



*A Comparison of  
Antibacterial Activity  
Against MRSA  
and Gramnegative  
Organisms for  
Antimicrobial  
Compounds in a  
Unique Composite  
Wound Dressing*

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## Introduction

The treatment of wounds has evolved from the usage of traditional dressings, such as gauze and bandages, to the implementation of more modern dressings that promote a moist environment in order to accelerate the wound healing process and reduce the frequency of and resulting pain from dressing changes.<sup>1-3</sup> TheraGauze™ is a modern dressing comprised of an inert breathable polymer integrated into a non-woven polyester/rayon substrate. It is hydrated with a 50/50 mixture of normal saline and propylene glycol, a non-toxic humectant. TheraGauze™ provides a balance between delivering fluids and oxygen to the wound and extracting exudates from the wound surface, while avoiding maceration.<sup>4</sup>

TheraGauze™ has been successfully implemented in the treatment of diabetic foot ulcers, venous stasis ulcers, vasculitis, skin grafts, burns, and surgical incisions.<sup>4</sup> The risk of infection for these types of wounds has led to the development of several antimicrobial wound dressings, such as those impregnated with dialkylcarbonylchloride, povidone iodine, and silver.<sup>1</sup> The unique hydrodynamic properties of TheraGauze™ suggest that it could provide an optimal platform for the development of an antimicrobial wound dressing that would retain excellent wound healing properties. The topical delivery of antibiotics minimizes the risk of systemic toxicities, reduces the chance of bacterial resistance, and increases the local concentration of the antimicrobial agent achievable in the wound.<sup>1</sup> The current study tests 16 antimicrobial agents incorporated into TheraGauze™ using disk diffusion susceptibility testing against a wide variety of clinical isolates of organisms that commonly cause wound infections and are associated with antibiotic resistance, including: *Acinetobacter* species, *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella* species, *Pseudomonas aeruginosa*, and methicillin-resistant *Staphylococcus aureus* (MRSA). The MRSA tested included community-associated and hospital-associated isolates.

Several previous studies have also used disk diffusion susceptibility testing for specialized wound dressings.<sup>5-6</sup> Martineau et al tested disks of a hydrogel/foam dressing to evaluate the antimicrobial activity of 3% cerium nitrate, 1% chlorhexidine digluconate, 1% chloramphenicol, and 1% chloroxylenol against *Pseudomonas aeruginosa*.<sup>6</sup> Gallant-Behm et al assayed disks of a number of commercially available wound dressings against 17 microorganisms, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*.<sup>5</sup>

The current study identified several antimicrobial agents that, when added to TheraGauze™, exhibited excellent broad-spectrum growth inhibition, including against MRSA. A review of the tissue toxicity and tissue absorption of the various antimicrobial agents, based on prior studies, is also provided.

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## Materials and Methods

### Bacterial Isolates and Preparation of Inoculum

Clinical strains were obtained as discarded de-identified isolates from the Clinical Microbiology Laboratory of The Children's Hospital of The King's Daughters (Eastern Virginia Medical School IRB protocol #06-04-WC-0040). Thirty-five isolates were tested and comprised of: 5 *Acinetobacter* species, 5 *Klebsiella* species, 10 *P. aeruginosa*, 10 community-associated MRSA, and 5 hospital-associated MRSA. In addition, 1 isolate each of *E. cloacae* and *E. coli* was tested in an experiment with Silver-impregnated TheraGauze™. Community-associated MRSA isolates were defined as MRSA susceptible to clindamycin, while hospital-associated MRSA were defined as resistant to clindamycin. All isolates were recovered from sites of active infection. Bacteria were grown on Mueller-Hinton II agar plates overnight at 37°C. Colonies were suspended in Mueller-Hinton II broth and adjusted to match the 0.5 McFarland turbidity standard of approximately 1.2 x 10<sup>8</sup> CFU/mL prior to plating.

