

Coagulase-negative staphylococci from healthy human skin: a potential source of antimicrobial compounds against multidrug-resistant pathogens

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Abstract

*Coagulase-negative staphylococci (CoNS) are common skin commensals, with some strains exhibiting antimicrobial activity against pathogenic bacteria. This study aimed to characterize the distribution of microbial groups on healthy human skin and to evaluate the antibacterial properties of CoNS isolates. A total of 420 microbial strains were isolated from 166 participants, with CoNS being the most prevalent at 96.19%. Among these, two strains (18.2 and 11.2) demonstrated antimicrobial activity, particularly against methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus faecalis* (VRE). Strain 18.2 exhibited broad-spectrum inhibitory effects, making it a promising candidate for further antimicrobial research.*

The study highlights the role of CoNS in skin microbiota and their potential as a source of novel antimicrobial agents. Therefore, further research should focus on optimizing the production of antimicrobial compounds and characterizing their mechanisms of action for therapeutic applications.

Keywords: Antimicrobial activity, coagulase-negative staphylococci, human skin microbiome, methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus faecalis*.

Introduction

Antimicrobial resistance (AMR) has emerged as a global health challenge, prompting the search for novel antimicrobial agents. According to the WHO Report¹⁷, the rapid rise in multidrug-resistant pathogens such as MRSA and VRE, necessitates the urgent development of alternative strategies¹⁷. Among microorganisms with potential therapeutic uses, coagulase-negative staphylococci (CoNS) found on healthy human skin have recently drawn interest due to their antimicrobial capabilities¹³. The skin is the largest human organ and plays a major role in protecting humans against pathogens. It not only acts as a barrier, but is also a huge ecosystem with many bacteria and fungi¹⁸.

Many of these symbiotic bacteria contribute to human health either directly or indirectly. Certain commensal bacteria on the skin can influence host health through biochemical or

immunological mechanisms⁴. Microorganisms are affected by various internal and external factors such as age² gender¹⁰ and geography¹⁵.

Besides, humans from different environments such as rural-urban¹¹ or different countries⁸ have distinct skin microbiomes. In a study conducted in Japan, the skin microbiomes of adult and elderly Japanese women showed differences in microbial diversity, with the older group exhibiting greater alpha diversity. Additionally, the effects of age on the composition and diversity of skin microorganisms have also been identified in women from Western Europe and Thai males¹⁶.

Humans from different environments, such as rural-urban¹¹ or different countries⁸, have distinct skin microbiomes. For example, geography significantly affects composition of skin microbes and abundances of *Trabulsella* and *Propionibacterium* differ due to the different contact environments for hands and other body parts⁷. Moreover, hand washing affects the composition of microorganisms on the surface of the palm⁶.

Finally, a recent study has shown that the above factors including demographics, lifestyle and physiological factors of the skin itself, together explain part of the variability in microbial composition³. Among these factors, body location is essential. As skin microbial communities vary among all ethnicities¹⁵, results obtained on Asian skin cannot be extrapolated to worldwide populations. It would therefore be of interest to identify potential age-related changes in cutaneous microbiota for each ethnic origin.

Staphylococcus cohnii and other CoNS strains have demonstrated inhibitory effects against pathogens, making them promising candidates for novel antimicrobial applications¹⁴. Although traditionally regarded as commensal organisms, studies suggest that certain strains of CoNS may produce bioactive compounds with significant antibacterial activity. In general, Vietnamese researchers today have shown considerable interest in microorganism biological antagonists. This study aimed to characterize the distribution of microbial groups on healthy human skin and to determine the antibacterial properties of CoNS isolates from healthy human skin. Results of this study may provide a scientific foundation for optimizing the biotechnological applications of CoNS from the skin of healthy humans, paving the way for novel antimicrobial solutions.

