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A Novel Topical Combination Ointment with Antimicrobial Activity against Methicillin-Resistant *Staphylococcus aureus*, Gram-Negative Superbugs, Yeasts, and Dermatophytic Fungi

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ABSTRACT

Background: The use of topical antimicrobial agents for management of minor skin infections is a clinical strategy that is commonly practiced in the community. Coupled with the use of topical antimicrobial agents is the emergence of antibiotic-resistant strains of pathogens leading to the need for alternative treatments.
Objective: A novel topical combination ointment consisting of salicylic acid, oak bark extract, benzoic acid, and polyethylene glycol (Bensal HP, Sonar products Inc., Carlstadt, NJ) with antimicrobial properties was assessed to determine its spectrum of activity.
Methods: One hundred eighty-four bacterial and fungal isolates from culture collections that included multidrug-resistant bacteria such as methicillin-resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Acinetobacter* spp, and gram-negative so-called superbugs, as well as yeasts and filamentous fungi, were investigated by cylinder diffusion and agar dilution assays.
Results: All 184 bacterial and fungal isolates were susceptible to the combination ointment at the clinically applied concentration and there was no evidence of cross-resistance between Bensal HP and other classes of antimicrobials. In time-kill tests, Bensal HP was rapidly bactericidal against *P aeruginosa* ATCC 27853 and methicillin-resistant *S aureus* SA179 at 4 × the MIC, a concentration that is applied clinically.
Conclusions: The results of this study suggest that this combination ointment has a broad in vitro spectrum of antimicrobial activity against both more common bacterial and fungal pathogens and may be particularly useful for treatment of infections by multidrug-resistant organisms. Additional studies are warranted to investigate the full clinical utility as a therapeutic agent and also for possible infection control interventions.
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Introduction

Antibiotic resistance is a serious health threat and has the potential for dire consequences. The Centers for Disease Control and Prevention estimate that more than 2 million individuals in the United States develop illness resulting from antibiotic-resistant infections on an annual basis and published *Antibiotic Resistance Threats in the United States, 2013*,[1] which provides a snapshot of the complex problem of antibiotic resistance. The threats were prioritized as urgent, serious, and concerning. Of particular concern is increasing multidrug resistance coupled with cessation of antibiotic discovery programs by most major pharmaceutical companies. This situation has created a major global health crisis in which there are few or no effective agents to treat common bacterial infections or infections caused by less common pathogens, including *Mycobacterium* spp,[2,3] filamentous fungi, and yeasts.[4–6] Furthermore, alternative second- and third-line agents that are effective are also associated with safety issues. Most current concerns about antibiotic resistance focus on infections in hospital

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Table I
Summary of cylinder test zone sizes for test isolates.

Organism	No. tested	Range of zone (mm)	Drug-sensitive zone*
Escherichia coli (M)	17	11–16	12
Klebsiella pneumoniae (M)	13	12–18	13
Serratia marcescens (M)	10	13–19	13
Pseudomonas aeruginosa (M)	11	13–18	13
Acinetobacter baumannii (M)	13	14–18	16
Methicillin-sensitive *Staphylococcus aureus*	12	16–23	16
Methicillin-resistant *Staphylococcus aureus* (M)	11	20–22	
Enterococcus faecalis (M)	11	16–21	17
Streptococcus pyogenes	12	10–15	0
Nocardia brasiliensis	10	18–42	0
Mycobacterium fortuitum	10	22–36	0
Candida albicans	10	14–19	0
Candida glabrata	10	12–17	0
Trichophyton rubrum	12	21–31	0
Trichophyton tonsurans	10	18–37	0
Trichophyton mentagrophytes	10	22–27	0
Propionibacterium acnes	1	27	0
Cryptococcus neoformans	1	18	0
Total	184		

M = multidrug-resistant organisms included.

* Zone of 0 mm indicates resistant. Note that zones for drug-sensitive isolates of *Escherichia coli*, *K pneumoniae*, *Serratia marcescens*, *Pseudomonas aeruginosa,* and *Enterococcus faecalis* tended to be smaller (suggesting they were more resistant) than those of their multidrug-resistant counterparts.

settings requiring parenteral agents.[3] Little is known about the activity of topical agents against multidrug-resistant organisms (MDROs), some of which are likely to be compromised because they contain agents to which resistance has already been reported. These include neomycin, polymyxin B, bacitracin, and mupirocin.[7,8] Bensal HP (Sonar Products Inc., Carlstadt, NJ) is a combination topical ointment with antimicrobial properties with activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and common bacterial and fungal skin pathogens.[9–11] The current study was designed to assess the in vitro activity of Bensal HP against a broad range of contemporary pathogens, including MDROs such as MRSA, vancomycin-resistant *Enterococcus*, gram-negative so-called superbugs, *Mycobacterium fortuitum*, *Nocardia brasiliensis*, yeasts, and filamentous fungi.