### Antimicrobial Agents and Preparation of Disks

Sixteen antimicrobial agents were assayed and are listed in Table 1. Propylene glycol, a component of TheraGauze™, was used as a control. Liquid antimicrobial agents were diluted with saline to their respective concentrations (% v/v) and solid antimicrobial agents were solubilized in saline to their respective concentrations (total mg or U/disk) according to previous literature (TABLE 1). TheraGauze™ disks were 6 mm in diameter, the same as standard Kirby-Bauer disks. The disks were loaded with 5 µL of propylene glycol and 6 µL of each antimicrobial solution. The disks were fully saturated within 5 minutes.

### Silver-impregnated TheraGauze™

Silver-impregnated TheraGauze™ (Ag-TheraGauze) disks were prepared by Polymer Solutions, Incorporated. Disks were dry when applied to the agar plate.

### Kirby-Bauer Disk Diffusion Method

Bacterial isolates were inoculated as a lawn onto 150 x 15 mm Mueller-Hinton II agar plates using sterile cotton swabs. This was repeated three times per plate, rotating 60° between streaking to assure confluence. Disks were applied onto the surface of the inoculated agar plates using sterile forceps with a minimum space of 24 mm from center to center between each disk. Plates were inverted and incubated overnight at 37°C. Zones of inhibition were measured manually and recorded to the nearest whole millimeter. Our pilot studies showed that measured zones of inhibition resulted in day-to-day variability within 1 mm for a given isolate tested with the same antimicrobial agent (data not shown). Because of this high degree of reproducibility, single values were measured for each isolate within a species from which means were calculated for the species.

**Table 1 — Information on Antimicrobial Agents**

	<b>Manufacturer</b>	<b>Source for Concentration Data</b>	<b>Source for Tissue Toxicity Data</b>	<b>Source for Tissue Absorption Data</b>
Propylene Glycol	Post Apple Scientific	provided	14	14
Hydrogen Peroxide	Thermo Fisher Scientific, Inc.	10	10, 12, 13	12
Povidone Iodine	First Priority, Inc.	12	9, 10, 12, 14	9, 10, 12
Bacitracin	USB Corporation	Commercial Kirby-Bauer Disk	11, 12, 13	14
Clindamycin	MP Biomedicals	Commercial Kirby-Bauer Disk	14	19
Polymyxin B	MP Biomedicals	Commercial Kirby-Bauer Disk	11, 12, 13, 14	11, 12, 13
Tobramycin	MP Biomedicals	Commercial Kirby-Bauer Disk	18	20
Vancomycin	MP Biomedicals	Commercial Kirby-Bauer Disk	15, 16	15, 16
Chlorhexidine Digluconate	Sigma-Aldrich, Inc.	9, 14	9, 11, 12	9, 11, 12
Chlorhexiine Gluconate	Spectrum Chemical Mfg. Corp.	10, 11	11, 12	11, 12
Mupirocin	Applichem, Inc.	Commercial Kirby-Bauer Disk	13	13
Trimethoprim	MP Biomedicals	Commercial Kirby-Bauer Disk	22	23
Doxycycline	MP Biomedicals	Commercial Kirby-Bauer Disk	14, 18	24
Levofloxacin	Sigma-Aldrich, Inc.	as sold in IQUIX	14, 18	21
Linezolid	ChemPacific Corp.	Commercial Kirby-Bauer Disk	17	17
Ag-TheraGauze	Soluble Systems/Polymer Solutions, Inc.	provided	25	25
SilvaSorb Gel	Medicine Industries, Inc.	as sold	25	25

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## Antimicrobial Activity Index

Mean zones of inhibition are listed in Table 2 and were categorized as follows:  $\leq 6.0$  mm (0), 6.1-9.9 mm (+), 10.0-19.9 mm (++), 20.0-29.9 mm (+++), or 30.0 mm or more (++++).

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## Evaluation of Tissue Toxicity and Tissue Absorption

Tissue toxicity and tissue absorption values for each antimicrobial agent were obtained from previously published literature and are referenced for each agent (TABLE 1). Tissue toxicity and tissue absorption values for each antimicrobial agent were indexed on a relative scale: low (+), moderately low (++), moderately high (+++), or high (++++).

