AAC Accepted Manuscript Posted Online 2 March 2015
Antimicrob. Agents Chemother. doi:10.1128/AAC.04624-14

Efficacy of Skin and Nasal Povidone-Iodine Preparation against mupirocin resistant MRSA and *Staphylococcus aureus* within the anterior nares

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Running title: Skin and Nasal Povidone-Iodine Preparation efficacy for MRSA

ABSTRACT

Mupirocin decolonization of nasal *Staphylococcus aureus* prior to surgery decreases surgical site infections, however, treatment requires 5 days, compliance is low and resistance occurs. In 2010, 3M™ Company introduced povidone-iodine (PVP-I) based Skin and Nasal Prep (SNP). SNP has rapid, broad spectrum antimicrobial activity. We tested SNP's efficacy using full-thickness tissue (porcine mucosal (PM) and human skin) explant models and in human subjects. Prior to or following infection with methicillin-resistant *Staphylococcus aureus* (MRSA, mupirocin sensitive and resistant), explants were treated with Betadine® Opthalmic Preparation (Bet), SNP, mupirocin (Bactroban Nasal®, BN) or untreated. One hour post-treatment, explants were washed with PBS + 2% mucin. One, 6 or 12 h later, bacteria were recovered and enumerated. Alternatively, following baseline sampling, human subjects applied two consecutive applications of SNP or saline to their anterior nares. One, 6 and 12 h post-prep, nasal swabs were obtained and *S. aureus* enumerated. We observed that treatment of infected PM or human skin explants with SNP resulted in >2.0 Log_{10} CFU reduction in MRSA regardless of mupirocin sensitivity, which was significantly different from BN, Bet and untreated controls 1 h, 6 h and 12 h post-wash. Swabbing the anterior nares of human subjects with SNP significantly reduced resident *S. aureus* compared to saline 1, 6 and 12 h post-prep. Finally, pre-treatment of PM explants with SNP, followed by a mucin rinse prior to infection completely prevented MRSA infection. We conclude that SNP may be an attractive alternative for reducing the bioburden of anterior nares prior to surgery.

INTRODUCTION

Methicillin resistant *Staphylococcus aureus* (MRSA) has emerged as one of the most important pathogens in nosocomial or hospital acquired infections (HAI) (1). Surgical site infections (SSIs), a class of HAI, are defined by the National Healthcare Safety Network (NHSN) as those infections that occur up to 30 days post-surgery or up to 90 days post-surgical implantation of a medical device, and remain a significant clinical problem despite advances made in reducing risk of SSI (2). The frequency of SSI can be as high as 20%, depending on the type of surgery (3). *S. aureus* is the leading cause of SSI, accounting for 30% of all SSI with almost half (49.2%) of those caused by MRSA (4). Preventing a single case of MRSA SSI can reduce hospital costs by as much as $42,300 and reduce the length of stay by 50% (median 2 weeks) (5-7).

S. aureus colonizes the anterior nares, skin and mucosal surfaces of approximately 30% of the population (8-10). Nasal colonization with *S. aureus* is a well known risk factor for acquisition of SSIs (11-13). Decolonization of the anterior nares is one strategy for reducing risk of SSIs. Intra-nasally applied mupirocin has been the therapy of choice since the 1980s (14). The most efficacious regimen for *S. aureus* eradication from the anterior nares is twice daily applications of mupirocin ointment for 5 days for a total of 10 doses (15-16). In a prospective study, Yano et al. saw a reduction in the rate of *S. aureus* SSI in patients decolonized with mupirocin prior to upper gastrointestinal surgery compared to the control group (0.71% vs. 11.7%, $p<0.001$) (17). Others have observed >60% reduction in SSI in a cohort of cardiothoracic patients treated with mupirocin prior to surgery (18-19). Furthermore, there was a significant cost savings, considering that the average cost of mupirocin

66 treatment was $12.47 compared to $10,428 ± $9,125 for a superficial sternal infection
67 or $81,018 ± $41,567 for a deep sternal infection(18). Finally, in a review by T. Perl,
68 although not statistically significant, a subset of surgical patients decolonized with
69 mupirocin experienced approximately 50% fewer SSIs compared to those who did not
70 receive treatment (20).

71 In contrast to these studies, double-blinded, randomized, placebo controlled
72 clinical trials have failed to demonstrate a significant impact of nasal decolonization by
73 mupirocin on SSI. For example, intranasal mupirocin administered to *S. aureus* carriers
74 did not reduce the rates of overall cardiac surgical SSI caused by this organism (21). In
75 2002, Perl et al. failed to demonstrate a significant difference in the rate of SSI patients
76 treated prophylactically with mupirocin vs. placebo (22).

77 Treatment failure has been associated with increased mortality (23). Patient
78 non-compliance may contribute to treatment failure (24). However, there is growing
79 evidence that treatment failure may also be due to acquired antibiotic resistance (25-
80 27). The prevalence rates of high-level mupirocin resistance (mupR) increased from
81 1.6% of MRSA strains during the 5-year period of 1995 to 1999 to 7% for 2000-2004
82 (28). The increasing prevalence of mupR has important implications for institutions
83 where decolonization is the standard of care. While there are no standardized
84 guidelines for screening or decolonization, most clinicians attempt to decolonize at risk
85 patients with a combination of chlorhexidine gluconate applied to the skin and intranasal
86 mupirocin (29). Mupirocin use enhances selective pressure, decreasing its
87 effectiveness as a decolonization strategy (30). This has prompted the evaluation of
88 alternative strategies for reducing the risk of MRSA SSI.

