Supplementary Methods

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed in accordance with the established guidelines by microdilution assay to determine minimal inhibitory concentrations (MICs) of the following antibiotics: vancomycin (0.5-256 μg/ml), erythromycin (0.5-256 μg/ml), trimethoprim (0.5-256 μg/ml), gentamicin (0.5-1024 μg/ml), norfloxacin (0.5-256 μg/ml), ampicillin (0.5-1024 μg/ml), oxacillin (0.5-1024 μg/ml), tetracycline (0.5-1024 μg/ml) and mupirocin (0.5-512 μg/ml). Also, benzalkonium chloride, a commonly used wound disinfectant, was tested (0.5-256 μg/ml). A 96 well plate was used to perform an 11-fold serial dilution of each antibiotic in triplicates with bacterial suspensions of each strain at 5×10^5 CFU. Plates were incubated at 37°C for 18 hours and absorbance measured using a GloMax plate reader (Promega) at 600 nm.

In vitro biofilm assay

Biofilm formation of *S. epidermidis* isolates was performed following the established protocol[4]. In brief, the optical density (OD600) of all overnight cultures was adjusted to 0.5 and 10 μl of suspension was inoculated into 190 μl TSB in a 96 well flat bottom polystyrene microtiter plate. After incubation at 37°C for 72 h, biofilm production was quantified using 0.2% crystal violet and the absorbance was measured using a GloMax plate reader at 560 nm.

Fibronectin and collagen binding assays

Assessment of adhesion properties of selected isolates was performed as previously described[5]. Briefly, 96well plates were coated with human fibronectin (100 μg/ml, Thermo Scientific) or type I bovine collagen (100 μg/ml, Nutragen). After overnight incubation at 4 °C,

wells were washed with sterile PBS and blocked with 2 % bovine serum albumin (BSA) at room temperature for 1 h. Following the removal of BSA, wells were washed three times with PBS, and 100 µl of each *S. epidermidis* isolate (10^8 CFU/ml), was added to wells. Plates were subsequently incubated at 37 °C for 2 h, after which, planktonic bacteria were washed out with PBS and adhered cells collected with 125 µl of PBS supplemented with 0.5 % Triton X-100 (Sigma). Serial dilutions of lysates were then plated onto blood agar plates and incubated at 37 °C overnight to determine CFU.

Ex vivo human skin wound infection model and histological analysis

The potential of *S. epidermidis* isolates to form biofilm on wounded human skin was examined by using previously described *ex vivo* wound infection model[6-8]. Isolates were cultivated at 37°C in TSB overnight and bacterial number were adjusted to 10^7 CFU/ml in DMEM supplemented with 10% fetal bovine serum (FBS). Either 10 µl of media or bacterial inoculum (10^5 CFUs) was applied to the wound. *Ex vivo* wounds were cultured at the air-liquid interface for 4 days, with media changes every 24 h to select for adherent bacteria and facilitate biofilm formation. After 4 days, wounds were collected, homogenized, and plated onto blood agar plates to quantify CFU. To evaluate wound healing rates, the *ex vivo* tissue explants were fixed and processed for paraffin embedding followed by sectioning and staining with hematoxylin and eosin (H&E). Histological assessment of healing rates were analyzed for epithelialization by histology assessment using a BZ-X700 microscope (Keyence) and and quantified by ImageJ.

Immunofluorescence staining

Paraffin-embedded tissue sections of *ex vivo* wounds were used for staining with anti–*S. epidermidis* antibody (Thermo Scientific, #MA1-35788, 1:50). *S. epidermidis* was visualized with

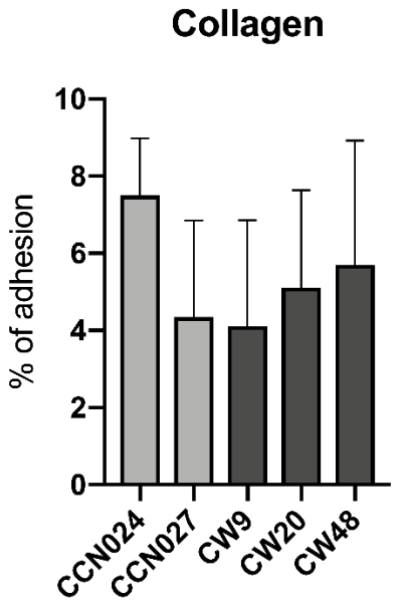
Alexa Fluor 594–conjugated goat anti-mouse antibody (Invitrogen, #A32742, 1:500) and mounted with Prolong DAPI Gold antifade reagent (Invitrogen) to visualize epidermal cell nuclei. Images were obtained with BZ-X700 microscope.