Material and Methods

Sample Collection from Healthy Human Skin:

Participants in this study were students and trainees at Hue University of Medicine and Pharmacy, aged between 19 and 41. They had no dermatological conditions, no skin infections at the time of sampling and no skin-transmissible diseases. Participants were categorized into two age groups based on their status as students or working trainees: those under 25 years old (junior trainees) and those 25 years or older (senior trainees). Additionally, participants belonged to different ethnic groups including Kinh, Cham, Bana, Ede and Thai. This categorization was done to evaluate potential differences in skin microbiota among these groups.

Sampling procedure: Participants were instructed not to bathe immediately before sampling. A sterile cotton swab pre-moistened with sterile saline was used to collect skin samples from seven body sites: behind the ear, palm, finger web spaces, fingernail tips, nasal wings, neck folds and between the toes. Each participant was sampled at all these sites using a single swab.

Isolation and Identification of Coagulase-Negative Staphylococci (CoNS) from Human Skin: Skin swabs were transported immediately to the laboratory of Department of Microbiology at Hue University of Medicine and Pharmacy Hospital within 15 minutes. Samples were then cultured on Brain Heart Infusion Agar (BHA) (Merk, Germany) and incubated at 37°C for 18–24 hours. Representative colonies were selected based on morphological characteristics such as color, shape and size.

The isolated colonies were subjected to Gram staining to differentiate cocci, bacilli and fungi. Coccal isolates underwent catalase and tube coagulase tests, as well as oxidase testing, to distinguish between coagulase-positive *Staphylococcus* (CoPS), coagulase-negative *Staphylococcus* (CoNS) and *Micrococcus* spp. CoPS strains were identified as coagulase-positive whereas CoNS and *Micrococcus* spp. were coagulase-negative. CoNS isolates were oxidase-negative while *Micrococcus* spp. showed oxidase-positive reactions¹.

Screening for bacteriocin and antimicrobial compound production in CoNS isolates: Single typical colonies were selected and subcultured in Brain Heart Infusion (BHI) broth (Merck, Germany) in 5 mL volumes at 37°C for 24 hours. The cultures were centrifuged at 10,000 rpm for 10 minutes and bacterial cells were removed. The supernatant was then adjusted to pH 6.9–7.2 using 1M NaOH and stored at 4°C.

Antimicrobial Activity Assay: The antimicrobial activity tests were conducted using indicator bacteria. For each assay, 50 µL of the cell-free supernatant was introduced into wells on an agar plate pre-inoculated with the indicator bacteria. The plates were first incubated at 4°C for 1 hour to facilitate diffusion, followed by 24-hour incubation at 37°C¹³. The antibacterial activity was assessed by measuring

the diameter of inhibition zones surrounding the wells. Coagulase-negative Staphylococci (CoNS) isolates obtained from healthy human skin were screened for the production of bacteriocins and antimicrobial compounds by evaluating their inhibitory effects against *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922.

Isolates that demonstrated antibacterial activity, were further tested against six additional indicator strains including *Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus* ATCC 43300, *Enterococcus faecalis* ATCC 29212, *Enterococcus faecalis* ATCC 51299, *Enterococcus casseliflavus* ATCC 700327 and *Staphylococcus saprophyticus* ATCC BAA-750.

Results

Demographic Characteristics of the Study Group

General demographic characteristics of the study group:

With a total of 166 study participants, the average age was 21.46 ± 2.795 years, with the majority (94.58%) falling within the age range of 19 to 24 years. The youngest participant was 19 years old, while the oldest was 41 years old. Among the 166 study participants, 31 were male (18.67%) and 135 were female (81.33%), with females outnumbering males (81.33% compared to 18.67%). Among the 166 study participants, 160 were of Kinh ethnicity (96.39%), while only 6 participants (3.61%) belonged to other ethnic groups (*Cham*, *Bana*, *E-de*, *Thai*). Thus, the majority of the study participants were of Kinh ethnicity (Table 1).

