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The effect of nail sticker use on hand surface bacterial counts after surgical scrubbing

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Objective
We hypothesized that applying nail stickers to nails shorter than 2 mm would increase bacterial load and species abundance compared to nails of the same length without stickers. The aim of our study was to investigate aerobic bacterial load and species abundance on fingernails before and after nail sticker application over a 2-week period.

Methods
50 participants were enrolled, with 48 completing the entire study. Participants wore study-provided nail stickers (solid or patterned) for 7 days. All nails were trimmed to ≤ 2 mm. Standardized surgical scrubs with 4% chlorhexidine were performed, and swabs from fingernail surfaces and subungual spaces were collected at 3 time points: day 0 (before application), day 7 (immediately after application), and day 14 (1 week after application). We quantified bacterial load, and species were identified with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.

Results
Median $\log_{10}$ bacterial loads were 1.48 at day 0, 1.00 at day 7, and 2.01 at day 14. Bacterial load increased from days 7 to 14 but did not differ between days 0 and 7 or days 0 and 14. *Staphylococcus epidermidis* abundance rose over time, while sticker design had no effect. *Enterococcus faecalis* was detected only once at day 14.

Conclusions
Wearing nail stickers for 1 week significantly increased bacterial load despite surgical scrubbing, likely due to adhesive surfaces promoting bacterial adhesion.

Clinical Relevance
These findings suggest that nail stickers may compromise hand hygiene and increase infection risks in veterinary clinical settings. Avoiding nail stickers during procedures may reduce bacterial persistence and potential zoonotic transmission.

Keywords: sterility, scrub, hand, nail, *Staphylococcus epidermidis*

Research on fingernail properties (eg, nail length) and the effects of nail polish and acrylic nails on bacterial load is well documented in human medicine.[1–3] However, there is limited research on veterinary professionals. Hardy et al[2] studied bacterial accumulation in a veterinary teaching hospital and found that while unchipped nail polish does not increase bacterial counts, nail lengths over 2 mm significantly contributed to increases in bacterial load despite thorough surgical scrubbing in veterinary students, small animal surgery technicians, interns, residents, and faculty.

The WHO and CDC have recommended that healthcare workers maintain natural nails < 5 mm (WHO) or < 6.35 mm (CDC) in length, but even shorter nails (under 2 mm) have been shown to harbor fewer bacteria.[2,4–7] The popularity of artificial acrylic nails and nail polish has grown in recent years. New nail cosmetics, including acrylics and flexible nails that can be cut to any length, have prompted research into the effects of nail length, acrylic use, and nail polish on bacterial load in human healthcare workers.[2,3,8–10] Research suggests that while there is no significant difference in bacterial load between chipped and unchipped polished nails,[1–3,11] artificial nails and longer nails harbor significantly more bacteria.[8,10] The impact of nail polish on surgical site infections remains unclear.[11,12]

Research on nail characteristics and bacterial pathogens leading to lower patient survival has predominantly focused on human medicine. In veterinary settings, professionals handle diverse species with the potential for harboring different and potentially zoonotic pathogens.[9,13–15] Veterinary professionals also encounter occupational hazards such as bites, scratches, and trauma from larger

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animals.[16] These injuries, coupled with the use of nail cosmetics, may contribute to bacterial build-up, creating infection risks that differ from those observed in human healthcare settings.

Specific bacterial species including *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Enterococcus faecalis* are often the most frequently isolated organisms in postoperative and implant-associated infections in both human and veterinary medicine.[17–24]

