Bensal HP (BHP-410), a Novel Antimicrobial Agent with Activity against MRSA, VRE, Gram-negative MDROs, Yeasts, and Dermatophytic Fungi

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Abstract

Background:
Increasing multidrug resistance and a dwindling antibiotic pipeline have created a major global health crisis. Little is known about the activity of topical agents against multidrug resistant organisms (MDROs) or about their therapeutic or infection control relevance in meeting this challenge. With this in mind, a study was designed to assess the activity of a novel topical antimicrobial BHP-410 containing salicylic acid, benzoic acid and QRB-7 (oak bark extract) against a broad range of MDRO's including MRSA, VRE, ESBL and carbapenemase-producing isolates. In addition its activity against selected isolates of *Mycobacterium fortuitum*, *Nocardia brasiliensis*, yeasts and filamentous fungi was also assessed.

Materials and Methods:
Activity against 181 isolates comprising 12 bacterial species (5 gram-negative, 7 gram-positive), 3 yeast species, and 3 dermatophyte species was assessed. The 129 bacterial isolates included well characterized non-MDRO and MDRO isolates of Enterobacteriaceae *P. aeruginosa*, *A. baumannii*, *S. aureus*, and *Enterococcus faecalis* and routine clinical isolates of Group A streptococci, *Propionibacterium acnes*, *M. fortuitum* and *N. brasiliensis*. Twenty one isolates of *Candida albicans*, *C. glabrata*, *Cryptococcus neoformans* and 30 isolates of *Trichophyton rubrum*, *T. tonsurans*, and *T. mentagrophytes* were also tested. Susceptibility to BHP-410 was determined by the cylinder diffusion and CLSI agar dilution methods. Bactericidal activity was assessed by time-kill methodology.

Results:
In cylinder diffusion tests, all bacterial and fungal isolates were inhibited by BHP-410 and no resistance was detected. There was no apparent reduction in inhibition zone when comparing MDRO-isolates to non-MDRO (wild type) isolates. In MIC tests non-MDRO and MDRO isolates were equally susceptible with all gram-positive isolates (including MRSA) inhibited by an 80-fold dilution of BHP-410. Gram-negative isolates were all susceptible within a range of 40 to 80-fold dilutions. BHP-410 was rapidly bactericidal against *P. aeruginosa* and MRSA.

Conclusion:
BHP-410 has an extremely broad spectrum of antimicrobial activity and is unaffected by the resistance mechanisms of MDROs. Further study is warranted to investigate its full clinical utility.

Background

The combination of increasing multidrug resistance and a dwindling antibiotic pipeline has created a major global health crisis in which there are few or no effective agents to treat bacterial infections. Little is known about the activity of topical agents against multidrug resistant organisms (MDROs) or about the therapeutic or infection control potential of topical agents in meeting this challenge. Bensal HP (BHP-410) is a broad spectrum topical antimicrobial agent containing salicylic acid (30 mg/gm), benzoic acid (60 mg/gm), QRB-7 (oak bark extract, 30 mg/gm) and vehicle polyethylene glycol 400 and polyethylene glycol 3350. The current study was designed to assess its activity against a broad range of contemporary pathogens including MDROs such as MRSA, vancomycin-resistant *Enterococcus* (VRE), MDR producers of AmpC, extended spectrum β-lactamase (ESBL) and carbapenemase β -lactamases, *Mycobacterium fortuitum*, *Nocardia brasiliensis*, yeasts and filamentous fungi.

Materials & Methods

Test Agent: Bensal HP marketed by EPI Health (Charleston SC)

Organisms: *In vitro* activity was investigated against 184 bacterial and fungal isolates from the culture collections of Creighton University, Omaha, NE, the Alegent Creighton Hospital Microbiology Laboratory, Omaha, NE, and the University of Louisville Hospital Microbiology Lab, Louisville, KY.

