Decolonization of Human Anterior Nares of *Staphylococcus aureus* with Use of a Glycerol Monolaurate Nonaqueous Gel

Patrick M. Schlievert, a Marnie L. Peterson

aDepartment of Microbiology and Immunology, University of Iowa Carver College of Medicine, Iowa City, Iowa

bHennepin Life Sciences, Minneapolis, Minnesota, USA

**ABSTRACT**  *Staphylococcus aureus* is a highly significant infection problem in health care centers, particularly after surgery. It has been shown that nearly 80% of *S. aureus* infections following surgery are the same as those in the anterior nares of patients, suggesting that the anterior nares is the source of the infection strain. This has led to the use of mupirocin ointment being applied nasally to reduce infections; mupirocin resistance is being observed. This study was undertaken to determine whether gel composed of 5% glycerol monolaurate solubilized in a glycol-based, nonaqueous gel (5% GML gel) could be used as an alternative. In our study, 40 healthy human volunteers swabbed their anterior nares for 3 days with the 5% GML gel. Prior to swabbing and 8 to 12 h after swabbing, *S. aureus* and coagulase-negative staphylococcal CFU per milliliter were determined by plating the swabs on mannitol salt agar. Fourteen of the volunteers had *S. aureus* in their nares prior to 5% GML gel treatment, most persons with the organisms present in both nares; five had pure cultures of *S. aureus*. All participants without pure culture of *S. aureus* were cocolonized with *S. aureus* and coagulase-negative staphylococci. Five of the *S. aureus* strains produced the superantigens commonly associated with toxic shock syndrome, though none of the participants became ill. For both *S. aureus* and coagulase-negative staphylococci, the 5% GML gel treatment resulted in a 3-log-unit reduction in microorganisms. For *S. aureus*, the reduction persisted for 2 or 3 days.

**IMPORTANCE** In this microflora study, we show that a 5% glycerol monolaurate nonaqueous gel is safe for use in the anterior nares. The gel was effective in reducing *Staphylococcus aureus* nasally, a highly significant hospital-associated pathogen. The gel may be a useful alternative or additive to mupirocin ointment for nasal use prior to surgery, noting that 80% of hospital-associated *S. aureus* infections are due to the same organism found in the nose. This gel also kills all enveloped viruses tested and should be considered for studies to reduce infection and transmission of coronaviruses and influenza viruses.

**KEYWORDS** *Staphylococcus aureus*, coagulase-negative staphylococci, decolonization, glycerol monolaurate, nose

*Staphylococcus aureus* bacteria are common commensal bacteria in the nose and other mucosal surfaces of humans (1–4). Estimates of colonization rates are from 30 to 40% depending on age and underlying conditions. As many as 70% of humans may be transiently colonized. For nearly 80% of patients being treated for hospital-associated infections, the infecting *S. aureus* bacteria are the same as those in the anterior nares (3). This has led to the use of agents, such as mupirocin, to be applied to the nose prior to surgery (5–7). As might be expected, there is the appearance of mupirocin-resistant *S. aureus* (8).

Glycerol monolaurate (GML) is generally recognized as a safe compound by the Food and Drug Administration (FDA) for oral consumption and for use in cosmetics.
This molecule is broadly antimicrobial for Gram-positive bacteria, including both methicillin-resistant \textit{S. aureus} (MRSA) and methicillin-susceptible \textit{S. aureus} (MSSA) (9). At approximately 50-fold-lower concentrations than the minimum bactericidal and minimum inhibitory concentrations, which are essentially the same for GML, the compound inhibits production of exotoxins (9). In human use studies, GML has been added to tampons and was shown to be safe (10). GML-coated tampons have been marketed in Europe to reduce the incidence of menstrual toxic shock syndrome (OptiBalance).