Nail stickers are a newer nail accessory and have not been studied in terms of bacterial load. These stickers can be cut to any length and allow for nails to be shorter, which may reduce bacterial accumulation as suggested by the Hardy et al study.[2] To our knowledge, no published studies have evaluated nail stickers in bacterial accumulation. To offer personnel working in the field of veterinary medicine an opportunity to understand the effects of nail stickers on bacterial load that has not been investigated, the objectives of our study were to (1) determine the effect of nail stickers on postscrub bacterial load by comparing fingernails with and without stickers over 3 time points and (2) identify aerobic bacterial species cultured from fingernail surfaces after nail stickers were worn for a week. The findings from our study could improve patient outcomes, with fewer postsurgical complications, as well as provide insight on changing infection-control protocols regarding workers and their interest in wearing nail cosmetics. We hypothesized that the application of nail stickers would increase aerobic bacterial load on nails under 2 mm and by default increase the overall abundance of aerobic bacterial types compared to nails of the same length without nail stickers. Given the aforementioned evidence of bacterial load on nail length, participants in this study were required to maintain their nails under 2 mm to minimize bacterial load and keep that variable constant.[2]

Methods

Our study assessed aerobic bacterial counts and species on fingernail surfaces and subungual spaces before and after applying nail stickers and performing surgical scrubbing over a 2-week period in veterinary professionals from October 8, 2024, until October 23, 2024. Each participant took part in the 2-week study period, with staggered start dates on either a Tuesday or Wednesday.

Volunteer recruitment

Volunteers were recruited from the University of Calgary Faculty of Veterinary Medicine (UCVM) community, including veterinary students, veterinary technicians, and veterinarians. The inclusion criteria consisted of participants being able to consistently wear nail stickers for 7 days. The exclusion criteria included having a known allergy to 4% chlorhexidine scrub brush chemicals, which could cause lesions on the skin, reducing the effective skin barrier and causing proliferation of other secondary bacteria that could affect the study. The study was approved by the University of Calgary's Conjoint Faculties Research Ethics Board (REB24-0074).

Volunteer participation and sample collection

A step-by-step video on the UCVM surgical scrubbing procedure was emailed to all participants a week prior to the first sample collection. Steps of this scrub protocol are highlighted in **Supplementary Material S1**. Each participant performed a scrub according to the same protocol once at baseline (labeled day 0, before application of nail stickers), without nail stickers, using 4% chlorhexidine surgical scrub. In brief, all participants were required to remove all jewelry (eg, rings, watches, hair ties, and bracelets). All participants began by washing their hands and arms with antimicrobial soap, and all nails were measured and cut to 2 mm or shorter. The lengths of all 10 nails were measured by inserting a nonsterile stainless steel ruler (150 X 10 mm) under each nail. A 4% chlorhexidine scrub brush package was opened (BD E-Z preoperative surgical scrub brush No. 30; Becton, Dickinson and Co), and the protocol began with creating suds and scrubbing all surfaces of the fingers, hands, and arms to begin contact time for the soap. The nail pick included in the scrub package was used to pick under each fingernail, and a timer was set for 5 minutes for each participant to begin scrubbing with the scrub brush. Participants began by using the plastic scrub side on their fingernail surfaces and subungual spaces of each finger, then proceeded to their palms and dorsal hand surfaces. They finished the scrub with their arms. Water was used to rinse off suds with hands held so that gravity pulled water to the elbows, rather than back over finger surfaces (ie, elbows were held low when running through the water). The primary researcher was present throughout all scrubbing procedures and nail measurements to ensure consistency in scrubbing technique for all participants. Following the scrubbing procedure, each hand of every participant had fingernail surfaces and subungual spaces swabbed and cultured with the use of a sterile swab and toothpick. One week later, all participants applied study-provided nail stickers (gloss nail stickers; Dashing Diva) in their choice of sticker type and characteristics (ie, with designs vs a solid color), and within 24 hours of application, they performed another surgical scrub according to the designated protocol. Second postscrubbing samples (fingernail surface and subungual space) were collected from each individual (labeled day 7, immediately following application). Each individual wore their nail stickers for 7 days and performed a third surgical scrub followed by sample collection in the same way mentioned after scrubbing (labeled day 14, a week after application). Swab collections were chiefly performed by the same primary researcher at each time point or by the same assisting researcher under the primary researcher's direct observation to maintain consistency in sampling technique. Samples were submitted to the UCVM Diagnostic Services unit for aerobic bacterial culture and identification. The primary outcome of interest was bacterial load and aerobic species type at the 3 designated time points. If a nail sticker started peeling or detaching to the point where it would normally be removed,

either from natural wear or participant preference, participants were instructed to remove it and not replace it until after their final scrub on day 14.

