A) represent standard curve for HBD-2, (B) represent standard curve for IL-8 and (C) represent standard curve for LL-37.

3.3 Statistical Analysis

IBM SPSS Statistics software, version 26.0 (IBM Corp., Armonk, NY, USA), was used for statistical analysis of immunological parameters. In the case of all quantitative data, descriptive statistics were presented as mean $\pm$ standard deviation (SD) and standard error of the mean (SEM). Data were normally distributed and the normality of the data distribution was first evaluated using the Shapiro-Wilk test to determine the appropriate statistical approach. For data that was normally distributed, parametric tests were used: the Unpaired t-test was used to compare continuous data between two independent groups (e.g. untreated control group versus pre-treatment group) and the Paired t-test used to compare two paired samples (before and after topical application of the treatment). Non parametric alternatives were used instead for data that failed to meet the assumptions of normality: Mann Whitney U test for independent group comparisons and Wilcoxon matched pairs signed rank test for baseline and post-treatment comparisons.

In all analyses, a P-value of less than 0.05 ($p < 0.05$) was considered statistically significant (Daniel, 2018).

Chapter Four

Results and Discussion

4.1 Isolation and identification of *Staphylococcus epidermidis*

A total of 110 skin swabs were collected from the forearms of healthy human volunteers who had not received any systemic or topical antimicrobial treatments for three months prior to sampling. Initially, the swabs were inoculated onto Mannitol Salt Agar (MSA) plates and incubated aerobically at 37 °C for 24 hours. Colonies exhibiting the typical morphological characteristics of *Staphylococcus epidermidis* appearing as small, moist, creamy, pink-to-white colonies with no discoloration of the surrounding medium were selected. These suspected colonies were then subcultured onto fresh MSA plates to obtain pure cultures. Figure (4-1) shows the growth of *S. epidermidis* isolates as well as the growth of *S. aureus* on MSA agar after aerobic incubation of 24 h at 37 °C.

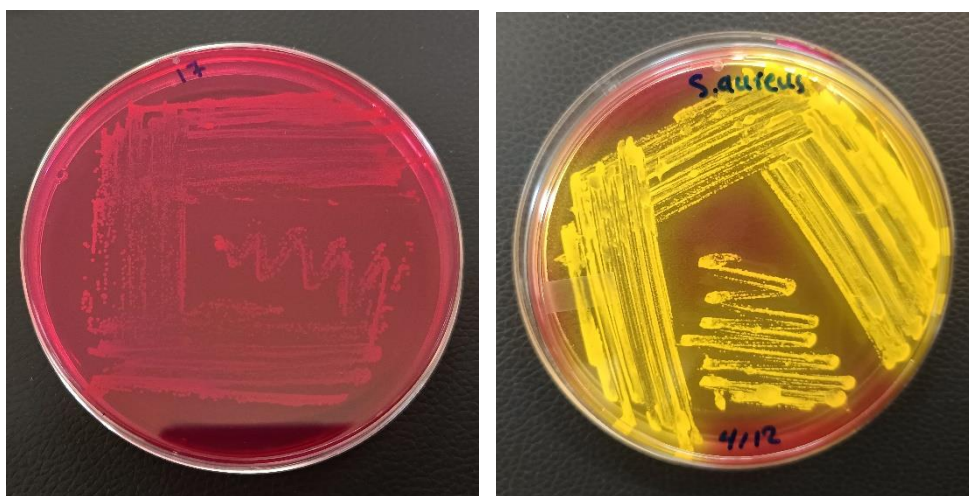


Figure (4-1): Growth of *Staphylococcus epidermidis* and *Staphylococcus aureus* on MSA after aerobic incubation of 24 h at 37 °C

For presumptive identification, the purified isolates were evaluated microscopically using Gram stain, revealing Gram-positive cocci arranged in irregular clusters. Further preliminary characterization included cultivating the isolates on Blood Agar which reveal gamma type analysis, alongside primary biochemical tests, which confirmed the isolates were

catalase-positive and oxidase-negative. Finally, the definitive identification of the isolates at the species level was conducted using the automated VITEK 2 system.

The VITEK 2 results revealed that the recovered isolates belonged to the Coagulase-Negative Staphylococci (CoNS) group, which included *Staphylococcus epidermidis*, alongside other normal skin microbiota residents such as *Staphylococcus hominis* and *Staphylococcus lentus* as shown in table (4-1). For the purpose of this study, only the confirmed *S. epidermidis* isolates were selected and preserved for further downstream applications.

Table (4-1): Distribution and percentage of Coagulase-Negative staphylococci (CoNS) isolate

Isolate type	Number of isolates	Percentage
<i>S. epidermidis</i>	51	46.36
<i>S. Hominis</i>	20	18.1
<i>S. lentus</i>	5	4.5

4.2 Antibiotic Susceptibility Testing for *S. epidermidis*

Considering that *S. epidermidis* is rarely employed as a probiotic (Yang et al., 2019), it was essential to evaluate the antibiotic sensitivity profile of the isolate *S. epidermidis* n7, especially given the increasing prevalence of multidrug-resistant (MDR) infections worldwide. This assessment also acts as a precautionary step should any symptoms arise. In this study, the choice of antibiotics and the analysis of inhibition zones were meticulously performed following the Clinical and Laboratory Standards Institute (CLSI) guidelines for *Staphylococcus* species, in conjunction with standard clinical protocols for managing bacterial skin

infections. The antibiotic panel specifically included those commonly prescribed by dermatologists for the effective management of bacterial skin infections (James et al., 2019).

Table (4-2): Antibiotic susceptibility of some antibiotics against the selected isolate *S. epidermidis* n7

Antibiotic	Symbol	Inhibition zone in mm	CLSI (2026) Breakpoints (mm)
Cefoxitin	FOX	34mm	≥ 25
Erythromycin	E	30mm	≥ 23
Clindamycin	DA-10	30mm	≥ 21
Trimethoprim	TR	33mm	≥ 16
Ciprofloxacin	CIP-10	35mm	≥ 21
Tetracycline	TE-10	24mm	≥ 16
Doxycycline	DO-10	27mm	≥ 16
Gentamicin	CN-10	25mm	≥ 15
Vancomycin	VA	20mm	–
Linezolid	LZ	30mm	≥ 26
Levofloxacin	LE	33mm	≥ 19
Rifampin	RA-6	35mm	≥ 20

During the preliminary antibiotic screening of the recovered *S. epidermidis* isolates, the majority exhibited phenotypic resistance to cefoxitin, indicating an unexpectedly high prevalence of Methicillin-Resistant *S. epidermidis* (MRSE) even among commensal strains. Notably, only three isolates demonstrated complete phenotypic susceptibility. From these rare susceptible strains, isolate (n7) was deliberately selected for all subsequent downstream applications and postbiotic formulation to ensure maximum safety and avoid any MDR-associated risks.

As presented in Table (4-1), The local *Staphylococcus epidermidis* isolate had an extremely sensitive profile on the antibiotic sensitivity tests. The isolate was sensitive to 12 antibiotics. The existence of this large response (as evidenced by an accurate measurement of the inhibition zones) suggests that this isolate does not have the phenotype of multidrug-resistant (MDR) bacteria. This observation further emphasizes the commensal nature of this particular strain, thus elevating its potential as a suitable strain to apply in the restoration of a healthy skin microbiome.

Thus, it is concluded that *S. epidermidis* n7 was multi-drug sensitive, which corroborates the results of Bora *et al.* (2018). In their study, *S. epidermidis* was the most prevalent species (56.7%) among coagulase-negative staphylococci (CoNS) and demonstrated a 100% susceptibility rate to critical antibiotics like linezolid and vancomycin, alongside an 80.2% susceptibility rate to doxycycline.

The high susceptibility profile observed in the local *S. epidermidis* isolate (n7) in the current study is well-aligned with these findings. However, this stands in stark contrast to the alarming global trend of increased resistance among clinical isolates, particularly against critical antibiotics such as glycopeptides and oxazolidinones (namely vancomycin and linezolid). For instance, Szemraj *et al.* (2023) noted a confirmed heterogeneous resistance profile to vancomycin in 6 out of 65 *S. epidermidis* strains. Furthermore, their investigations showed that 56 of these 65 clinical strains were phenotypically resistant to methicillin, and all tested multi-resistant strains genetically harbored the *mecA* gene. Similarly, Shabana *et al.* (2022) demonstrated that 70% of *S. epidermidis* strains isolated from prosthetic joint infections (PJI) exhibited reduced susceptibility to vancomycin ($\text{MIC} \geq 2 \mu\text{g/mL}$). Furthermore, Methicillin-

Resistant *S. epidermidis* (MRSE) has become highly prevalent in clinical settings.

According to Xu et al. (2020), 76.5% of the *S. epidermidis* isolates recovered from healthcare workers were identified as MRSE and were 100% resistant to cefoxitin, with the majority harboring the mobile genetic element *SCCmec* type IV.

Behshood et al. (2020) reported a 60% methicillin resistance rate among nosocomial isolates, alongside an 80% prevalence of the β -lactamase gene (*blaZ*). Corroborating this alarming trend, the aforementioned study by Shabana et al. (2022) reported a strikingly high methicillin resistance rate of 82% among their PJI cases.

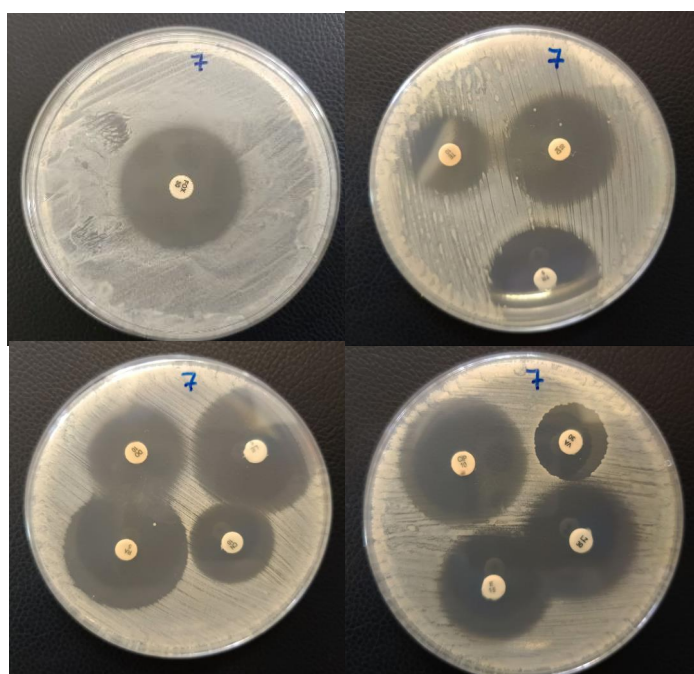


Figure (4-2): Antibiotic susceptibility of some antibiotics against the selected isolate *S. epidermidis* n7

4.3 Molecular detection of the *mecA* gene and phenotypic-genomic variation

Molecular analysis via polymerase chain reaction (PCR) and subsequent gel electrophoresis revealed a surprising genomic profile the selected local isolate (n7) harbored the *mecA* gene, which encodes the variant penicillin-binding protein (PBP2a) responsible for methicillin resistance. This is in contradiction with the phenotypical results obtained in which the isolate was highly sensitive to the antibiotic cefoxitin as shown in Figure (4-3).

This is called oxacillin-susceptible *mecA*-positive *S. epidermidis* (OS-MRSE). Typical laboratory conditions are believed to cause the *mecA* gene to be in an unexpressed or silent state, resulting in a discrepancy between genotype and phenotype. It has been shown that this dormancy could be related to mutations in the promoter region or the presence of repressor genes (e.g. *mecI* and *blaI*) that prevent expression of the PBP2a protein unless the bacteria are subjected to high levels of selective pressure from beta-lactam antibiotics.

The *mecA* gene is present in the genome of the isolate and is a latent risk factor, but the isolate is phenotypically susceptible and is a commensal organism. The skin microbiome is highly dynamic, and if live bacteria with a silent resistance gene are introduced onto human skin, they can be picked up by other naturally occurring strains of bacteria that live on the skin, like *Staphylococcus aureus*, and thereby create highly resistant strains (MRSA) to which it is difficult to find treatments.

Additionally, future exposure to an antibiotic on the skin could act as an "inducer" to trigger the dormant gene in the formulation of the hydrogel. Considering the above-mentioned molecular information, one of

the most important methodological decisions which had to be made in this study was to avoid employing live bacterial cells as a 'live' ingredient in the topical formulation. The research focused on the recovery of cell-free supernatants (postbiotics) as a biosafety approach.

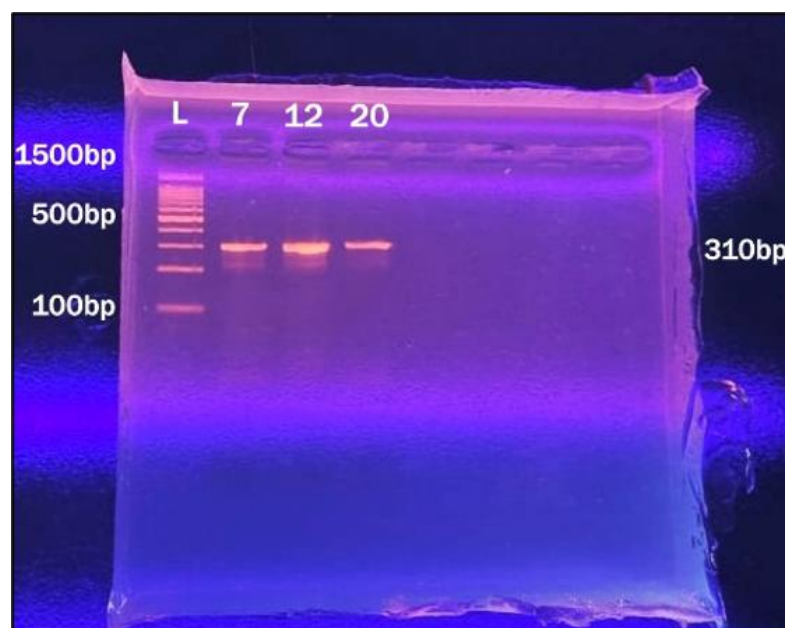


Figure (4-3): Agarose gel electrophoresis of mecA PCR amplicon (310 bp) from cefoxitin-susceptible *S. epidermidis* isolates, electrophoresed on [1.5%] agarose gel in [1× TBE] buffer at [80 V] for [60 min] and stained with [ethidium bromide]. Lane L: DNA ladder; Lanes 7, 12, and 20: Positive bands of mecA gene at 310 bp.

In 2023, Abdelwahab et al. studied 43 clinical isolates of Coagulase-Negative Staphylococci (CoNS) isolated in Egypt and found that 30.2% were mecA-positive and oxacillin-susceptible (OS-CoNS). The authors emphasized the need for screening both with the standard phenotypic test and with a genotypic test, in addition to phenotypic testing.