In early 2010, 3M™ Company launched Skin and Nasal Prep (3M™ SNP), an alternative to topical antibacterial therapy with mupirocin, as a topical patient preoperative antiseptic prep for the reduction of microbial bioburden on the skin and in the anterior nares. The preparation is povidone-iodine (PVP-I) based, which has broad spectrum antibacterial activity, as well as activity against fungi, protozoa, viruses and some bacterial spores (31-32). PVP-I has rapid *in vitro* activity (bactericidal within 15-20 s) and the duration of the effect on skin has been reported to be 12-14 h due to a phenomenon called back-diffusion (33-34). In contrast to the use of antibiotics, there is minimal potential for the development of resistance to PVP-I due to multiple cellular targets (35-36). Finally, excipients in the 3M™ SNP formulation protect PVP-I from inactivation by organic compounds such as blood or mucin and increase muco-adhesion.

Recently, Philips et al. conducted a prospective, open-label, randomized clinical study to evaluate 3M™ SNP as an alternative to intranasal mupirocin because of lack of patient compliance and increasing mupirocin resistance (37). They demonstrated that 3M™ SNP resulted in significantly fewer *S. aureus* SSI than mupirocin. Therefore, we conducted an analysis of 3M™ SNP and mupirocin and determined their ability to reduce MRSA burden on mucosal tissues and human skin. The aims of this study were 3-fold: 1) to demonstrate that 3M™Skin and Nasal Prep is efficacious at reducing microbial bioburden and in prevention of methicillin resistant *S. aureus* infections in an *ex vivo* model; 2) to show efficacy against mupR MRSA in a novel *ex vivo* human skin MRSA infection model; and 3) to validate reduction in the anterior nares *S. aureus* bioburden of human subjects.

METHODS

Bacteria Growth and Explant Inoculation

MRSA strain Xen30 was purchased from Caliper Life Sciences (Hopkinton, MA). Mupirocin resistant clinical MRSA isolates were received from the Minnesota Department of Health's repository. Mupirocin susceptibility was evaluated using E test strips (bioMerieux, Durham, NC) according to manufacturer's instructions. Strips were interpreted according to literature and opinion, as they were for research use only. Bacterial strains described in these studies are maintained in our laboratories as frozen glycerol stocks. Prior to experimentation, Tryptic Soy Agar containing 5% sheep's blood (TSB, Beckton-Dickenson, Franklin Lakes, New Jersey) is inoculated from frozen stocks. On the afternoon prior to initiation of experiment, Todd Hewitt broth (Becton-Dickenson Biosciences, San Jose, CA) is inoculated with colonies from the fresh Tryptic Soy Agar with 5% sheep's blood (TSB, Becton-Dickenson Biosciences, San Jose, CA). Stationary phase (overnight) cultures are washed in RPMI 1640 (Invitrogen, Carlsbad, CA) and resuspended to a concentration of approximately 5 x 10^8 CFU/mL. Two µl of this suspension are used to inoculate explants on the mucosa or stratum corneum surface (1 x 10^6 CFU/explants). Explants are returned to 37°C, 7% CO_2 and incubated for 1-24 h.

Explants of normal porcine vaginal mucosa (5 mm, full-thickness squamous epithelium) or human skin (5 mm, full-thickness) were infected with methicillin-resistant *S. aureus* (MRSA). Prior to or following infection, explants were treated with Betadine® Solution (Opthalmic Preparation, Alcon, Fort Worth, TX), 3M™ Skin and Nasal Prep (St. Paul,

MN), mupirocin ointment (Bactroban Nasal® Glaxo Smith Kline, Research Triangle Park, NC) or left untreated (controls). Bacteria were enumerated by transferring explants to 2x Dey-Engley (DE) broth (Beckton Dickenson) for neutralization, vortex mixing then plating onto TSB plates neat or serially diluted in PBS.

Ex vivo porcine vaginal mucosa culture

In a previous publication, we describe a novel model for determining efficacy of antimicrobials (38). Briefly, specimens of normal porcine vaginal mucosa are excised from animals at slaughter in the U of MN Andrew Boss Laboratory of Meat Science. The tissue is a by-product of the slaughter of animals for human consumption and therefore Institutional Animal Care and Use Committee (IACUC) exempt. Specimens are then transported to the laboratory in RPMI 1640 media supplemented with 10% fetal calf serum (Invitrogen, Carlsbad, CA), on ice. Tissue was utilized within 3 h of excision. Tissue explants of uniform size were obtained from the porcine vagina using a 5mm biopsy punch. Excess muscle tissue was trimmed away with a scalpel. The explants are washed in serum and antibiotic-free media 3 times and then placed mucosal side up on a 0.4 µm cell culture insert (BD Bioscience, San Jose, CA) in 6-well plates containing fresh serum and antibiotic-free RPMI 1640. The mucosal surface was continually exposed to air.

