

# Berberex Antimicrobial Performance: Assay For Antibiotic Susceptibility In Resistant Micro-Organisms.

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Strains of resistant microorganisms were collected over the course of 6 weeks and given unique identifiers (Table 1). This group contained 7 unique MRSA strains, and 1 strain each of VRE, B-strep, and E. coli as noted below.

Table 1:

Culture Identification	MRSA	VRE	B-strep	E. coli
	280-972		280-0731	798-13-873
	286-1356			
	288-1206			
	267-2846			
	279-865			
	278-778			
	276-2114			

Bacteria were suspended in normal saline and approximated using a McFarland Nephelometer at 600 nm (Table 2). Using a 0.01-ml calibrated loop, agar surfaces were inoculated by standard techniques. Each of these cultures was found to be resistant to standard antibiotics and selected as a test organism to evaluate the ability of Berberex to suppress bacterial growth in virulent organisms.

Table 2: \*at wavelength of 600 nm; McFarland Nephelometer Standards

McFarland Standard No.	0.5	1	2	3	4
Approx. cell density (1X10 <sup>8</sup> CFU/ml)	1.5	3.0	6.0	9.0	12.0
% Transmittance*	74.3	55.6	35.6	26.4	21.5
Absorbance*	0.08 to 0.1	0.257	0.451	0.582	0.669

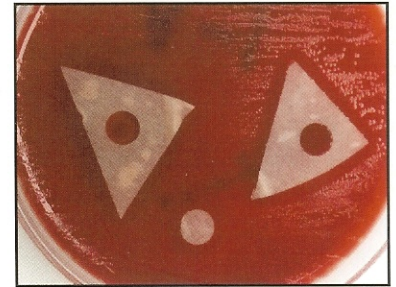
Whatman filter papers were punched to create discs, and cut into triangles. These materials were saturated in Berberex in an empty, sterile, culture dish and then transferred with sterile forceps to the surface of the agar. Plates were incubated overnight at 37° with 80% humidity, and photographed. Each of the test cultures was coded without identity and only after reading and photography were the identities of the cultures made available to the photographer.

While all cultures were photographed, only representative images of the original culture are presented.

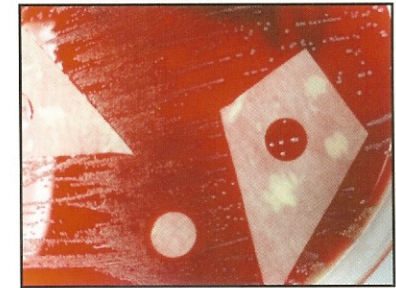
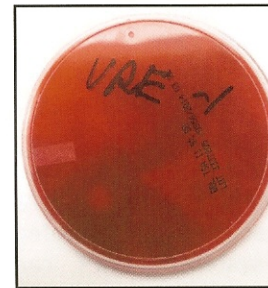
## 276-2114 – MRS



## B-Strep 1



## VRE



Resistant strains of MRSA, B-strep, VRE, and E coli were standardized in plating based on McFarland turbidity standardization. At this standard, approximately 150 million CFU/bacteria per ml would be expected. An inoculation loop of 0.01-ml was used to distribute the organisms and they were spread evenly over a culture plate. Expectations of approximately 1.5 million CFU.

There has been an enormous amount of scientific effort dedicated to quantifying the resistance to wound care, and assigned specific metrics to the risk factors. Wounds thrive and progress only above 10<sup>6</sup> CFU per ml of exudate. Another study evaluated log CFU with regard to area of the ulcers. After 28 days of treatment, ulcers that had reduced in size showed a strong inverse relationship with log CFU (r = 0.46, P = 0.008). For each log order of CFU increase, ulcer healing was delayed by 44%. Poor wound healing, indicated by either no significant change or an increase in wound area over the 28 days, was observed in 13% of patients, and all of these had a CFU in the order of at least 10<sup>4</sup> CFU.

The bacterial species present in the wound fluid of the second study were typical of those commonly found in diabetic foot ulcers. *S. aureus* and *Staphylococcus epidermidis* were present in 41 and 47% of the cases, respectively. None of the *S. aureus* isolates were methicillin-resistant *S. aureus* positive.

Given the MRSA of all strains evaluated in this laboratory analysis, and the inhibition shown in log<sup>6</sup> inoculation in this short experiment, even the most resistance of wounds cited in these references would have been 2 orders of magnitude less under our test conditions. In summary; highly virulent strains of bacteria collected in a hospital environment from patient wounds were susceptible to contact inhibition with the Berberex treatment, and demonstrated peripheral antibiotic properties as well.

## References

1. P. G. Bowler, B. I. Duerden, and D. G. Armstrong Wound Microbiology and Associated Approaches to Wound Management *Clin Microbiol Rev.* 2001 April; 14(2): 244–269. doi: 10.1128/CMR.14.2.244-269.2001
2. Ling Xu, Susan V. McLennan, Lisa Lo, Anas Natfaji Bacterial Load Predicts Healing Rate in Neuropathic Diabetic Foot Ulcers. *Diabetes Care* 30: 378-380, 2007