RNA isolation and quantitative real-time PCR (qRT-PCR)

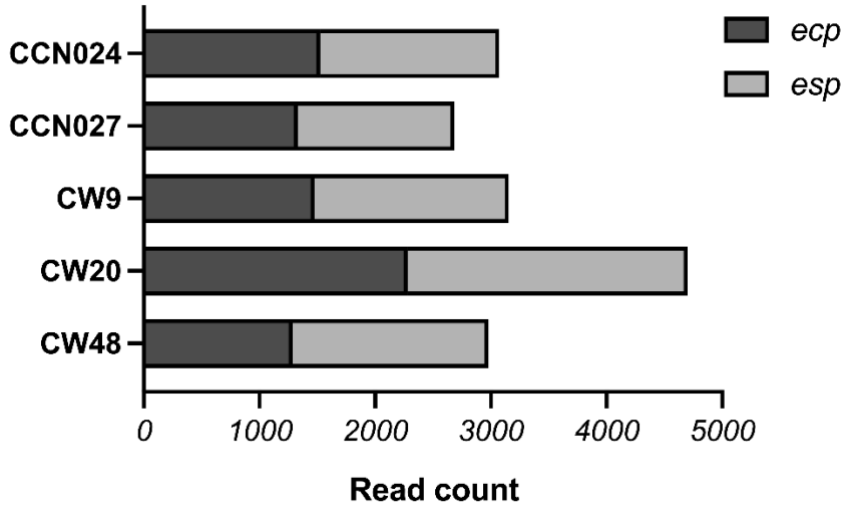
Total RNA from *S. epidermidis* strains was isolated by using RNeasy Mini Kit (Qiagen), with a modified lysis step using 1 mg/ml of lysostaphin in 20 mM TE buffer pH=8 for 1h at 37°C followed by addition of proteinase K and incubation for additional 30 min on 56°C. After the lysis step, the isolation was done according to manufacturer′s protocol. DNase treatment was performed with Turbo DNA-free Kit (Thermo Scientific) followed by reverse transcription of 200 ng of RNA template with a QuantiTect Reverse Transcription Kit (Qiagen). RNA from human *ex vivo* wounds was extracted using Direct-zol RNA Miniprep Kit (Zymo Research); 500 ng of RNA was transcribed into cDNA after DNase treatment. qPCR was done using the CFX Connect thermal cycler and detection system (Bio-Rad) in triplicates by using PerfeCTa SYBR Green SuperMix (Quanta BioSciences) and primers listed in **Supplementary Table 1**. Normalization was done against the *rpoB* for bacterial genes and the *ARPC2* for human genes by using the $2^{-\Delta\Delta CT}$ method[9].

Supplementary Table 1. List of primers used in this study.