Procurement Culture of Human Skin Explants

Normal human skin (de-identified) is procured by the National Disease Research Interchange (NDRI) and is exempt from Institutional Review Board review. Normal healthy specimens are excised from cadavers and transported to the laboratory in HypoThermosol® (BioLife Solutions.Bothell, WA), a cryopreservation media. Tissue is utilized within 24 h of excision. Decolonization of normal flora is achieved by drying the surface of the specimen and swabbing the area with ChloraPrep® (CareFusion, San Diego, CA) twice. Explants of uniform size are then obtained from the specimen using a 5mm biopsy punch. Tissue explants are washed in RPMI 1640 media containing 2% v/v normal human serum. The explants are then placed on a PET track-etched 0.4µm cell culture insert (Becton-Dickenson Biosciences, San Jose, CA) in 6-well plates containing fresh RPMI 1640 + 2% normal human serum (Invitrogen, Carlsbad, CA) and incubated at 37°C, 7% CO_2. An air interface is maintained with the stratum corneum.

Application of test formulations or comparators

The model was developed to be a semi high-throughput screen of full strength formulations in the form of liquids, pastes, gels, foams or dressings. One hundred µl of Betadine® Opthalmic Preparation, 3M™ Skin and Nasal Prep or mupirocin (Bactroban Nasal®) ointment are applied topically to the explants and incubated for 1 – 24 h at 37°C, 7% CO_2.

Mucin wash

To mimic mucocilliary clearance, 1 h following application of treatments to the explants, 1 mL of 2% w/v mucin (Sigma, St. Louis, MP) in PBS was pipetted into each well containing explants. The plate was swirled gently to wash and suspension was aspirated. The explants were then returned to culture for the indicated time periods.

Bacterial (colony forming units, CFU) enumeration

Bacteria are enumerated from infected explants by vortex mixing (medium-high setting, 4 m) in 250 µl sterile anti-microbial neutralizing DE broth at twice the manufacturer's recommended concentration. Suspensions are serially diluted in PBS (or plated neat) and spread on TSB plates using a spiral plater (Biotek, Microbiology International).

Nasal normal flora reduction in human subjects

Baseline samples (n=70) were taken from normal healthy human subjects prior to the application of prep or saline. Only subjects with baselines > 5 x 10^3 CFU/swab were included in this efficacy study. Depending on the sampling time, seven to eighteen subjects applied 3M™ Skin and Nasal Prep or 0.9% saline control to their nostrils for 30 s each, followed by an immediate repeat application, for a total application time of 1 min per nare (3M™ Study #EM-05-011100). Quantitative cultures were obtained from the anterior nares using a standardized swabbing procedure. Briefly, one dry, sterile rayon swab was used to sample both the right and left nostril. For each nostril the rayon swab was inserted carefully into the anterior, apex portion of the nostril and rotated 2 times with slight pressure. This swabbing procedure was used to collect Study Day baselines,

and 1, 6, and 12 h post-prep nasal samples. Following collection, the swab sample was immediately immersed into a tube containing 1 mL of a neutralizer solution (NS) described previously (39). The sample tube was capped tightly and vortex mixed for ~1 min, then serially diluted in phosphate buffered dilution water. Duplicate 0.1 mL aliquots were spread on HardyCHROM™ *Staph aureus* (CSA, Hardy Diagnostics) and Trypticase Soy Agar with 5% Sheep Blood (SBA). All samples were plated within 20 min of collection. After 20 to 28 h of aerobic incubation at 35 - 37ºC, CSA plates were evaluated for differentially selective growth (smooth, deep pink to fuchsia colonies) and SBA plates were evaluated for total growth using a Quebec Colony Counter (Reichert Technologies, Depew, NY). Data presented are M±SD Log_{10} reductions of *S. aureus* from baseline.

Statistical analysis

Each ex vivo experiment was repeated a minimum of 3 times. Data presented are mean of triplicates ± SEM. Analysis of variance (ANOVA) followed by Bonferroni's post-test were performed using the GraphPad PRISM software (GraphPad Software, Inc., California). The human subject study was performed once and data presented are mean Log_{10} reduction from baseline±SD. Students t-test was used to evaluate significant differences at each time point.

RESULTS

Efficacy of 3M™ SNP, Betadine® Opthalmic and Bactroban Nasal® in treatment of MRSA infected *ex vivo* porcine vaginal mucosa. We tested the antimicrobial activity of two povidone-iodine based preparations (5% w/v) and 2% mupirocin, Bactroban Nasal® (BN) using a modified version (Fig. 1a) of a previously developed ex vivo full thickness tissue model of MRSA infection (38). PVM explants were infected with MRSA (strain Xen30) for 2 h, then treated with antimicrobials for 1 h. Treated and untreated control explants were washed in PBS + 2% w/v porcine mucin to mimic mucociliary clearance. CFU were enumerated from neutralized explants following 1, 6 or 12 h (to mimic short, average and long surgeries) incubation at 37°C. One hour post-washing, MRSA bacterial densities of infected PVM explants treated with 3M™ SNP or Betadine® Opthalmic were significantly less compared to untreated controls (Log_{10} 1.09±0.57, 2.51±0.20, and 5.30±0.06 CFU/explant, respectively). Treatment with BN had no effect on CFU recovered at this time point (Log_{10} 5.14±0.09 CFU/explant). There was bacterial growth in untreated control explants and Betadine® Opthalmic treated explants 6 h post-wash (Log_{10} 6.86±0.01, 5.12±0.48 CFU/explant), whereas the bacterial burden from BN treated explants remained static and not different from untreated control (Log_{10} 5.07±0.06 CFU/explant). 3M™ SNP exerted a significant persistent effect at this time point, further reducing bacterial densities (Log_{10} 0.43±0.43 CFU/explant).