Sterile cotton swabs were first moistened in 1 mL of buffered peptone water (BPW) in sterile vortex tubes before being used to swab the surfaces of all 10 fingernails, ensuring coverage of all nail edges and the dorsal surface. A sterile toothpick was then used to scrape the subungual region of the same 10 nails. Samples from both the swabs and toothpicks were combined and immediately placed into the BPW prior to being plated on agar.

Bacterial culture and analysis

A serial 10-fold dilution from 10^{-1} to 10^{-5} was prepared from the combined swab and toothpick samples of each participant collected in BPW (Oxoid; Nepean). All dilutions were vortexed for 10 to 15 seconds then plated onto Columbia blood agar (Oxoid) and MacConkey agar (Oxoid). Aerobic cultures were performed with blood agar plates that were incubated at 35 °C in 5% CO_2, and the MacConkey agar plates were incubated at 35 °C in ambient air after initial plating. Aerobic bacterial colonies were examined at 24, 48, and 72 hours, with colony counts performed at 48 hours to ensure sufficient growth, expressed as CFUs per milliliter. Colonies were counted on all plates, with a maximum limit of 300 colonies per plate for accurate counting. The CFUs per milliliter for each sample were calculated by multiplying the number of colonies on each plate by the reciprocal of the dilution factor and dividing by the volume plated (in milliliters). This process was repeated for each dilution, and the results were averaged to determine the final CFUs per milliliter for each sample.

Bacterial identification

Bacterial isolates were identified with the use of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI Biotyper Sirius; Bruker Daltonics). This method ionizes bacterial proteins and measures their mass-to-charge ratio, which is then generated into a characteristic mass spectrum. Spectra matching against the reference library database was performed with MBT Compass software (version 4.1; Bruker Daltonics GmbH). Bacterial identification criteria were as follows: 2.00 to 3.00 indicated high-confidence species identification, 1.70 to 1.99 indicated low-confidence species identification, and 0.00 to 1.69 indicated no organism identification.

Statistical analysis

Data transformation and normality assessment—A logarithmic base 10 transformation was used on bacterial load (log_{10} CFUs/mL), as their values were highly skewed. To avoid undefined results in log transformation due to 0 bacterial load values, 1 CFU/mL was added to all observations prior to log_{10} transformation. A Shapiro-Wilk test was used to check the normality assumption of the transformed bacterial load. Summary statistics of mean, median, SD, minimum, and maximum of bacterial load were reported. Distinct bacterial species for each participant were counted per time point. Results of bacterial counts for each bacterial species can be found in **Supplementary Table S1**.

Analysis of bacterial load over time—Differences in bacterial load across the 3 time points (days 0, 7, and 14) were analyzed with the Friedman test to account for the nested data structure from repeated measurements. Post hoc pairwise comparisons between time points were conducted with the Wilcoxon signed rank test with Bonferroni correction. A Wilcoxon rank sum test was conducted to investigate whether nail sticker characteristics (ie, having designs vs a solid color) would influence the difference in bacterial load between days 7 and 14.

Bacterial type distribution analysis—A Fisher exact or χ^2 test was used to assess differences in aerobic bacterial type distributions over the 3 time points. Specifically, we focused on *S aureus*, *S epidermidis*, and *E faecalis*, as these bacterial species are commonly associated with postoperative infections in both human and veterinary medicine.

Power analysis—Prior to data collection, based on an effect size of 0.43 estimated from Hardy et al,[2] a power analysis performed with G*Power (version 3.1; Heinrich-Heine-Universität Düsseldorf) suggested a sample size of 44 participants was required in order to reach a power of 0.803 at an α level of 0.05.

All data analyses were performed in R[25] and RStudio.[26] A *P* value of < .05 was considered statistically significant in all tests.