In subsequent studies, GML was mixed with a nonaqueous glycol-based gel at 5% GML (11–14). This gel has been referred to as 5% GML gel. In studies with 5% GML gel, it has been shown to be safe vaginally in chronic-use studies in rhesus macaques (6-month study) (12) and women (3-month study; unpublished data). The gel also reduces the transmission vaginally of multiple-challenge, high-dose simian immunodeficiency virus (13, 14). The 5% GML gel is highly active at killing both Gram-positive and Gram-negative bacteria, except lactobacilli, bifidobacteria, and certain enterococci (9, 12, 15). Resistant bacteria contain an immunity gene to GML, where GML acts as a quorum-sensing growth stimulant (15, 16). The 5% GML gel also prevents biofilm formation and removes preformed biofilms (9). The mechanism of action of the gel depends on GML dissipation of the potential difference across bacterial plasma membranes, with accompanying synergy by the nonaqueous gel component (9). As shown in vaginal studies, the glycol-based gel spreads laterally quickly to coat the vagina and other parts of the genital tract (17, 18). Because of the myriad of potential targets of 5% GML gel to kill bacteria, resistance to antimicrobial effects is limited (9).

Staphylococcal superantigens are a large family of secreted toxins that cause massive T lymphocyte proliferation (19, 20). Three of these toxins, notably toxic shock syndrome toxin 1 (TSST-1) and staphylococcal enterotoxins B and C (SEB and SEC), are the major causes of TSS (21). TSST-1 is the exclusive cause of menstrual TSS, occurring with mucosal colonization of \textit{S. aureus} (22). In recent studies, it has been shown that there has been a significant increase in strains producing the six-member enterotoxin gene cluster of superantigens, at least since 2008 (23, 24). These six superantigens, including SEG and SE-like I, M, N, O, and U, appear to be more important for \textit{S. aureus} colonization than overt disease causation (25).

This study was undertaken with institutional review board (IRB) approval to test the ability of 5% GML gel to reduce \textit{S. aureus} colonization of the anterior nares of 40 humans. We confirmed that 35% of healthy volunteers were colonized with \textit{S. aureus}. Strains were identified by the ability to produce the major superantigens that cause TSS, although no participants developed TSS. As in our prior studies, the enterotoxin gene cluster of superantigens was commonly present in \textit{S. aureus} isolates. Five percent GML gel reduced \textit{S. aureus} colonization significantly. Its antimicrobial effect persisted for up to 3 days.

RESULTS

Of greatest importance, when queried upon completion of the study, none of the participants reported any adverse events with use of the 5% GML gel. Of the 40 participants, 14 were positive for \textit{S. aureus} (35%) in the pre-GML gel treatment. Twelve of 14 individuals had \textit{S. aureus} isolated from both nares. Five persons had pure cultures of \textit{S. aureus} in both nares. The remaining nine individuals had mixtures of both \textit{S. aureus} and coagulase-negative staphylococci in both nares.

The \textit{S. aureus} isolates were analyzed for the presence of superantigen genes by PCR. All (100%) of the isolates contained the genes for one or more superantigens. Two strains had the ability to produce TSST-1, and the other 12 had the ability to produce SE-like X. There were no strains that had the genes for both TSST-1 and SE-like X. These two superantigens are usually not produced by the same strains (26). The reason for the exclusion remains unknown. Another notable feature of the superantigen profile was that 9/14 isolates contained components of the enterotoxin gene cluster of six superantigens, including SEG, SE-like I, M, N, O, and U (25, 27). This is consistent with the increased presence of these six superantigens in strains isolated at least since 2008 (24).
None of the strains were positive for the SEB gene, while three were positive for the SEC gene. This means that at least five of the strains contained superantigen genes, where the superantigens are produced in high enough concentration to cause TSS (21). None of the individuals developed any sign of disease.

The pre-GML gel and post-GML gel CFU per milliliter (CFU/ml) values were determined on the 14 individuals for both *S. aureus* and coagulase-negative staphylococci (Fig. 1); the data from both nares were included in the analysis, essentially giving 28 data points. As seen in Fig. 1, there were more than $10^5$ CFU/ml of *S. aureus* on average pre-GML gel treatment (log CFU/ml approximately 5.5). In contrast, after GML gel treatment for 3 days, the *S. aureus* counts fell to just over $10^2$/ml (log CFU/ml was approximately 2.2). Thus, there was a 3-log-unit reduction in *S. aureus* CFU/ml. Eight of the 14 participants (60%) had no detectable *S. aureus* in the nares after GML gel treatment.