Efficacy of 3M™ SNP and Bactroban Nasal® against mupirocin resistant, MRSA infections of *ex vivo* porcine vaginal mucosa. We next evaluated the antimicrobial effect of 3M™ SNP compared to Bactroban Nasal® on 10 mupR MRSA isolates [both hi-level (HLR) and lo-level resistance (LLR), see Table 1 for mupirocin sensitivity data]

using the PVM infection model described above. As expected, 1 h following 2% mucin wash, 3M™ SNP had significantly less LLR MRSA bacteria on infected PVM explants, compared to untreated controls and BN treated explants (Log_{10} 1.63±0.44 vs. 5.30±0.30 and 5.71±0.57 CFU/explant, respectively, Fig 2a). Although some re-growth was observed at 6 h (Fig. 2c) and 24 h (Fig. 2e), 3M™ SNP treated explants were associated with significantly lower bacterial densities (Log_{10} 2.56±1.60 and 3.62±0.50 CFU/explant, respectively) than untreated controls (Log_{10} 6.60±0.75, 7.76±0.22 CFU/explant) or BN treated explants (Log_{10} 5.08±0.39, 5.99±0.73 CFU/explant). BN appeared to be bacteriostatic, but had no significant effect on LLR MRSACFU recovered at any time point compared to control. Similar results were observed with the HLR MRSA isolates as well (Fig. 2b, d and f). At all 3 time points examined, HLR MRSA infected explants treated with 3M™ SNP had significantly less bacterial densities compared to untreated or BN treated explants (Log_{10} 1.55±0.29 vs. 5.68±0.29, 6.03±0.32 CFU/explant, 1 h, Log_{10} 2.98±0.23 vs. 7.04±0.26, 5.99±0.43 CFU/explant, 6 h, Log_{10} 3.24±0.36 vs. 7.66±0.19, 6.88±0.24 CFU/explant, 24 h). Although not significantly different from control, some growth was observed in the HLR MRSA isolates at 24 h compared to 1 h.

Efficacy of 3M™ SNP, Betadine® Opthalmic and Bactroban Nasal® in the treatment of MRSA infected *ex vivo* human skin. We adapted our MRSA PVM infection model to fresh, full-thickness human skin which is more representative of the tissue type in the anterior nares and therefore more translational. Explants of normal human skin were prepared as described above and infected with MRSA (strain Xen30)

for 2 h (Fig. 3a). Similar to the above experiment on PVM, explants were treated with 3M™ SNP, Betadine Opthalmic® or BN for 1 h, then washed with 2% w/v mucin in PBS. Explants were incubated for 1, 6 or 12 h prior to neutralization and bacteria recovery. One hour post-washing, MRSA burden of infected PVM explants treated with 3M™ SNP or Betadine® Opthalmic was significantly reduced compared to untreated controls (Log_{10} 0.33±0.33, 2.09±0.60 and 4.06±0.19 CFU/explant, respectively, Fig. 3b). Further, CFU recovered from 3M™ SNP treated explants were significantly lower than the Betadine® Opthalmic treated group. Treatment with BN had no effect on CFU recovered at this time point (Log_{10} 4.50±0.08 CFU/explant). Six hours post-washing, there was evidence of growth in the untreated controls (Log_{10} 5.50±0.40 CFU/explant) which continued through the 12 h time point (Log_{10} 6.57±0.35 CFU/explant). Again, 3M™ SNP was significantly more effective at reducing MRSA burden than the Betadine® Opthalmic or BN treated groups (0 CFU recovered vs. Log_{10} 1.72±0.0.40 or 4.20±0.24 CFU/explant). BN treatment appeared bacteristatic at 6 h post-wash; however, this was not statistically different from control. By 12 h post-washing, the difference between untreated controls and BN treatment was significant (Log_{10} 6.57±0.35 vs. 3.92±0.11 CFU/explant, respectively). Zero CFU were recovered from the 3M™ SNP treated explants 12 h post-wash, which was significantly lower than BN treated and untreated controls. At this time point, no statistically significant difference between CFU recovered from the 3M™ SNP treated explants and the Betadine® Opthalmic treated explants was observed, however, numerically, 3M™ SNP treatment was more effective at eradicating MRSA (0 vs. Log_{10} 0.63±0.34 CFU/explant).