Distribution of isolated microbial groups: From 420 strains isolated from skin swab samples of 166 study participants, the results were as follows: Coagulase-negative staphylococci (CoNS) were overwhelmingly dominant, accounting for 404 out of 420 strains (96.19%), indicating that this bacterial group was the primary component of the microbiota on healthy human skin. In contrast, *Micrococcus* spp. and fungi had the lowest proportions at 0.95% and 0.24% respectively. Thus, the study results suggest that the microbiota on healthy human skin was predominantly composed of CoNS, constituting nearly the entire microbial population (Fig. 1).

General demographic characteristics of among isolated microbial groups:

The distribution of isolated microbial strains on the skin of two age groups was: < 25 years and ≥ 25 years. A total of 420 microbial strains were isolated from 166 study participants with coagulase-negative staphylococci (CoNS) being the predominant group in both age categories. Other microbial groups, including coagulase-positive Staphylococci, Bacilli, *Micrococcus* spp. and fungi were only found in the < 25 years' group with a low prevalence.

Out of 420 microbial strains isolated from swab samples taken from the skin of study participants we found that

coagulase-negative staphylococci (CoNS) were predominant in both males and females at very high rates, accounting for 96.44% in females and 95.18% in males. Other microorganisms such as coagulase-positive Staphylococci, Bacilli, *Micrococcus* spp. and fungi had relatively low occurrence rates. Thus, CoNS were the dominant microbial strain in both genders at approximately equal rates, while other microorganisms appeared at much lower rates. The difference between males and females in the proportion of different microbial strains was insignificant, indicating that gender does not significantly influence the distribution of microorganisms on the skin (Table 2).

Antimicrobial activity of isolated CoNS Strains against indicator bacteria: All 404 coagulase-negative staphylococcal strains isolated from skin swab samples of 166 study participants were screened for antimicrobial

activity against 3 indicator strains (*S. aureus* ATCC 25923, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853). The results showed that the proportion of strains with antimicrobial activity was low (only 2 out of 404 coagulase-negative staphylococcal strains exhibited antimicrobial activity, accounting for 0.5%) (Table 3).

2 coagulase-negative staphylococcal strains with antimicrobial activity (11.2 and 18.2) were tested for their ability to inhibit 6 other indicator bacterial strains including *S. aureus* ATCC 29213, *S. aureus* ATCC 43300, *E. faecalis* ATCC 29212, *E. faecalis* ATCC 51299, *E. casseliflavus* ATCC 700327 and *S. saprophyticus* ATCC BAA-750 (Fig. 2). The results in fig. 2 show that strain 18.2 exhibited antimicrobial activity against all 6 indicator bacterial strains. However, strain 11.2 did not show any antagonistic activity against 6 indicator strains.