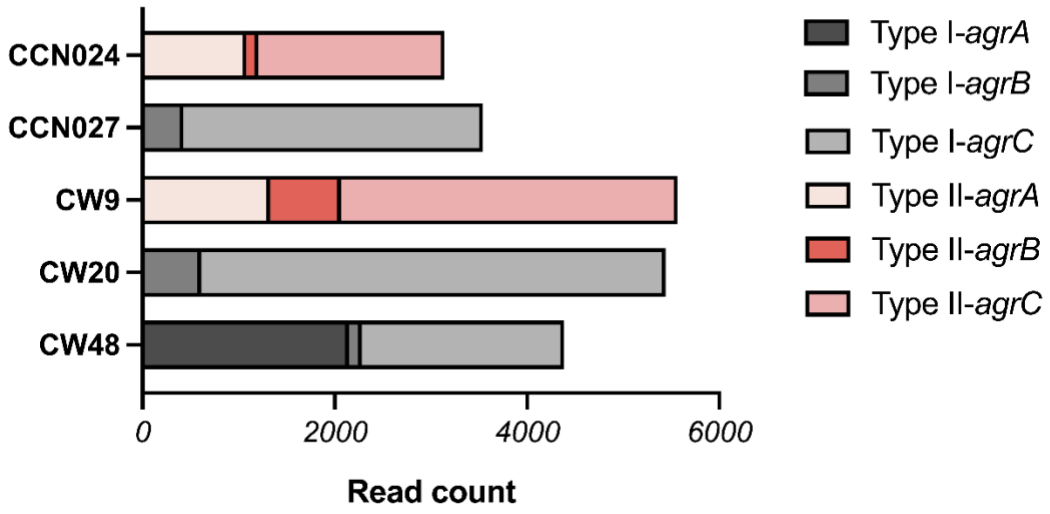
Primer name	Primer sequence 5′–3′	Reference
rpoB forward	ACGTGCGCTAGGTTTCTCAA	This work
rpoB reverse	TCTACTGTTGGTGGTTCGCC	
embp forward	ACAACAAGCAAGTGCAACAAGT	This work
embp reverse	GTTGCAAGTCGTTGTTCACCA	
sdrG forward	AAGACGGCCCTTTCATCAGT	This work
sdrG reverse	CCTTCATCGCCATTCCCTGA	
ARPC2 forward	TCCGGGACTACCTGCACTAC	[10]
ARPC2 reverse	GGTTCAGCACCTTGAGGAAG	
IL-1β forward	GGCTTATTACAGTGGCAATGAGGA	[10]
IL-1β reverse	TCCATGGCCACAACAACTGA	
TNF-α forward	CCGAGGCAGTCAGATCATCCT	[8]
TNF-α reverse	AGGATGATCTGACTGCCTCGG	
IL-8 forward	TTTTGCCAAGGAGTGCTAAAGA	[8]
IL-8 reverse	AACCTCTGCACCCAGTTTTC	
IL-6 forward	GCTACATCCTCGACGGCATCT	This work
IL-6 reverse	AGATGCCGTCGAGGATGTAGC	
S100A7 forward	ACGTGATGACAAGATTGAGAAGC	This work
S100A7 reverse	GCGAGGTAATTTGTGCCCTTT	
LL-37 forward	GGCTGGTGAAGCGGTGTAT	[1]
LL-37 reverse	TGGGTACAAGATTCCGCAAAAA	



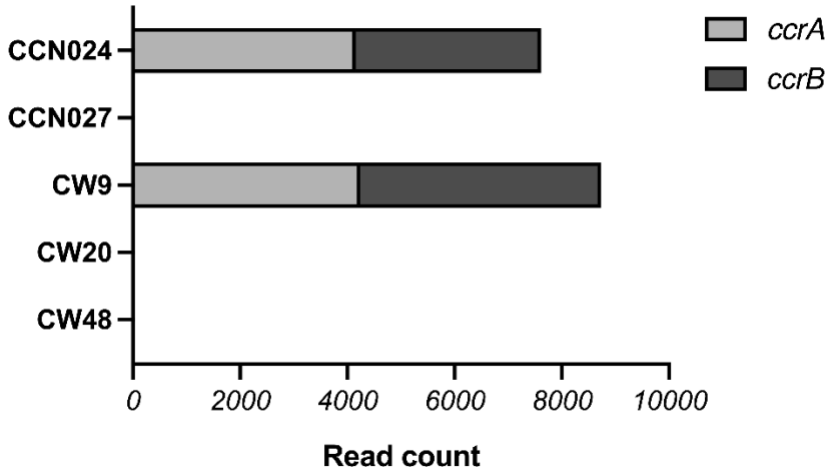
Supplementary Figure 1. Percentage (%) of adhesion of all isolates to collagen, *in vitro*. Data are presented as the mean ± SD from results obtained from three independent experiments (n=6-12). One-way ANOVA followed by Dunnett's *post hoc* test was used to compare the results.



Supplementary Figure 2. Abundance of the cysteine (*ecp*) and serine (*esp*) exoprotease genes in *S. epidermidis* isolates. All strains regardless of the origin contained *ecp* and *esp* genes.



Supplementary Figure 3. Distribution of the *agr* genes in *S. epidermidis* isolates from VLUs and healthy skin. Heathy skin isolate CCN024 and chronic wound isolate CW9 contained genes encoding Type I agr; type II agr was detected in heathy skin isolate CCN027 and chronic wound isolates CW20 and CW48.



Supplementary Figure 4. Abundance of the genes encoding cassette chromosome recombinase A and B (*ccrA* and *ccrB*) in *S. epidermidis* isolates. *CcrA* and *ccrB* were presents only in healthy skin isolate CCN024 and chronic wound isolate CW9.

Supplementary References

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