The isolates were from U.S. and international sources and included well characterized non-MDRO and MDRO isolates of Enterobacteriaceae (n=40), *Pseudomonas aeruginosa* (n=11), *Acinetobacter baumannii* (n=13), *Staphylococcus aureus* (n=23) including MRSA and methicillin-susceptible *S. aureus* (MSSA), and *Enterococcus faecalis* (n=11) including VRE. Also tested were routine clinical isolates of Group A *Streptococcus* (*S. pyogenes*, n=12), *Propionibacterium acnes* (n=1), *Mycobacterium fortuitum* (n=10) and *Nocardia brasiliensis* (n=10). The fungal isolates included *Candida albicans* (n=10), *C. glabrata* (n=10), *Cryptococcus neoformans* (n=1), *Trichophyton rubrum* (n=12), *T. tonsurans* (n=10), and *T. mentagrophytes* (n=10). The MDROs were previously characterized for resistance mechanisms by phenotypic, biochemical and molecular methods (1) and included isolates producing the ESBLs TEM-52, SHV ESBLs, 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 that included FOX-like and CMY-2 enzymes, and carbapenemases of the IMP, VIM, KPC, and NDM families. Especially challenging MDROs included carbapenemase-producing isolates of *P. aeruginosa* and *A. baumannii* and *P. aeruginosa* isolates with upregulated MexAB, MexEF, and MexXY efflux pumps, and down-regulation of the OprD porin.

Investigations Susceptibility was determined by the cylinder diffusion (2) and CLSI agar dilution (3) methods. Examples of cylinder diffusion tests are shown in Figure 1. Bactericidal activity was assessed by time-kill methodology following exposure of *P. aeruginosa* ATCC 27853 and MRSA SA179 to concentrations of 1x and 4x the MIC.

Results

All isolates, bacterial and fungal, were inhibited by Bensal HP. No resistance was detected. No MDRO isolates exhibited cross resistance to Bensal HP. That is, susceptibility was unaffected by the innate or acquired resistance mechanisms of the isolates. Zone diameters were generally larger for gram-positive bacteria and filamentous fungi than for gram-negative bacteria (Table 1). Some MDROs had larger inhibition zones than their wild type counterparts. Representative isolates exhibiting this trend are shown in Table 2.

In MIC tests with 73 selected isolates that included both MDROs and non-MDROs in each species tested, all gram-positive isolates were inhibited by an 80-fold dilution of Bensal HP (MIC = 0.375/ 0.75/ 0.375 mg/gm of salicylic acid/benzoic acid/QRB-7 respectively) and the gram-negative isolates were all susceptible to a 40-fold dilution (MIC = 0.75/ 1.5/ 0.75 mg/gm of salicylic acid/benzoic acid/QRB-7 respectively), with most gram-negatives being susceptible to an 80-fold dilution (0.375/ 0.75/ 0.375 mg/gm).

In time-kill tests, BHP-410 was rapidly bactericidal against *P. aeruginosa* ATCC 27853 and MRSA SA179 at 4x the MIC (1:20 dilution) with no regrowth occurring in the 24 hour incubation period (Figures 2 and 3).

Conclusions

1. Bensal HP has an extremely broad spectrum of activity that is not compromised by mechanisms of antibiotic resistance occurring in contemporary multidrug resistant bacteria.
2. All gram-positive and gram-negative bacteria, yeasts, and filamentous fungi in this study were susceptible to the clinically used concentration of Bensal HP (i.e. inhibited by undiluted ointment).
3. No resistance to Bensal HP was detected.
4. Bensal HP was rapidly bactericidal in time-kill studies with an isolate of *Pseudomonas aeruginosa* and an isolate of MRSA.
5. Further study is warranted to investigate its full clinical utility.