Results

A total of 50 participants volunteered for this study; however, 2 did not finish the final scrub (following application of nail stickers, day 14) and were removed from the study, resulting in a total of 48 participants included in the analysis. Of the 48 participants, 18.75% chose plain (solid-color) nail stickers and 81.25% chose designs with glitter and/or ridges. In total, 144 fingernail swab samples were analyzed for the study. Summary statistics of bacterial load for each time point are presented in **Table 1**.

Analysis of bacterial load

There was a significant increase in bacterial load at day 14 compared to day 7 ($P < .001$), but no significant differences were observed between days 0 and 7 ($P = 1.00$) or days 0 and 14 ($P = .059$; **Figure 1**).

Table 1—Descriptive statistics for bacterial load (log_{10} CFUs/mL).

Time	Mean	Median	SD	Min	Max	Sample size (n)
Day 0	1.67	1.49	1.27	0	4.59	48
Day 7	0.85	1.04	0.97	0	4.06	48
Day 14	2.06	2.01	1.06	0	4.31	48

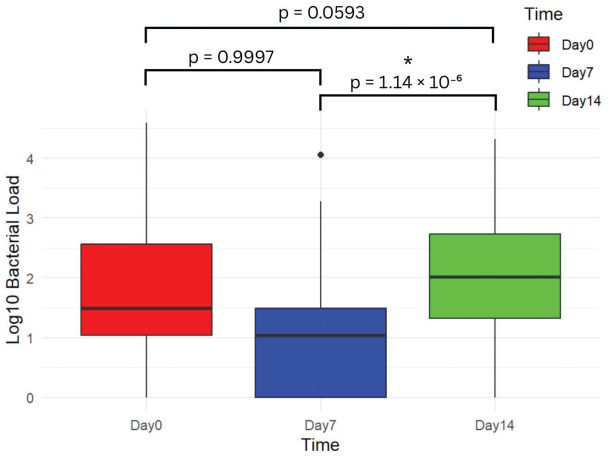

Figure 1—Box plot of bacterial load across 3 time points. Bacterial load was compared with a Friedman test, followed by Wilcoxon signed rank tests for pairwise comparisons. *P* values are noted between time points, with bacterial load significantly increasing between days 7 and 14 (asterisk).

Median $\log_{10}$ bacterial loads were 1.48 at day 0, 1.00 at day 7, and 2.01 at day 14. There was no significant effect of nail sticker type on bacterial load (P = .94; r = 0.013; 95% CI, 0.0018 to 0.31).

Although comparing bacterial load between nail stickers with designs (eg, grooves and glitter) versus no designs (eg, solid color) was not the focus of our project, there were no significant changes found between days 7 and 14 (1 week of wearing the nail stickers) between participants who chose to wear nail stickers without a design compared to those who wore nail stickers with a design.

Analysis of bacterial type

There were no significant changes in overall bacterial composition across all time points (P = .418). Similar results were observed when bacteria were categorized by genus (P = .423). However, a significant change over time was observed only in *S epidermidis* species (χ^2 = 23.17; P < .001), but not in *S epidermidis* (P = .62) or *E faecalis* (P = 1.00). Bacterial types by species were plotted on a stacked bar chart to visualize distribution across 3 time points (days 0, 7, and 14), as shown in **Figure 2**.

Enterococcus faecalis was cultured only once throughout the study, from a single participant at day 14 (1 week after nail application). It was not detected at day 0 or 7 for this or any other participant. At the time of detection, the CFU count for *E faecalis* was 0.5 X 10^1 CFUs/mL, which was markedly lower than the highest bacterial loads observed for other species in the study, which reached up to 8.07 X 10^2 CFUs/mL.

Discussion

Our study replicated the design of the Hardy et al study[2] of the effects of nail characteristics on bacterial count following surgical scrubbing, with modifications to fit our specific research objectives. The objectives of our study were to determine whether nail stickers had an effect on the bacterial load and aerobic bacterial types present on fingernails following a standard surgical scrub procedure. The results of this study indicated that bacterial load does increase significantly after a week of wearing nail stickers despite performing a surgical scrub procedure. Overall, the distribution of bacterial species abundance across the 3 time points (day 0, before application of nail stickers; day 7, immediately after application; and day 14, a week after application) did not differ significantly. However, we revealed a significant difference specifically in the abundance of *S epidermidis* across the 3 time points. This distinction indicates that while the overall composition of bacterial species remained similar, *S epidermidis* showed a time-dependent shift in abundance, with an increase noted after participants wore nail stickers for 1 week.

