

Application of a Biomodulator for Enhancing the Efficacy of Antibiotics Against Wound Pathogens

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In this study we used a Biomodulator technology* which generates microcurrent, electrical impulses that are transmitted by electrodes in the device through the skin to interface with the body's internal peripheral nervous system for various therapeutic intervention. This technology is FDA approved for pain management. This research was focused on evaluation of the Biomodulator and determine if synergistically enhances efficacy of antibiotics for treating wound infections. We studied the synergistic effect of the Biomodulator and antibiotics by employing various testing parameters. We conducted testing for Untreated Control, Antibiotic Treated Control, Treated with Biomodulator alone, Treated with Biomodulator and 1 µg antibiotic. The antibiotic control and antibiotic treated groups were tested at four time points (0, 1, 2, 4, and 24 hours). Antibiotics were added to the culture on glass cover slip after two treatments with microcurrent on the pad with contact basis applied for 30 minutes, waited for 30 minutes, and applied again for 30 minutes. Untreated control, and non-antibiotic treated groups were treated with phosphate buffered saline (PBS) at 24-hour time point. The glass cover slips were removed from petri dishes and the number of surviving organisms (ex. *P. aeruginosa*, MRSA, *A. baumannii*, and *C. albicans*). were determined by serial dilutions and plating. Results showed that the antibiotic and Biomodulator combination treatment leads to an enhanced synergistic killing effect. There was a significant difference (10 times less) between treated cultures vs. control in Biomodulator treatment with no antibiotic (1 µg/ml of Tobramycin) at 0, 1, 2-hour for 10⁶ cultures. We are currently conducting studies with additional wound pathogens. We have developed a novel therapeutic tool for potentially increasing the efficacy of antibiotics used for treatment of wound infections.

Objective

- Due to the emergence of antibiotic and multi-drug resistant pathogens, there is a growing need for development of non-antibiotic based therapeutics and devices.
- The objective of this study is to evaluate antimicrobial efficacy of the Tennant Biomodulators® and determine if synergistically enhances the efficacy of antibiotics for treating wound infections. This FDA-approved technology generates microcurrents/electrical impulses that are transmitted by electrode in the device through the skin.
- This handheld device is ideal for field application due to its size, portability, and precision.

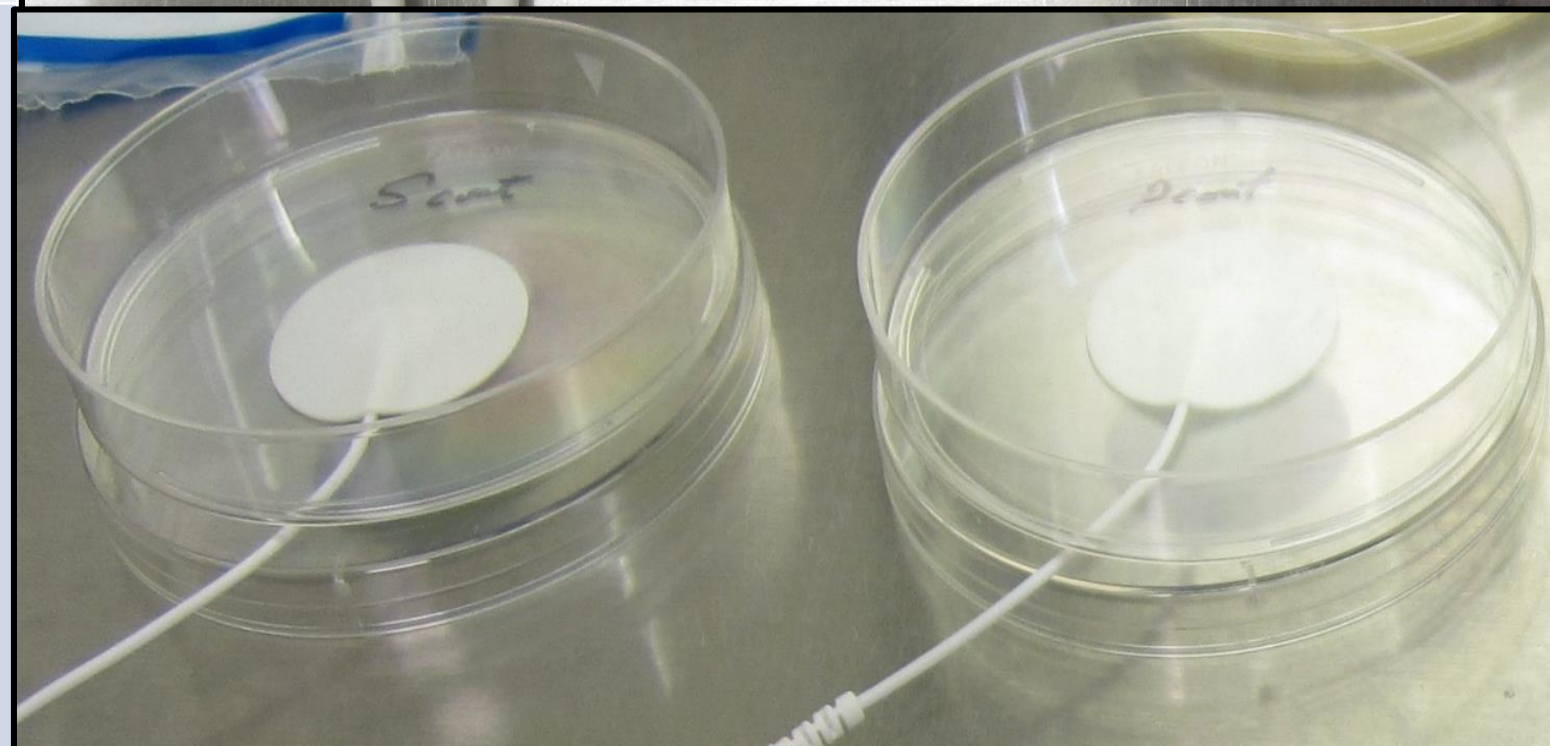
Biomodulator Pad Applied to Bacterial Cultures and Treated with Antibiotics