Materials and Methods

Test agent

Bensal HP contains salicylic acid (30 mg/g), benzoic acid (60 mg/g), QRB-7 (oak bark extract) (30 mg/g), and vehicle polyethylene glycol 400 and polyethylene glycol 3350. The test agent was provided by SMG Pharmaceuticals, Cary, North Carolina.

Organisms

In vitro activity was investigated against 184 bacterial and fungal isolates from the culture collections of Creighton University, Omaha, Nebraska; the Alegent Creighton Hospital Microbiology Laboratory, Omaha, Nebraska; and the University of Louisville Hospital Microbiology Laboratory, Louisville, Kentucky. The bacterial isolates were from US and international sources and included well characterized non-MDRO and MDRO isolates of Enterobacteriaceae (n = 40), *Pseudomonas aeruginosa* (n = 11); *Acinetobacter baumannii* (n = 13); *S aureus* (n = 23), including MRSA and methicillin-susceptible *S aureus*; and *Enterococcus faecalis* (n = 11), including vancomycin-resistant *Enterococcus*, Group A *Streptococcus* (*Streptococcus pyogenes* [n = 12]), *Propionibacterium acnes* (n = 1), *M fortuitum* (n = 10), and *N brasiliensis* (n = 10). The fungal isolates were *Candida albicans* (n = 10), *Candida glabrata* (n = 10), *Cryptococcus neoformans* (n = 1), *Trichophyton rubrum* (n = 12), *T tonsurans* (n = 10), and *T mentagrophytes* (n = 10). The gram-negative bacteria were previously characterized for resistance mechanisms by phenotypic, biochemical, and molecular methods.[2] These included isolates of Enterobacteriaceae, *Pseudomonas* spp, and *Acinetobacter* spp producing the extended spectrum β-lactamases TEM-52, SHV-4, SHV-12, OXA-45, CTX-M-1, CTX-M-9, CTX-M-12, CTX-M-14, CTX-M-15, CTX-M-17, CTX-M-18, and CTX-M-19; chromosomal and plasmid-mediated AmpC β-lactamases, including FOX-like and CMY-2 enzymes; and carbapenemases of the IMP, VIM, KPC, OXA, and NDM families. The *Pseudomonas aeruginosa* isolates included some with upregulated MexAB, MexEF, and MexXY efflux pumps, and downregulation of the OprD porin. The isolates included organisms described in the media as superbugs because of their resistance to most available antibacterial agents. ATCC reference isolates included in the study were *Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, and *T mentagrophytes* ATCC 9533.

Cylinder diffusion susceptibility testing

All isolates were tested by a cylinder diffusion procedure[12,13] that was a modification of the Clinical and Laboratory Standards Institute (CLSI) disk diffusion method.[14,15] In this procedure a cylinder containing Bensal HP was substituted for the impregnated filter paper disks of the CLSI method. Bensal HP liquefied by heating to 56°C for 10 minutes and 40 μL was pipetted into a sterile metal cylinder placed on a lawn culture of the test organism. The lawn culture of the test isolate was prepared according to CLSI methodology and inoculated onto appropriate media (see Media). The tests with gram-negative pathogens, staphylococci, streptococci, and enterococci were incubated as recommended by CLSI; that is, overnight, typically 18–20 hours. All other isolates were incubated for as long as necessary to be able to visualize sufficient growth to allow measurement of an inhibition zone; that is, 48–72 hours. After incubation, inhibition diameters around the cylinders were measured and recorded according to the CLSI method. In the absence of CLSI interpretive criteria, any zone of inhibition was interpreted to indicate susceptibility and the absence of an inhibition zone indicated resistance. This interpretation was adopted to correlate with the occurrence or absence of activity at the undiluted concentration of Bensal HP that is used therapeutically.

MIC testing

Bensal HP MICs were determined by CLSI agar dilution methodology.[14,15] The test isolates were 73 representative bacterial isolates that were capable of overnight growth at 35°C on Mueller-Hinton agar.

Time-kill testing

Using concentrations based on the agar dilution MICs, the bactericidal activity of Bensal HP against *Pseudomonas aeruginosa* ATCC 27853 and MRSA SA179 was determined by time-kill methodology. The Bensal HP concentrations tested were 4 × the MIC and 1 × the MIC. Drug-free and antibiotic-supplemented Mueller-Hinton broths were inoculated to provide an initial inoculum of $\geq 5 \times 10^5$ CFU/mL of each isolate. Growth rates

and killing were determined by comparing viable counts at 0, 1, 2, 4, and 24 hours. Samples for the counts were plated on Roswell Park Memorial Institute (RPMI) 1640 medium (Remel, Lenexa, Kansas). This medium inhibits Bensal HP activity and is therefore suitable to inactivate drug carryover in the samples. Bactericidal activity was interpreted as ≥ 3 $\log_{10}$ CFU/mL decrease after 24 hours of incubation.