Furthermore, Katkowska et al. (2025) have specifically studied the OS-MRCoNS phenomenon employing phenotypic methods (disk

diffusion, latex agglutination, agar dilution, chromogenic agar) alongside molecular techniques (*mecA*, *mecB*, *mecC*) in a study involving 101 CoNS strains. Their results showed that 6.9% of all CoNS were identified as *mecA*-positive (OS-MRCoNS) and 43.8% of *mecA*-positive strains were classified as OS-MRCoNS. Notably, OS-MRCoNS had much lower resistance to non-beta-lactam antibiotics than phenotypically MRCoNS, which suggests that the *mecA* under-expression in OS-MRCoNS correlated with a less extensively resistant background.

Moreover, this phenomenon is not limited to localized isolates but is consistently reported across various clinical settings. In a comprehensive study of 412 staphylococcal blood cultures, Williams et al. (2020) corroborated these findings. While they reported a 100% concordance between the absence of the *mecA* gene and oxacillin susceptibility in monomicrobial CoNS, they crucially identified that 7.1% of *mecA*-positive monomicrobial CoNS cultures exhibited an unexplained oxacillin-susceptible phenotype (OS-MRCoNS). The presence of this phenomenon even among severe bloodstream infections globally reinforces the necessity of the dual phenotypic-genotypic screening approach adopted in the current study.

4.4 Antimicrobial activity of Cell-free Supernatant of *S. epidermidis*

The inhibitory evaluation indicated that the cell-free supernatant (CFS) derived from *S. epidermidis* (isolate n7) effectively suppressed the proliferation of pathogenic *S. aureus*, resulting in a notable zone of inhibition measuring 10 mm in diameter as shown in figure (4-4). This quantitative result is significant; it substantiates that the antagonistic influence of the local isolate transcends simple physical competition for

space and nutrients among living organisms. Rather, it illustrates a robust chemical antagonism facilitated by the release of extracellular metabolic products (postbiotics) that can induce a direct lethal or inhibitory effect on the target pathogen.

It is extensively recognized that commensal strains of *S. epidermidis* synthesize a wide variety of extracellular antimicrobial peptides (AMPs), particularly bacteriocins, which exhibit strong activity against closely related Gram-positive pathogens, including *S. aureus*.



Figure (4-4): Antagonistic effect of the *Staphylococcus epidermidis* cell-free supernatant against *Staphylococcus aureus*.

Janek et al. (2016) conducted a comprehensive investigation demonstrating that 84% of the tested nasal *Staphylococcus* isolates were capable of producing antimicrobial substances, particularly under habitat-specific stress conditions. The researchers identified a representative bacteriocin, related to the nukacin peptide family, produced by the *Staphylococcus epidermidis* strain IVK45. Furthermore, they confirmed that the genetic inactivation of this specific bacteriocin significantly

reduced the strain's capacity to inhibit the growth of other competing nasal bacteria.

Building upon this commensal protective role, Nakatsuji et al. (2017) provided groundbreaking in vivo evidence demonstrating that healthy human skin is heavily colonized by CoNS, prominently *S. epidermidis*, which actively secrete highly potent, strain-specific antimicrobial peptides (AMPs). Their high-throughput screening revealed that these AMP-producing strains selectively kill *S. aureus* and are significantly depleted in patients suffering from skin dysbiosis. Crucially, their study highlighted a profound immunological interaction: these bacterial AMPs do not act in isolation, but rather synergize potently with the human innate immune antimicrobial peptide (LL-37) to amplify the clearance of *S. aureus*. While Nakatsuji utilized live bacterial applications to achieve this microbiome restoration, the current study innovatively captures these identical extracellular metabolites within the cell-free supernatant (postbiotics).

More directly, Jang et al. (2020) observed clear inhibition zones when live planktonic *S. epidermidis* was used in an agar well diffusion assay against *S. aureus* and MRSA. The authors isolated a novel, thermolabile, cytoplasmic bacteriocin (40-70 kDa) from *S. epidermidis* that specifically targets *S. aureus* and MRSA, proposing its potential for topical atopic dermatitis treatment. However, their research is particularly interesting because their non-lysed cell-free supernatant (CFS) completely failed to show any inhibitory activity, suggesting that their specific bacteriocin was strictly retained intracellularly. In stark contrast, the current study successfully demonstrated potent inhibitory activity using the directly recovered cell-free supernatant of isolate (n7) without requiring cell lysis. This crucial functional difference indicates that the local isolate

actively secretes its antimicrobial metabolites (postbiotics) into the extracellular environment, making it a vastly superior, safer, and more scalable candidate for non-living topical formulations.

Recently, Newstead et al. (2020) conducted a systematic review of staphylococcal derived bacteriocins, and found that many of these were isolated from commensal coagulase negative staphylococci (CoNS), such as *S. epidermidis*, with a number showing strong inhibitory properties against *S. aureus* in vitro and in vivo. Such bacteriocins are designed to target the ability of bacteria to form biofilms, and show novel modes of action that are not affected by antibiotic resistance. Beyond direct bactericidal activity, Canovas et al. (2016) demonstrated a sophisticated non-lethal mechanism acting as an inter-species communication system: an auto-inducing peptide (AIP) produced by *S. epidermidis* is capable of cross-inhibiting the accessory gene regulator (agr) quorum-sensing system of *S. aureus*. This cross-inhibition effectively down-regulates the expression of critical virulence factors governed by the agr system. Consequently, the authors proposed utilizing such natural staphylococcal interactions as a novel anti-virulence therapy. Thus, the overall antagonistic effect of *S. epidermidis* is multifaceted, combining both direct lethal action and virulence attenuation.

4.5 Characterization of the Lyophilized Postbiotic Extract Based on Appearance, pH and Mass

A total of 10.5 g of fine, yellowish powder was successfully lyophilized from the cell free supernatant of 600 mL. The color and texture of the resulting powder are consistent with standard lyophilized aqueous microbial extracts as shown in Figure (4-5). Crucially, the lyophilization (freeze-drying) process was highly effective in converting the crude, unstable liquid CFS into a stable, concentrated solid product. This phase

transition is vital for preserving the integrity of the thermolabile antimicrobial peptides and extending their shelf life. Furthermore, converting the liquid postbiotic into a dry powder allows for precise gravimetric standardization of the active ingredient during the subsequent formulation of the topical hydrogel.



Figure (4-5): lyophilized *S. epidermidis* cell-free supernatant

Several studies have successfully applied lyophilization to cell-free supernatants and obtained comparable solid powders, supporting the feasibility and reproducibility of this approach.

Bari *et al.* (2018) conducted a pilot production of freeze dried MSC-secretome using around 500 mL of conditioned culture media in each batch. They obtained a free soluble powder containing extracellular vesicles and proteins after ultrafiltration and lyophilization with mannitol as the cryoprotectant. They found yields of about 882 to 1150 mg per batch,

in three production runs, showing that lyophilization of culture supernatants consistently yields recoverable solid material.

A study comparing freeze-drying (FD) and spray-drying (SD) of *Pediococcus acidilactici* cell-free extracts reported that FD achieved an efficiency of approximately 98%, defined as the ratio of mass of solutes in the final powder to the mass of solutes in the starting mixture (Noguerol *et al.*, 2021). The high recovery rate is indicative of the fact that the lyophilization process should preserve the bulk of the dissolved solids in the CFS.

Hossain *et al.* (2021) prepared cell-free freeze-dried supernatants from two LAB genera, *Lactobacillus sakei* and *Lactobacillus plantarum*, and found that the lyophilized extracts had antimicrobial activity against *Listeria monocytogenes*, with minimum effective amounts of 55 mg/mL and 40 mg/mL for *L. sakei* and *L. plantarum*, respectively, in a milk model. This is a confirmation of the bioactivity of lyophilized CFS powders after reconstitution.

The lyophilized microbial culture supernatant yield of 10.5g in this study is within the range expected for microbial culture supernatants, which contain a combination of secreted metabolites, proteins, organic acids, salts and residual media components.

4.6 Formulation and Characterization of the Hydrogel Base

The results achieved confirmed the effective creation of a homogeneous aqueous gel through the application of a Carbopol polymer base. The observed consistency is explained by the cross-linking and extension of polymer chains that occurred with the addition of Triethanolamine (TEA), which neutralized the medium and ensured a stable pH of 5.5 as shown in Figure (4-6).

A pH of 5.5 is considered optimal as it closely aligns with the natural acid mantle of the skin. This minimizes irritation risks and provides suitable physiological conditions for the activity of the postbiotics contained within the cell-free extract of *Staphylococcus epidermidis*. Moreover, glycerin was effective in ensuring the moisture content of the gel and preventing its drying, which further enhanced its spread ability on skin. No phase separation or sedimentation was also noticed, which proved the efficiency of the cold incorporation method to keep the composition homogeneous without subjecting bacterial extract to heat stress that could cause inactivation of the active proteins in the bacterial extract.

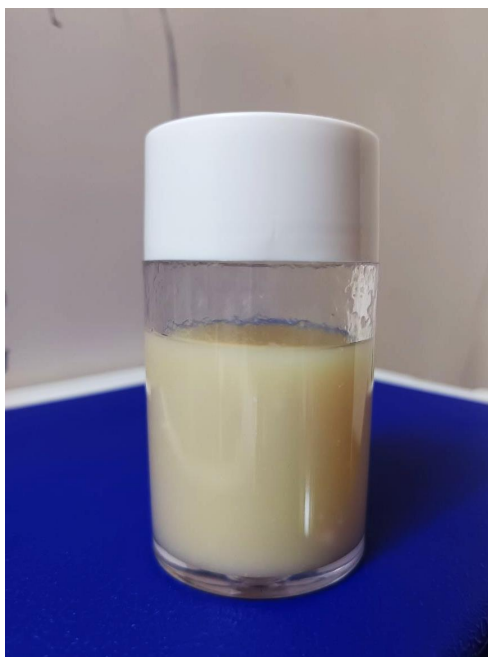


Figure (4-6): Hydrogel containing *S. epidermidis* cell-free supernatant

This finding is consistent with the results reported by Ran *et al.* (2024), who have shown that carbomer-glycerol-TEA hydrogel systems can form three-dimensional network structures that are suitable for topical use. Likewise, Houllberghs *et al.* (2022) found that the neutralization of TEA in Carbopol can be used to fine-tune the pH value and the viscosity

of the gel by cross-linking, with the best gel formation result in ranges of acidic to neutral pH.

Most important quality attribute is the stabilization of the formulation at pH 5.5, which is in the natural range of the acid mantle of the skin (pH 4.5-6.0). The acidic pH of the skin's outer layer, the stratum corneum, is crucial for preserving epidermal barrier function, facilitating corneocyte shedding, and facilitating antimicrobial defense, (Kumar *et al.* 2023). Additionally, following a detailed review, Lukić *et al.* (2021) found that the topical formulations should be acidified to pH 4-6 for skin homeostasis, where pH 5.5 is the optimum value, considering the balance between skin compatibility and the stability of the formulation.

This range of pH minimizes the potential for skin irritation and provides beneficial physiological conditions for the action of postbiotics produced by the cell-free supernatant of *Staphylococcus epidermidis*.

The incorporation of glycerin as a humectant worked well in maintaining moisture content and in preventing the gel drying, improving the spread ability on skin. This is consistent with Joukhadar *et al.* (2024) who found that glycerol containing hydrogels had better moisture holding capacities and skin hydration properties and with Gómez-Farto *et al.* (2024) who reported that glycerol containing topicals reduced TEWL and increased hydration of SC.

The lack of phase separation or sedimentation indicates the efficiency of cold incorporation method which does not impose any thermal stress on the bacterial extract and ensures compositional homogeneity. This is especially significance for maintaining the bioactivity of the proteins and metabolites in the Postbiotic Extract. The rationale to use cold incorporation was demonstrated by Duarte *et al.*

(2023), who showed that antioxidant, anti-inflammatory, and antimicrobial activity is not altered by processing the extracts in a gentle manner.

The use of postbiotics of *Staphylococcus epidermidis* is also justified by De Almeida *et al.* (2023) who comprehensively demonstrated that postbiotics applied to the skin have the beneficial effects of improving the barrier function, anti-inflammatory properties and modulation of the skin microbiome.

Furthermore, Kim *et al.* (2023) have shown that the postbiotic ferment filtrates enhance the barrier function, elasticity and dermal density of the skin, while increasing the amount of useful commensal bacteria, such as *Staphylococcus* species.

The findings of the present study collectively support the formulation approach used in this study, which resulted in the development of a stable and skin compatible formulation for delivering bioactive postbiotic components without compromising their functional integrity, using “postbiotic” cell-free culture supernatant and cold incorporation method into the Carbopol-TEA formulation at pH 5.5.

4.7 Antimicrobial Activity of Lyophilized *S. epidermidis* CFS Against *S. aureus*

The agar well diffusion method showed that lyophilized cell-free supernatant of *S. epidermidis* possessed strong antimicrobial activity and concentration-dependent activity against *S. aureus*.

The inhibition zone was 31 mm at the highest concentration tested (0.1 g/mL) and is interpreted as strong activity based on the standard interpretive criteria for the agar well diffusion method. The inhibition zone was gradually reduced as the concentration was lowered: 25 mm at 0.05

g/mL, 20 mm at 0.025 g/mL and 14 mm at 0.0125 g/mL as shown in (4-7) figure.

The regular decrease of zone diameter that was observed for each serial dilution is consistent and demonstrates a direct dose-response relationship between lyophilized extract concentration and antimicrobial activity. Interestingly, the lowest concentration tested (0.0125 g/mL) showed measurable inhibitory activity (14 mm), indicating that the bioactive metabolites of the lyophilized CFS are effective over a wide range of concentrations.

The concentration was reduced by about twofold, giving a corresponding reduction in zone diameter, as would be expected with the diffusion of a mixture of antimicrobial agents through the medium of the agar.

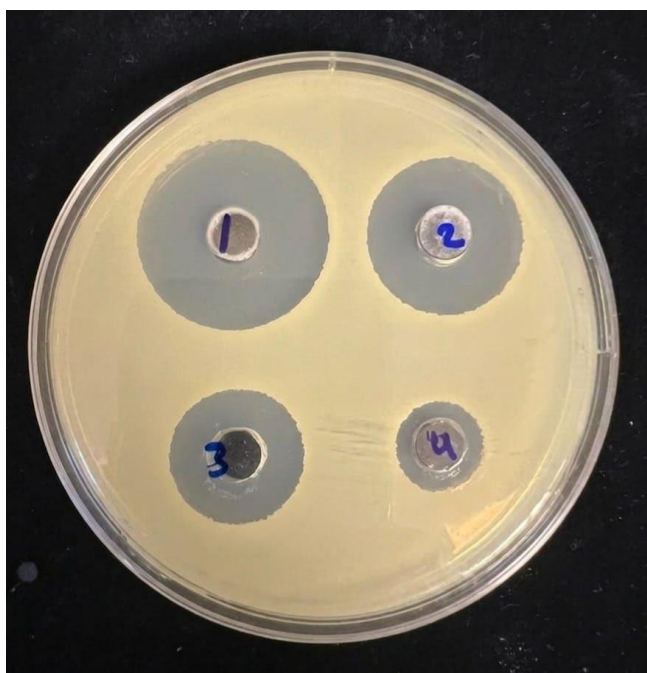


Figure (4-7): Antimicrobial Activity of lyophilized cell-free supernatant of *S. epidermidis* against *S. aureus*. (1) represent 0.1g/mL of CFS, (2) represent 0.05g/m L of CFS, (3) represent 0.025g/mL of CFS and (4) represent 0.0125g/mL of CFS.