- Place diluted bacterial culture on a cover glass and let it dry.
- Place pad onto the dried culture and applied microcurrent for 30 minutes, after 30 minutes applied microcurrent again for another 30 minutes.
- Treat with antibiotics (see table for conditions) and incubate for 0, 1, 2, 4, or 24 hours at room temperature.
- Add phosphate buffered saline (PBS) on the cultures for control and treatment without antibiotics and incubate for 24 hours at room temperature.
- Place cover glass into a tube containing PBS.
- Vortex and sonicate tube for 1 minute.
- Make serial dilutions and plate onto tryptic soy agar (TSA) or potato dextrose agar (PDA) plates.
- Incubate at appropriate conditions and count bacterial or fungal colonies.



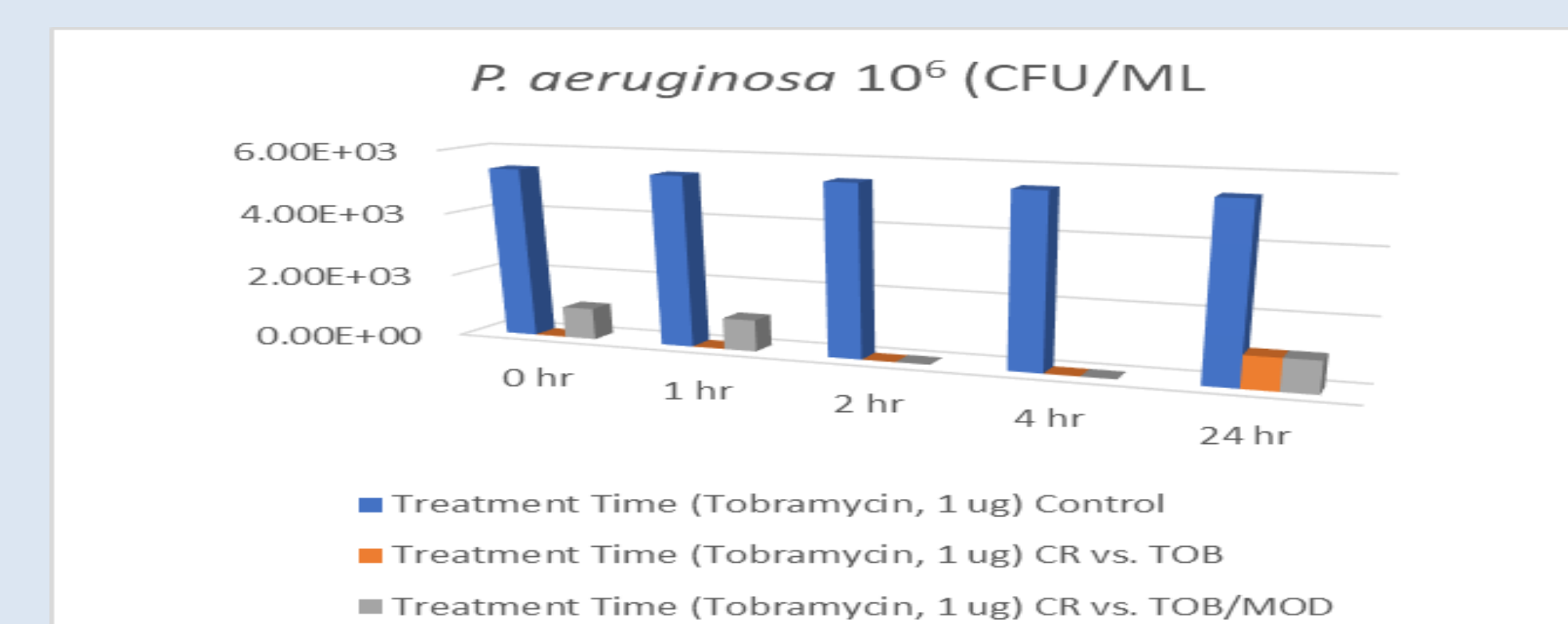
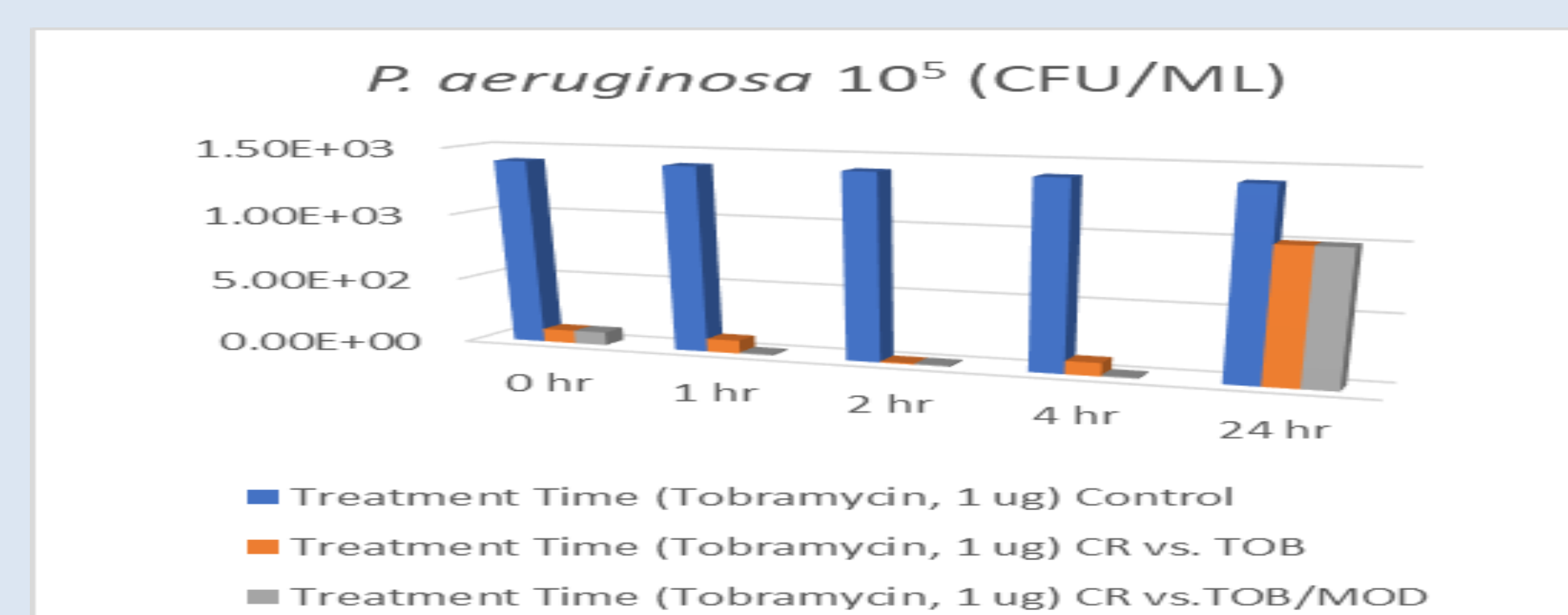
Treatment of Air-Dried Bacterial Cultures

