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Original Communication

Photo-biologic inhibition of biofilm formation in MRSA as a potential amplification of mupirocin nasal carriage eradication therapy

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ABSTRACT

Nasal decolonization of *Staphylococcus aureus* has been accepted as an established prophylactic therapy to prevent post-operative infection, as colonization with *S. aureus* or MRSA (Methicillin-resistant *Staphylococcus aureus*) is now a known independent risk factor linked to increasing incidence and severity of infection after surgery. The principal topical antimicrobial in use today for decolonization (mupirocin) was approved by the US FDA nearly two decades ago for 'the eradication of nasal colonization with MRSA' and completed its 'microbiology review' in July 1997 for the treatment of small wounds. In the twenty years since its approval for nasal decolonization, there have been ever increasing reports of *S. aureus* eradication failure and antimicrobial resistance to mupirocin from around the world, including eradication failures with mupirocin susceptible species based solely on biofilm production. This article attempts to cover three aspects of *S. aureus* nasal decolonization: (a) present a brief history of the data that has established *S. aureus* in the human nares as a locus for distal infection; (b) do a review of published photo-biologic data showing attenuation of efflux pumps and generic antimicrobial potentiation against MRSA; (c) do an examination of new data concerning the potential benefit of a novel photo-biologic 'minimum biofilm inhibitory concentration' of near infrared light (PMBIC). This third aspect will be discussed as a possible pre-treatment for overcoming antimicrobial resistance in MRSA, and restoring a higher efficacy for mupirocin as a nasal decolonization therapy.

KEYWORDS: nasal decolonization, near-infrared, MRSA, mupirocin, biofilm, efflux pump

INTRODUCTION

I. *Staphylococcus aureus* in the nares as a commensal pathogen

Staphylococcus aureus is a Gram-positive cocci in the phylum Firmicutes that is in the human commensal microbiome, and is also a potential pathogen. *S. aureus* colonizes the skin and various mucosal surfaces in several parts of the body, including the nasal cavity (nares) of roughly 30% of the human populace [1]. In 1882, Sir Alexander Ogston, a surgeon from Aberdeen, Scotland first isolated *S. aureus* from purulence in a human abscess. He observed the bacteria's typical 'grape-like' clusters which he termed *Staphylococci* from the Greek word staphylé (bunch of grapes) [2]. In 1884, Rosenbach further classified the *Staphylococci "aureus"* when he observed their characteristic yellow or gold pigmentation [3]. Commensurate with his discovery, Ogston was also a firm believer in the nascent science of 'antisepsis' put forward by Joseph Lister in 1867. The lack of inflammation Ogston observed in the surgical wounds of Lister's post-operative patients, because of his antiseptic protocols, led him to

praise Lister for changing the nature of surgery from an 'art' to a scientifically based discipline [4]. Ogston's early praise of Lister's teachings on the benefits of antiseptic surgery were prescient, as the next century produced voluminous data describing the transmissibility of *S. aureus* from the human nares to other parts of the body, thereby causing distal infection.

II. Tracing infection of *S. aureus* from the nares

As early as 1931, Danbolt reported that 35 patients with recurring furunculosis (boils) had *Staphylococci* with the same biochemical properties both in the nose and in infected hair follicle lesions. Danbolt suggested that the nasal staphylococcal colonization was accountable for the recurrent furunculosis infection, representing auto-infection (i.e. infection from a source within the patient itself) [5].

In 1948, Moss, *et al*. deduced that skin carriage of *S. aureus* is dependent on nasal carriage in patients with normal skin. When Moss employed local penicillin treatment (not systemic treatment) he reduced nasal *S. aureus* carriage from 97% to 37% and simultaneously reduced skin carriage from 57% to 38% after 15 days of treatment [6].

In 1961 and 1963, Varga and White found an important relationship between nasal carriage of *Staphylococci* and contaminated air samples, by showing that nasal administration of oxacillin not only decreased nasal colonization, but also decreased culturable colonies from the air or 'aerial *Staphylococci*'. White and Smith suggested that the nose is the primary focus of multiplication and dissemination of organisms onto the skin and into the air, and that the multiplication and dissemination from the skin itself is a minor source of *Staphylococci* in the environment [7, 8].

In 1997 and 2005, Kluytmans, *et al*. found that the elimination of nasal carriage of *S. aureus* reduced infection rates in surgical patients and those on hemodialysis, and found *S. aureus* to be a risk factor for skin and soft tissue infections in patients undergoing surgery, and in those colonized with MRSA [9, 10]. In 2008, Munoz, *et al*. found that among MRSA carriers, the incidence of surgical site infection (SSI) reached 33%. Multivariate analysis showed that the largest independent risk factor for SSI was *S. aureus* nasal carriage with a 95% confidence interval. Munoz concluded that nasal carriage of *S. aureus* significantly increases the rate of nosocomial SSI and decolonization strategies should be implemented in this population [11].