There are no published studies investigating the impact of nail stickers on bacterial load in either human or veterinary medicine. Nail characteristics such

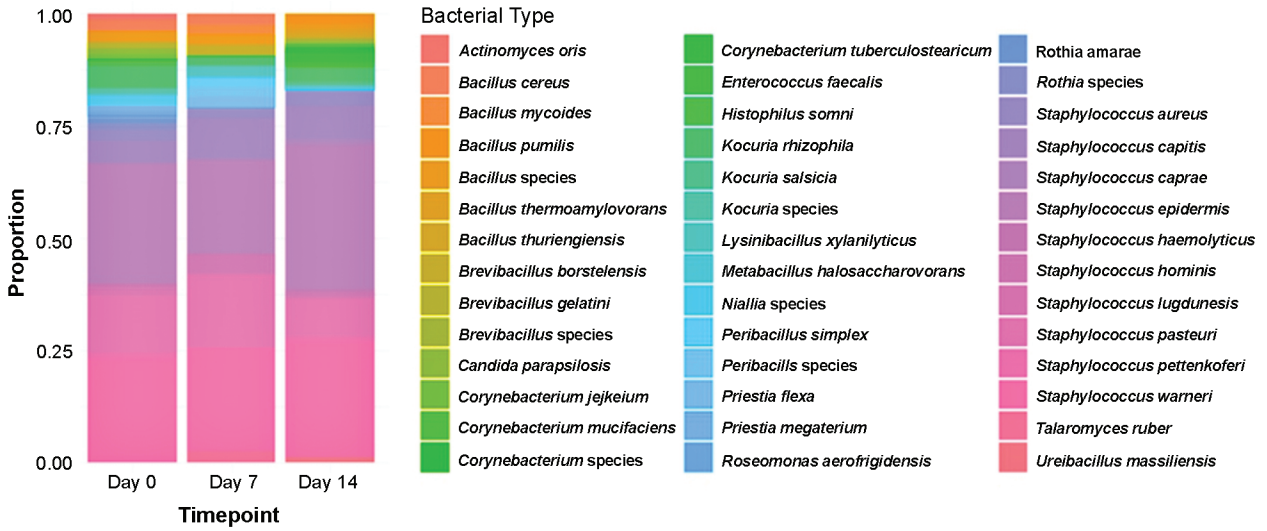

Figure 2—Bacterial species distribution across 3 time points (proportion).

as length and various accessories (eg, acrylic nails, gel nails, and nail polish) have been widely studied; however, their effects on bacterial accumulation remain under debate.[1–3,11] We enforced that participants volunteering for this study had to cut their nails to ≤ 2 mm prior to scrubbing and swabbing, as existing research strongly suggests that surgical personnel should maintain natural nails shorter than 2 mm to minimize bacterial load on fingernail surfaces.[2,7] Our study recruited participants from the field of veterinary medicine who had knowledge and practice in performing a surgical scrub; however, they were not required to have recently performed a surgical scrub. Our initial hypothesis proposed that bacterial load would be higher at days 7 and 14 compared to day 0. However, the results of this study only showed a significant increase in bacterial load from days 7 to 14. These findings suggest that bacterial accumulation on nail stickers may not be solely due to duration of wear and could be influenced by additional factors. Firstly, the adhesive properties of the Dashing Diva nail stickers are likely made up of polyurethane-11 and acrylate copolymers as stated in their ingredients list.[27] Although direct studies on nail adhesives containing polyurethane-11 and acrylate copolymers are limited, research on similar materials suggests that polyurethane and acrylate copolymer modifications can influence bacterial adhesions.[28,29] These studies demonstrate that altering the polymer structure with hydrophilic compounds can enhance antibacterial properties by reducing bacterial attachment. However, the nail stickers used in our study contained adhesives composed of polyurethane and acrylate copolymers without any structural modifications and that naturally possess adhesive properties. These adhesives likely make the nail stickers more prone to bacterial adhesion by providing a surface that facilitates bacterial attachments. The results of our study indicate that bacterial load significantly increased from days 7 to 14 (Figure 1), which may be explained by more bacteria being cultured from the adhesive surfaces of the nail stickers. In addition, on visual inspection of nail stickers that were removed after the scrub and swab protocol on day 14, there was distinct debris left on the edges of the nail stickers, as shown in **Figure 3**. This finding corresponds with the bacterial load differences cultured between days 7 and 14.