Several studies are consistent with the anti-staphylococcal activity of *S. epidermidis* cell-free supernatants demonstrated here.

A marine strain of *S. epidermidis* (S14) was isolated from marine products by Zhang et al. (2016) that showed broad-spectrum antimicrobial activity against *S. aureus*, *E. coli*, *B. cereus* and *Shigella castellani*. These antibacterial metabolites were pepsin, trypsin, and lysozyme resistant, and survived heating at 100 degrees C for 30 minutes, demonstrating that *S. epidermidis* strains can produce thermostable antimicrobial products in the extracellular environment similar to the observation reported in current study regarding the lyophilized extract.

This finding is consistent with reports that many *S. epidermidis* strains secrete diffusible antimicrobial peptides and bacteriocins active against *S. aureus*, a trait implicated in its role as a protective skin commensal (Burke et al., 2024). Notably, this activity is strain-dependent rather than a universal species trait: CFS from the reference strain *S. epidermidis* ATCC12228 showed no inhibitory activity against *S. aureus* by the same method, with only live cells and a purified intracellular bacteriocin fraction active (Jang et al., 2020), underscoring that the extracellular, dose-responsive activity observed here reflects a specific property of the isolated strain rather than an expected species-wide result.

Although diffusion tests do not accurately identify the inhibitory agent, *S. epidermidis* is known to secrete heat-resistant antibiotic bacteriocins that directly affect *S. aureus*. An example of this is the clinical strain KSE112, which secretes the pore-forming bacteriocin (Pep5), which has proven effective against various cocci, including *S. aureus*. This effectiveness is influenced by the stress response systems of the target bacteria (Suzuki *et al.*, 2024)."

Researchers investigated the active role of staphylococcal structures (CoNS), specifically the novel bacterium *S. epidermidis*, in the culture of (MRSA). They discovered that this bacterium is capable of producing bacteriocins. They were able to remove and identify an antimicrobial peptide secreted by the *S. epidermidis* strain, which demonstrated potent and direct effects on MRSA, inhibiting biofilm formation. These findings highlight the importance of *S. epidermidis* as a promising alternative therapeutic source in the context of antibiotic resistance (Twomey *et al.*, 2024).

4.8 Histological evaluation of the effect of hydrogel containing cell-free Supernatant (CFS) of *S. epidermidis* bacteria on mice.

For the purpose of assessing the histological safety and biological effect of the topical preparation, tissue sections of mouse skin were examined after the application of hydrogel containing cell-free supernatant (CSF) of *S. epidermidis* bacteria at different concentrations. Microscopic examinations showed the following results:

- **Group 1 (Control)**

Microscopic examination of the skin in the control group showed normal skin structure, with a normal-thickness epidermis consisting of its four layers. The dermis contained regular collagen fibers with a normal distribution of blood vessels, sebaceous glands, and hair follicles, without any inflammatory infiltration or obvious pathological changes as showed in Figure (4-8).

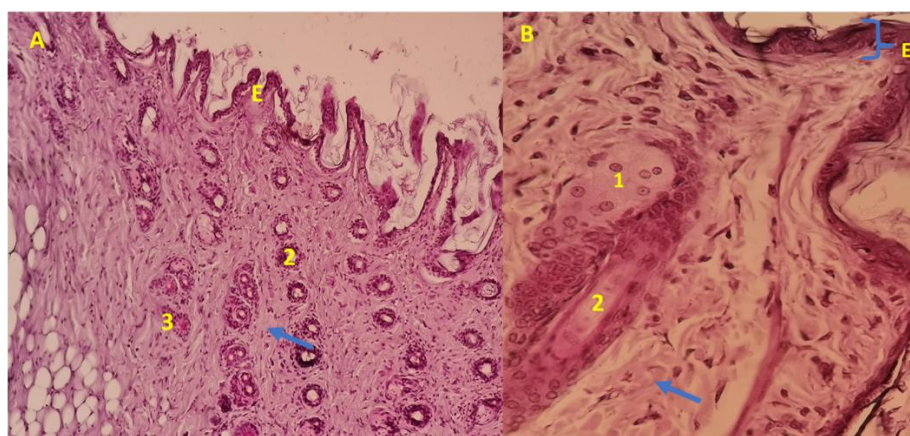


Figure (4-8): Microscopic histological sections of the skin in the control group showed the normal skin structure, characterized by a normal-thickness epidermis composed of its four layers. (E) The dermis contained regular collagen fibers (blue arrow) with a normal distribution of blood vessels (3), sebaceous glands, and hair follicles (2). Hematoxylin and eosin staining. Magnification: Figure A: 100X, B: 400X

- **Group 2 (5% gel)**

Histological sections revealed slight improvement in epidermal integrity characterized by more uniform keratinocytes and a slight thickening of the thickness of stratum spinosum without pathological hyperplasia.

There was a reduction in the amount of inflammatory cells scattered throughout the dermis compared with the control group, a slight rise in the number of fibroblasts and improvements in the organization of the collagen fibers. The blood vessels were not dilated or congested.

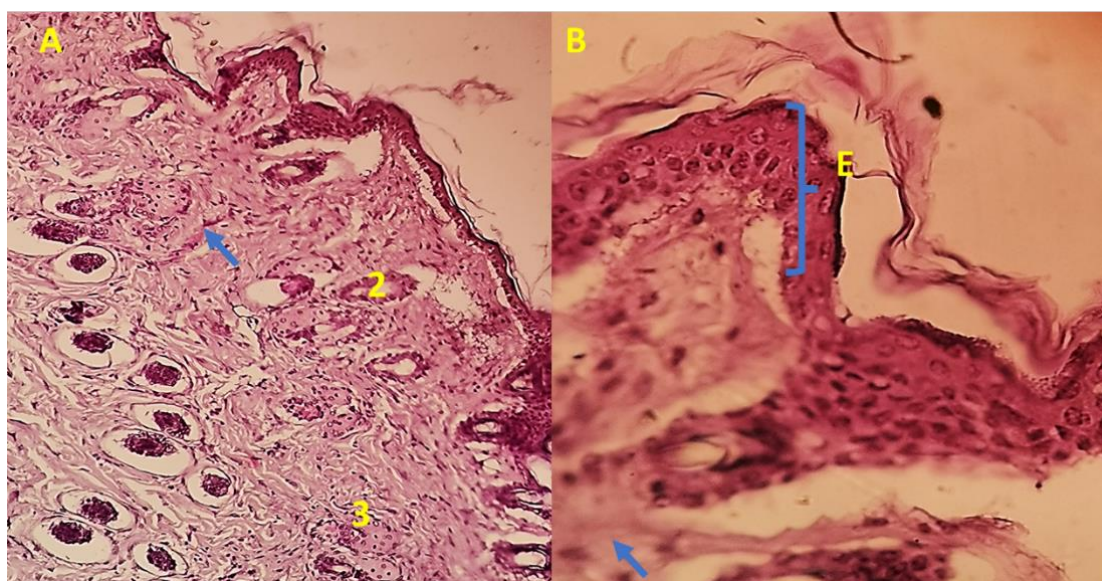


Figure (4-9): Histological sections revealed that the epidermal integrity improved (slightly) with more regularly arranged keratinocytes and without pathological hyperplasia of the stratum, slightly thicker than usual (blue arrow), and normal blood vessels, not dilated nor congested (3). Hematoxylin and eosin staining: A: x200; B: x400

- **Group 3 (10% gel)**

Among these groups, the best histological improvement was observed in this group, where the epidermis became more uniform, with activation of the proliferation of keratinocytes, with moderate increase in thickness. The regularity of all the epidermal layers was preserved and there was no hyperkeratosis noted.

The dermis was characterized by a higher density of organized and parallel collagen fibers, with a marked increase in fibroblast activity. Inflammatory infiltration was minimized with no area of degeneration or tissue edema observed. A marked regularity in the structure of hair follicles and sebaceous glands was noted.

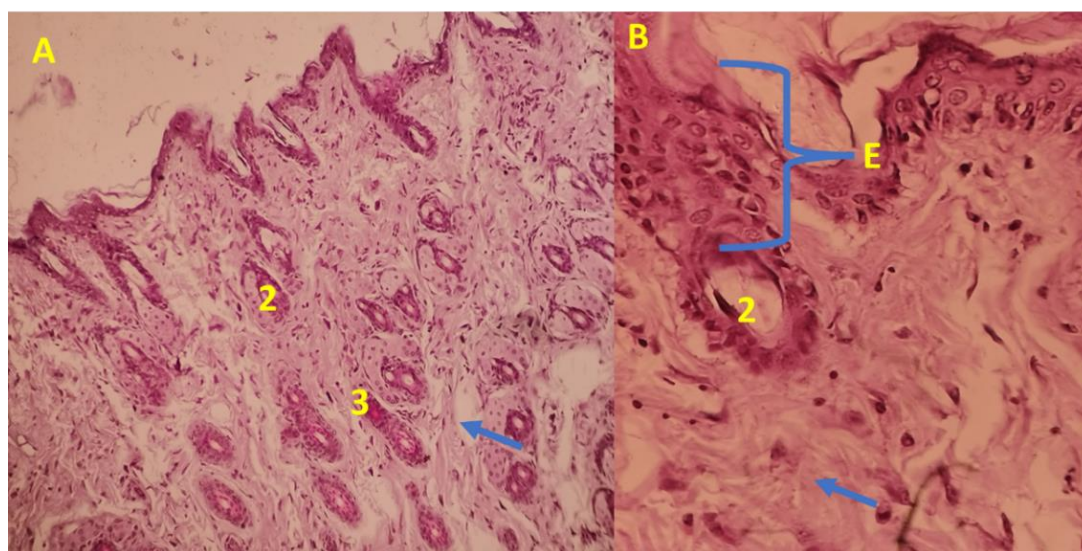


Figure (4-10): This group showed the best histological improvement, with the epidermis becoming more regular (E) and exhibiting a moderate increase in thickness due to activation of keratinocyte proliferation (E). The dermis was characterized by a higher density of organized and parallel collagen fibers (blue arrow) with clear regularity in the structure of hair follicles (2) and blood vessels (3). Hematoxylin and eosin staining: A: x200; B: x400

The observed thicker dose-dependent increase in the thickness of the spinous layer without any pathological hyperplasia, and the observation of more regular keratinocytes, is in line with recent observations on the skin-protective fractions.

The current histological evaluation results, specifically the marked increase in fibroblast activity and the density of parallel and organized collagen fibers in the 10% gel group, are consistent with the molecular mechanisms elucidated by recent studies on the metabolic activity of *S. epidermidis*. These studies have shown that the metabolites secreted by this bacterium (such as short-chain fatty acids, specifically butyric acid) have the ability to restore and stimulate the gene expression of type I collagen in dermal fibroblasts. These metabolites act by binding to the free fatty acid receptor (FFaR2), leading to activation of the p-ERK cell signaling pathway, which stimulates collagen synthesis. This maybe related to mechanism clearly explains the significant histological improvement, increased extracellular matrix (ECM) density, and improved skin layering observed in mice treated with the hydrogel containing the highest concentration of bacterial filtrate (Negari *et al.*, 2021).

On the other hand, the improvement in skin integrity (epidermal integrity) and the reduction of cumulative cells in the dermis tissue of mice treated with hydrogel can be attributed to a regulatory gain in immunity characterized by bacterial infiltrate. It was subsequently shown that extracellular vehicles (EVs) extracted from commensal strains of *S. epidermidis* do not play a significant role in the effect of wounds and inflammation. These components reduce infiltration to thin the thickening. The social group (acanthosis) is well-known from the clove group, creating an anti-inflammatory environment that supports the dermal tissues and

maintains the organization of its layers, as clearly enhanced in the 10% concentration group (Gómez-Chávez et al., 2021).

Likewise, Landemaine *et al.* (2023) demonstrated that strains from healthy skin induce the AhR/OVOL1 differentiation pathway that helps maintain the regularity and moderate thickness of the epidermis in 3D reconstructed skin models, which are non-pathogenic proliferative signals.

Recent study demonstrated that a topical gel preparation containing *S. epidermidis* extract significantly accelerated healing time in an incision wound rat model. Mechanistically, this enhanced regenerative capacity can be attributed to the rich composition of the cell-free supernatant. As highlighted in recent literature, *S. epidermidis* secretes bioactive compounds such as lipoteichoic acid (LTA) and lipopeptide 78, which modulate the local immune response at the wound site. These metabolites interact with keratinocytes via a TLR2-dependent mechanism to inhibit excessive inflammatory cytokine release triggered by the injury, while simultaneously promoting re-epithelization (Mochtar *et al.* 2024).

Recent evidence further supports the profound protective role of this microbiome member against severe environmental insults. For instance, Wang *et al.* (2026) demonstrated that *S. epidermidis* effectively prevents ultraviolet (UV)-induced skin aging and photo-damage by suppressing TLR3-mediated cellular senescence. These advanced mechanistic insights align perfectly with the overarching objective of our formulated topical powder/hydrogel. By rebuilding the skin microbiome balance and delivering active microbial metabolites, this formulation does not merely soothe the skin, but actively stimulates its natural defense mechanisms against both inflammatory triggers and external oxidative stressors. Consequently, the utilization of this local *S. epidermidis* isolate represents a highly promising, next-generation prophylactic and therapeutic approach

in dermatological care, paving the way for advanced treatments aimed at preserving skin barrier integrity and preventing premature cellular senescence."

4.9 Immunological Evaluation of the Topical Formulation

In vivo application of the formulated *Staphylococcus epidermidis* cell-free supernatant targeted to assess its immune system interaction, especially immunomodulatory safety and efficacy, in the skin.

In the clinical study, 15 human volunteers were treated with the formulation on their volar forearms. Upon macroscopic inspection of the application sites, the extract was found to be very well tolerated, with no signs of allergic reaction, redness (erythema) or swelling (edema) being observed. The evaluation was carried out using quantitative immunological assays, measuring three important immunological biomarkers, namely the pro-inflammatory cytokine Interleukin-8 (IL-8), the innate antimicrobial peptides Human Beta-Defensin 2 (HBD-2) and Cathelicidin (LL-37), to further substantiate these clinical observations.

4.9.1 *In vivo* Effect of CFS on the Pro-inflammatory Cytokine (IL-8)

The statistical data analysis showed a significant decrease in the amount of IL-8 after 20 days of topical application of the formulation compared to the baseline before treatment.

IL-8 is a powerful chemoattractant and one of the key markers of inflammatory response and irritation of the skin barrier. This formulation is found to have significant anti-inflammatory and soothing effects, due to the marked reduction of this cytokine. Furthermore, the pure nature of the freeze-dried cell-free supernatant, allowed for a direct and unhindered

interaction between the active bacterial metabolites and the host cells. This absence of an induced inflammatory cascade is good proof of the high biocompatibility and safety-profile of this topical preparation.