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## Statistical Analysis

Means and standard deviations were calculated for each species of microorganisms using Excel (Microsoft). Significance testing was performed by Student's T-test and  $P < 0.05$  was considered significant.<sup>7</sup> A power analysis suggests that 10 organisms in each group are necessary for an alpha error of 0.05, a power of 0.8, and an expected difference of the means of 1 standard deviation.<sup>8</sup>

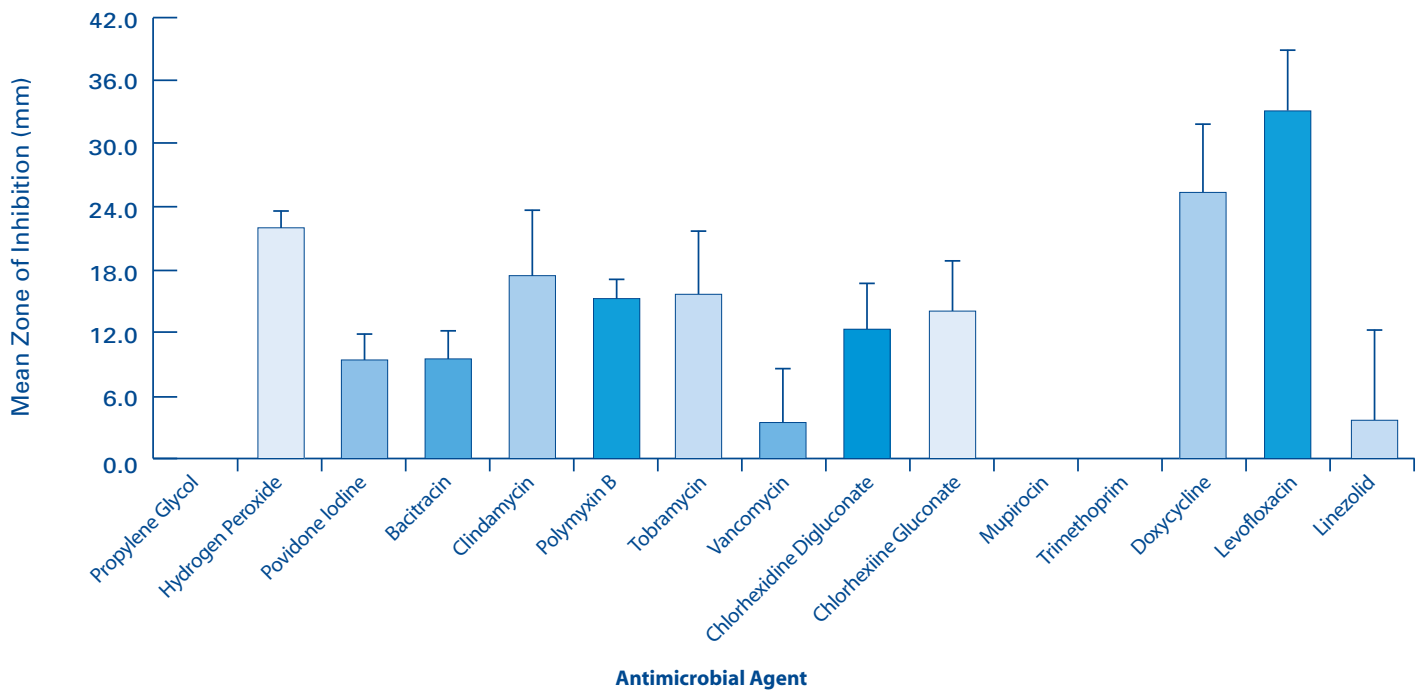
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## Results

### Gram-Negative Rods

Testing of antimicrobial-containing TheraGauze™ for 5 clinical *Acinetobacter* species isolates showed mean zones of inhibition greater than 10.0 mm for: hydrogen peroxide, clindamycin, polymyxin B, tobramycin, chlorhexidine digluconate, chlorhexidine gluconate, doxycycline, and levofloxacin (FIGURE 1).