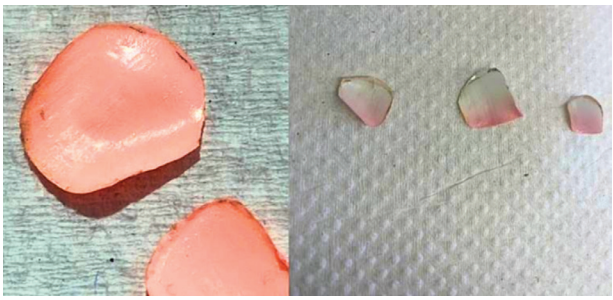

Figure 3—Visual inspection of the adhesive side of the removed nail stickers showing distinct debris along the lateral and distal edges. The stickers were examined after the scrub and swab protocol on day 14.

When comparing changes in bacterial load following a surgical scrub for the 2 different types of nail stickers (nail stickers that were solid or with no designs vs those with designs), no significant change was found between days 7 and 14 (1 week of wearing the nail stickers). These results can be related to the study by Hardy et al,[2] identifying no significant differences in bacterial load between chipped and nonchipped nail polish. A proper surgical scrub is likely able to disinfect within grooves or crevices that may act as a nidus for bacteria to replicate.

Several bacterial types were cultured from our study at different time points (Figure 2). *Staphylococcus epidermidis* is a common biofilm-forming pathogen identified in implantation procedures involving biomaterials that cause several fatal surgical site infections in humans.[17,18] One study[30] examined how the surface chemistry of different polyurethane-based polymers that were modified with either hydrophilic or hydrophobic end groups influenced bacterial adhesion. Polyurethane is naturally slightly hydrophobic, making it an appropriate adhesive with strong, durable bonding while resisting excessive moisture absorption that could weaken the nail sticker adhesion properties. Research by Patel et al[30] found increased initial bacterial adhesion on polyurethane modified with hydrophobic end groups. Since *S epidermidis* is a relatively hydrophobic bacterium,[31] this characteristic may have contributed to the higher quantities of *S epidermidis* cultured after the application of nail stickers (day 7) and again after they had been worn for a full week (day 14).

Another bacterial species cultured in this study that stood out as being impactful in the field of medicine is *S aureus*. This pathogen is highly opportunistic in humans and has several deleterious effects on animal health, particularly causing major economic losses in livestock production. For example, *S aureus* is a causal pathogen for mastitis in ruminants and horses.[22,23] A property of staphylococci that is becoming increasingly problematic in the field of human and veterinary medicine is their ability to become resistant to antimicrobials, creating methicillin-resistant strains. More specifically, methicillin-resistant *S aureus* (MRSA) and methicillin-resistant *S pseudintermedius* (MRSP) are 2 important bacterial species causing significant problems in the field of veterinary medicine and from a public health perspective. Both MRSA and MRSP are important because of their zoonotic potential and for the possibility of companion animals to act as carriers of the methicillin-resistant strains. While both are relevant in this field, our study only cultured *S aureus* rather than *S pseudintermedius* and we did not test for methicillin resistance. *Staphylococcus aureus* has been associated with mastitis in ruminants and superficial to deep infections involving skin (dermatitis), urinary tracts (cystitis), prosthetics, or biomaterial implants.[22,23] However, it is important to recognize its role in clinical and epidemiological contexts.