Efficacy of 3M™ SNP on normal flora in the anterior nares of human subjects. Having demonstrated that 3M™ SNP significantly reduced MRSA in two ex vivo full thickness models, we next determined whether it could reduce normal flora of human anterior nares. Baseline samples (n=70) were taken by swabbing the anterior nares. The baseline *S. aureus* mean of subjects included in this study was Log_{10} 4.77±0.62 CFU. The anterior nares of subjects were then sampled at 1, 6 or 12 h following application of SNP (n=13-18) or saline control (n=7-9). At all 3 time points, the *S. aureus* Log_{10} reduction from baseline in 3M™ SNP treated subjects was significantly greater than that observed in the saline control subjects (2.3±1.68 vs. 0.86±0.73, 1 h, 2.79±1.52 vs. 0.76±0.58, 6 h and 2.37±1.77 vs. 0.6±0.9 12 h).

3M™ SNP prevents MRSA infection of PVM explants. Since the intended outcome of 3M™ SNP use is to prevent SSI, we next determined whether treatment could prevent PVM explants from becoming infected with MRSA. We slightly modified the PVM infection model described above, as depicted in Fig. 5a. Explants were treated for 5 min with 3M™ SNP, Betadine Opthalmic®, mupirocin or left untreated, incubated for 15 min, washed with 2% mucin in PBS (w/v), infected with MRSA (Xen30), incubated for 1 h, then neutralized and CFU were recovered. CFU recovered from untreated control explants were Log_{10} 4.19±0.12 CFU/explant(Fig. 5b). Treatment with either 5% povidone-iodine based product resulted in a reduction in the ability of MRSA to infect explants, with 3M™ SNP being far superior to the Betadine® Opthalmic formulation (Log_{10} 0.00±0.00 vs. 2.34±0.12 CFU/explant). CFU recovered from mupirocin (2%)

311 treated explants was equivalent to that recovered from controls (Log_{10} 4.53±0.05

312 CFU/explant).

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DISCUSSION

There are numerous commercially manufactured PVP-I containing preoperative skin preps that meet FDA requirements, including the recently developed 3M™ SNP. To our knowledge, this is the first study in translational, full thickness tissue models, which directly compares PVP-I formulations and topical application of 2% mupirocin ointment (BN). Using an *ex vivo* porcine mucosal (PVM) infection model, we demonstrated that treatment with 3M™ SNP or Betadine® Opthalmic was bactericidal against MRSA within 2 h of application. This is consistent with the reported rapid activity of PVP-I based antimicrobials in the ophthalmic surgical setting (40), which, in turn, is associated with a decreased risk of post-operative infections (41). In contrast, no change in the MRSA burden of explants treated with BN was observed out to 14 h post-application(12 h post-mucin wash), which is consistent with its known slow mode of action (42). While we recognize the mupirocin decolonization strategy includes twice daily application for 5 days intra-nasally prior to surgery, there is also a risk of patient non-compliance. Therefore, our studies are intended to mimic mupirocin efficacy in a non-compliant patient being decolonized prior to surgery.

PVP-I kinetics are well understood and it is well known that iodine is the active antimicrobial component. For example, Schenck et. al. showed that povidone complexes with hydrogen triiodide through hydrogen bonding with the proton (43). Triiodide is in equilibrium with iodine (I_2) and iodide (I^-) shown by the reaction: $I_3^- = I_2 + I^-$. There are other excipients in 3M™ SNP which buffer the composition and protect the

iodine from reduction to inactive iodide which happens rapidly at increased pH (above 4.5) and reaction with organic matter.

Treatment with 3M™ SNP resulted in sustained bactericidal activity for up to 8 h post-application (6 h post-mucin wash), which was a significant improvement over the Betadine® Opthalmic treated explants where >5.0 Log_{10} growth was observed. We believe the sustained activity is a result of increased adhesion to the net negatively charged mucus on the tissue surface. This muco-adherence results from the presence of a proprietary cationic polymer in the formulation (44-45). We acknowledge that re-colonization after 8 h is a possibility and that is the subject of ongoing studies.

Recently, universal decolonization was shown to be more effective than targeted decolonization in the prevention of MRSA ICU infections (46). This, along with the increased use of mupirocin for prevention of recurrent skin and soft tissue MRSA infections (47-49), and growing interest in peri-operative eradication of MRSA for the prevention of SSI has important implications for the spread of mupirocin resistance (50). In 2002, Deshpande et al. performed an extensive study of mupirocin resistance and noted marked increase globally, compared to an earlier study (51). Widespread use of mupirocin is commonly associated with increased incidence of resistance (25, 52-54). The short, defined course (5 days) of mupirocin prescribed in clinical trials of efficacy do not appear to select for mupirocin resistance in one study (22). In contrast, Watanabe et al. reported the development of low-level mupirocin resistance after intranasal treatment to reduce nasal carriage (55). This same research group also presented a case study in 2001 which described the development of mupirocin resistance in the pharynx of a patient during the course of intranasal application (56). Mupirocin resistance has been

associated with decolonization failure, which correlates with increased SSI (57-59). We evaluated 10 mupR MRSA clinical isolates, both HLR and LLR, in our PVM infection treatment model. We saw that both HLR and LLR MRSA isolates were sensitive to 3M™ SNP. This is not surprising due the differences in mechanism of action by the two agents. Mupirocin reversibly binds to the isoleucyl t-RNA synthetase, resulting in inhibition of protein synthesis (60). Resistance is mediated by the acquisition of a plasmid-encoded *mupA* gene (HLR) (61) or a mutation in the native *ileS* gene (LLR) (62). In contrast, iodine has many cellular targets, including fatty acids, nucleotides and the free-sulfur amino acids cysteine and methionine in proteins (63). This makes the development of resistance unlikely, and in fact, has not been reported (35).