**Figure 1: *Acinetobacter* spp. (n=5)**



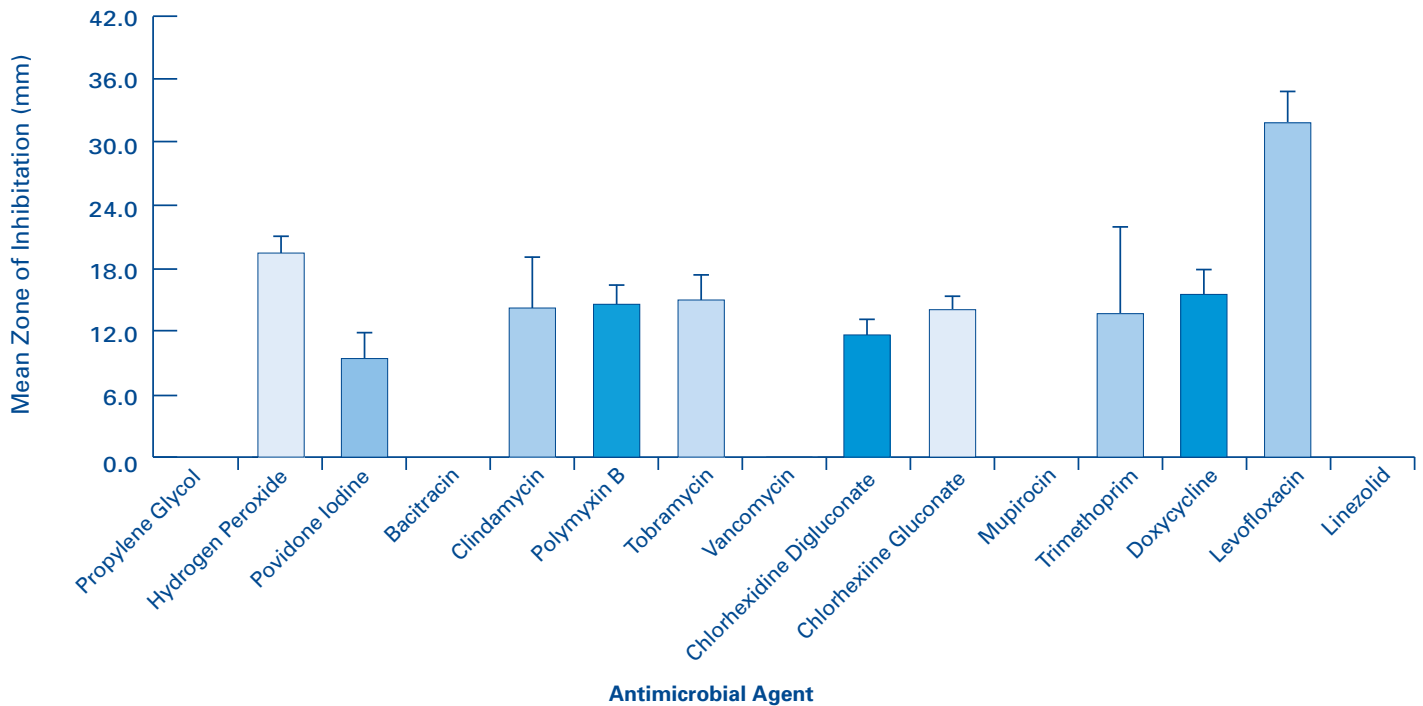
Testing of 5 clinical *Klebsiella* species isolates showed mean zones of inhibition greater than 10.0 mm for: hydrogen peroxide, polymyxin B, tobramycin, chlorhexidine digluconate, chlorhexidine gluconate, trimethoprim, doxycycline, and levofloxacin (FIGURE 2).