In this study, *S epidermidis* was cultured 20 times at day 0, 7 times at day 7, and 30 times at day 14, while *S aureus* appeared only once at day 0, once at

day 7, and 3 times at day 14. Because *S aureus* was cultured so infrequently, statistical comparisons of its bacterial load across time points would not have been meaningful. However, given its importance in veterinary medicine, we conducted a test to determine whether its presence in cultures varied significantly over time. The results did not show a significant difference. Lerebour et al[31] found differences in the physicochemical characteristics between *S epidermidis* and *S aureus*, with the latter being more hydrophilic. This finding may explain the differences in bacterial counts observed in our study, with far fewer participants culturing *S aureus*. Given these factors and the results obtained from our study, the adhesive properties of nail stickers may contribute to the persistence of bacteria like *S epidermidis* and *S aureus* on fingernail surfaces and may complicate efforts to remove pathogens from fingernail surfaces following a standard surgical scrubbing. Thus, the results from our study suggest that wearing these nail stickers may not be advisable for surgical personnel in medical settings.

Lastly, *Enterococcus* spp were cultured in our study and have been known to cause nosocomial infections in animals, leading to this bacterial type to become more intrinsically resistant to antimicrobial agents.[19,21,24] More specifically, *E faecalis* was cultured in our study and historically has had zoonotic potential with impacts in human health settings.[19,24] This species was only cultured from 1 participant at day 14, after the nail stickers had been worn for a week. Colony counts from our serial tenfold dilutions indicated a very low bacterial load, with growth of only 1 colony at the undiluted level. In comparison, other bacterial species isolated across all participants yielded a larger quantity of colonies, indicating a substantially higher bacterial load. Considering that *E faecalis* is a problematic pathogen with zoonotic potential, it raises concerns about the adhesive properties of the nail stickers. These properties might cause bacteria to adhere more strongly to the nails, potentially making it harder for a surgical scrub to remove pathogens. The presence of this bacterium, known to cause skin, urinary tract, and other types of infections in animals and humans,[20,24] further suggests that wearing these nail stickers may not be ideal from an infection-control perspective.

The SD of bacterial load measurements at day 0 (initial scrub/swab without nail stickers) indicated considerable variation between participants. Consequently, no significant difference in bacterial load was observed between days 0 and 14 (following the scrub/swab protocol after 1 week of nail sticker application). There seemed to be a general trend where bacterial counts decreased from days 0 to 7 (after the scrub/immediately after nail sticker application) that was not statistically significant. This finding may be due to variations in the participants' experience with proper surgical scrubbing techniques. Although we provided participants with an instructional video before the initial scrub and had access to a written protocol during the procedure, an initial practice session was not scheduled. It is possible that the initial scrub session on day 0 served as a reminder and scrubbing was improved on day 7. We would have expected a flat line between days 0 and 7 or a slight increase in bacterial counts and not the dip noted (Figure 1). To address this variation, future studies should incorporate a practice scrub session for all participants before collecting culture swabs from subungual and fingernail surfaces to ensure greater consistency in technique.

In addition, exclusion factors such as history of dermatitis, occupation (veterinarian, veterinary student, veterinary technician), and quantified values of animal contact per day or week were not recorded. Each participant served as their own internal control, which likely helped reduce interindividual variability by accounting for personal hygiene habits and baseline bacterial load differences. However, the inclusion of participants from diverse roles and varying levels of animal exposure may have introduced additional variability in our results. Future studies could aim to further control these factors by recruiting a more homogenous study population, such as exclusively surgical personnel or individuals working strictly with small or large animals. Nonetheless, this study represents a preliminary investigation in the area of nail sticker use in medical personnel. Our broader recruitment approach was intended to reflect real-world conditions in veterinary medicine and enhance the generalizability of the findings.