We previously developed a semi-high throughput *ex vivo* PVM infection model for determining efficacy and toxicity of antimicrobial agents which is translational in nature (38). Its use as a substrate for bacterial attachment and nutrition provides an alternative, cost-effective method that more closely mimics *in vivo* conditions than classic *in vitro* studies. The indications for use of 3M™ SNP for nasal bacterial reduction is application to the anterior nares, which more closely resembles skin in terms of cell type and microflora (64). The *ex vivo* PVM explant infection model does not entirely mimic the host factors or microbial components found in skin, therefore, we extended the principles of the PVM infection model to a model which uses fresh human skin as the substrate. Using this *ex vivo* human skin MRSA infection model, we observed results similar to those obtained in the *ex vivo* PVM model. This not only confirms that the PVM infection model is a valuable tool for the preclinical evaluation of

topical biocides for infection prevention or treatment, but suggests that 3M™ SNP could reduce skin or anterior nares flora.

3M™ SNP rapidly achieved a significant reduction in the resident *S. aureus* from the anterior nares of human test subjects, demonstrating clinical translation of the PVM and *ex vivo* human skin models. This is consistent with a recent report of a prospective, open-label, randomized clinical trial where bacterial reduction with 3M™ SNP resulted in significantly fewer *S. aureus* SSI than mupirocin treated patients (twice daily x 5 days) undergoing primary or revision arthroplasty or spinal fusion (37).

In conclusion, given the issues with medication compliance and evolving mupirocin resistance, and the importance of reducing risk of *S. aureus* SSI, the benefits of 3M™ SNP should be considered. They include rapid efficacy, broad spectrum activity against multiple opportunistic pathogens, lack of development of antimicrobial resistance, and ease of use. 3M™ SNP provides just in time, health-care provider directly-observed preventive application and may be an attractive alternative for reducing the bioburden of anterior nares prior to surgery.

ACKNOWLEDGEMENT

This work was supported by a grant from 3M™ Company.

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Figure Legends

Figure 1. Efficacy of PVP-I formulations and Bactroban Nasal® against MRSA infection in an *ex vivo* PVM model. A. Schematic of experimental design. B. Explants of normal PVM were infected with *S. aureus* Xen30, treated, washed with sterile PBS containing 2% mucin, then incubated as described in a. Following incubation for 1, 6, or 12 h, explants were transferred to a neutralization buffer containing sodium thiosulfate and vortex mixed to release surviving bacteria. Serial dilutions were made in sterile PBS and plated onto Tryptic Soy Agar supplemented with sheep's blood, using a spiral plater. The number of viable bacterial cells is expressed as mean±SEM Log_{10} CFU/explants recovered (y-axis) over time (x-axis). Untreated control, open circles, Bactroban Nasal, closed circles, Betadine Opthalmic, triangles, 3M SNP, squares.

Figure 2. Efficacy of 3M™ SNP or Bactroban Nasal® against low and high level mupirocin resistant MRSA isolates. Explants of normal PVM were infected with ~ 1 X 10^6 CFU low-level mupR MRSA isolates (a, c, e) or high-level mupR MRSA isolates (b, d, f) for 2 h. Infected explants were then treated with SNP, BN or left untreated (control) for 1 h, followed by washing with sterile PBS supplemented with 2% mucin. Washed explants were then returned to the incubator for 1 h (a, b), 6 h (c, d) or 24 h (e, f). Following incubation, explants were transferred to a neutralization buffer containing sodium thiosulfate and vortex mixed to release surviving bacteria. Serial dilutions were made in sterile PBS and plated onto Tryptic Soy Agar supplemented with sheep's blood, using a spiral plater. The number of viable bacterial cells is expressed as mean±SEM Log_{10} CFU/explants (y-axis).

603 **Figure 3.** Efficacy of PVP-I formulations and Bactroban Nasal® against MRSA infection
604 in an *ex vivo*, full-thickness, fresh human skin model. A. Schematic of experimental
605 design. B. Explants of normal human skin were infected with *S. aureus* Xen30, treated,
606 washed with sterile PBS containing 2% mucin, then incubated as described in a.
607 Following incubation for 1, 6, or 12 h, explants were transferred to a neutralization buffer
608 containing sodium thiosulfate and vortex mixed to release surviving bacteria. Serial
609 dilutions were made in sterile PBS and plated onto Tryptic Soy Agar supplemented with
610 sheep's blood, using a spiral plater. The number of viable bacterial cells is expressed
611 as mean±SEM Log_{10} CFU/explants recovered (y-axis) over time (x-axis). Untreated
612 control, open circles, Bactroban Nasal®, closed circles, Betadine® Opthalmic, triangles,
613 3M™ SNP, squares.