Media

Susceptibility tests were performed on Mueller-Hinton agar (BD Diagnostic Systems, Sparks, Maryland) exception for microorganisms that did not grow well on this medium. Group A streptococci were tested on Mueller-Hinton agar supplemented with 5% sheep's blood (BD Diagnostic Systems), and fungal isolates were tested on Sabouraud dextrose agar (Remel). Initial tests of antifungal activity with RPMI 1640 medium determined that this medium antagonized the activity of Bensal HP and was unsuitable for susceptibility testing. Time-kill testing was performed in Mueller-Hinton broth (BD Diagnostic Systems) with viable counts determined on RPMI 1640 medium.

Results

All 184 bacterial and fungal isolates were susceptible to this combination ointment in the cylinder diffusion tests. No resistance was detected. The susceptibility of both wild type and MDRO isolates indicated that the mechanisms of resistance to other antimicrobial agents of the isolates did not compromise Bensal HP activity. Inhibition zone diameters were generally larger for gram-positive bacteria and filamentous fungi than for gram-negative bacteria (Table I). Curiously, some MDROs had larger inhibition zones than their wild type counterparts (Table I). Figure 1 shows 3 cylinder tests on 1 plate in which isolates of MRSA, *Escherichia coli*, and *Pseudomonas aeruginosa* were inhibited by this combination ointment.

The 73 isolates in the MIC tests included both MDROs and non-MDROs for each species tested. All gram-positive isolates were inhibited by an 80-fold dilution of Bensal HP, which corresponded to salicylic acid/benzoic acid/QRB-7 concentrations of 0.375/0.75/0.375 mg/g, respectively; that is, identical MIC_{50} and MIC_{90} values of 80-fold dilution of Bensal HP. The gram-negative isolates were all susceptible to a 40-fold dilution of Bensal HP (0.75/1.5/0.75 mg/g), whereas MIC_{50} and MIC_{90} values were 80-fold and 40-fold dilutions, respectively.

In time-kill tests, the combination ointment was rapidly bactericidal against *Pseudomonas aeruginosa* ATCC 27853 and MRSA SA179 at 4 × MIC. This concentration was a 20-fold dilution of the concentration that is applied clinically. No regrowth occurred during the 24-hour incubation period. The *Pseudomonas aeruginosa* isolate was killed very rapidly. The initial count of $\geq 2 \times 10^6$ CFU/mL was reduced to 800 CFU/mL by the time the inoculum was sampled and plated for the time zero reading. That is, the bactericidal criterion of at least a 3-log reduction in viable count was achieved within approximately 5 minutes of exposure to Bensal HP. The MRSA isolate was killed with a >4 log kill attained within an hour of exposure to 4 × MIC. At 1 × MIC (ie, an 80-fold dilution of the clinical concentration) the viable counts were unchanged after 24 hours. On sampling the tests after 24 hours, there was no evidence of reduced susceptibility to Bensal HP in cylinder diffusion; that is, mutational resistance did not emerge during prolonged exposure to Bensal HP.

Bensal HP was active against 10 isolates each of *Candida albicans* and *Candida glabrata* when tested on Sabouraud agar but was inactive against both species on RPMI 1640 medium. This indicated that RPMI 1640 medium antagonized the activity of Bensal HP and susceptibility tests on RPMI 1640 medium were discontinued. On Sabouraud agar, Bensal HP was also active against 12 isolates of *T rubrum*, 10 isolates of *T tonsurans*, 10 isolates of *T mentagrophytes*, and a single isolate of *Cryptococcus neoformans*. Figures 2 and 3 show the inhibition of *Candida albicans* and *T mentagrophytes,* respectively, by Bensal HP.

Discussion

Bensal HP is currently marketed and is indicated for treatment of the inflammation and irritation associated with many common forms of dermatitis, including certain eczematous conditions. These conditions include complications associated with pyodermas. It is also used for the treatment of insect bites, burns,