In 2011, Lamers, *et al*. compared seven multilocus sequence typing gene fragments and the hyper-variable regions from 56 nasal carriage isolates, 28 clinical isolates and 15 complete *S. aureus* genomes from the National Center for Biotechnology Information database. They found that nasal strains are genetically related to those responsible for high levels of morbidity and mortality. Lamers described that nasal carriage strains and clinical strains exhibited the same genetic makeup in housekeeping and virulence genes, which suggests that any *S. aureus* isolate could either simply colonize or act as an invasive pathogen, and that the degree of success would be defined by the host [12]. Most recently, Gjodsbol, *et al*. (2013) found that patients were colonized by the same *S. aureus* type (characterized by pulse-field gel electrophoresis) in chronic leg ulcers and nasal cavities, which indicated cross-contamination between the ulcers and the nasal cavity [13].

III. Contemporary resistance of *S. aureus* to mupirocin

Today, mupirocin resistant *S. aureus* and MRSA, whether it be hospital or community acquired, can cause a wide range of infections. These can include skin infections, pneumonia, osteomyelitis, endocarditis, septicemia, medical implants and postsurgical wounds [14-19]. In 2013 alone, multiple studies were published discussing this dangerous trend in Singapore [20], France [21], Spain [22], India [23], Qatar [24] and the United States [25].

Most recently, Melake, *et al*. (2014) published a study of 106 patients that were found to have *S. aureus* nasal carriage in a university hospital outside of Cairo. Most disturbing in this study was the report that half of these *S. aureus* isolates were found to be MRSA, and 80% of the MRSA isolates were also found to be positive for biofilm production [26].

IV. *S. aureus* biofilm production as a mechanism of antibiotic resistance

Biofilm producing bacteria are protected by aggregation and dwell within the shielding of the biofilm's carbohydrate matrix. This extracellular polymeric substance (EPS) allows the aggregate communities to (a) adhere to a wound surface and (b) substantially block the phagocytic activity of neutrophils. *S. aureus* that produce biofilm are no longer considered 'planktonic' as they have evolved the capability to survive in the adverse environments of the host immune response. Similarly, *S. aureus* in biofilms are also resistant to topical or systemic antibiotics [27-29].

There are many different mechanisms that biofilm bacteria employ to resist antibiotics. These include:

- subpopulations of specialized survivor cells
- drug target modification or non-expression
- less susceptible slow-growing bacteria
- inadequate antibiotic penetration into the biofilm

Biofilms will assist *S. aureus* colonies in evading the host's immune system, and some studies have estimated that bacteria in biofilms survive the use of antibacterial agents at concentrations 1,000-1,500 times higher than needed to eradicate planktonic bacteria of the same species. Sedlacek and Walker examined sub-gingival bacteria in biofilm, and growing in planktonic states, and found that the vast majority of the bacteria in biofilms displayed higher minimum inhibitory concentrations (MICs) to antibiotics than planktonic cells [30-32].

Biofilms present challenges to traditional dosing regimens for antimicrobials, as the majority of conventional antibiotics have been created for the treatment of planktonic bacteria. Antibiotic treatment of planktonic bacteria had been the foremost application of microbiology research since the advent of penicillin, until the modern characterization of biofilms in 1978 [33]. Clinically, these implications of biofilms are serious, as bacteria in biofilms exhibit tolerance to most clinically relevant pharmacokinetic/pharmacodynamic dosing regimens of antibiotics, in spite of susceptibility of the same pathogens as planktonic cells [34].

In 2007, Kostenko, *et al*. reported that certain biofilm-producing strains of *S. aureus* exhibited resistance survival rates against antibiotics > 500 times the dose that is bactericidal against planktonic population [35].

V. *S. aureus* biofim production as a mechanism of mupirocin resistance

In 2008, Davis, *et al*. investigated biofilm resistance to mupirocin by applying mupirocin or triple antibiotic ointment after 15 minutes (to planktonic bacteria) or 48 hours (to biofilm bacteria) in a partial-thickness porcine wound that was inoculated with *S. aureus*. They found that both treatments reduced planktonic bacteria but had decreased efficacy on *S. aureus* biofilms [36].

In 2013, Ogura, *et al*. published a comparative analysis of 38 MRSA isolates from a nasal carriage eradication study. They tested all isolates for susceptibility to mupirocin and for biofilm production, and recorded 14 successfully eradicated strains of MRSA, and 24 failures. The successfully eradicated strains all tested as susceptible to mupirocin, with only one of the eradicated strains classified as a biofilm producer. Of the 24 strains that failed eradication, Ogura found 21 of them were tested as susceptible to mupirocin. However, of these 21 'susceptible failures', seventeen of them were classified as biofilm producers. The other 3 failures were tested as 'low-level' resistance to mupirocin, and two of them were biofilm producers. With this data, Ogura concluded that biofilm production was the only significant difference between MRSA isolates recovered from the successful and unsuccessful nasal carriage eradications in his study [37].