Table (4-3): Statistical analysis of Interleukin-8 (IL-8) levels in human volunteers before and after a 20-day application of 5% *S. epidermidis* cell-free supernatant hydrogel.

Marker	Group	N	Mean	SD	SEM	P-Value	Test/Distribution
IL-8	Control	10	200	129	41	0.001>	Non-Normal
IL-8	Before Treatment	15	436	332	86	0.001>	Non-Normal
IL-8	After Treatment	15	397	302	78	0.003	Non-Normal
IL-8	Control vs Before	10 vs 15				0.006	Mann-Whitney U
IL-8	Before vs After	15 vs 15				0.055	Paired t-test
IL-8	Before vs After	15 vs 15				0.026	Wilcoxon signed-rank

IL-8 levels were assessed across three groups: untreated control skin (n = 10), treatment sites before topical application of *S. epidermidis* cell-free supernatant (n = 15), and the same sites after treatment (n = 15). Because the Shapiro-Wilk test indicated significant departures from normality in each of the three raw group distributions ($p < 0.01$ for all), the unpaired comparison between Control and Before Treatment was assessed using the Mann-Whitney U test, revealing a significant baseline reduction in IL-8 in skin destined for treatment relative to control ($U = 25.0$, $p=0.006$).

For the paired Before-vs-After comparison, normality was evaluated on the distribution of individual differences ($d_i = \text{Before} - \text{After}$) rather than on the raw group values, consistent with the assumption underlying the paired t-test. The difference between pre- and post-treatment IL-8 levels was

examined with a paired t-test (the primary analysis, as no evidence to reject the normality assumption was found using Shapiro-Wilk testing of the differences ($W = 0.888$, $p = 0.064$), revealing a reduction in mean IL-8 levels from 436 ± 332 pg/mL (Before) to 397 ± 302 pg/mL (After) that approached, but did not reach, conventional statistical significance ($t = 2.091$, $p = 0.055$). Given the borderline result and modest sample size ($n = 15$), a Wilcoxon signed-rank test was also conducted as a non-parametric robustness check and confirmed the directionality and bolstered the significance of the effect ($W = 21.0$, $p = 0.026$), with 13 of 15 volunteers (86.7%) showing a reduction in IL-8 after treatment.

Overall, results indicate a consistent reduction of IL-8 following the topical application of *S. epidermidis* CFS in both parametric and non-parametric analyses, and the non-parametric test further supports the robustness of this effect in a small and borderline-normal sample.

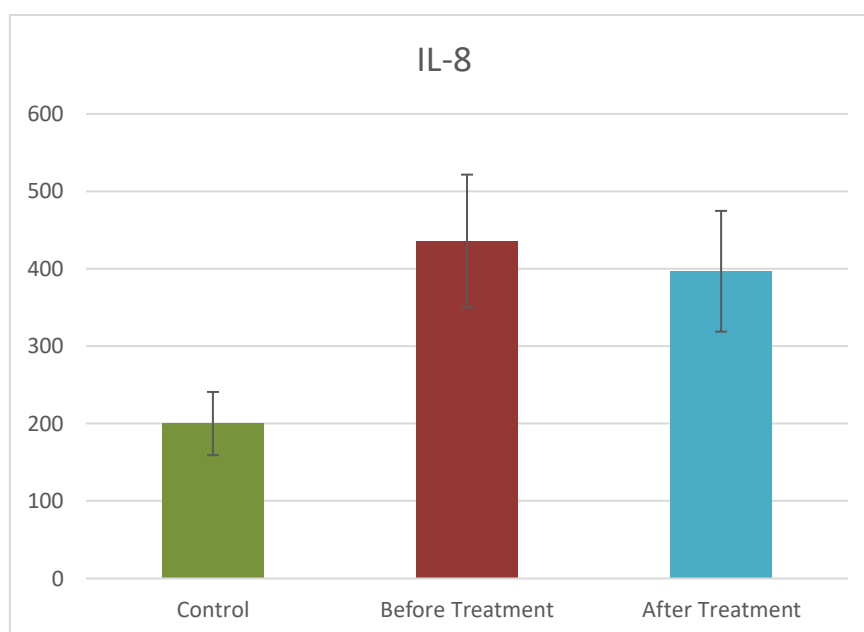


Figure (4-11): Comparison of mean Interleukin-8 (IL-8) levels among the healthy control group and volunteers before and after a 20-day treatment with 5% *S. epidermidis* cell-free supernatant hydrogel. Data are presented as mean $\pm$ SD.

Recent data suggest that *S. epidermidis* secretome has a complex and context-dependent immunomodulatory effect on skin cells.

Baquer *et al.* (2023) have shown that the secretome of *S. epidermidis* has differential effects on the expression of cytokines depending on the cell type; they found that *S. epidermidis* induces expression of IL-8 in fibroblasts and undifferentiated keratinocytes but inhibits the expression of IL-8 in differentiated keratinocytes. The incomplete restoration of IL-8 to control levels in the present study may be a reflection of this type of cell specificity because the topical formulation was potentially applied to more than one cell subpopulation in the skin.

A study by (Shao *et al.*, 2025) demonstrated that the CFS of *S. epidermidis* significantly downregulates the expression of multiple pro-inflammatory cytokines, including IL-8, at both the protein and mRNA levels in a HaCaT cell model. Mechanistically, this immunomodulation is mediated through the inhibition of the MAPK/NF- κ B signaling pathway. The significant decrease in IL-8 levels observed in our human volunteers perfectly aligns with these cellular findings, suggesting that the active metabolites within the CFS of our local isolate effectively suppress inflammatory cascades, thereby promoting skin homeostasis without inducing irritation.

Additionally, Landemaine *et al.* (2023) reported that *S. epidermidis* strains from healthy skin were able to activate the aryl hydrocarbon receptor (AHR) pathway, resulting in epidermal differentiation and barrier integrity, but *S. epidermidis* strains from atopic dermatitis skin did not have the same ability. The results highlight the need to consider strain selection in the formulation of topical therapies containing *S. epidermidis*.

However, it is important to note that *S. epidermidis* is not always anti-inflammatory. Williams *et al.* (2023) demonstrated that the phenol-soluble modulins (PSMs) secreted by some *S. epidermidis* strains are able to induce expression of cytokines in keratinocytes and cause inflammation in the skin, especially in the context of allergic inflammation.

Likewise, Dagnelie *et al.* (2021) showed that imbalances in the ratio of *Cutibacterium acnes* to *S. epidermidis* could trigger inflammatory markers such as IL-8 and IL-6, indicating that the immunological effects of the fractions released depends on the molecular fraction and host microenvironment. The results show the importance of the proper characterization of the composition of CFS to be used as a topical formulation.

4.9.2 *In vivo* Effect of CFS Hydrogel on Antimicrobial Peptide (HBD-2) Levels

The concentration levels of the antimicrobial peptide index (HBD-2) were investigated in 15 volunteers to evaluate the efficiency of a topical gel containing cell-free supernatant (CFS) of *Staphylococcus epidermidis* in triggering a systemic immune response. The trial protocol included topical application of the gel to the forearm once daily for 20 consecutive days.

Table (4-4): Statistical analysis of HBD-2 levels before and after 20 days of topical application of *S. epidermidis* CFS gel.

Marker	Group	N	Mean	SD	SEM	P-Value	Test/Distribution
DEFB2	Control	10	750.94	150	47.5	0.378	Normal
DEFB2	Before Treatment	15	706.31	117	30.23	0.729	Normal
DEFB2	After Treatment	15	818.5	101	26.18	0.547	Normal
DEFB2	Control vs Before	10 vs 15				0.413	Independent t-test
DEFB2	Before vs After	15 vs 15				0.017	Paired t-test

Prior to comparative analysis, normality of DEFB2 (hBD-2) values was assessed for each group. The control group ($p = 0.378$), pre-treatment group ($p = 0.729$) and post-treatment group ($p = 0.547$) were not significantly different from a normal distribution, so parametric tests could be used for subsequent comparisons. Baseline levels of hBD-2 for the pre-treatment group (706.31 ± 117 pg/mL) were not significantly different from the untreated control group (750.94 ± 150 pg/mL; independent-samples t test, $p = 0.413$), indicating that the level of antimicrobial peptides

was comparable to the untreated control group prior to any intervention. A 15.9% increase (mean from 706.31 (SD = 117) to 818.5 (SD = 101)), was found to be statistically significant (paired t-test, $p = 0.017$) after 20 days of daily application of the *S. epidermidis* CFS-loaded hydrogel to the volar forearm.

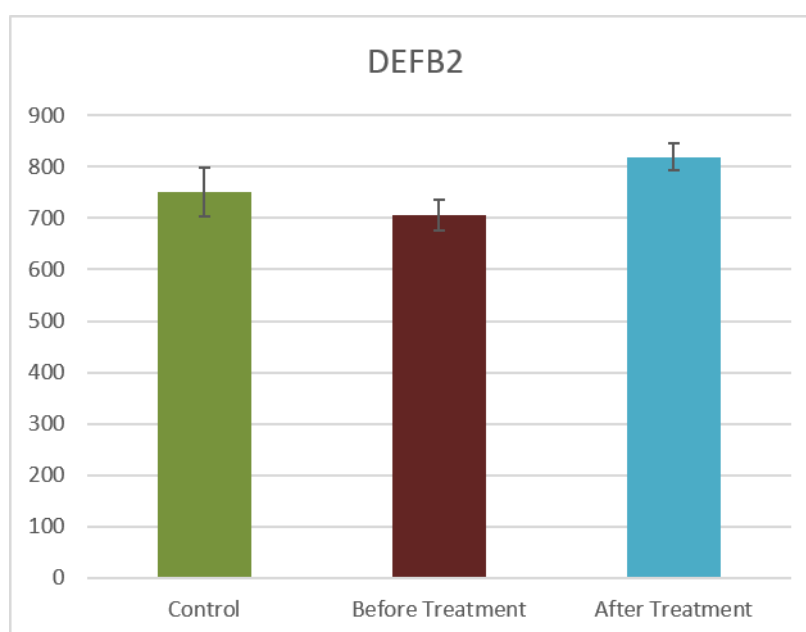


Figure (4-12): Effect of the *S. epidermidis* CFS topical gel on HBD-2 levels among the study groups.

The skin commensal bacteria *S. epidermidis* activates innate defense mediators such as the antimicrobial peptide hBD-2 and IL-1 beta in keratinocytes. Simanski et al., (2018) shows that excessive production of these defense mediators can be controlled by the induction of the host molecule A20 by *S. epidermidis*. A20 is known to inhibit inflammatory signaling and is thought to be associated with chronic inflammatory diseases, including psoriasis. Increasing the expression of A20 could help maintain the balance of *S. epidermidis* as a commensal bacterium on human skin, which would prevent exacerbation of inflammatory responses.

Inducing A20 in specific cutaneous inflammatory diseases could be beneficial.

Mohammed saeed and Manzoor's work has confirmed that cell-free products of *S. epidermidis* are effective in protecting human epidermal keratinocytes from pathogenic skin damage, as well as having protective properties against pathogen-induced toxicity, while stabilising the epidermis in cells in vitro, our in vivo trial focused on the active stimulation of innate immunity through the upregulation of the antimicrobial peptide hBD-2, identifying another mechanism by which cell-free products of *S. epidermidis* protect the skin (Mohammedsaeed & Manzoor, 2022). Together, these two complementary protective mechanisms, competitive exclusion of pathogens at the cell level and concurrent upregulation of the epidermis's innate immunity, demonstrate the effectiveness of these *S. epidermidis* cell-free products as highly safe and multifaceted therapeutic approach to skin infections and promote homeostasis of the epidermis's microbiome.

At the secretome level, soluble factors from skin commensals such as phenol-soluble modulins via TLR2 are enough to induce AMP and cytokine production in keratinocytes without the presence of viable bacteria (Baquer *et al.*, 2023).

All these data contribute to the biological plausibility of the observed in vivo effect and are in line with the rationale for using a postbiotic product (i.e. a cell-free preparation) instead of a live product (bacterial product) to trigger this response.

4.9.3 *In vivo* Effect of CFS Hydrogel on Antimicrobial Peptide (CAMP-LL37) Levels

Following 20 days of once-daily application of the 5% *S. epidermidis* CFS gel, CAMP-LL37 levels were compared across the Control, Before Treatment, and After Treatment groups.

Table (4-5): Statistical analysis of CAMP-LL37 levels before and after 20 days of topical application of *S. epidermidis* CFS hydrogel

Marker	Group	N	Mean	SD	SE M	P-Value	Test/Distribution
CAMP-LL37	Control	10	4.7873	1.95	0.615	0.353	Normal
CAMP-LL37	Before Treatment	15	5.362	1.96	0.507	0.975	Normal
CAMP-LL37	After Treatment	15	5.981	1.89	0.490	0.413	Normal
CAMP-LL37	Control vs Before	10 vs 15				0.479	Unpaired t-test
CAMP-LL37	Before vs After	15 vs 15				0.332	Paired t-test

The quantitative measurements of CAMP/LL-37 peptide levels were generally in the same direction after 20 days of the 5% CFS bacterial extract gel (increasing from 5.36 ± 1.96 to 5.98 ± 1.89 before treatment to after treatment) but 9 out of 15 of the volunteers (60%) had elevated levels, which was not statistically significant as judged by a paired t-test ($p = 0.332$). The normality assumption for the data and the absence of significant outliers indicates that the modest elevations are not likely to represent a strong treatment effect, but instead may represent biological variation in the baseline immune status of the volunteers' skin.

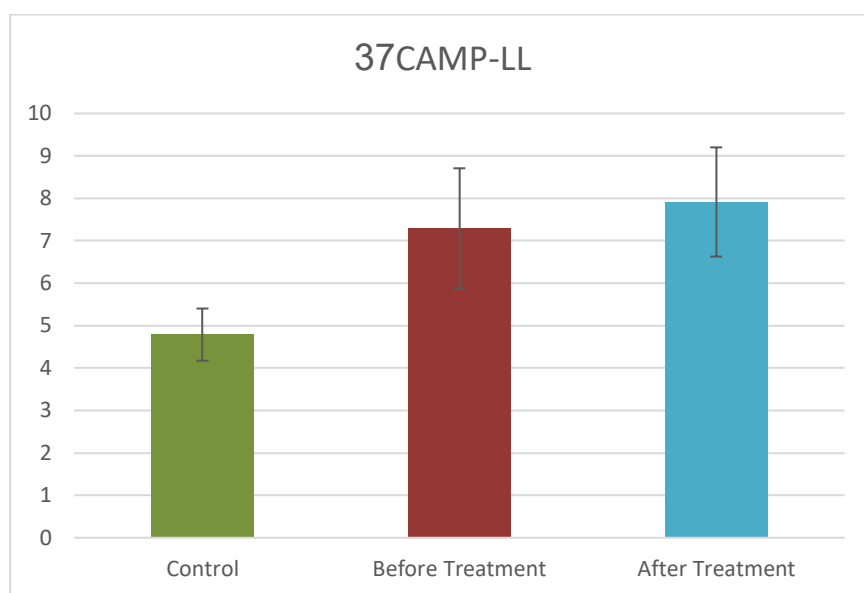


Figure (4-13): Effect of the *S. epidermidis* CFS topical gel on CAMP-LL 37 levels among the study groups.

cathelicidin (LL-37) varies considerably among healthy individuals, even within the same ethnicity. Its concentration in humans varies only slightly, with an average concentration of 1 micromolar in normal individuals. This peptide is structurally expressed in neutrophils, lung tissue, and skin, but it only appears at significant levels in critical situations such as wound healing. Even in healthy, non-inflamed forearm skin from volunteers, the "normal ceiling" for the LL-37 response is inherently limited compared to hBD-2, which is primarily expressed in normal skin (Murakami et al., 2002).