Testing of 10 clinical *P. aeruginosa* isolates showed mean zones of inhibition greater than 10.0 mm for: hydrogen peroxide, polymyxin B, tobramycin,

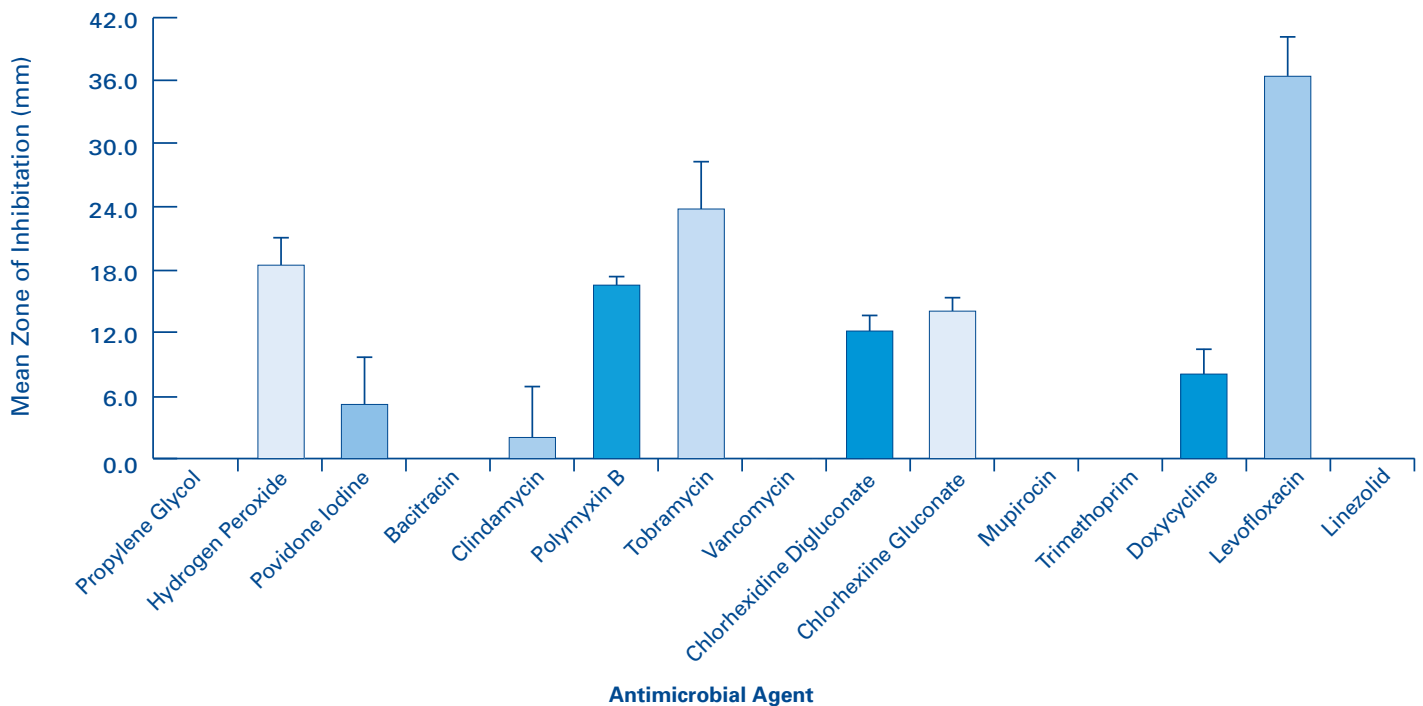
chlorhexidine digluconate, chlorhexidine gluconate, and levofloxacin (FIGURE 3).

Several antimicrobial agents produced mean zones of inhibition greater than 10.0 mm for all three groups of Gram-negative rods tested. These included: hydrogen peroxide, polymyxin B, tobramycin, chlorhexidine digluconate, chlorhexidine gluconate, and levofloxacin. Doxycycline produced mean zones of inhibition greater

**Figure 2: *Klebsiella* spp (n=5)**



**Figure 3: *Pseudomonas aeruginosa* (n=10)**



than 10.0 mm for *Acinetobacter* species and *Klebsiella* species isolates, but not for *P. aeruginosa* isolates. Povidone iodine, bacitracin, vancomycin, mupirocin, and linezolid did not show significant antimicrobial activity against any of the three groups of Gram-negative rods.