GML gel also significantly reduced coagulase-negative staphylococci as present in the anterior nares (Fig. 1). Except for five individuals, where *S. aureus* was present in pure culture, all nine other persons had coagulase-negative staphylococci in the anterior nares with *S. aureus*. Additionally, all 26 individuals who did not have cultured *S. aureus* were positive for coagulase-negative staphylococci. Thus, 35 of the 40 participants had coagulase-negative staphylococci in their anterior nares before GML gel treatment.

In three individuals (six total data points at each time point), the persistence of reduction in *S. aureus* CFU/ml was evaluated (Fig. 2). The reduction in *S. aureus* CFU/ml persisted for 2 days before regrowth commenced as seen on day 3 after GML gel application.

**DISCUSSION**

*S. aureus* causes more than 500,000 hospital-associated infections yearly in the United States. It has been shown that as much as 80% of the time, the *S. aureus* in the hospital-associated infection are the same as in the anterior nares (3). The data suggest that the anterior nares is the reservoir for the majority of hospital-associated *S. aureus* infections (2, 3).

The above observations have led to mupirocin in ointment form to be added to the anterior nares prior to surgery to reduce infections (5–7). Despite this, there remain a large number of infections. With these data in mind, the ability of 5% GML in a nonaqueous gel to reduce nasal *S. aureus* was evaluated in 40 humans.

The data showed that approximately 35% of adult humans had nasal *S. aureus*, and the percentage of persons positive is consistent with data from many other studies. The
data also show that, when *S. aureus* was present, they were generally but not always present in both nares. In this study, 12.5% of healthy adults had *S. aureus* bacteria with the capability of producing large amounts of superantigens present, and thus, under the right conditions to cause TSS. For example, we described postinfluenza TSS in 1987 where 8/9 children succumbed to postinfluenza TSS, with 100% succumbing when TSST-1 was present (28); the other TSS isolate produced SEB. Additionally, TSST-1 is exclusively the cause of menstrual, vaginal TSS (22). Nine of 14 isolates contained components of the enterotoxin gene cluster of six superantigens. These six superantigens appear to be common in isolates, at least since 2008 (24). They appear to be more like colonization factors, as opposed to causing TSS (23, 25).

The current study is most significant because it shows that the 5% GML gel can be used to reduce *S. aureus* in the anterior nares significantly, and the effect lasts for at least 48 h posttreatment. There were no adverse events reported by any study participant. A prior study with rats, colonized nasally with *S. aureus*, obtained similar findings (29). The current data are significant for at least three reasons. (i) GML is generally recognized as safe by the FDA as a food and cosmetic additive. It is found in human breast milk at concentrations of about 3,000 μg/ml (30). Some underserved countries have used human breast milk to treat atopic dermatitis where *S. aureus* is commonly present (31). The gel component of the current mixture is nonaqueous, but the gel is already an approved class II medical device by FDA for human mucosal use. (ii) The GML gel as formulated has the ability to spread laterally to other parts of the nose. Although not tested in this study, K-Y warming gel, related to the gel used in this study, was shown in women to spread laterally after vaginal application to coat the genital tract (17, 18). Thus, if the movement of GML gel in the nares functions similarly, it would be expected to provide extensive coverage of the nose. (iii) The 5% GML gel is potently virucidal for all tested enveloped viruses, including influenza viruses and coronaviruses (13, 14, 32, 33). This makes 5% GML gel a possible preventative for viral transmission and nasal carriage. Subsequent studies will need to assess this in vivo in humans. However, in other studies, we have shown >90% effectiveness in preventing simian immunodeficiency virus transmission vaginally in rhesus macaques (13, 14).

For many years, mupirocin has been used topically, including nasally to reduce *S. aureus* colonization. For example, in one study of 68 health care workers, up to 6 months of treatment resulted in an 87% reduction in colonization rate (34). After only two treatments, there was a 58% reduction in colonization rate. Recolonization oc-
Colonies that grew as bright yellow were then tested using catalase and slide coagulase tests to confirm staphylococci. Based on prior observation, it was assumed that each swab contained 0.1 ml of saline. The prewetted saline (0.15 M NaCl) up to the nasal bones. The swabs were rotated three to five times during the study, and 100% of enrollees completed the study. Each participant had their nares swabbed over a 2-week time period. There were 40 healthy volunteers, aged 18 to 64 years old, who completed the study.