The differential expression patterns observed between hBD-2 and LL-37 following the topical application of the *S. epidermidis* CFS hydrogel can be mechanistically explained by their distinct transcriptional requirements. While hBD-2 is directly induced via innate signaling pathways such as NF- κ B, the expression of the cathelicidin LL-37 is strictly governed by a Vitamin D-dependent pathway.

As elucidated by Liu *et al.* (2006), Toll-Like Receptor (TLR) activation by staphylococcal products does not directly transcribe the LL-37 gene, but rather upregulates the Vitamin D receptor (VDR) and associated hydroxylases. Building upon this molecular framework, Gombart *et al.* (2005) demonstrated that the human cathelicidin (*CAMP*) gene is a direct genomic target of the VDR, requiring the active metabolite 1,25-dihydroxyvitamin D3 to initiate transcription. Consequently, although the applied CFS effectively triggers the initial innate signaling cascades on the skin surface, the subsequent physiological translation into elevated LL-37 levels *in vivo* is inherently restricted by the baseline systemic Vitamin D status of the volunteers. This strict hormonal dependency provides a robust molecular rationale for the limited induction ceiling of LL-37 compared to the robust upregulation of hBD-2.

While other studies have shown rising in LL-37 levels, In an ex vivo human skin model, *S. epidermidis* colonization for 24 hours significantly up-regulated the expression of cathelicidin (LL-37) and this up-regulation persisted after 48 hours post colonization—the expression of this marker is a direct precedent to that of *S. epidermidis* or its products upregulating exactly this marker (Pastar et al., 2020).

In conclusion, the clinical and immunological findings of this study position the formulated *S. epidermidis* CFS hydrogel at the forefront of modern dermatological therapeutics. The escalating global challenge of antimicrobial resistance necessitates a critical shift toward sustainable, non-antibiotic alternatives, particularly host-directed and microbiome-based therapies, as advocated by (Lang *et al.* 2024).

Addressing this global need, our cell-free formulation embodies a highly practical postbiotic approach. As highlighted by Chia *et al.* (2024), utilizing postbiotics (such as sterile CFS) rather than live probiotics

successfully circumvents significant formulation, stability, and safety challenges in skincare. Ultimately, by synergizing the pharmaceutical stability of a postbiotic delivery system with the capacity to safely upregulate endogenous antimicrobial peptides like hBD-2, this CFS hydrogel emerges as a promising, resistance-evasive, and sustainable strategy for reinforcing skin barrier immunity and restoring microbiome homeostasis.

Conclusions and Recommendations

Conclusions

- A local isolate of *Staphylococcus epidermidis* was successfully isolated and identified using conventional microbiological methods and VITEK 2 system. Molecular analysis demonstrated the presence of the *mecA* gene in selected isolate, highlighting the importance of molecular screening for resistance determinants when selecting bacterial isolates intended for postbiotic applications.
- The selected *S. epidermidis* isolate produce a cell free supernatant with detectable antimicrobial activity against *S. aureus*. The observed activity supports the potential of strain derived extracellular products as a source of bioactive compounds with antagonistic activity against a skin associated pathogen.
- The CFS was successfully lyophilized and incorporated into a Carbopol base topical hydrogel. The formulated preparation provided a suitable topical delivery matrix for the tested CFS and retained detectable antimicrobial activity after the processing and formulation.
- Histological examination of mouse skin indicates good tissue tolerance of the CFS containing hydrogel under the experimental conditions. The 10% formulation showed more pronounced histological changes, including increase keratinocyte activity, greater fibroblast activity and more organized collagen fibers, without evident pathological hyperplasia or hyperkeratosis.
- Topical application of the 5% of CFS hydrogel for 20 days was associated with significant reductions in the levels of IL-8.
- Topical applications of the 5% of CFS hydrogel for 20 days was associated with a significant increase in HBD-2 levels
- Balanced effect stable level of LL-37 without over stimulation.

Recommendations

- Further molecular characterization of the selected *S. epidermidis* isolate should be performed to identify additional antimicrobial resistance determinants and functional gene relevant to postbiotic production and safety
- Conducting advanced analytical studies (such as HPLC or GC-MS) to purify and characterize the micro-compounds and metabolites (peptides, enzymes, organic acids) within the cell-free supernatant (CFS) that are directly responsible for this immunomodulatory effect.
- The antimicrobial activity of CFS should be evaluated against a border panel of clinically relevant skin associated microorganism, including different strain of *S. aureus*, particularly MRSA, as well as other relevant bacterial and fungal species.
- Applying the developed hydrogel to a broader range of volunteers, specifically testing it on patients with impaired skin barrier function or inflammatory skin conditions (such as acne, eczema, or atopic dermatitis) to evaluate its therapeutic efficacy.
- Expanded in the future to include measuring the effect of the extract on other cytokines (both pro-inflammatory and anti-inflammatory, such as IL-10 and TNF- α) to create a comprehensive immunological map of the product's mechanism of action.
- Direct assessment of the skin microbiome before and after treatment is recommended using culture independent approach, particularly 16S rRNA gene sequencing to determine whether the formulation alter microbial community composition or diversity.

References

References

-A-

- Abdelwahab, M. A., Amer, W. H., Elsharawy, D., Elkolaly, R. M., Helal, R. A. E. F., El Malla, D. A., Elfeky, Y. G., Bedair, H. A., Amer, R. S., Abd-Elmonsef, M. E., & Taha, M. S. (2023). Phenotypic and Genotypic Characterization of Methicillin Resistance in Staphylococci Isolated from an Egyptian University Hospital.
- Abdurrahman, G., Pospich, R., Steil, L., Salazar, M. G., González, J. J. I., Normann, N., Mrochen, D., Scharf, C., Völker, U., Werfel, T., Bröker, B. M., Roesner, L. M., & Gómez-Gascón, L. (2024). The extracellular serine protease from *Staphylococcus epidermidis* elicits a type 2-biased immune response in atopic dermatitis patients. *Frontiers in Immunology*, 15, 1352704.
- Aimon Khan, Young-Sang Koh. (2024). The Role of Skin Microbiome in Human Health and Diseases. *JOURNAL OF BACTERIOLOGY AND VIROLOGY*, 54(3), 191-202.
- Akaza, N., Takasaki, K., Nishiyama, E., Usui, A., Miura, S., Yokoi, A., ... & Yagami, A. (2022). The microbiome in comedonal contents of inflammatory acne vulgaris is composed of an overgrowth of *Cutibacterium* spp. and other cutaneous microorganisms. *Clinical, Cosmetic and Investigational Dermatology*, 2003-2012.
- Alkhawaja, E., Hammadi, S., Abdelmalek, M., Mahasneh, N., Alkhawaja, B., & Abdelmalek, S. M. (2020). Antibiotic resistant *Cutibacterium acnes* among acne patients in Jordan: a cross sectional study. *BMC dermatology*, 20(1), 17.

References

- Alonso, L. and Mizuno, K. (2021) ‘Epidermal lamellar body biogenesis: insight into the roles of Golgi, endosomes and lysosomes’, *Frontiers in Cell and Developmental Biology*, 9, 722898.
- Andriessen, A., & Lynde, C. W. (2014). Antibiotic resistance: shifting the paradigm in topical acne treatment. *Journal of drugs in dermatology: JDD*, 13(11), 1358-1364.
- Aryal, S., PhD. (2024, November 21). *Staphylococcus epidermidis- An Overview - Microbe Notes*. Microbe Notes.

-B-

- Baker, L. B. (2019). Physiology of sweat gland function: The roles of sweating and sweat composition in human health. *Temperature*, 6(3), 211–259.
- Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*, 6(2), 71-79.
- Baquer, F., Jaulhac, B., Barthel, C., Paz, M., Wolfgramm, J., Müller, A., Boulanger, N., & Grillon, A. (2023). Skin microbiota secretomes modulate cutaneous innate immunity against *Borrelia burgdorferi* s.s. *Scientific Reports*, 13(1), 16393.
- Bari, E., Perteghella, S., Di Silvestre, D., Sorlini, M., Catenacci, L., Sorrenti, M., Marrubini, G., Rossi, R., Tripodo, G., Mauri, P., Marazzi, M., & Torre, M. L. (2018). Pilot Production of Mesenchymal Stem/Stromal Freeze-Dried Secretome for Cell-Free Regenerative Nanomedicine: A Validated GMP-Compliant Process. *Cells*, 7(11), 190.

References

- Becker, K., Heilmann, C., & Peters, G. (2014). Coagulase-Negative staphylococci. *Clinical Microbiology Reviews*, 27(4), 870–926.
- Behshood, P., Tajbakhsh, E., & Momtaz, H. (2020). Recognition of (Sesc) for easy identification of *Staphylococcus epidermidis* and molecular and phenotypic study of β -lactam resistance in *Staphylococcus epidermidis* isolates in Isfahan. *Reports of Biochemistry & Molecular Biology*, 9(1).
- Benson, H. J. (2002). *Microbiological applications: Laboratory manual in general microbiology* (8th ed.). McGraw-Hill.
- Berdyshev, E. (2024) 'Skin lipid barrier: structure, function and metabolism', *Allergy, Asthma & Immunology Research*, 16(5), pp.445–461.
- Betts, J. G., Kelly, K. A., Desaix, P., Johnson, E., Johnson, J. E., Korol, O., Kruse, D., Poe, B., Wise, J., & Womble, M. D. (2022). *Anatomy and physiology 2e*. OpenStax.
- Betts, J. G., Young, K. A., Wise, J. A., Johnson, E., Poe, B., Kruse, D. H., Korol, O., Johnson, J. E., Womble, M., & DeSaix, P. (2022, April 20). 5.1 Layers of the skin - Anatomy and Physiology 2E |
- Bhadade, A., Yadav, J., Veeramachaneni, V., Asati, D., Biswas, D., & Nema, S. (2026). Emerging antimicrobial resistance among *Cutibacterium acnes* isolated from inflammatory acne vulgaris patients. *Journal of Family Medicine and Primary Care*, 15(1), 173-176.
- Biedermann, T., Skabytska, Y., Kaesler, S., & Volz, T. (2015). Regulation of T cell immunity in atopic dermatitis by microbes:

References

- the yin and yang of cutaneous inflammation. *Frontiers in Immunology*, 6, 353.
- Bora, P., Datta, P., Gupta, V., Singhal, L., & Chander, J. (2018). Characterization and antimicrobial susceptibility of coagulase-negative staphylococci isolated from clinical samples. *Journal of Laboratory Physicians*, 10(4), 414–419.
 - Borrego-Ruiz, A., & Borrego, J. J. (2024). Microbial dysbiosis in the skin microbiome and its psychological consequences. *Microorganisms*, 12(9), 1908.
 - Bouwstra, J.A., Krien, P. and Gooris, G.S. (2023) ‘The skin barrier: an extraordinary interface with an exceptional lipid organization’, *Progress in Lipid Research*, 92, 101252.
 - Brandi, J., Cheri, S., Manfredi, M., Di Carlo, C., Vita Vanella, V., Federici, F., ... & Cecconi, D. (2020). Exploring the wound healing, anti-inflammatory, anti-pathogenic and proteomic effects of lactic acid bacteria on keratinocytes. *Scientific reports*, 10(1), 11572.
 - Brescó, M. S., Harris, L. G., Thompson, K., Stanic, B., Morgenstern, M., O’Mahony, L., Richards, R. G., & Moriarty, T. F. (2017). Pathogenic Mechanisms and Host Interactions in *Staphylococcus epidermidis* Device-Related Infection. *Frontiers in Microbiology*, 8, 1401.
 - Broussard, J.A., Getsios, S. and Green, K.J. (2021) ‘Desmosomes polarize and integrate chemical and mechanical signaling to govern epidermal tissue form and function’, *Current Biology*, 31(15), pp.3275–3291.e5.

References

- Brown, G. C. (1995). Reversible binding and inhibition of catalase by nitric oxide. *European Journal of Biochemistry*, 232(1), 188–191.
- Brown, M. M., & Horswill, A. R. (2020). *Staphylococcus epidermidis*—Skin friend or foe?. *PLOS Pathogens*, 16, e1009026.
- Brown, M.M. and Horswill, A.R. (2020) ‘*Staphylococcus epidermidis*—skin friend or foe?’ , PLoS Pathogens, 16(11), e1009026.
- Burke, Ó., Zeden, M. S., & O’Gara, J. P. (2024). The pathogenicity and virulence of the opportunistic pathogen *Staphylococcus epidermidis*. *Virulence*, 15(1), 2359483.
- Byrd, A. L., Belkaid, Y., & Segre, J. A. (2018). The human skin microbiome. *Nature Reviews Microbiology*, 16(3), 143–155.

-C-

- Cambier, S., Gouwy, M., & Proost, P. (2023). The chemokines CXCL8 and CXCL12: molecular and functional properties, role in disease and efforts towards pharmacological intervention. *Cellular and Molecular Immunology*, 20(3), 217–251.
- Canovas, J., Baldry, M., Bojer, M. S., Andersen, P. S., Gless, B. H., Grzeskowiak, P. K., Stegger, M., Damborg, P., Olsen, C. A., & Ingmer, H. (2016). Cross-talk between *Staphylococcus aureus* and other staphylococcal species via the agr quorum sensing system. *Frontiers in Microbiology*, 7, 1733.
- Carvalho, S. M., & Saraiva, L. M. (2026). Metabolic and regulatory adaptations of *Staphylococcus epidermidis* to host stresses. *FEMS Microbiology Reviews*, 50.