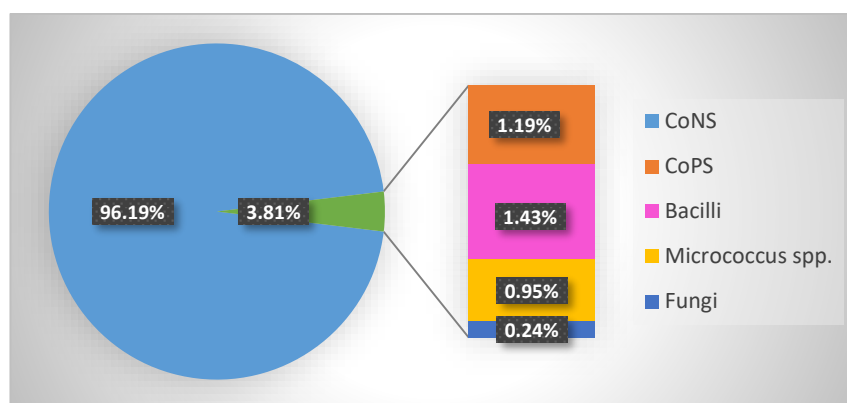


Fig. 1: Distribution of isolated microbial groups

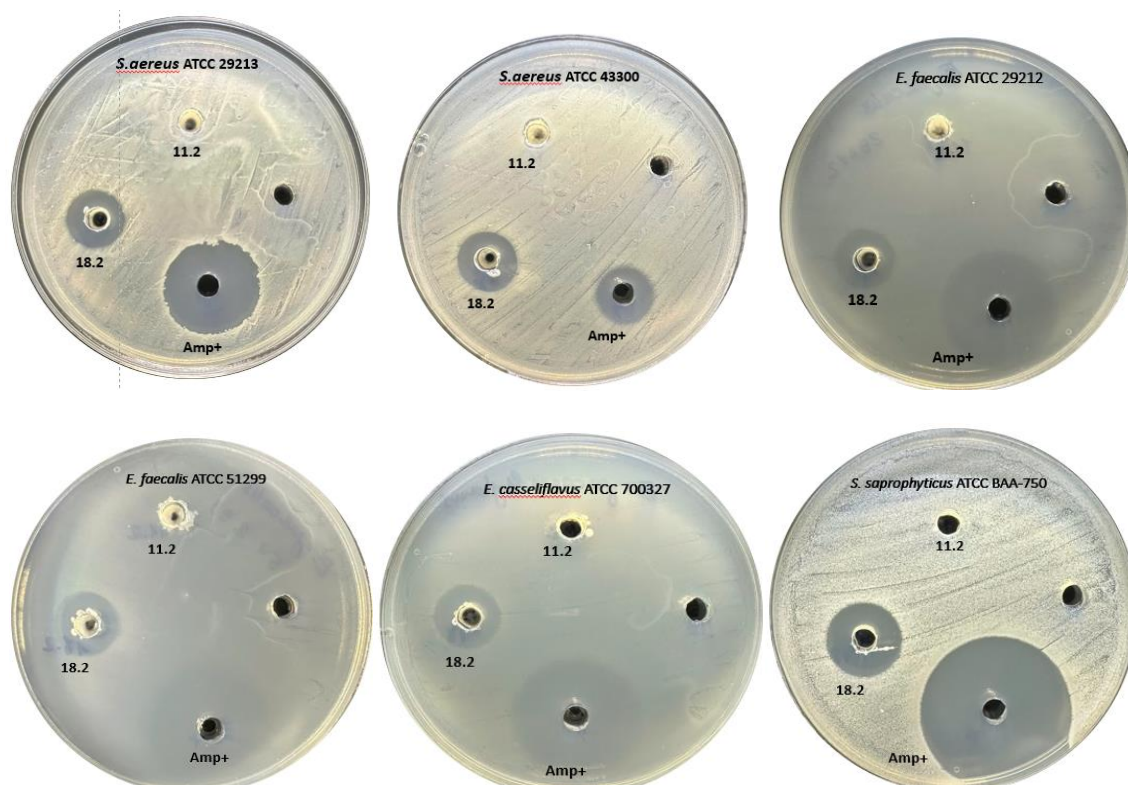


Fig. 2: Results of antimicrobial activity with the different indicators bacteria

Discussion

In this study, we analyzed the distribution of coagulase-negative staphylococci (CoNS) and other microorganisms on the skin of healthy individuals while also evaluating the antibacterial activity of the isolated CoNS strains. The results revealed that CoNS were the predominant microbial group, highlighting their significant role in the skin microbiota.

The distribution of microbial groups on healthy human skin:

The distribution of coagulase-negative staphylococci (CoNS) and other microorganisms on human skin is influenced by various demographic factors including age, gender and ethnicity. Our findings indicate that CoNS were the dominant microbial group in both age groups (<25 years and ≥25 years), consistent with previous studies that highlight the ubiquitous presence of CoNS on human skin. Other microbial groups such as *Micrococcus* spp., fungi and Gram-positive bacilli were exclusively detected in the younger age group (<25 years). This observation aligns with Howard et al⁹ who found that bacterial diversity on human skin increases with age, potentially due to age-related changes in skin physiology, such as reduced sebaceous gland area and increased skin lipids, natural moisturizing factors and antimicrobial peptides.