VI. Triggers for biofilm formation

A species of bacteria that has an elevated potential for pathogenicity can readily adapt to negative changes in its growth environment such as exposure to toxic substances. One example of such an adaption is the ability of an organism to alter its metabolism under the adverse conditions of antibiotic administration, so as to form small colony variants (SCV). This phenotypic shift by the organism is performed to enhance preservation, is practiced by *Staphylococci*, and transforms *S. aureus* into a more robust phenotype [38-41].

If this SCV transformation is then combined with a genetic ability to produce biofilm, the bacteria can be especially difficult to (a) eradicate in the nares and (b) treat in an infection at distal body sites [42-44]. Pathogenic strains that are capable of enhanced biofilm formation and the transformation to the SCV phenotype are known as persister cells, which have adapted to specifically survive harsh conditions such as antibiotic administration [45, 46].

As early as 2002, Drenkard and Ausubel [47] reported that chronic infections with SCV phenotype bacteria also had the ability to produce biofilm that was created at a higher rate, and was more dense than what was observed in wild type colonies. They concluded that this combination of phenotypic changes in the organisms resulted in a highly adept mass of bacteria for sustaining relapsing implant-related infections. These data highlight the fact that (a) *S. aureus* has the ability to produce biofilms under stress, and (b) infections with this species are often difficult to treat independent of where they are found in the human body [48].

VII. Photo-biologic potentiation of multiple classes of generic antibiotics against MRSA

Bornstein, *et al*. previously reported on the selective photo-damage of Gm+ and Gm- bacterial pathogens when pre-irradiated with a multiplexed dual wavelength (870 nm/930 nm) near-infrared laser at physiologic temperatures, before an antibiotic challenge [49, 50]. In these experiments, the photo-biologic pre-treatment of MRSA and *E. coli* led to significant potentiation of erythromycin, tetracycline and ciprofloxacin in archived and modern clinical strains. These samples were exposed to a blended laser energy (4584 J/cm^2) and then 930 nm energy alone (2037 J/cm^2) before exposure to sub-lethal concentrations of multiple classes of antibiotics.

Erythromycin, tetracycline and ciprofloxacin are known to succumb to efflux pumps present in resistant MRSA and *E. coli* strains. We postulated in 2008-2009 that an optically mediated reduction of $\Delta\Psi p$ (plasma membrane potential) in a SCCmec IIIA (staphylococcal cassette chromosome with a mecA gene coding for methicillin resistance) MRSA clone would cause an attenuation of the energy-dependent efflux systems, normally active against these generic antibiotics. This reduction in $\Delta\Psi p$ was quantified with fluorescent dye assays.

We tested erythromycin and tetracycline side-by-side to rifampin and trimethoprim (with identical potentiation protocols) and found substantial potentiation to both the generic macrolide and ketolide with the pre-irradiation of the MRSA colonies. Of equal consequence was the lack of a net benefit (beyond the laser photo-damage) seen with the latter two antimicrobials, rifampin and trimethoprim, when laser pre-irradiation of the colonies was performed (cf. Tables 1 and 2).

The noteworthy point is that MRSA does not have $\Delta\Psi p$ or ATP-driven efflux pumps that act on rifampin and trimethoprim. Trimethoprim inhibits the synthesis of tetrahydrofolic acid and rifampin inhibits bacterial RNA polymerase by binding to the enzyme's beta-subunit, and the hydrolysis of ATP is not vital to either function. All of the predicate *in vitro*, and follow-up human clinical data generated from these prior experiments can be reviewed in the literature [49-51].

Our experiments with 870 nm/930 nm suggested that bacterial photo-damage occurs via the perturbation of $\Delta\Psi p$, with concomitant generation of endogenous reactive oxygen species (ROS) measured as a shift in the equilibrium of reduced and oxidized glutathione to the oxidized form (i.e. reduced glutathione % of total glutathione). The prospective chromophores absorbing 870 nm/930 nm energy in the bacteria are the C–H covalent bonds of phospholipids in the plasma membranes and cytochrome c in the electron transport systems [49-53].

Similarly, in two separate Investigational Review Board (IRB) approved human pilot studies, this photo-biologic laser system successfully eradicated human nasal carriage of erythromycin resistant Methicillin-sensitive *S. aureus* (MSSA) and MRSA with two simple seven-minute irradiation cycles in the nose (day one and day three), followed by application of generic 2% erythromycin topical gel 3x/day for five days.