References

- Celoria, V., Rosset, F., Pala, V., Dapavo, P., Ribero, S., Quaglino, P., & Mastorino, L. (2023). The skin microbiome and its role in psoriasis: a review. *Psoriasis: Targets and Therapy*, 71-78.
- Chan, A. A., Tran, P. T., & Lee, D. J. (2024). Quantitative aggregation of microbiome sequencing data provides insights into the associations between the skin microbiome and psoriasis. *JID Innovations*, 4(1), 100249.
- Chang, H. W., Yan, D., Singh, R., Liu, J., Lu, X., Ucmak, D., ... & Liao, W. (2018). Alteration of the cutaneous microbiome in psoriasis and potential role in Th17 polarization. *Microbiome*, 6(1), 154.
- Cheung, G. Y. C., & Otto, M. (2025). *Staphylococcus epidermidis*—key to understanding biofilms, commensalism, and more. *Journal of Bacteriology*, 207(9), e00165-25.
- Chia, J., Carma, A., Alwyn, A., Cho, R., Hill, D. S., & Borrello, M. T. (2026). The Skin Microbiome Revolution: The science and challenges of prebiotics, probiotics, and postbiotics in skincare. *Cosmetics*, 13(1), 43.
- Christensen, G. J. M., & Brüggemann, H. (2014). Bacterial skin commensals and their role as host guardians. *Beneficial microbes*, 5(2), 201-215.
- Cogen, A. L., Yamasaki, K., Muto, J., Sanchez, K. M., Crotty Alexander, L., Tanios, J., ... & Gallo, R. L. (2010). *Staphylococcus epidermidis* antimicrobial δ -toxin (phenol-soluble modulins- γ) cooperates with host antimicrobial peptides to kill group A *Streptococcus*. *PloS one*, 5(1), e8557.
- Collee, J. G., Miles, R. S., & Watt, B. (1996). Tests for the identification of bacteria. In J. G. Collee, B. P. Marmion, A. G.

References

Fraser, & A. Simmons (Eds.), Mackie & McCartney practical medical microbiology (14th ed., pp. 131–151). Churchill Livingstone.

-D-

- da Silva Vale, A., de Melo Pereira, G. V., de Oliveira, A. C., de Carvalho Neto, D. P., Herrmann, L. W., Karp, S. G., Soccol, V. T., & Soccol, C. R. (2023). Production, Formulation, and Application of Postbiotics in the Treatment of Skin Conditions. *Fermentation*, 9(3), 264.
- Daniel, W. W., & Cross, C. L. (2018). *Biostatistics: A foundation for analysis in the health sciences* (11th ed.). John Wiley & Sons.
- Dagnelie, M., Corvec, S., Timon-David, E., Khammari, A., & Dréno, B. (2021). *Cutibacterium acnes* and *Staphylococcus epidermidis*: the unmissable modulators of skin inflammatory response. *Experimental Dermatology*, 31(3), 406–412.
- De Almeida, C. V., Antiga, E., & Lulli, M. (2023). Oral and Topical probiotics and postbiotics in skincare and dermatological therapy: A concise review. *Microorganisms*, 11(6), 1420.
- De Tomassi, A., Reiter, A., Reiger, M., Rauer, L., Rohayem, R., Group, C. S., Traidl-Hoffmann, C., Neumann, A. U., & Hülpüsch, C. (2023). Combining 16S Sequencing and qPCR Quantification Reveals *Staphylococcus aureus* Driven Bacterial Overgrowth in the Skin of Severe Atopic Dermatitis Patients. *Biomolecules*, 13(7), 1030.

References

- Deckers, J., Hammad, H. and Hoste, E. (2018) ‘Langerhans cells: sensing the environment in health and disease’, *Frontiers in Immunology*, 9, 93.
- Dessinioti, C., & Katsambas, A. (2022, December 22). Antibiotics and antimicrobial resistance in acne: Epidemiological trends and clinical practice considerations.
- Dinić, M., Gonzalez, T., Hernandez, M. C., Radojević, D., Fernandez, L., Golić, N., & Pastar, I. (2025). Postbiotics as microbial-derived therapeutics for wound healing disorders: from molecular mechanisms to future application. *Cell Communication and Signaling*, 23(1), 504.
- Drumond, M. M., Tapia-Costa, A. P., Neumann, E., Nunes, Á. C., Barbosa, J. W., Kassuha, D. E., & Mancha-Agresti, P. (2023). Cell-free supernatant of probiotic bacteria exerted antibiofilm and antibacterial activities against *Pseudomonas aeruginosa*: A novel biotic therapy. *Frontiers in Pharmacology*, 14, 1152588.
- Duarte, M., Carvalho, M. J., De Carvalho, N. M., Azevedo-Silva, J., Mendes, A., Ribeiro, I. P., Fernandes, J. C., Oliveira, A. L. S., Oliveira, C., Pintado, M., Amaro, A., & Madureira, A. R. (2023). Skincare potential of a sustainable postbiotic extract produced through sugarcane straw fermentation by *Saccharomyces cerevisiae*. *BioFactors*, 49(5), 1038–1060.

-F-

- Fernández-Calderón, M. C., Fernández-Babiano, I., Navarro-Pérez, M. L., Pazos-Pacheco, C., & Calvo-Cano, A. (2025). Biofilm formation and role of other pathogenic factors in the virulence of *Staphylococcus epidermidis* clinical isolates. *Frontiers in Cellular and Infection Microbiology*, 15, 1630341.

References

- Fournière, M., Latire, T., Souak, D., Feuilloley, M. G. J., & Bedoux, G. (2020). *Staphylococcus epidermidis* and *Cutibacterium acnes*: Two Major Sentinels of Skin Microbiota and the Influence of Cosmetics. *Microorganisms*, 8(11), 1752.
- Francino, M. P. (2015). Antibiotics and the human gut microbiome: dysbioses and accumulation of resistances. *Front Microbiol* 6: 1543.
- Fukuda, K., Li, X. and Chen, Y. (2025) ‘The acid mantle plays a significant role in the skin barrier, pH homeostasis, and microbiome composition’, *Journal of Integrative Dermatology*, 3(1), pp.1–12.

-G-

- Glatthardt, T., De Mello Campos, J. C., Chamon, R. C., De Sá Coimbra, T. F., De Almeida Rocha, G., De Melo, M. a. F., Parente, T. E., Lobo, L. A., Antunes, L. C. M., Santos, K. R. N. D., & Ferreira, R. B. R. (2019). Small Molecules Produced by Commensal *Staphylococcus epidermidis* Disrupt Formation of Biofilms by *Staphylococcus aureus*. *Applied and Environmental Microbiology*, 86(5).
- Gómez-Chávez, F., Cedillo-Peláez, C., Zapi-Colín, L. A., Gutiérrez-González, G., Martínez-Torres, I., Peralta, H., Chavez-Galan, L., Avila-Calderón, E. D., Contreras-Rodríguez, A., Bartolo-Aguilar, Y., Rodríguez-Martínez, S., Cancino-Díaz, M. E., & Cancino-Díaz, J. C. (2021). The Extracellular Vesicles from the Commensal *Staphylococcus Epidermidis* ATCC12228 Strain Regulate Skin Inflammation in the Imiquimod-Induced Psoriasis Murine Model. *International Journal of Molecular Sciences*, 22(23), 13029.

References

- Gómez-Farto, A., Jiménez-Escobar, A. L., Pérez-González, N., Castán, H., Clares, B., Arias-Santiago, S., & Montero-Vílchez, T. (2024). Development of an emulgel for the effective treatment of atopic dermatitis: biocompatibility and clinical investigation. *Gels*, 10(6), 370.
- Gombart, A. F., Borregaard, N., & Koeffler, H. P. (2005). Human cathelicidin antimicrobial peptide (CAMP) gene is a direct target of the vitamin D receptor and is strongly up-regulated in myeloid cells by 1,25-dihydroxyvitamin D₃. *The FASEB Journal*, 19(9), 1067–1077.
- Grice, E. A., & Segre, J. A. (2011). The skin microbiome. *Nature reviews microbiology*, 9(4), 244-253.
- Gupta, R. K., Wasnik, P., Mondal, D., & Shukla, D. (2024). Critical role of keratinocytes in cutaneous immune responses. *Exploration of Immunology*, 4(4), 502-522.

-H-

- Habeebuddin, M., Karnati, R. K., Shiroorkar, P. N., Nagaraja, S., Asdaq, S. M. B., Khalid Anwer, M., & Fattepur, S. (2022). Topical Probiotics: More Than a Skin Deep. *Pharmaceutics*, 14(3), 557.
- Hamann, T., Brüggemann, H., Feidenhansl, C., Rruci, E., Gallinger, J., Gallinat, S., & Hüpeden, J. (2025). Distinct Intraspecies Variation of *Cutibacterium acnes* and *Staphylococcus epidermidis* in Acne Vulgaris and Healthy Skin. *Microorganisms*, 13(2), 299.
- Harris-Tryon, T. A., & Grice, E. A. (2022). Microbiota and maintenance of skin barrier function. *Science*, 376(6596), 940–945.

References

- Hashemi, S. S., Rafati, A., Roohinejad, S., Yaghoobi, F., & Salehi, A. (2025). Postbiotics as Emerging Therapeutics for Skin Wound Healing and Dermatological Care: Clinical Trends and Mechanistic Insights. *International Wound Journal*, 22(12), e70799.
- Hossain, M. I., Kim, K., Rahaman Mizan, M. F., Tousehik, S. H., Ashrafudoulla, M., Roy, P. K., Nahar, S., Jahid, I. K., Choi, C., Park, S. H., & Ha, S.-D. (2021). Comprehensive molecular, probiotic, and quorum-sensing characterization of anti-listerial lactic acid bacteria, and application as bioprotective in a food (milk) model. *Journal of Dairy Science*, 104(6), 6516–6534.
- Hou, Y., & Huttenlocher, A. (2024). Advancing chemokine research: the molecular function of CXCL8. *Journal of Clinical Investigation*, 134(10).
- Houllberghs, M., Verheyden, L., Voorspoels, F., Chandran, C. V., Duerinckx, K., Radhakrishnan, S., Martens, J. A., & Breynaert, E. (2022). Magneto-Hydrodynamic Mixing: a New Technique for Preparing Carbomer Hydrogels. *ChemRxiv*.

-I-

- Iyer, V., Raut, J., & Dasgupta, A. (2021). Impact of pH on growth of *Staphylococcus epidermidis* and *Staphylococcus aureus* in vitro. *Journal of Medical Microbiology*, 70(9).

-J-

- Janek, D., Zipperer, A., Kulik, A., Krismer, B., & Peschel, A. (2016). High frequency and diversity of antimicrobial activities

References

- produced by nasal *Staphylococcus* strains against bacterial competitors. *PLOS Pathogens*, 12(8), e1005812.
- Janek, D., Zipperer, A., Kulik, A., Krismer, B., & Peschel, A. (2016). High Frequency and Diversity of Antimicrobial Activities Produced by Nasal *Staphylococcus* Strains against Bacterial Competitors. *PLoS Pathogens*, 12(8), e1005812.
 - Jang, I.-T., Yang, M., Kim, H.-J., & Park, J.-K. (2020). Novel Cytoplasmic Bacteriocin Compounds Derived from *Staphylococcus epidermidis* Selectively Kill *Staphylococcus aureus*, Including Methicillin-Resistant *Staphylococcus aureus* (MRSA). *Pathogens*, 9(2), 87.
 - James, W. D., Elston, D. M., Treat, J. R., Rosenbach, M. A., & Neuhaus, I. M. (2019). *Andrews' diseases of the skin: Clinical dermatology* (13th ed.). Elsevier.
 - Jo, J. H., Harkins, C. P., Schwardt, N. H., Portillo, J. A., NISC Comparative Sequencing Program, Zimmerman, M. D., ... & Kong, H. H. (2021). Alterations of human skin microbiome and expansion of antimicrobial resistance after systemic antibiotics. *Science translational medicine*, 13(625), eabd8077.
 - Joukhadar, R., Nodilo, L. N., Lovrić, J., Hafner, A., Pepić, I., & Jug, M. (2024). Functional nanostructured lipid Carrier-Enriched hydrogels tailored to repair damaged epidermal barrier. *Gels*, 10(7), 466.
- K-**
- Katkowska, M., Kosecka-Strojek, M., Wolska-Gębarzewska, M., Kwapisz, E., Wierzbowska, M., Międzobrodzki, J., & Garbacz, K. (2025). Emerging Challenges in Methicillin Resistance of Coagulase-Negative *Staphylococci*. *Antibiotics*, 14(1), 37.

References

- Kim, H. B., Alexander, H., Um, J. Y., Chung, B. Y., Park, C. W., Flohr, C., & Kim, H. O. (2025). Skin microbiome dynamics in Atopic dermatitis: Understanding Host-Microbiome interactions. *Allergy Asthma and Immunology Research*, 17(2), 165.
- Kim, J., Lee, Y. I., Mun, S., Jeong, J., Lee, D., Kim, M., Jo, H., Lee, S., Han, K., & Lee, J. H. (2023). Efficacy and Safety of *Epidermidibacterium keratini* EPI-7 derived postbiotics in skin aging: a Prospective Clinical study. *International Journal of Molecular Sciences*, 24(5), 4634.
- Kong, H. H., Oh, J., Deming, C., Conlan, S., Grice, E. A., Beatson, M. A., ... & Segre, J. A. (2012). Temporal shifts in the skin microbiome associated with disease flares and treatment in children with atopic dermatitis. *Genome research*, 22(5), 850-859.
- Kumar, P., & Das, A. (2023). Acid mantle: What we need to know. *Indian Journal of Dermatology, Venereology and Leprology*, 89(5), 729-732.

-L-

- Landemaine, L., Da Costa, G., Fissier, E., Francis, C., Morand, S., Verbeke, J., Michel, M., Briandet, R., Sokol, H., Gueniche, A., Bernard, D., Chatel, J., Aguilar, L., Langella, P., Clavaud, C., & Richard, M. L. (2023). *Staphylococcus epidermidis* isolates from atopic or healthy skin have opposite effect on skin cells: potential implication of the AHR pathway modulation. *Frontiers in Immunology*, 14, 1098160.
- Lang, J. C., Shahata, M., & Melican, K. (2024). Towards sustainable antimicrobial therapies for *Staphylococcus aureus* skin infections. *Sustainable Microbiology*, 1(1).

References

- Lee, H. J., & Kim, M. (2022). Skin barrier function and the microbiome. *International journal of molecular sciences*, 23(21), 13071.
- Lee, H.J. and Kim, M. (2022) ‘Skin barrier function and the microbiome’, *International Journal of Molecular Sciences*, 23(21), 13071.
- Lefèvre Utile, A., Braun, C., Haftek, M. and Aubin, F. (2021) ‘Five functional aspects of the epidermal barrier’, *International Journal of Molecular Sciences*, 22(21), 11676.
- Lesiak, A., Paprocka, P., Wnorowska, U., Mańkowska, A., Król, G., Głuszek, K., Piktel, E., Spałek, J., Okła, S., Fiedoruk, K., Durnaś, B., & Bucki, R. (2024). Significance of host antimicrobial peptides in the pathogenesis and treatment of acne vulgaris. *Frontiers in Immunology*, 15, 1502242.
- Li, X. et al. (2023) ‘Dissecting skin microbiota and microenvironment for the development of microbiome-targeted therapies’, *Current Opinion in Microbiology*, 71, 102227.
- Li, Z., Zhang, J., Zhang, Y., Chen, H., & Bao, Y. (2025). Skin Microbiome in Health and Disease: Mechanisms and Emerging Therapeutic Strategies. *Clinical, Cosmetic and Investigational Dermatology*, 3443-3455.
- Liu, P. T., Stenger, S., Li, H., Wenzel, L., Tan, B. H., Krutzik, S. R., Ochoa, M. T., Schaubert, J., Wu, K., Meinken, C., Kamen, D. L., Wagner, M., Bals, R., Steinmeyer, A., Zügel, U., Gallo, R. L., Eisenberg, D., Hewison, M., Hollis, B. W., . . . Modlin, R. L. (2006). Toll-Like receptor triggering of a vitamin D-Mediated human antimicrobial response. *Science*, 311(5768), 1770–1773.