614

615 **Figure 4.** 3M™ SNP reduces normal flora of human anterior nares. Thirteen to
616 eighteen human subjects (3M™ Study #EM-05-011100) applied 3M™ Skin and Nasal
617 Prep and seven to nine human subjects applied 0.9% saline control to their nostrils for
618 30 s each, followed by an immediate repeat application, for a total application time of 1
619 minute per nare. Post-prep samples were taken via swabbing the nares at 1 h, 6 h and
620 12 h. Baseline samples were taken prior to the application of prep or saline. The
621 baseline *S. aureus* mean of subjects included in this study was Log_{10} 4.77±0.62 CFU.
622 The numbers of viable bacterial cells are expressed as mean±SEM Log_{10} reduction from
623 baseline (y-axis) over time (x-axis). Saline control, open squares, 3M™ SNP, squares.

624

Figure 5. 3M™ SNP prevents MRSA infection in an *ex vivo* model. A. Schematic of experimental design. B. Explants of normal PVM were treated with PVP-I formulations or mupirocin ointment (2%) for 5 minutes at RT, followed by 15 m incubation at 37^{o}C. Explants were then washed with sterile PBS containing 2% mucin. Post-wash, explants were infected with ~ 1 x 10^{6} CFU MRSA Xen30 and incubated at 37^{o}C for 1 h. Explants were transferred to a neutralization buffer containing sodium thiosulfate and vortex mixed to release surviving bacteria. Serial dilutions were made in sterile PBS and plated onto Tryptic Soy Agar supplemented with sheep's blood, using a spiral plater. The number of viable bacterial cells is expressed as mean±SEM Log_{10} CFU/explants (y-axis).

Figure 1. Efficacy of PVP-I formulations and Bactroban Nasal® against MRSA infection in an *ex vivo* PVM model.

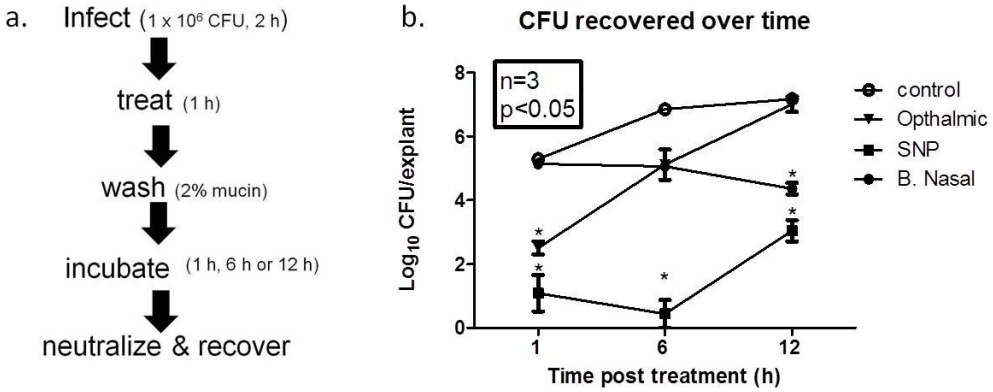

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Figure 2. Efficacy of 3M™ SNP or Bactroban Nasal® against low and high level mupirocin resistant MRSA isolates.