Myers et al¹² found that skin microbial diversity grows with age, which differs from the extra microbial groups we saw only in younger people. Regarding gender distribution, our findings indicated nearly equal prevalence of CoNS among males (95.18%) and females (96.44%), suggesting minimal influence of gender on the prevalence of CoNS. Ying et al¹⁹ identified gender-related differences in bacterial composition, noting higher relative abundances of *Propionibacterium* and *Corynebacterium* in males, while *Staphylococcus* and *Alloiococcus* were more prevalent in females. The discrepancy between these results and our findings could be due to differences in sample size, gender ratio (our study had significantly more female participants) and methods used for microbial isolation and identification.

Our study included participants from various ethnic groups including Kinh, Cham, Bana, Ede and Thai. The results showed that CoNS predominated across these groups, with slightly higher isolation rates among minority ethnicities (100%) compared to the Kinh majority (96.05%). Although the number of minority ethnic participants was limited, this result aligns with other studies indicating that ethnicity may influence skin microbiome composition. Potbhare et al¹⁵ highlighted differences in skin microbial communities among various ethnic groups, emphasizing the need for further investigation into how ethnicity-related factors such as genetics, lifestyle and environmental exposure shape microbial diversity.

Overall, CoNS consistently emerged as the predominant microbial group across all demographic categories analyzed, underscoring their central role in the human skin microbiota.

Despite variations observed in other microbial groups, the widespread presence of CoNS suggests their fundamental and stable association with healthy human skin, relatively independent of age, gender and ethnicity.

Antimicrobial activity of isolated CoNS Strains against indicator bacteria:

A total of 420 bacterial strains were isolated, with CoNS comprising of 96.19% of the microbial population. Among these, two strains (18.2 and 11.2) exhibited antimicrobial activity. Compared to previous studies, the detection rate of antimicrobial-producing CoNS in our study was notably lower.

For instance, a study conducted by Fernández-Fernández et al⁵ reported that approximately 6% of CoNS strains exhibited antimicrobial activity, whereas our findings indicate a detection rate of only 0.5%. The lower prevalence observed in our study could be attributed to differences in sampling locations, culture conditions, or interindividual variability in skin microbiota composition.

A critical aspect of this study was the selection of ATCC reference strains for antimicrobial testing. ATCC 25923 (*S. aureus*) was chosen as a representative Gram-positive pathogen due to its role in skin and soft tissue infections. ATCC 25922 (*E. coli*) was included as a standard Gram-negative control strain, often used in antimicrobial susceptibility testing. ATCC 27853 (*P. aeruginosa*) was selected because of its intrinsic resistance mechanisms, making it a crucial model for evaluating the potency of new antimicrobials. The Gram-negative bacteria (*E. coli* and *P. aeruginosa*) showed no susceptibility to both strains, indicating that the antimicrobial compounds produced by CoNS may primarily target Gram-positive bacteria. This aligns with previous research suggesting that many bacteriocins produced by staphylococci have a stronger effect on closely related Gram-positive bacteria rather than Gram-negative species⁵.

To further characterize the antimicrobial potential of strains 18.2 and 11.2, they were tested against six additional ATCC strains: *S. aureus* ATCC 29213: A standard methicillin-sensitive *S. aureus* strain used for antibiotic susceptibility testing, *S. aureus* ATCC 43300 (MRSA): A methicillin-resistant *S. aureus* strain, representing a major antibiotic-resistant pathogen, *E. faecalis* ATCC 29212: A vancomycin-sensitive *E. faecalis* strain included to assess the efficacy of CoNS antimicrobials against enterococci, *E. faecalis* ATCC 51299 (VRE): A vancomycin-resistant *E. faecalis* strain, selected to evaluate potential activity against drug-resistant enterococci, *E. casseliflavus* ATCC 700327: A strain with intrinsic low-level vancomycin resistance, included to assess its susceptibility to CoNS-derived antimicrobials, *S. saprophyticus* ATCC BAA-750: A CoNS species frequently associated with urinary tract infections included to test whether antimicrobial activity extends to other CoNS strains (Fig. 2). Strain 18.2 showed strong inhibition against all indicator strains including *S. aureus* (ATCC 29213, ATCC