Table 1

MRSA (ATCC BAA-43) control					870 nm/930 nm pre-irradiation			
Test	AGAR alone	Rifampin 90 μg/ml	Tetracycline 4 μg/ml			AGAR alone	Rifampin 90 μg/ml	Tetracycline 4 μg/ml
Total	1632	1129	1515		Total	804	765	69

Table 2

MRSA (ATCC BAA-43) control					870 nm/930 nm pre-irradiation			
Test	AGAR alone	Trimethoprim 2 μg/ml	Erythromycin 4 μg/ml			AGAR alone	Trimethoprim 2 μg/ml	Erythromycin 4 μg/ml
Total	1089	1016	911		Total	523	588	168

- Tables represent total aggregate colony forming units from quintuplicate experiments

Figure 1

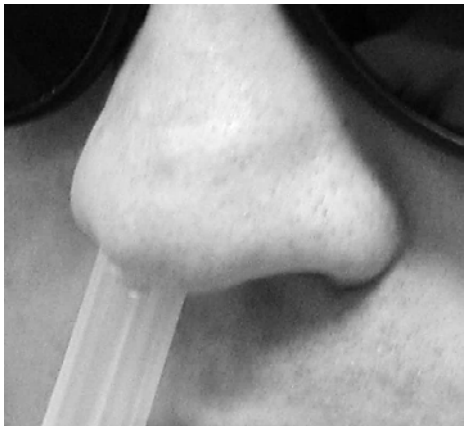

Figure 2

Prototype "flat-top" diffusion probe

In the second human pilot study, quantitative cultures from each nostril were obtained from seven patients of known MRSA carriers and plated in triplicate on chromogenic agar. These cultures were taken before, and 20 min after seven minute 870 nm/930 nm exposures (190 J/cm^2) in the nose. Irradiation was performed with a prototype (1-cm barrel shaped flat-top) dispersion probe (Figures 1 and 2). All patient cultures were pretested as erythromycin-resistant MRSA or erythromycin-resistant MSSA, and all patients were cleared of *S. aureus* and MRSA (measured by follow-up triplicate nasal culture) after two laser treatments and topical 2% erythromycin applications to the nares (Figures 3 and 4) [49, 50].

VIII. Photo-biologic inhibition of biofilm production in MRSA

Continuing to build on the antibiotic potentiation data in our aforementioned *in vitro* and human studies, further *in vitro* experiments (previously non-published) were also performed to determine the effect of 870 nm/930 nm laser pre-irradiation on MRSA when challenged with generic topical antibiotics.

MATERIALS AND METHODS

Methicillin-Resistant *Staphylococcus aureus* (ATCC BAA-43) liquid cultures were grown in Tryptic Soy Broth (TSB) medium with overnight