Results

Figure 1: Representative Cylinder Diffusion Tests

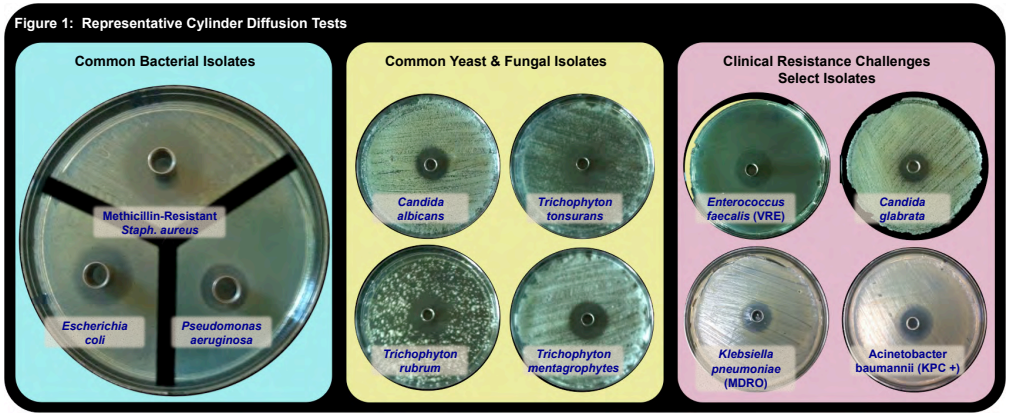


Table 1: Summary of Cylinder Diffusion Tests

Organism	No. Tested	Range of Zone Diameters (mm)*	Wild type Zone	MDROs Included?
MSSA	12	16-23	16	y
MRSA	11	20-22	NA*	Y
Enterococcus faecalis	11	16-21	17	Y
Streptococcus pyogenes	12	10-15	ND*	NA
Nocardia brasiliensis	10	18-42	ND	NA
Mycobacterium fortuitum	10	22-36	ND	NA
Escherichia coli	17	11-16	12	Y
Klebsiella pneumoniae	13	12-18	13	Y
Serratia marcescens	10	13-19	13	Y
Pseudomonas aeruginosa	11	13-18	13	Y
Acinetobacter baumannii	13	14-18	16	Y
Candida albicans	10	14-19	ND	NA
Candida glabrata	10	12-17	ND	NA
Trichophyton rubrum	12	21-31	ND	NA
Trichophyton tonsurans	10	18-37	ND	NA
Trichophyton mentagrophytes	10	22-27	ND	NA
Propionibacterium acnes	1	27	ND	NA
Cryptococcus neoformans	1	18	ND	NA
Total	184			

Table 2: Representative Examples of Wild Type Strains with Smaller Inhibition Zones than MDROs

MDRO	Organism	Strain Code	Zone (mm)	Wild Type / Resistance Mechanism
	MSSA	ATCC 29213	16	Wild type
✔	MSSA	SA266	21	*MecA*
	Enterococcus faecalis	ATCC 29212	17	Wild type
✔	*Enterococcus faecalis*	Entc141	21	Van B
	Escherichia coli	ATCC 25922	13	Wild type
✔	*Escherichia coli*	Ecoli257	16	CTX-M-18 ESBL
	Klebsiella pneumoniae	Kleb23	13	Wild type
✔	*Klebsiella pneumoniae*	GM267	18	SHV-12, SHV-1, OXA-9, KPC, FOX, PSE-1, TEM-1, TEM-30 β-lactamases
	Serratia marcescens	Misc223	13	Wild type
✔	*Serratia marcescens*	Serr126	19	IMP-1 metallo-β-lactamase
	Pseudomonas aeruginosa	ATCC 27853	13	Wild type
✔	*Pseudomonas aeruginosa*	Ps369	18	Overexpressed MexEF-OprM, OprD Diminished
✔	*Pseudomonas aeruginosa*	Ps332	22	VIM-7 metallo-β-lactamase, OXA-45 ESBL

Figure 2: Time-Kill Curves - MRSA

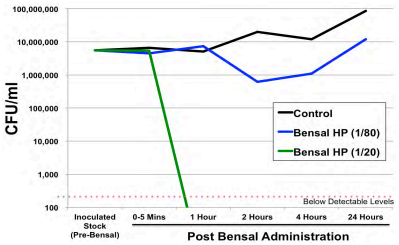
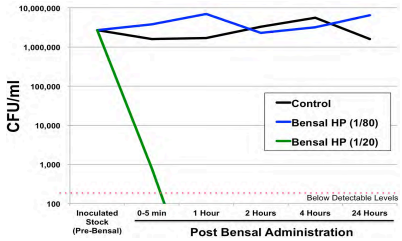


Figure 3: Time-Kill Curves – *Pseudomonas aeruginosa*



References

1. **Moland, E. S., N. D. Hanson, J. A. Black, A. Hossain, W. Song, and K. S. Thomson.** 2006. Prevalence of newer β-lactamases in gram-negative clinical isolates collected in the United States from 2001 to 2002. J Clin Microbiol **44:**3318-24.
2. **Barry, A. L.** 1991. Procedures and Theoretical Considerations for Testing Antimicrobial Agents in Agar Media, p. 1-16. *In* V. Lorian (ed.), Antibiotics in Laboratory Medicine, 3rd ed. Williams & Wilkins, Baltimore.
3. **Clinical and Laboratory Standards Institute.** 2013. Performance standards for antimicrobial susceptibility testing; twenty-third informational supplement M100-S23. Clinical and Laboratory Standards Institute, Wayne, PA.

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