43300), enterococci (*E. faecalis* ATCC 29212, ATCC 51299 and *E. casseliflavus* ATCC 700327), making it a promising candidate for further antimicrobial studies. The ability to inhibit MRSA and VRE was particularly significant, as it suggests that strain 18.2 may produce bacteriocin-like compounds effective against antibiotic-resistant *S. aureus* and vancomycin-resistant *E. faecalis* infections. Besides, strain 18.2 showed inhibition against the strain *S. saprophyticus* ATCC BAA-750, which was CoNS species; so, antimicrobial activity extends to other CoNS strains which should be tested further to confirm its application validation.

However, strain 11.2 had much weaker inhibition across all four ATCC strains including *S. aureus* (ATCC 29213, ATCC 43300), enterococci (*E. faecalis* ATCC 29212, ATCC 51299 and with no measurable effect against *E. casseliflavus* ATCC 700327 and *S. saprophyticus* ATCC BAA-750. This suggests that the antimicrobial mechanism of strain 11.2 may be strain-specific and less effective against multidrug-resistant pathogens.

These findings contribute to the growing interest in CoNS as potential sources of novel antimicrobials, particularly for targeting multidrug-resistant Gram-positive pathogens, especially MRSA, vancomycin-resistant *E. faecalis* and *S. saprophyticus*. However, the lack of broad-spectrum activity, particularly against Gram-negative bacteria, suggests that CoNS-derived antimicrobials may be most useful in niche applications rather than as broad-spectrum antibiotics.

The lower rate of antimicrobial-producing CoNS in our study compared to others raises questions regarding the factors influencing bacterial antimicrobial expression. Environmental conditions, genetic variability and host factors may contribute to differences in antimicrobial production across studies. Future investigations should focus on sequencing and characterizing the genetic elements involved in antimicrobial compound synthesis to determine whether specific CoNS strains possess distinct biosynthetic pathways. Furthermore, the purification and molecular characterization of the antimicrobial compounds produced by strain 18.2 should be prioritized to evaluate their clinical and pharmaceutical applications.

There were some limitations in our research. The exact molecular structure of the antimicrobial compounds was not determined, necessitating further research on their chemical properties and mechanisms of action. Additionally, this study did not assess the *in vivo* efficacy of the antimicrobial compounds, which was essential for determining their therapeutic potential.

The low detection rate of antimicrobial-producing CoNS compared to other studies suggests that additional environmental and genetic factors may play a role, warranting further research. However, one of the major

strengths of this study was its detailed evaluation of CoNS strains isolated from human skin, with a specific focus on their antimicrobial potential against clinically relevant pathogens including MRSA and VRE. The use of multiple ATCC reference strains provides a comprehensive understanding of antimicrobial efficacy and specificity. Future research should explore the development of CoNS-derived antimicrobial formulations for clinical applications, particularly focusing on strain 18.2 potential against MRSA and VRE.

Conclusion

CoNS strains isolated from healthy skin exhibit antimicrobial properties, with both strains 18.2 and 11.2 demonstrating activity under certain conditions. Notably, strain 18.2 exhibited strong inhibitory activity against MRSA and VRE, making it a particularly promising candidate for further study. These findings highlight the potential of CoNS as a source of novel antimicrobial agents. Despite some limitations, this study provides a foundation for further exploration of CoNS-derived antimicrobials, paving the way for potential therapeutic and industrial applications.

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