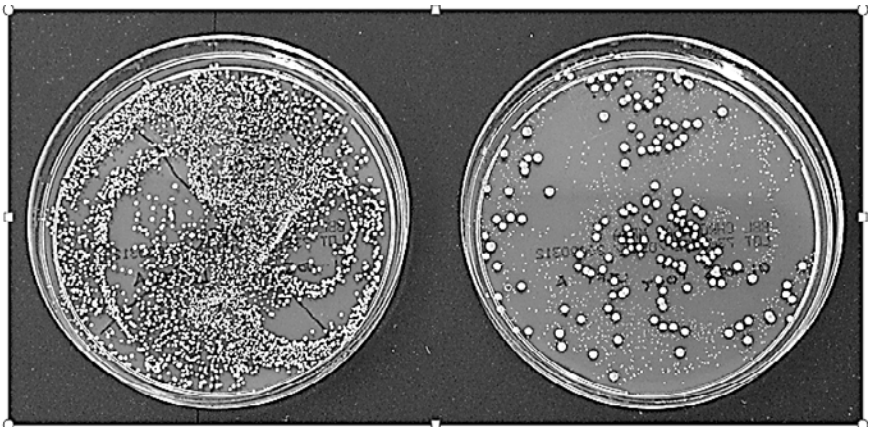

Figure 3. Day 1

Pre treatment culture **30 min post treatment culture**

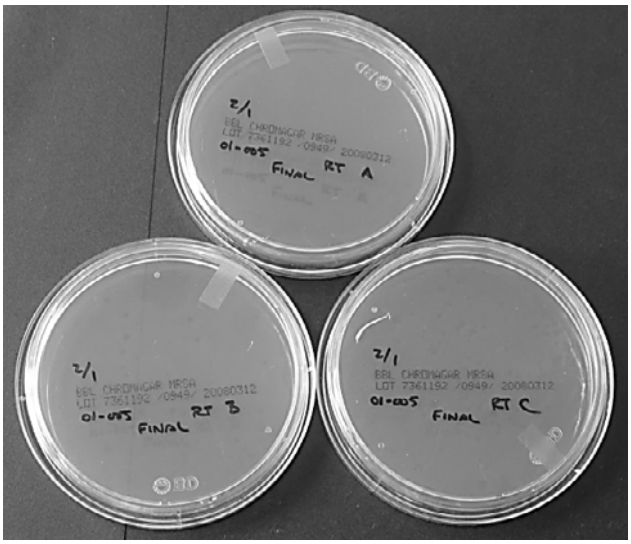

Figure 4. Day 5

Post treatment cultures

cultures containing 40 µg/ml methicillin. Culture dilutions were performed using phosphate-buffered saline (PBS), pH 7.4. On the day prior to laser experimentation, a 50 ml culture was inoculated using a glycerol stock of MRSA (stored at -80 ^{o}C), and allowed to grow for approximately 16 hrs. It was then sub-cultured, and growth was monitored by measuring the optical density at 600 nm until the culture reached an optical density (OD) of approximately 1. An aliquot of 1 ml was removed from the subculture and serially diluted to 1:300 in PBS. This dilution was allowed to incubate at room temperature for approximately 1 hour. 2 ml of this suspension was aliquoted into selected wells of 24-well tissue culture plates and presented for laser irradiation experiments. The laser was a multi-functional Class IV CDRH 20 W CW dual wavelength (10 W at 870 nm, 10 W at 930 nm) laser. The laser energy was shaped as a ‘flat-top’ profile with a custom-fabricated infrared microlens. The colonies were irradiated with sub-lethal 870 nm/930 nm

blended laser energy (4584 J/cm^2) and then 930 nm energy alone (2037 J/cm^2). Following laser treatments, 100 µl was removed from each well (control and treatment) and spread to form a uniform lawn of growth on pre-made control agar plates. This was immediately followed by the application in a "W" pattern of various generic topical antibiotics. The plates were then incubated at 37 °C for approximately 24 hrs for follow-up observation.

RESULTS

MRSA challenged with generic 2% erythromycin topical showed (Figure 5B) complete resistance to the antibiotic in the control (non-irradiated) sample. However, at 24 hours, in the pre-irradiated MRSA sample (Figure 5C), there was a moderate potentiation effect with generic 2% erythromycin topical.

MRSA then challenged with generic mupirocin topical showed (Figures 6A and 6B.) high susceptibility to the antibiotic in both the pre-irradiated and control samples. However, at 24 hours, the control cells challenged with the mupirocin produced substantial biofilm in response to the insult. In the pre-irradiated sample, the MRSA exhibited greater susceptibility to mupirocin and there was an unforeseen effect of substantially suppressed biofilm production in response to the laser and mupirocin.

Figure 5A

Figure 5B **Figure 5C**

Figure 5A. Topical antibiotic application graphic. **Figure 5B.** 2% Erythromycin topical application to MRSA (no pre-laser treatment) with full resistance seen in control plate. **Figure 5C.** Laser pre-treatment plate shows moderate 2% Erythromycin potentiation.

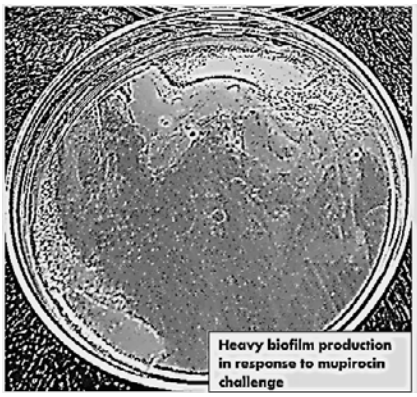

Figure 6A
Control with mupirocin

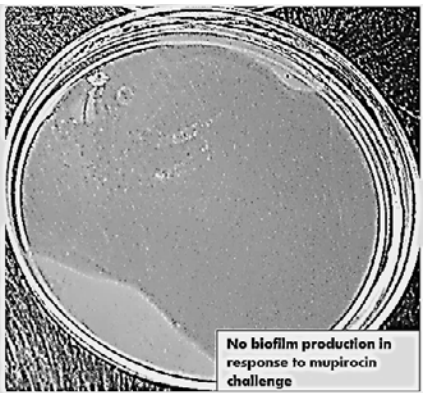

Figure 6B
Laser pre-treatment with mupirocin

Figure 6A. Heavy biofilm production evident of control (non-irradiated colonies) with mupirocin challenge.
Figure 6B. Pre-treatment with laser of planktonic cells inhibited biofilm production on agar plate.

Inhibition of multidrug efflux pumps as a strategy to prevent biofilm formation

In 2008, Kvist, *et al*. showed that efflux pump inhibitors (EPIs) used against *E. coli*, *Klebsiella pneumonia*, *S. aureus* and *Pseudomonas putida* significantly reduced biofilm formation when used singly, and almost abolished biofilm formation completely when used in combinations against certain species. Kvist suggested that efflux pump inhibitors could be exploited for prevention of biofilms in general [54]. Also in 2008, Ikonomidis, *et al*. evaluated the effect of the proton motive force (PMF) inhibitor Carbonyl cyanide m-chlorophenyl hydrazone (CCCP) on *Pseudomonas aeruginosa* biofilm development, and found that the inhibition of PMF-dependent efflux pumps may inhibit the formation of biofilm in *P. aeruginosa* [55].