References

- Lueschow-Guijosa, S. R., Stanford, A. H., Berger, J. N., Gong, H., Boly, T. J., Jensen, B. A., Nordkild, P., Leegwater, A. J., Wehkamp, J., Underwood, M. A., & McElroy, S. J. (2024). Host defense peptides human β defensin 2 and LL-37 ameliorate murine necrotizing enterocolitis. *iScience*, 27(6), 109993.
- Lukić, M., Pantelić, I., & Savić, S. D. (2021). Towards optimal pH of the skin and topical formulations: from the current state of the art to tailored products. *Cosmetics*, 8(3), 69.

-M-

- Malachowa, N., Kobayashi, S. D., Lovaglio, J., & DeLeo, F. R. (2019). Mouse Model of *Staphylococcus aureus* Skin Infection. In *Methods in molecular biology* (pp. 139–147).
- Martínez-García, S., Rodríguez-Martínez, S., Cancino-Díaz, M. E., & Cancino-Díaz, J. C. (2018). Extracellular proteases of *Staphylococcus epidermidis*: roles as virulence factors and their participation in biofilm. *Apmis*, 126(3), 177-185.
- Mochtar, C. F., Bakhtiar, M. I., Hamzah, H., Pratama, A., Rasyidah, J., Indriyani, J. R., Alfiannur, M. R., Amalia, N. S., Putri, N. O., & Damis, N. L. (2024). Topical gel preparation from *Staphylococcus epidermidis* isolate extract accelerated healing on incision wound model in rats. *Indonesian Journal of Pharmacology and Therapy*, 5(1).
- Mohammedsaeed, W., & Manzoor, N. (2022). An in vitro investigation of the protective role of *Staphylococcus Epidermidis* extracts on *Staphylococcus Aureus* induced toxicity in human keratinocytes. *Indian Journal of Medical Microbiology*, 40(2), 239–244.

References

- Murphrey, M. B., Miao, J. H., & Zito, P. M. (2022). Histology, stratum corneum. In StatPearls. StatPearls Publishing
- Murakami, M., Ohtake, T., Dorschner, R. A., Schitteck, B., Garbe, C., & Gallo, R. L. (2002). Cathelicidin anti-microbial peptide expression in sweat, an innate defense system for the skin. *Journal of Investigative Dermatology*, 119(5), 1090-1095.

-N-

- Nakamura, K., O'Neill, A. M., Williams, M. R., Cau, L., Nakatsuji, T., Horswill, A. R., & Gallo, R. L. (2020). Short chain fatty acids produced by *Cutibacterium acnes* inhibit biofilm formation by *Staphylococcus epidermidis*. *Scientific reports*, 10(1), 21237.
- Nakatsuji, T., Chen, T. H., Butcher, A. M., Trzoss, L. L., Nam, S. J., Shirakawa, K. T., ... & Gallo, R. L. (2018). A commensal strain of *Staphylococcus epidermidis* protects against skin neoplasia. *Science Advances*, 4(2), eaao4502.
- Nakatsuji, T., Chen, T. H., Narala, S., Chun, K. A., Two, A. M., Yun, T., Shafiq, F., Kotol, P. F., Bouslimani, A., Melnik, A. V., Latif, H., Kim, J., Lockhart, A., Artis, K., David, G., Taylor, P., Streib, J., Dorrestein, P. C., Grier, A., . . . Gallo, R. L. (2017). Antimicrobials from human skin commensal bacteria protect against *Staphylococcus aureus* and are deficient in atopic dermatitis. *Science Translational Medicine*, 9(378).
- Nakatsuji, T., Chen, T. H., Two, A. M., Chun, K. A., Narala, S., Geha, R. S., ... & Gallo, R. L. (2016). *Staphylococcus aureus* exploits epidermal barrier defects in atopic dermatitis to trigger cytokine expression. *Journal of Investigative Dermatology*, 136(11), 2192-2200.

References

- Negari, I. P., Keshari, S., & Huang, C. (2021). Probiotic Activity of *Staphylococcus epidermidis* Induces Collagen Type I Production through FFaR2/p-ERK Signaling. *International Journal of Molecular Sciences*, 22(3), 1414.
- Nemes, Z., & Steinert, P. M. (1999). Bricks and mortar of the epidermal barrier. *Experimental & molecular medicine*, 31(1), 5-19.
- Newstead, L. L., Varjonen, K., Nuttall, T., & Paterson, G. K. (2020). Staphylococcal-produced bacteriocins and antimicrobial peptides: their potential as alternative treatments for *Staphylococcus aureus* infections. *Antibiotics*, 9(2), 40.
- Ngo, Q. V., Faass, L., Sähr, A., Hildebrand, D., Eigenbrod, T., Heeg, K., & Nurjadi, D. (2022). Inflammatory response against *Staphylococcus aureus* via intracellular sensing of nucleic acids in keratinocytes. *Frontiers in immunology*, 13, 828626.
- Niedźwiedzka, A., Micallef, M. P., Biazzo, M., & Podrini, C. (2024). The Role of the Skin Microbiome in Acne: Challenges and Future Therapeutic Opportunities. *International Journal of Molecular Sciences*, 25(21), 11422.
- Noguerol, A. T., Igual, M., & Pagán, M. J. (2021). Comparison of biopreservatives obtained from a starter culture of *Pediococcus acidilactici* by different techniques. *Food Bioscience*, 42, 101114.

-O-

- Okamoto, H., & Nakamura, Y. (2026). Dysbiosis in the Pathogenesis of Atopic Dermatitis. *The Journal of Dermatology*, 53(3), 388-398.

-P-

References

- Paranthaman, K., Wilson, A., Verlander, N., Rooney, G., Macdonald, N., Nsonwu, O., Hope, R., Fleming, P., Hatcher, J., Ogundipe, E., Ratnaraja, N., Wan, Y., Pichon, B., Westrop, S. J., & Brown, C. S. (2023). Trends in coagulase-negative staphylococci (CoNS), England, 2010–2021. *Access Microbiology*, 5(6), acmi000491.v3.
- Pastar, I., O'Neill, K., Padula, L., Head, C. R., Burgess, J. L., Chen, V., ... & Strbo, N. (2020). *Staphylococcus epidermidis* boosts innate immune response by activation of gamma delta T cells and induction of perforin-2 in human skin. *Frontiers in immunology*, 11, 550946.
- Patangia, D. V., Anthony Ryan, C., Dempsey, E., Paul Ross, R., & Stanton, C. (2022). Impact of antibiotics on the human microbiome and consequences for host health. *Microbiologyopen*, 11(1), e1260.
- Pérez-Losada, M., & Crandall, K. A. (2023). Spatial diversity of the skin bacteriome. *Frontiers in microbiology*, 14, 1257276.

-Q-

- Qi, P., Gong, F., Leng, M., & Wei, Z. (2025). Beneficial perspective on *Staphylococcus epidermidis*: a crucial species for skin homeostasis and pathogen defense. *Frontiers in immunology*, 16, 1674392.

-R-

- Rahmdel, S., & Götz, F. (2021). The Multitasking Surface Protein of *Staphylococcus epidermidis*: Accumulation-Associated Protein (Aap). *mBio*, 12(5), e0198921.

References

- Rademacher, F., Simanski, M., Hesse, B., Dombrowsky, G., Vent, N., Gläser, R., & Harder, J. (2018). Staphylococcus epidermidis Activates Aryl Hydrocarbon Receptor Signaling in Human Keratinocytes: Implications for Cutaneous Defense. *Journal of Innate Immunity*, 11(2), 125–135.
- Rajkumar, J., Chandan, N., Lio, P., & Shi, V. (2023). The skin barrier and moisturization: function, disruption, and mechanisms of repair. *Skin Pharmacology and Physiology*, 36(4), 174–185.
- Ran, F., Mu, K., Liu, G., Liu, Y., Pang, Y., Feng, G., Zhou, L., & Peng, L. (2024). Preparation, Characterization, and Wound Healing Promotion of Hydrogels Containing Glucosyloxybenzyl 2-Isobutylmalates Extract from *Bletilla striata* (Thunb.) Reichb.f. *International Journal of Molecular Sciences*, 25(19), 10563.
- Rawlings, A. V., Lane, M. E., & Voegeli, R. (2024). The importance of stratum corneum ω -linoleoyloxyacylceramides in human skin barrier health: their biochemistry, processing enzymes and metabolites involved in corneocyte lipid envelope maturation. *International Journal of Cosmetic Science*, 46(4), 623–642.
- Riebelmann, J., Kienzle, N., Sauer, B., & Schitteck, B. (2025). Commensal Staphylococci attenuate Staphylococcus aureus skin colonization and inflammation via AHR-dependent signaling. *Frontiers in Immunology*, 16, 1726557.
- Rizo-Téllez, S. A., & Filep, J. G. (2024). Beyond host defense and tissue injury: the emerging role of neutrophils in tissue repair. *American Journal of Physiology-Cell Physiology*, 326(3), C661–C683.

References

- Rohde, H. et al. (2015) ‘Structural basis of Staphylococcus epidermidis biofilm formation’, *Frontiers in Cellular and Infection Microbiology*, 5, 14.

-S-

- sabaté Brescó, M., Harris, L. G., Thompson, K., Stanic, B., Morgenstern, M., O'Mahony, L., Richards, R. G., & Moriarty, T. F. (2017). Pathogenic mechanisms and host interactions in Staphylococcus epidermidis device-related infection. *Frontiers in Microbiology*, 8, 1401.
- Salminen, S., Collado, M. C., Endo, A., Hill, C., Lebeer, S., Quigley, E. M. M., Sanders, M. E., Shamir, R., Swann, J. R., Szajewska, H., & Vinderola, G. (2021). The International Scientific Association of Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of postbiotics. *Nature Reviews Gastroenterology & Hepatology*, 18(9), 649–667.
- Sambrook, J., & Russell, D. W. (2006). Alkaline Agarose Gel Electrophoresis. *Cold Spring Harbor Protocols*, 2006(1), pdb. prot4027.
- SanMiguel, A. J., Meisel, J. S., Horwinski, J., Zheng, Q., & Grice, E. A. (2017). Topical antimicrobial treatments can elicit shifts to resident skin bacterial communities and reduce colonization by Staphylococcus aureus competitors. *Antimicrobial agents and chemotherapy*, 61(9), 10-1128.
- Scheuer, P. J., & Chalk, B. T. (1986). Clinical tests: Histopathology. Wolfe Medical Publications.

References

- Severn, M. M., & Horswill, A. R. (2023). Staphylococcus epidermidis and its dual lifestyle in skin health and infection. *Nature Reviews Microbiology*, 21, 97-111.
- Severn, M.M. and Horswill, A.R. (2022) ‘Staphylococcus epidermidis and its dual lifestyle in skin health and infection’, *Nature Reviews Microbiology*, 21(2), pp.97–111.
- Severn, M.M. et al. (2023) ‘Sweat and sebum preferences of the human skin microbiota’, *mSystems*, 8(1), e01234-22.
- Shabana, N. S., Seeber, G., Soriano, A., Jutte, P. C., Westermann, S., Mithoe, G., Pirii, L., Siebers, T., Have, B. t., Zijlstra, W., Lazovic, D., & Wouthuyzen-Bakker, M. (2023). The Clinical Outcome of Early Periprosthetic Joint Infections Caused by Staphylococcus epidermidis and Managed by Surgical Debridement in an Era of Increasing Resistance. *Antibiotics*, 12(1), 40.
- Shao, L., Liu, G., Li, Y., Zhou, Z., Jiang, S., Jiang, H., Ma, L., Bai, T., & Yang, S. (2025). The Anti-inflammatory, Antioxidant Effects and Protective Barrier Function of Cell-Free Supernatant of Potential Probiotic Staphylococcus epidermidis CCSM0322 in HaCaT Cell Induced by UVB. *Probiotics and Antimicrobial Proteins*
- Sharma, S., Sharma, A., Sodhi, G. K., George, N., Alarjani, K. M., Mukherjee, A., ... & Dwibedi, V. (2025). Staphylococcus epidermidis SAS1: new probiotic candidate for obesity and allergy treatment their mechanistic insights and cytotoxicity evaluation. *Frontiers in Microbiology*, 16, 1546687.
- Shrestha, L. B., Bhattarai, N. R., Rai, K., & Khanal, B. (2020). Antibiotic Resistance and mecA Gene Characterization of

References

- Coagulase-negative Staphylococci Isolated from Clinical Samples in Nepal. *Infection and Drug Resistance*, Volume 13, 3163–3169.
- Simanski, M., Erkens, A. S., Rademacher, F., & Harder, J. (2019). Staphylococcus epidermidis-induced interleukin-1 beta and human beta-defensin-2 expression in human keratinocytes is regulated by the host molecule A20 (TNFAIP3). *Acta Dermato-Venereologica*, 99(2), 181–187
 - Simpson, E. L., Schlievert, P. M., Yoshida, T., Lussier, S., Boguniewicz, M., Hata, T., Fuxench, Z., De Benedetto, A., Ong, P. Y., Ko, J., Calatroni, A., Spergel, A. K. R., Plaut, M., Quataert, S. A., Kilgore, S. H., Peterson, L., Gill, A. L., David, G., Mosmann, T., . . . Beck, L. A. (2023). Rapid reduction in Staphylococcus aureus in atopic dermatitis subjects following dupilumab treatment. *Journal of Allergy and Clinical Immunology*, 152(5), 1179–1195.
 - Skowron, K., Bauza-Kaszewska, J., Kraszewska, Z., Wiktorczyk-Kapischke, N., Grudlewska-Buda, K., Kwiecińska-Piróg, J., Gospodarek-Komkowska, E., & ... (2021). Human skin microbiome: Impact of intrinsic and extrinsic factors on skin microbiota. *Microorganisms*, 9(3), 543.
 - Smythe, P., & Wilkinson, H. N. (2023). The skin microbiome: current landscape and future opportunities. *International journal of molecular sciences*, 24(4), 3950.
 - Sornsenee, P., Chatatikun, M., Mitsuwan, W., Kongpol, K., Kooltheat, N., Sohbenalee, S., Pruksaphanrat, S., Mudpan, A., & Romyasamit, C. (2021). Lyophilized cell-free supernatants of Lactobacillus isolates exhibited antibiofilm, antioxidant, and

References

- reduces nitric oxide activity in lipopolysaccharide-stimulated RAW 264.7 cells. *PeerJ*, 9, e12586.
- Sugimoto, S., Iwamoto, T., Takada, K., Okuda, K., Tajima, A., Iwase, T., & Mizunoe, Y. (2013). *Staphylococcus epidermidis* Esp degrades specific proteins associated with *Staphylococcus aureus* biofilm formation and host-pathogen interaction. *Journal of bacteriology*, 195(8), 1645–1655.
 - Suzuki, Y., Kawada-Matsuo, M., Le, M. N., Eng, S., Hisatsune, J., Sugai, M., Sakaguchi, T., & Komatsuzawa, H. (2024). The two-component regulatory systems GraRS and SrrAB mediate *Staphylococcus aureus* susceptibility to Pep5 produced by clinical isolate of *Staphylococcus epidermidis*. *Applied and Environmental Microbiology*, 90(7), e0030024.
 - Swaney, M. H., Nelsen, A., Sandstrom, S., & Kalan, L. R. (2023). Sweat and sebum preferences of the human skin microbiota. *Microbiology spectrum*, 11(1), e04180-22.
 - Szemraj, M., Glajzner, P., & Sienkiewicz, M. (2023). Decreased susceptibility to vancomycin and other mechanisms of resistance to antibiotics in *Staphylococcus epidermidis* as a therapeutic problem in hospital treatment. *Dental Science Reports*, 13.