Sample Treatments	Mode ¹	Time	Settings
Control (PBS 24 hr)	-	-	-
Control Antibiotic, 0hr	Antibiotic only	-	n/a
Control Antibiotic, 1hr			
Control Antibiotic, 2hr			
Control Antibiotic, 4hr			
Control Antibiotic, 24hr			
Treatment (PBS + Mod. 24 hr)	Antibiotic + PG2500	30 min (2x)	•Power level: 50 •Frq: 2500 Hz
Treatment (Antibiotic + Mod. 0 hr)			
Treatment (Antibiotic + Mod. 1 hr)			
Treatment (Antibiotic + Mod. 2 hr)			
Treatment (Antibiotic + Mod. 4 hr)			
Treatment (Antibiotic + Mod. 24 hr)			



100 µl of culture (~10⁵ or 10⁶ CFU/ml) treated directly with Tennant Biomodulator® (MOD) two times for 30 minutes and treated with the following antibiotics: Tobramycin (TOB) for Gram negative bacteria, Vancomycin (VAN) for Gram positive bacteria, and Amphotericin B (AmpB) for fungal yeast, and incubated at different timepoints. Control (CR) untreated was also applied.

Efficacy Testing against *Pseudomonas aeruginosa*



Treatment Time (Tobramycin, 1 µg)	Applied CFU	TOB	TOB+MOD	Difference
		4.7 x 10 ²	0	100X
0 hr	1 x 10 ⁶			
1 hr	1 x 10 ⁶	2.9 x 10 ²	0	100X

Efficacy Testing against *Acinetobacter baumannii*

A. baumannii (10⁵ applied)

Treatment Time (Tobramycin, 1 µg)	Changes (CFU)		
	Control	CR vs. TOB	CR vs. TOB/ MOD
0 hr	~1 x 10 ⁵	100X	100X
1 hr	~1 x 10 ⁵	100X	100X
2 hr	~1 x 10 ⁵	100X	100X
4 hr	~1 x 10 ⁵	100X	100X
6 hr	~1 x 10 ⁴	10X	1000X
24 hr	~1 x 10 ⁵	No Effect	No Effect

A. baumannii (10⁶ applied)

Treatment Time (Tobramycin, 1 µg)	Changes (CFU)		
	Control	Cnt vs. TOB	Cnt vs. TOB/ MOD
0 hr	1 x 10 ⁶	100X	100X
1 hr	1 x 10 ⁶	100X	100X
2 hr	1 x 10 ⁶	100X	100X
4 hr	1 x 10 ⁶	10X	100X
6 hr	5.9 x 10 ⁴	10X	100X
24 hr	1 x 10 ⁶	No Effect	No Effect