In 2011, Matsumura, *et al*. studied multidrug efflux pumps and their effects on biofilm formation in *E. coli* by experimenting on 22 different mutant strains missing selected genes related to multidrug efflux pumps. They found that certain mutants showed extremely decreased biofilm formation, and suggested that certain multidrug efflux pumps significantly contribute to the biofilm formation of *E. coli* [56].

In 2013**,** Baugh, *et al*. evaluated the ability of the EPIs CCCP and chlorpromazine and phenyl-arginine-β-naphthylamide to prevent biofilm formation in *E. coli*, *P. aeruginosa* and *S. aureus.* They showed that chemical inhibition of efflux pumps will result in the transcriptional repression of certain biofilm matrix components and thereby inhibit biofilm formation. Baugh suggested that actively inhibiting efflux pumps is a promising anti-biofilm strategy [57].

DISCUSSION

When these data [54-57] are examined in light of (a) our previously published photo-biologic efflux attenuation data, (b) previously published photo-biologic potentiation of erythromycin topical against MRSA in the human nares, and (c) the newly presented *in vitro* data showing apparent inhibition of biofilm formation in MRSA, after 870 nm/930 nm sub-lethal irradiation with a mupirocin challenge, there is a likelihood that generating a photo-biologic minimum biofilm inhibitory concentration (PMBIC) of 870 nm/930 nm light in the human nares is conceivable.

The simplest basis for achieving a similar photo-biologic biofilm inhibition phenomenon in human therapy would theorize the minimal number of unknown causal powers. Hence, Occam's razor would present the most attractive explanation stating that the attenuation of efflux pumps shown in MRSA would also lead to a transcriptional repression of necessary biofilm components in MRSA. However, this apparent correlation between chemical, genetic and photo-biologic attenuation of bacterial efflux pumps and inhibited biofilm production does not prove causation, because there are many currently indistinguishable pathways that are yet to be studied.

Tempting as it may be to declare *post hoc ergo propter hoc* (after this, therefore because of this), there are presently not enough data to simply affirm 'As biofilm inhibition followed efflux pump attenuation, biofilm inhibition must have been caused by efflux pump attenuation', simply because the events appear to occur simultaneously in the present data and other contemporary studies [54-57]. In fact, the chronological ordering of these phenomena is at this time largely unknown. The causal intermediate pathways will most likely be studied in a bottoms-up manner, where there will be a 'piecing together' of the various biochemical interactions that ostensibly connect antibiotic efflux to biofilm generation in this complex system, as more data are generated.

If photo-biologic biofilm inhibition in human therapy prevents MRSA from being able to alter its phenotype (and produce biofilm) in response to a mupirocin challenge, adding such a pre-irradiation protocol to a typical nasal carriage eradication therapy would then theoretically extend the efficacy of mupirocin as a nasal therapeutic for carriage eradication. This would be a significant improvement upon current therapy, as we are now seeing eradication failures with mupirocin susceptible species, based solely on biofilm production [36, 37].

Finally, Miller *et al*. (2014) in the largest longitudinal study of *S. aureus* nasal carriage to

date (571 patients cultured every 60 days and followed for median two years) reported that those patients receiving anti-staphylococcal antibiotics "significantly increased the likelihood of losing *S. aureus* in the next swab, but also increased the likelihood of later acquisition". Miller suggested that their findings "question the validity of *S. aureus* eradication as a concept, and suggest that reducing *S. aureus* load around high-risk procedures (e.g. through decontamination/ prophylaxis pre-surgery) is a more biologically plausible approach to reducing *S. aureus* infection risk" [58].

CONCLUSION

The inherent challenges of MRSA and *S. aureus* decontamination and eradication are becoming more difficult every year. The difficulties that arise are in the form of numerous antibiotic resistance mechanisms, including biofilm formation. New data has been presented that suggests a correlation between the attenuation of efflux pumps in MRSA, and a transcriptional repression of biofilm components in MRSA. In the next 24 months multiple photobiologic protocols will be designed, and methods will be tested, examining a minimum biofilm inhibitory concentration of 870 nm/930 nm light for MRSA decontamination in pre-surgery nasal prophylaxis, with a series of *in vitro* and IRB approved human studies.

CONFLICT OF INTEREST STATEMENT

Dr. Eric Bornstein is the Chief Science Officer and Chief Medical Officer of Nomir Medical Technologies, the inventor of the 870 nm/930 nm phototherapy device. Dr. Bornstein is employed by Nomir and retains stock in the company.