-T-

- Takahashi, T., & Gallo, R. L. (2017). The Critical and Multifunctional Roles of Antimicrobial Peptides in Dermatology. *Dermatologic clinics*, 35(1), 39–50.
- Tan, I. J., & Lio, P. A. (2025). From discovery to modern understanding: the acid mantle in dermatology. *Journal of Integrative Dermatology*.

References

- Tricarico, P. M., Gratton, R., Santos-Silva, C. a. D., De Moura, R. R., Ura, B., Sommella, E., Campiglia, P., Del Vecchio, C., Moltrasio, C., Berti, I., D'Adamo, A. P., Elsherbini, A. M. A., Staudenmaier, L., Chersi, K., Boniotto, M., Krismer, B., Schitteck, B., & Crovella, S. (2022). A rare loss-of-function genetic mutation suggest a role of dermcidin deficiency in hidradenitis suppurativa pathogenesis. *Frontiers in Immunology*, 13, 1060547.
- Trivedi, B. (2012). Microbiome: The surface brigade. *Nature*, 492(7429), S60–S61.
- Twomey, E., O'Connor, P. M., Coffey, A., Kiste, M., Guinane, C. M., Hill, C., Field, D., & Begley, M. (2024). Inhibition of clinical MRSA isolates by coagulase negative staphylococci of human origin. *Antibiotics*, 13(4), 338.

-V-

- Vandecandelaere, I., Depuydt, P., Nelis, H. J., & Coenye, T. (2014). Protease production by *Staphylococcus epidermidis* and its effect on *Staphylococcus aureus* biofilms. *Pathogens and disease*, 70(3), 321-331.
- Vandepitte, J., Verhaegen, J., Engbaek, K., Rohner, P., Piot, P., & Heuck, C. C. (2003). *Basic laboratory procedures in clinical bacteriology* (2nd ed.). World Health Organization.
- Vinderola, G., Sanders, M. E., & Salminen, S. (2022). The concept of postbiotics. *Foods*, 11(8), 1077.

-W-

- Wang, X., Wang, M., Yang, J., Liu, W., Jia, H., Chen, Y., & Lai, Y. (2026). *Staphylococcus epidermidis* prevents UV-induced skin aging by suppressing TLR3-mediated senescence. *Frontiers in Immunology*, 17, 1796085.

References

- Wilkhoo, H. S., Islam, A. W., Hussain, S., Kadam, S. R., Rao, Z. K., & Singh, B. (2025). Skin microbiome and inflammatory dermatoses: A focused review. *Cosmoderma*, 5.
- Williams, M. C., Dominguez, S. R., Prinzi, A., Lee, K., & Parker, S. K. (2020). Reliability of *mecA* in predicting phenotypic susceptibilities of coagulase-negative staphylococci and *Staphylococcus aureus*. *Open Forum Infectious Diseases*, 7(12), ofaa553.
- Williams, M. R., Bagood, M. D., Enroth, T. J., Bunch, Z. L., Jiang, N., Liu, E., Almoughrabie, S., Khalil, S., Li, F., Brinton, S., Cech, N. B., Horswill, A. R., & Gallo, R. L. (2023). *Staphylococcus epidermidis* activates keratinocyte cytokine expression and promotes skin inflammation through the production of phenol-soluble modulins. *Cell Reports*, 42(9), 113024.
- Wojciechowska, K., & Szewczyk, K. D. S. (2025). The skin microbiome and bioactive compounds: mechanisms of modulation, dysbiosis, and dermatological implications. *Molecules*, 30(22), 4363.
- Wu, C., Lower, B. A., Moreira, R., Dorantes, D., Le, T., Giurgiu, C., ... & van der Donk, W. A. (2023). Investigation into the mechanism of action of the antimicrobial peptide epilancin 15X. *Frontiers in Microbiology*, 14, 1247222.
- Wu, F., Jiang, X., Chen, G., & Zhang, L. (2025). Integrated Microbiome and metabolome analysis reveals Microbial-Metabolic interactions in psoriasis pathogenesis. *BMC microbiology*, 25(1), 665.

-X-

References

- Xu, Z., Cave, R., Chen, L., Yangkyi, T., Liu, Y., Li, K., Meng, G., Niu, K., Zhang, W., Tang, N., Shen, J., & Mkrtchyan, H. V. (2020). Antibiotic resistance and molecular characteristics of methicillin-resistant *Staphylococcus epidermidis* recovered from hospital personnel in China. *Journal of Global Antimicrobial Resistance*, 22, 195–201.

-Y-

- Ye, H., Wong, A., Chen, X., Sun, W., He, H., Li, X., & Wu, J. (2026). Dynamic remodeling of skin microbiota during healthy homeostatic and wound repair conditions. *Frontiers in Microbiology*, 17, 1781606.
- Yousef, H., Alhajj, M., & Sharma, S. (2024). Anatomy, skin (Integument), epidermis. *StatPearls*.

-Z-

- Zanetta, P., Ballacchino, C., Squarzanti, D. F., Amoroso, A., Pane, M., & Azzimonti, B. (2023). *Lactobacillus johnsonii* LJO02 (DSM 33828) cell-free supernatant and vitamin D improve wound healing and reduce Interleukin-6 production in *Staphylococcus aureus*-infected human keratinocytes. *Pharmaceutics*, 16(1), 18.
- Zhang, J., Jiang, H., Jiang, J., & Huang, G. (2016). Isolation and Identification of *Staphylococcus epidermidis* S14 Screening Extracellular Antimicrobial Metabolites. *American Journal of Biochemistry & Biotechnology/American Journal of Biochemistry and Biotechnology*, 12(1), 56–63.
- Zhang, W., Du, J., Dong, L., Zhang, S., Song, G., Ao, L., Wang, Y., Ma, L., Sun, W., Ma, J., Tian, X., Qu, B., Long, T., Chen, L., Huang, Z., Qiao, J., Zhang, Y., Li, J., Lu, X., . . . Lu, Q. (2026). Comprehensive profiling of skin microbiome diversity and major

References

- determinants in a multi-regional Chinese population. *Cell Reports*, 45(3), 117097.
- Zhang, Y. et al. (2024) ‘Causal roles of skin and gut microbiota in skin appendage disorders’, *Frontiers in Immunology*, 15, 1427276.
 - Żółkiewicz, J., Marzec, A., Ruszczyński, M., & Feleszko, W. (2020). Postbiotics—a step beyond pre-and probiotics. *Nutrients*, 12(8), 2189.

Appendices

Appendix

(1)

bioMérieux Customer:		Microbiology Chart Report		Printed February 13, 2026 7:48:41 AM CST													
Patient Name:				Patient ID:													
Location:				Physician:													
Lab ID: Ra1				Isolate Number: 7													
Organism Quantity:																	
Selected Organism : Staphylococcus epidermidis																	
Source:				Collected:													
Comments:																	
Identification Information		Analysis Time:		Status:													
		4.85 hours		Final													
Selected Organism		99% Probability		Staphylococcus epidermidis													
		Bionumber:		010400056620211													
ID Analysis Messages																	
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	BGURr	-	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	MBdG	-	56	PUL	-
57	dRAF	-	58	O129R	+	59	SAL	-	60	SAC	+	62	dTRE	-	63	ADH2s	-
64	OPTO	+															

(2)

Ministry of Higher Education
& Scientific Research
AL-Qadisiyah University
Collage of Science



No: 1417
Date: 17/3/2026



ETHICS COMMITTEE CERTIFICATE OF APPROVAL

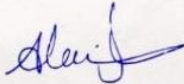
This is to certify that
Project No: 004
Project Title:
Phenotypic and Genotypic Characterization of mecA-Positive Multidrug-Resistant Staphylococcus epidermidis Isolated from Healthy Skin Carriers in Al-Qadisiyah, Iraq
Principal Researcher: Rafal Ali Hatef¹, Maitham Ghaly Yousif²
¹Department of biology, College of Science, AL-Qadisiyah University, AL-Diwania, Iraq
²Department of biology, College of Science, AL-Qadisiyah University, AL-Diwania, Iraq

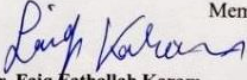
It was considered for an ethics review program by the Ethics Committee on 23 February 2026, to meet the requirements of the Ethical Principles for Research with Human Participants, and has had a continuing validity period since 3 March 2026 .

We wish you and your colleagues every success in your research.

Sincerely,


Assis.Prof. Dr. Lujain Ibrahim Hussain
Member


Prof. Dr. Alaa Mohammed Hasoon Al-Hussini
Member


Prof. Dr. Faiq Fathallah Karam
Chairman

Chairs Research Ethics Review Committee

الخلاصة

تواجه الميكروبيوتا الجلدية ضغوطاً بيئية وضغوطات ممرضة مستمرة، مما يؤدي غالباً إلى حالات من اختلال التوازن الميكروبي. وقد تؤدي التدخلات التقليدية بمضادات الميكروبات واسعة الطيف إلى تفاقم هذا الاختلال، مما يفرض ضرورة سريرية لإيجاد وسائل علاجية فعالة تحافظ على الميكروبيوم. وبناءً على ذلك، تبحث هذه الدراسة في الفعالية العلاجية للمكونات الحيوية اللاحقة (postbiotics) وتحديد الراشح الخالي من الخلايا (CFS) المشتقة من سلالة محلية لبكتيريا المكورات العنقودية البشرية، والمدمجة في تركيبة هيدروجيل موضعي، وذلك بهدف استعادة التوازن الفسيولوجي للجلد وتنظيم آليات المناعة الفطرية بفعالية.

أُجريت هذه الدراسة في قسم الأحياء بكلية العلوم في جامعة القادسية، خلال الفترة الممتدة من أكتوبر 2025 إلى أغسطس 2026. وتمثل الهدف الرئيسي في تطوير هيدروجيل فعال ومتوافق حيويًا مشتق من هذه المكونات الحيوية اللاحقة، وتقييم خصائصه في تجديد الأنسجة وتعديل الاستجابة المناعية. ولتحقيق هذه الأهداف، جرى الحصول على عزلات محلية لبكتيريا المكورات العنقودية البشرية من مسحات جلدية أُخذت من أفراد أصحاء، ثم استُزرعت وعُزلت باستخدام وسط مانيتول سولت أغار (Mannitol Salt Agar) واستُخلصت المكونات الحيوية النشطة الراشح الخالي من الخلايا وجرى ترشيحها وتحويلها إلى تركيبة مجففة بالتجميد. قبل التركيب، تم تقييم النشاط الحيوي في المختبر للراشح الخالي من الخلايا (CFS) باستخدام طريقة الانتشار في الآبار على وسط الأجار، مما أظهر فعالية ملحوظة لمضادة للبكتيريا ضد المكورات العنقودية الذهبية. بعد ذلك، تم تحضير المسحوق، حيث دمج الراشح في مصفوفة كاربوبول باستخدام تقنية دمج منخفضة الحرارة للحفاظ على سلامة بنية الجل وتعديل الأس الهيدروجيني (pH) عند 5.5 باستخدام ثلاثي إيثانول أمين.

قُيِّمت الفعالية العلاجية للهيدروجيل المُحضّر داخل الجسم الحي باستخدام نموذج تجريبي على الفئران. وأكد الفحص النسيجي المرضي التوافق الحيوي للهيدروجيل وتحسّن بنية الأنسجة بشكل يعتمد على التركيز المستخدم. كشف التحليل النسيجي المرضي أن الهيدروجيل المكون من CFS بتركيز (5%) أدى إلى تحسينات هيكلية طفيفة في نسيج الجلد بينما أظهر الهيدروجيل المحتوي على (10%) من الراشح الخالي من الخلايا (CFS) قدرة فائقة على تجديد الأنسجة، حيث سهّل التكاثر الأمثل لخلايا الكيراتين (keratinocytes)، وعزز وظيفة الخلايا الليفية

(fibroblasts)، وأدى إلى ترسب منظم للكولاجين دون ظهور أي علامات لتضخم نسيجي مرضي (hyperplasia).

في مرحلة لاحقة، أُجريت دراسة سريرية شملت 15 شخصاً سليماً على مدار 20 يوماً لتقييم التركيبة التي تحتوي على (5%) من الراشح الخالي من الخلايا (CFS). وأظهرت النتائج السريرية خصائص قوية مضادة للالتهاب، تجلت في انخفاض ذي دلالة إحصائية في تركيزات السيتوكين المسبب للالتهاب (IL-8) في المنطقة المعالجة ($p = 0.026$). علاوة على ذلك، عززت التركيبة بفعالية آليات المناعة الذاتية للجلد، مما أدى إلى ارتفاع ذي دلالة إحصائية بنسبة 15.9% في مستوى الببتيد المضاد للميكروبات (HBD-2 ($p = 0.017$). ورغم أن الارتفاع في تركيزات LL-37 كان طفيفاً ($p = 0.332$)، إلا أن 60% من المشاركين أظهروا استجابة إيجابية. وفي الختام، أثبت الهلام المائي (hydrogel) المطور والمحتوي على السائل الطافي المستخلص (CFS) من مزرعة بكتيريا المكورات العنقودية البشرية سلامته وفعاليته وخصائصه غير الممرضة كوسيلة علاجية لتعزيز تجديد الجلد وتقوية المناعة الجلدية الذاتية.



جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة القادسية
كلية العلوم
قسم علوم الحياة

تقييم هيدروجيل من الراشح الخالي من الخلايا لبكتريا *Staphylococcus epidermidis* من حيث السلامة عند التطبيق الموضعي وتأثيراته المعدلة للمناعة

رسالة

مقدمة الى مجلس كلية العلوم - قسم علوم الحياة - جامعة القادسية كجزء من متطلبات نيل درجة الماجستير في علوم الحياة

رفل علي هاتف

بكالوريوس في علوم الحياة / كلية العلوم / جامعة الكوفة

2018

بإشراف

أ.د. ميثم غالي يوسف