The results from our study highlight the need for caution when medical personnel use nail stickers, as the adhesive properties of the nail stickers, which do not require UV curing or additional methods of forming a tight seal, may facilitate bacterial attachment. Even after surgical scrubbing with 4% chlorhexidine solution and scrub brushes, bacteria persisted, which is likely because of their ability to adhere to the adhesive surfaces of the nail stickers. Additionally, bacteria like *S epidermidis*, known to form biofilms and cause implantation infections, may be particularly prone to adhering to adhesive areas of nail stickers. This is especially relevant in veterinary settings, where contact with animals recovering from implantation surgeries necessitates strict hygiene precautions. Outside the operating room, it is also important to note that nail stickers may serve as reservoirs for bacteria, including *S aureus* and *S pseudintermedius*. These species were cultured in our study and are associated with MRSA and MRSP infections, which could contribute to the spread of highly resistant and clinically damaging nosocomial infections in both human and veterinary hospital settings. Because of these factors, avoiding nail stickers during surgical procedures is advisable. Until further research is done, an additional recommendation to consider would be to wear gloves when handling patients that are susceptible to nosocomial infections.

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Disclosures

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Supplementary Materials

Supplementary materials are posted online at the journal website: avmajournals.avma.org.

Supplementary Material S1—Scrub Protocol.

1. Remove all jewelry.

2. Examine your fingernails. Trim them to <2 mm if necessary with sanitized nail clippers.

3. Start with clean hands. Wash your hands and arms with soap and towel dry them if they are not clean.

4. Open your brush pack. Hold the brush & nail pick in your hand versus setting it down in the scrub sink area.

5. Wet the brush with water and wet your hands and forearms with water. Squeeze the brush to generate suds. Using the scrub brush apply antiseptic soap over both hands and forearms. Try to generate a good lather and do not rinse.

6. Hold your hands higher than your elbows (to prevent dirty water from running down onto your hands).

7. Using nail pick, clean your nails under running water. You can hold your scrub brush while doing this to avoid setting it down. Drop nail pick into sink when done with it.

8. Scrub both hands with the brush. The idea is to scrub all surfaces of your hand, fingers, and thumb for a total of 3 minutes. Do not rinse.

9. Next, move on to scrubbing each forearm, starting near the wrist and progressing towards the elbow for a total of 1 minute each. Works best to divide the forearm into 1/3's, focusing on each 1/3 for 20 seconds. Do not rinse.
scrub.

10. Drop the scrub brush into the sink once you are done scrubbing.

11. Rinse hands and arms, starting at your hands and ensuring that you keep your elbows lower than your hands so that the water runs down your arm towards your elbows and not away.

Supplementary Table S1—Bacterial species counts.

Bacterial Species	Day 0	Day 7	Day 14
Actinomyces oris	1	0	0
Bacillus cereus	2	1	0
Bacillus mycoides	0	1	0
Bacillus pumilis	0	0	2
Bacillus species	2	1	1
Bacillus thermoamylovorans	0	0	1
Bacillus thuriengiensis	0	0	1
Brevibacillus borstelensis	0	1	0
Brevibacillus gelatini	1	0	0
Brevibacillus species	0	0	1
Candida parapsilosis	1	0	0
Corynebacterium jejkeium	1	0	0
Corynebacterium mucifaciens	0	0	1
Corynebacterium tuberculostearicum	0	0	2
Enterococcus faecalis	0	0	1
Histophilus somni	1	0	0
Kocuria rhizophila	4	1	2
Kocuria salsicia	0	0	1
Kocuria species	1	0	0
Lysinibacillus xylanilyticus	0	1	0
Metabacillus	0	0	1

halosaccharovorans			
Niallia species	2	0	0
Peribacillus simplex	0	1	0
Peribacillus species	0	1	0
Priestia flexa	0	1	0
Priestia megaterium	1	0	0
Roseomonas aerofrigidensis	1	0	0
Rothia species	1	0	0
Staphylococcus aureus	1	1	3
Staphylococcus capitis	4	4	7
Staphylococcus caprae	0	0	1
Staphylococcus epidermidis	20	7	30
Staphylococcus haemolyticus	0	0	1
Staphylococcus hominis	1	2	1
Staphylococcus lugdunesis	1	0	0
Staphylococcus pasteuri	9	6	8
Staphylococcus pettenkoferi	1	0	1
Staphylococcus warneri	16	8	24
Talaromyces ruber	0	1	0
Ureibacillus massiliensis	0	0	1