REFERENCES

1. J. R. Marchesi (Ed.). 2014, The Human Microbiota and Microbiome. CABI.
2. Ogston, A. 1882, J. Anat. Physiol., 16(Pt 4), 526-567.
3. Rosenbach, F. J. 1884, Microorganismen bei den Wund-Infections-Krankheiten des Menschen, J. F. Bergmann, Wiesbaden, 1-122.
4. Orenstein, A. 2011, The Discovery and Naming of *Staphylococcus aureus*, Gevonden op.
5. Solberg, C. O. 1965, Acta Med. Scand. Suppl., 436, 1-96.
6. Moss, B., Squire, J. R., Topley, E. and Johnston, C. M. 1948, Lancet, 251(6496), 320-325.
7. Varga, D. T. and White, A. 1961, J. Clin. Invest., 40(12), 2209.
8. White, A. and Smith, J. 1963, Antimicrob. Agents Chemother., 161, 679-683.
9. Kluytmans, J., van Belkum, A. and Verbrugh, H. 1997, Clin. Microbiol. Rev., 10(3), 505-520.
10. Kluytmans, J. A. J. W. and Wertheim, H. F. L. 2005, Infection, 33(1), 3-8.
11. Munoz, P., Hortal, J., Giannella, M., Barrio, J. M., Rodríguez-Créixems, M., Pérez, M. J. and Bouza, E. 2008, J. Hosp. Infect., 68(1), 25-31.
12. Lamers, R. P., Stinnett, J. W., Muthukrishnan, G., Parkinson, C. L. and Cole, A. M. 2011, PLoS One, 6(1), e16426.
13. Gjødsbøl, K., Skindersoe, M. E., Skov, R. L. and Krogfelt, K. A. 2013, The Open Microbiology Journal, 7, 6.
14. Coates, T., Bax, R. and Coates, A. 2009, J. Antimicrob. Chemother., 64, 9-15.
15. Babu, T., Rekasius, V., Parada, J. P., Schreckenberger, P. and Challapalli, M. 2009, J. Clin. Microbiol., 47, 2279-2280.
16. Huang, S. S., Septimus, E., Kleinman, K., Moody, J., Hickok, J., Avery, T. R, Lankiewicz, J., Gombosev, A., Terpstra, L., Hartford, F., Hayden, M. K., Jernigan, J. A., Weinstein, R. A., Fraser, V. J., Haffenreffer, K., Cui, E., Kaganov, R. E., Lolans, K., Perlin, J. B. and Platt, R. 2013, N. Engl. J. Med., 368(24), 2255-2265.
17. McDanel, J. S., Murphy, C. R., Diekema, D. J., Quan, V., Kim, D. S., Peterson, E. M., Evans, K. D., Tan, G. L., Hayden, M. K. and Huang, S. S. 2013, Antimicrob. Agents Chemother., 57(1), 552-558.
18. Abbasi-Montazeri, E., Khosravi, A. D., Feizabadi, M. M., Goodarzi, H., Khoramrooz, S. S., Mirzaii, M., Kalantar, E. and Darban-Sarokhalil, D. 2013, Burns, 39(4), 650-654.
19. Jones, J. C., Rogers, T. J., Brookmeyer, P., Dunne, W. M., Storch, G. A., Coopersmith, C. M., Fraser, V. K. and Warren, D. K. 2007, Clin. Infect. Dis., 45(5), 541-547.