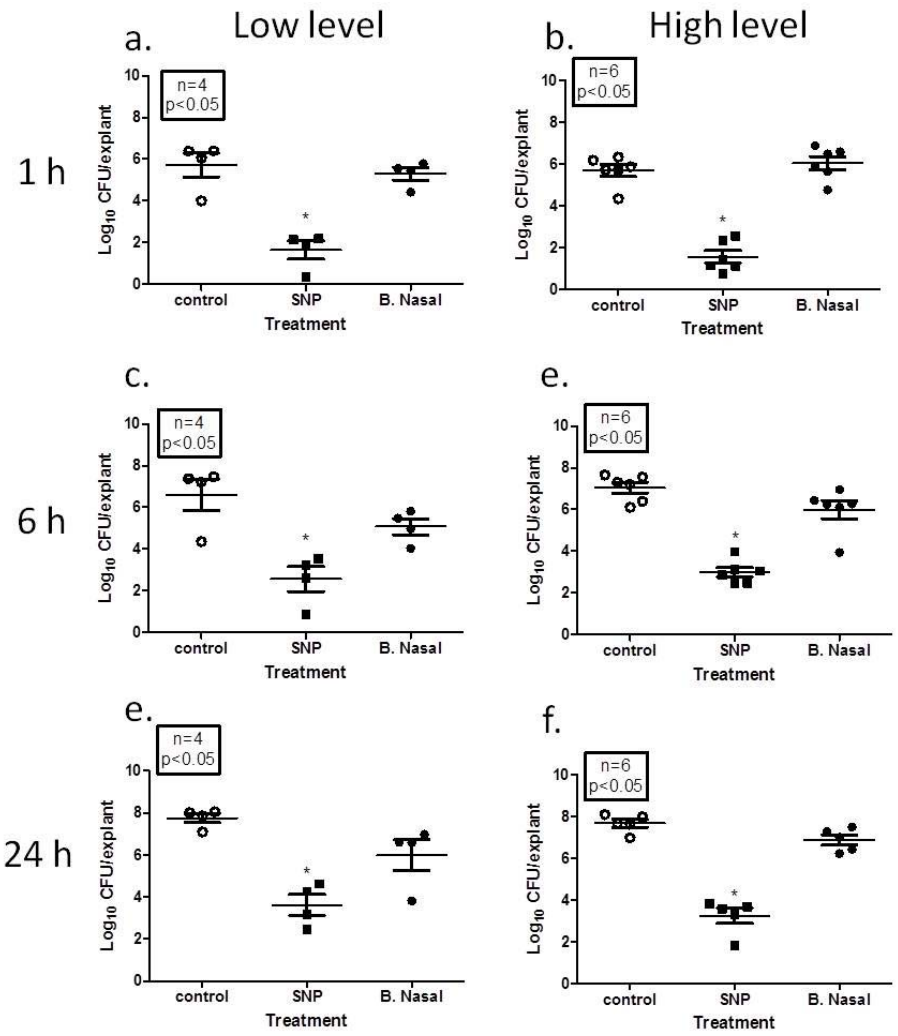

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Figure 3. Efficacy of PVP-I formulations and Bactroban Nasal® against MRSA infection in an *ex vivo*, full-thickness, fresh human skin model.

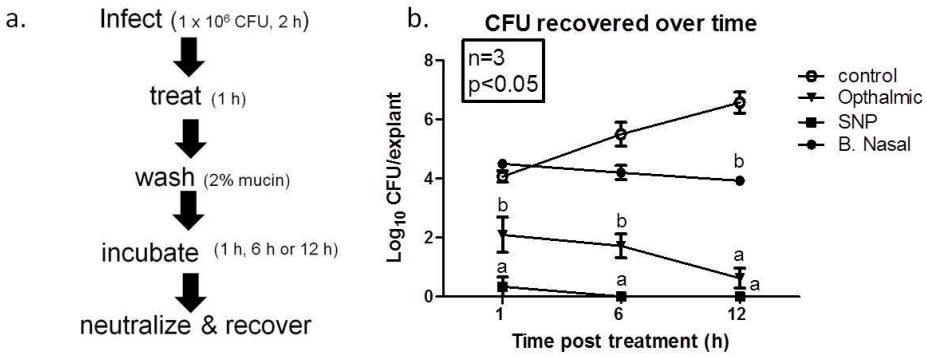

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Figure 4. 3M™ SNP reduces normal flora of human anterior nares.

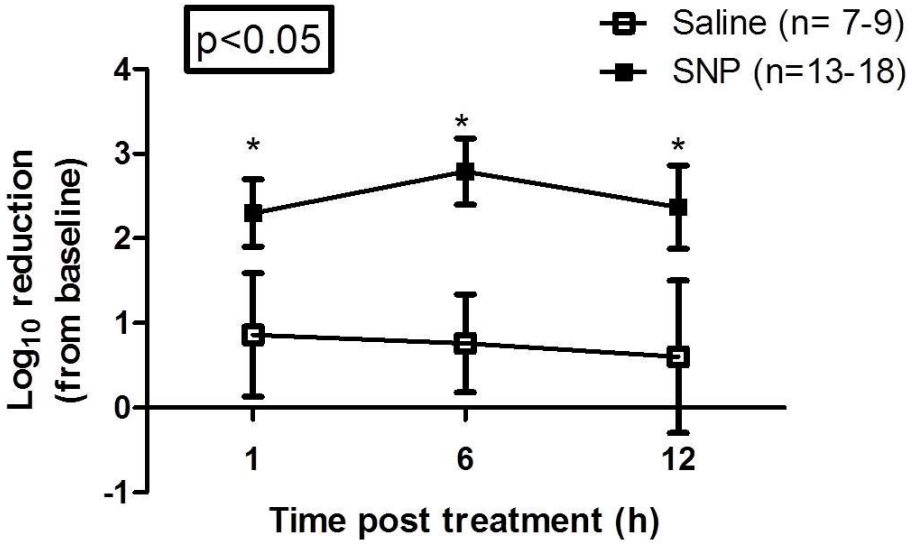

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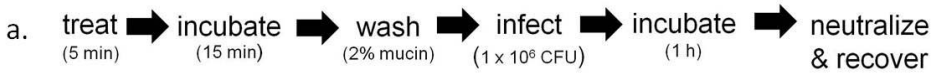
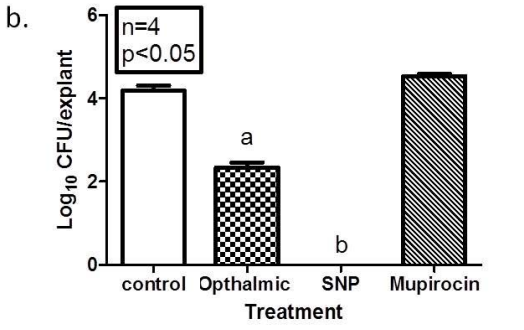
Figure 5. 3M™ SNP prevents MRSA infection in an *ex vivo* model.





Treatments not sharing a letter are significantly different.

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645 **Table I. Mupirocin susceptibilities of clinical MRSA isolates by E test**

Isolate	E test (µg/mL)	Mup R*
146	8	LLR
823	12	LLR
815	8	LLR
748	12	LLR
103	≥1024	HLR
920	≥1024	HLR
559	≥1024	HLR
476	≥1024	HLR
993	≥1024	HLR
329	≥1024	HLR

646 * mupirocin resistance (MupR); hi-level mupirocin resistance (HLR) and lo-level

647 mupirocin resistance (LLR)

648