Glycerol monolaurate (GML) was applied in the spring of 2011, and all participants were enrolled. The participants were next comparably swabbed with GML gel, for 3 days, approximately 12 h apart (twice per day). Finally, the participants returned to the laboratory 8 to 12 h after the last application to assess the nasal colonization. Data were analyzed by Student's paired t test by comparing log CFU/ml of S. aureus and coagulase-negative staphylococci in the pre-GML gel swabs compared to CFU/ml in the post-GML swabs.

ACKNOWLEDGMENTS

This work was supported by U.S. Public Health Service grants AI74283 and AI73366.

REFERENCES

1. Schlievert PM, Osterholm MT, Kelly JA, Nishimura RD. 1982. Toxin and enzyme characterization of Staphylococcus aureus isolates from patients with and without toxic shock syndrome. Ann Intern Med 96:937–940. https://doi.org/10.7326/0003-4819-96-6-937.

2. Lowy FD. 1998. Staphylococcus aureus infections. N Engl J Med 339:520–532. https://doi.org/10.1056/NEJM199808203390806.

3. von Eff C, Becker K, Machka K, Stammer H, Peters G. 2001. Nasal carriage as a source of Staphylococcus aureus bacteremia. N Engl J Med 344:11–16. https://doi.org/10.1056/NEJM200101043440102.

4. Egirir B, Guardabassi L, Esson J, Nielsen SS, Newman MJ, Addo KK, Larsen AR. 2014. Insights into nasal carriage of Staphylococcus aureus in an urban and a rural community in Ghana. PLoS One 9:e96119. https://doi.org/10.1371/journal.pone.0096119.

5. Perl TM, Cullen JJ, Wenzel RP, Zimmerman MB, Pfaller MA, Sheppard D, Twombley J, French PP, Herwaldt LA, Mupirocin and Risk of Staphylococcus aureus Study Team. 2002. Intranasal mupirocin to prevent postoperative Staphylococcus aureus infections. N Engl J Med 346:1871–1877. https://doi.org/10.1056/NEJMoa0203069.

6. Reagan DR, Doebbeling BN, Pfaller MA, Sheetz CT, Houston AK, Hollis RJ, Wenzel RP. 1991. Elimination of coincident Staphylococcus aureus nasal and hand carriage with intranasal application of mupirocin calcium ointment. Ann Intern Med 114:101–106. https://doi.org/10.7326/0003-4819-114-2-101.

7. Doebbeling BN, Breneman DL, Neu HC, Aly R, Yangco BG, Holley HP, Jr, Marsh RJ, Pfaller MA, McGowan JE, Jr, Scully BE, Reagan DR, Wenzel RP, Mupirocin Collaborative Study Group. 1993. Elimination of Staphylococcus aureus nasal carriage in health care workers: analysis of six clinical trials with calcium mupirocin ointment. Clin Infect Dis 17:466–474. https://doi.org/10.1093/clinids/17.3.466.

8. Patel JB, Gorwitz RJ, Jernigan JA. 2009. Mupirocin resistance. Clin Infect Dis 49:935–941. https://doi.org/10.1086/605495.

9. Schlievert PM, Peterson ML. 2012. Glycerol monolaurate antibacterial activity in broth and biofilm cultures. PLoS One 7:e40350. https://doi.org/10.1371/journal.pone.0040350.

10. Strandberg KL, Peterson ML, Schaefers MM, Case LC, Pack MC, Chase DJ, Schlievert PM. 2009. Reduction in Staphylococcus aureus growth and exotoxin production and in vaginal interleukin 8 levels due to glycerol monolaurate in tampons. Clin Infect Dis 49:1711–1717. https://doi.org/10.1086/644614.

11. Strandberg KL, Peterson ML, Lin YC, Pack MC, Chase DJ, Schlievert PM. 2010. Glycerol monolaurate inhibits Candida and Gardnerella vaginalis in vitro and in vivo but not Lactobacillus. Antimicrob Agents Chemother 54:597–601. https://doi.org/10.1128/AAC.01151-09.