20. Hon, P. Y., Koh, T. H., Tan, T. Y., Krishnan, P., Leong, J. W. Y., Jureen, R., Chan. J., Tee, N. W., Murugesh, J., Chan, K. S. and Hsu, L. Y. 2014, J. Glob. Antimicrob. Resist., 2(1), 53-55.
21. Desroches, M., Potier, J., Laurent, F., Bourrel, A. S., Doucet-Populaire, F., Decousser, J. W. and Vernet-Garnier, V. 2013, J. Antimicrob. Chemother., 68(8), 1714-1717.
22. Pérez-Roth, E., Potel-Alvarellos, C., Espartero, X., Constela-Caramés, L., Méndez-Álvarez, S. and Álvarez-Fernández, M. 2013, Int. J. Med. Microbiol., 303(4), 201-204.
23. Singh Amit, K., Vimala, V. and Mastan, S. 2013, Int. J. Med. Res. Health Sci., 2(4), 840-847.
24. El-Mahdy, T. S., El-Ahmady, M. and Goering, R. V. 2014, Clin. Microbiol. Infect., 20(2), 169-173.
25. Kavanagh, K. T., Saman, D. M. and Yu, Y. 2013, Antimicrob. Agents Chemother., 57(12), 5789-5791.
26. Melake, N. A., Zakaria, A. S., Ibrahim, N. H., Salama, M. A. and Mahmoud, A. Z. 2014, Intl. J., 5(2), 76-84.
27. Healy, B. and Freedman, A. 2006, BMJ, 332(7545), 838-841.
28. Mertz, P. M. 2003, Wounds, 15(5), 129-132.
29. Edwards, R. and Harding, K. G. 2004, Curr. Opin. Infect. Dis., 17(2), 91-96.
30. Tenke, P., Riedl, C. R., Jones, G. L., Williams, G. J., Stickler, D. and Nagy, E. 2004, Int. J. Antimicrob. Ag., 23, 67-74.
31. Sedlacek, M. J. and Walker, C. 2007, Oral Microbiol. Immunol., 22(5), 333-339.
32. Marsh, P. D. 2005, J. Clin. Periodontol., 32(s6), 7-15.
33. Costerton, J. W., Geesey, G. G. and Cheng, K. J. 1978, Scientific American, 238(1), 86-95.
34. Høiby, N., Ciofu, O., Johansen, H. K., Song, Z. J., Moser, C., Jensen, P. Ø. and Bjarnsholt, T. 2011, Int. J. Oral Sci., 3(2), 55.
35. Kostenko, V., Ceri, H. and Martinuzzi, R. J. 2007, FEMS Immunol. Med. Microbiol., 51(2), 277-288.
36. Davis, S. C., Ricotti, C., Cazzaniga, A., Welsh, E., Eaglstein, W. H. and Mertz, P. M. 2008, Wound Repair and Regeneration, 16(1), 23-29.
37. Ogura, M., Yano, H., Sato, M., Nakamura, A., Wakimoto, Y., Ohkusu, K. and Ezaki, T. 2013, J. Infect. Chemother., 19(2), 196-201.
38. Baumert, N., von Eiff, C., Schaaff, F., Peters, G., Proctor, R. A. and Sahl, H. G. 2002, Microbial Drug Resistance, 8(4), 253-260.
39. von Eiff, C. 2008, Int. J. Antimicrob. Ag., 31(6), 507-510.
40. Abu-Qatouseh, L. F., Chinni, S. V., Seggewiss, J., Proctor, R. A., Brosius, J., Rozhdestvensky, T. S. and Becker, K. 2010, J. Mol. Med., 88(6), 565-575.
41. Morikawa, K., Ohniwa, R. L., Ohta, T., Tanaka, Y., Takeyasu, K. and Msadek, T. 2010, Microbes and Environments, 25(2), 75-82.
42. von Eiff, C., Becker, K., Machka, K., Stammer, H. and Peters, G. 2001, N. Engl. J. Med., 344(1), 11-16.
43. Proctor, R. A., Kahl, B., von Eiff, C., Vaudaux, P. E., Lew, D. P. and Peters, G. 1998, Clin. Infect. Dis., 27(Suppl. 1), S68-S74.
44. Alreshidi, M. M., Dunstan, R. H., Onyango, L. A. and Roberts, T. K. 2013, Microbial pathogens and strategies for combating them: science, technology and education, Formatex Research Center, Spain, 690-701.
45. Lewis, K., Spoering, A. L., Kaldalu, N., Keren, I. and Shah, D. 2005, Biofilms, Infection, Nottingham, 241.
46. Gilbert, P., Allison, D. G. and McBain, A. J. 2002, J. Appl. Microbiol., 92(s1), 98S-110S.
47. Drenkard, E. and Ausubel, F. M. 2002, Nature, 416(6882), 740-743.
48. Watkins, R. R., David, M. Z. and Salata, R. A. 2012, J. Med. Microbiol., 61(Pt 9), 1179-1193.
49. Bornstein, E., Hermans, W., Gridley, S. and Manni, J., 2009, Photochem. Photobiol., 85(6), 1364-1374.
50. Bornstein, E., Gridley, S., Wengender, P. and Robbins, A. 2010, Photochem. Photobiol., 86(3), 617-627.
51. Bornstein E. and Gridley S. 2010, 9th International Mycological, Congress, Future strategies for the control of fungal diseases, Edinburgh UK. Aug 1-6.

52. Landsman, A. S., Robbins, A. H., Angelini, P. F., Wu, C. C., Cook, J., Oster, M. and Bornstein, E. S. 2010, J. Am. Podiatr. Med. Assoc., 100(3), 166-177.
53. Bornstein E. 2014, Podiatry Management, August, 137-140.
54. Kvist, M., Hancock, V. and Klemm, P. 2008, Appl. Environ. Microbiol., 74(23), 7376-7382.
55. Ikonomidis, A., Tsakris, A., Kanellopoulou, M., Maniatis, A. N. and Pournaras, S. 2008, Lett. Appl. Microbiol., 47(4), 298-302.
56. Matsumura, K., Furukawa, S., Ogihara, H. and Morinaga, Y. 2011, Biocontrol Science, 16(2), 69-72.
57. Baugh, S., Phillips, C. R., Ekanayaka, A. S., Piddock, L. J. and Webber, M. A. 2014, J. Antimicrob. Chemother., 69(3), 673-681.
58. Miller, R. R., Walker, A. S., Godwin, H., Fung, R., Votintseva, A., Bowden, R. and Knox, K. 2014, J. Infect., 68(5), 426-439.