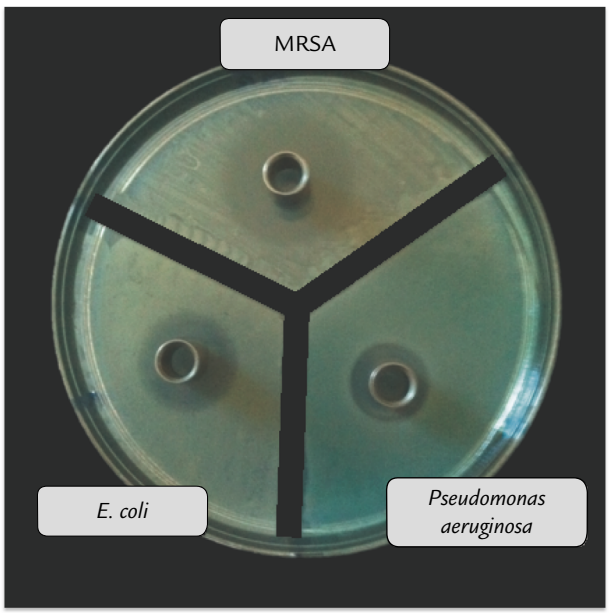

Figure 1. Three cylinder tests showing inhibition of methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli* (*E. coli*), and *Pseudomonas aeruginosa* on Mueller-Hinton agar after diffusion of Bensal HP (Sonar Products Inc., Carlstadt, NJ) from cylinders.

Figure 2. Inhibition of *Candida albicans* on Sabouraud agar by Bensal HP (Sonar Products Inc., Carlstadt, NJ).

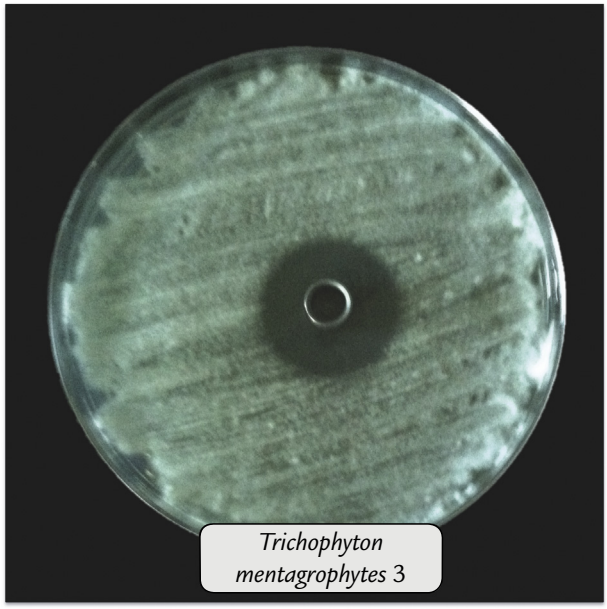

Figure 3. Inhibition of *Trichophyton mentagrophytes* on Sabouraud agar by Bensal HP (Sonar Products Inc., Carlstadt, NJ).

and fungal infections. It has been shown to accelerate reepithelialization.[10]

This study confirmed and extended previous information about the in vitro antimicrobial spectrum of Bensal HP.[10] The most important finding was that all 184 bacteria, yeasts, and filamentous fungi tested were susceptible to the clinically used concentration of Bensal HP, indicating that it has a very broad spectrum of activity compared with other topical agents. In addition, the finding that Bensal HP was not compromised by mechanisms of antibiotic cross-resistance between it and other classes of antimicrobials is of interest.

Of note, multidrug-resistant *Acinetobacter,* extended spectrum β-lactamase–producing Enterobacteriaceae, multiresistant *Pseudomonas aeruginosa,* and MRSA were inhibited by Bensal HP. These pathogens correspond to the categories of potential pathogen threats listed in the Centers for Disease Control and Prevention report.[1] The results from our study suggest that Bensal HP may provide an effective topical treatment in situations where MDROs are problematic. Additional study will be needed to substantiate this clinical utility.

The activity of Bensal HP against *M fortuitum* also raises the possibility of activity against other *Mycobacterium* species, some of which are highly drug-resistant. In addition, the absence of mutational resistance emerging during prolonged exposure in the time-kill tests suggested that pathogens may not easily develop resistance to Bensal HP.

Additional investigation is needed on the mechanism of action of Bensal HP and whether the antimicrobial activity results from the individual components or whether the combination is required to demonstrate these effects. Currently, the mechanism of action is not known.

Further studies are warranted to investigate the potential prophylactic, decolonization, and therapeutic uses of Bensal HP. It would also be useful to compare its activity to other topical agents such as the combinations of bacitracin/neomycin/polymyxin, and bacitracin/polymyxin, and the monocomponent agents, mupirocin, and silver sulfadiazine.

A limitation of this study is that there is neither a standardized susceptibility test method nor interpretative criteria for topical ointments such as Bensal HP, with the exception of mupirocin, which is water-soluble.[16] In the absence of such methodology, the cylinder test method using the clinically applied concentration provided useful information.

Conclusions

Bensal HP is a very-broad-spectrum topical antimicrobial agent with in vitro activity against important pathogens such as MRSA, *Pseudomonas aeruginosa*, gram-negative superbugs, yeasts, and filamentous fungi. Based on the in vitro findings in this study, additional studies are warranted to better understand the full clinical utility of this agent.

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Conflicts of Interest Statement

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