12. Schlievert PM, Strandberg KL, Brosnahan AJ, Peterson ML, Pambuccian SE, Nephew KR, Brunner KG, Schultz-Darken NJ, Haase AT. 2008. Glycerol monolaurate does not alter rhesus macaque (Macaca mulatta) vaginal colonization. Antimicrob Agents Chemother 52:4440–4444. https://doi.org/10.1128/AAC.01151-09.
lactobacilli and is safe for chronic use. Antimicrob Agents Chemother 52:4448–4454. doi:https://doi.org/10.1128/AAC.00989-08.

13. Li Q, Estes JD, Schlievert PM, Duan L, Brosnaham AJ, Southern PJ, Reilly CS, Peterson ML, Schultz-Darken N, Brunner KG, Nephew KR, Pambuc- cian S, Lifson JD, Carlis JV, Haase AT. 2009. Glycolcerol monolaurate pre- vents mucosal SIV transmission. Nature 458:1034–1038. doi:https://doi.org/10.1038/nature07831.

14. Haase AT, Rakaiz E, Schultz-Darken N, Nephew K, Weissgrau KL, Reilly CS, Li Q, Southern PJ, Rothenberger M, Peterson ML, Schlievert PM. 2015. Glycolcerol monolaurate microbiocide protection against repeat high-dose SIV vaginal challenge. PLoS One 10:e0129465. doi:https://doi.org/10.1371/journal.pone.0129465.

15. Brosnaham AJ, Merriman JA, Salgado-Pabón W, Ford B, Schlievert PM. 2013. Enterotoxigenic faecalis inhibits superantigen toxic shock syndrome toxin-1-induced interleukin-8 from human vaginal epithelial cells through tetracycic acids. PLoS One 8:e61255. doi:https://doi.org/10.1371/journal.pone.0061255.

16. Lin XB, Lohants C, Duan L, Brosnaham AJ, Southern PJ, Reilly CS, Su I, Page R, Barnhart K. 2008. Vaginal distribution of Replens and K-Y cellulose sulfate (2.5 and 3.5 mL). Contraception 72:65–70. doi:https://doi.org/10.1016/j.contraception.2005.02.006.

17. Barnhart KT, Pretorius ES, Shaunik A, Timbers K, Nasution M, Mauck C. 2005. Vaginal distribution of two volumes of the novel microbiocide gel. Contraception 72:65–70. doi:https://doi.org/10.1016/j.contraception.2005.02.006.

18. Mauck CK, Katz D, Sandefer EP, Nasution MD, Henderson M, Digenis GA, Li Q, Southern PJ, Rothenberger M, Peterson ML, Schlievert PM. 2015. Staphylococcal superantigen gene cluster is essential for infective endocarditis. PLoS One 11:e0154762. doi:https://doi.org/10.1371/journal.pone.0154762.

19. Schlievert PM, Davis CC. 2020. Device-associated menstrual toxic shock syndrome. Clin Microbiol Rev 33:e00032-19. doi:https://doi.org/10.1128/CMR.00032-19.

20. Marrack P, Kappler J. 1990. The staphylococcal enterotoxins and their relatives. Science 246:705–711. doi:https://doi.org/10.1126/science.2185544.

21. Su I, Page R, Barnhart K. 2008. Vaginal distribution of Replens and K-Y Jelly using three imaging techniques. Contraception 77:195–204. doi:https://doi.org/10.1016/j.contraception.2007.11.016.

22. Pointdexter NJ, Schlievert PM. 1985. Toxic-shock-syndrome toxin-1-induced proliferation of lymphocytes: comparison of the mitogenic response of human, murine, and rabbit lymphocytes. J Infect Dis 151:65–72. doi:https://doi.org/10.1093/infdis/151.1.65.

23. Mack C, Katz D, Mauck CK, Sanderfer EP, Nasution MD, Henderson M, Digenis GA, Su I, Page R, Barnhart K. 2008. Vaginal distribution of Replens and K-Y Jelly using three imaging techniques. Contraception 77:195–204. doi:https://doi.org/10.1016/j.contraception.2007.11.016.

24. Schlievert PM, Strandberg KL, Leung DY. 2008. Secreted virulence factor comparison between methicillin-resistant and methicillin-sensitive Staphylococcus aureus, and its relevance to atopic dermatitis. J Allergy Clin Immunol 125:39–49. doi:https://doi.org/10.1016/j.jaci.2009.10.039.