Efficacy Testing against MRSA

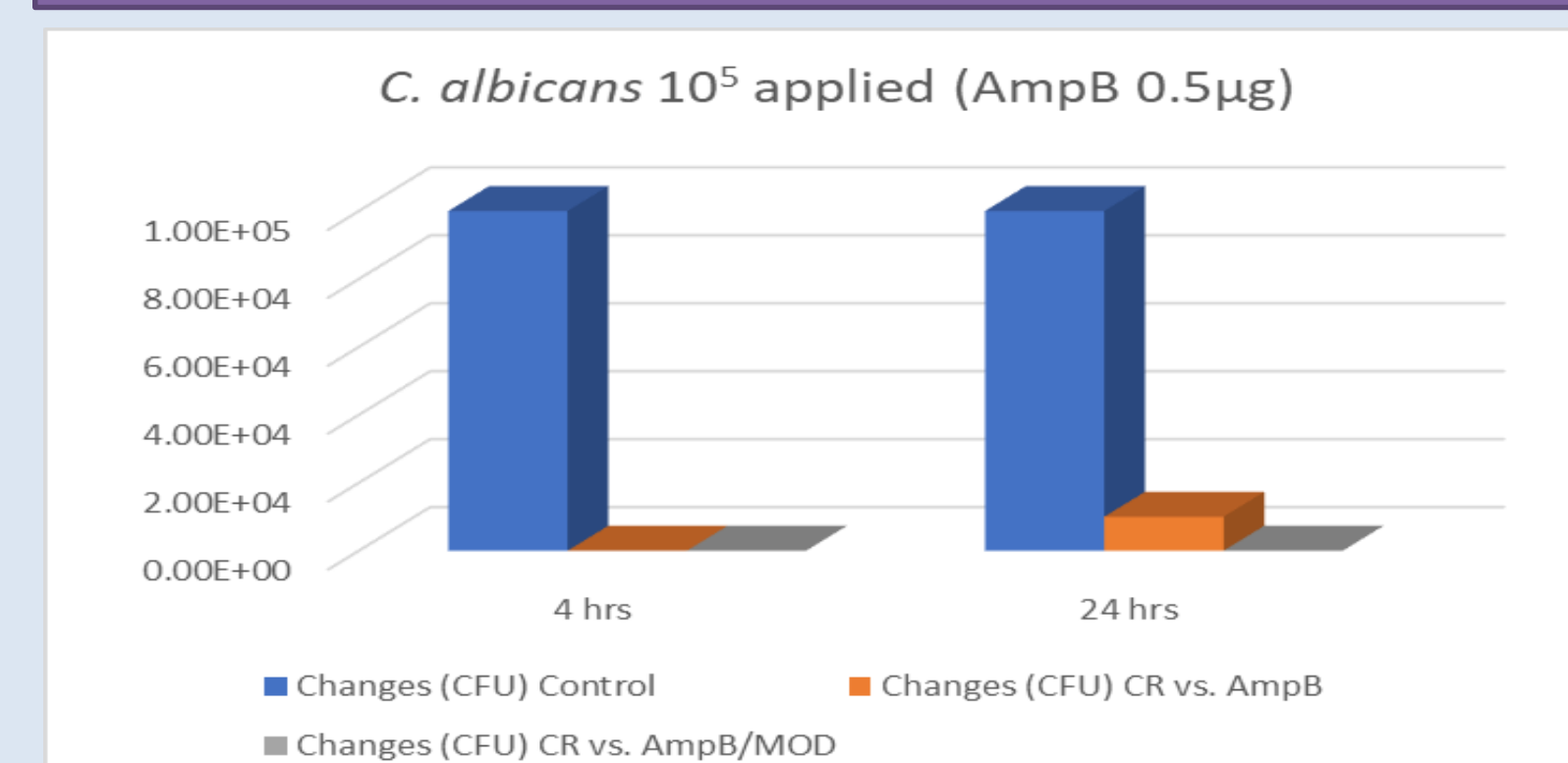
MRSA (10³ & 10⁴ applied)

Treatment Time (Vancomycin)	Changes (CFU)		
	Control	CR vs. VAN	CR vs. VAN/ MOD
24 hr (1 µg)	8.7 x 10 ³	No Effect	100X
	2.4 x 10 ⁴	10X	10X

MRSA (10⁵ applied)

Treatment Time (Vancomycin)	Changes (CFU)		
	Control	CR vs. VAN	CR vs. VAN/ MOD
24 hr (1 µg)	~1 x 10 ⁵	100X	10X
24 hr (2 µg)	~1 x 10 ⁵	10X	100X

Efficacy Testing against *Candida albicans*



C. albicans (10⁵ and 10⁶ applied)

Treatment Time (Amphotericin B)	Changes (CFU)		
	Control	CR vs. AmpB	CR vs. AmpB/ MOD
24 hr (0.25 µg)	~1 x 10 ⁵	1000X	100X
	1.34 x 10 ⁶	1000X	No Growth

Conclusion

- The Tennant Biomodulator® treatment showed antimicrobial efficacy against bacterial and fungal pathogens.
- Treatment of the Biomodulator synergistically enhances the efficacy of antibiotics against wound pathogens.
- Additional studies including antibiofilm efficacy testing are ongoing.

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