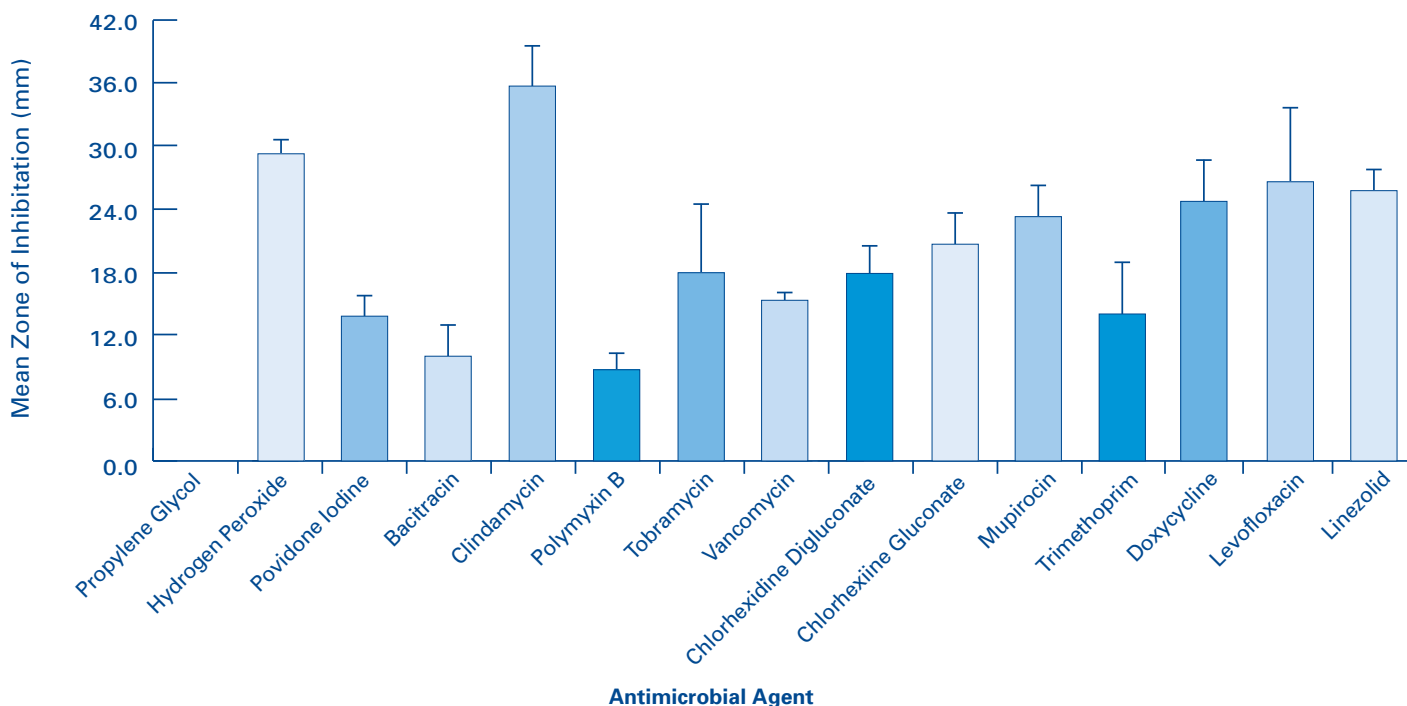
vancomycin, chlorhexidine digluconate, chlorhexidine gluconate, mupirocin, trimethoprim, doxycycline, levofloxacin, and linezolid (FIGURE 4).

Testing of 5 hospital-associated MRSA isolates showed mean zones of inhibition greater than 10.0 mm for: hydrogen peroxide, povidone iodine, bacitracin, clindamycin, tobramycin, vancomycin, chlorhexidine digluconate, chlorhexidine gluconate, mupirocin, trimethoprim, doxycycline, levofloxacin, and linezolid (FIGURE 5).

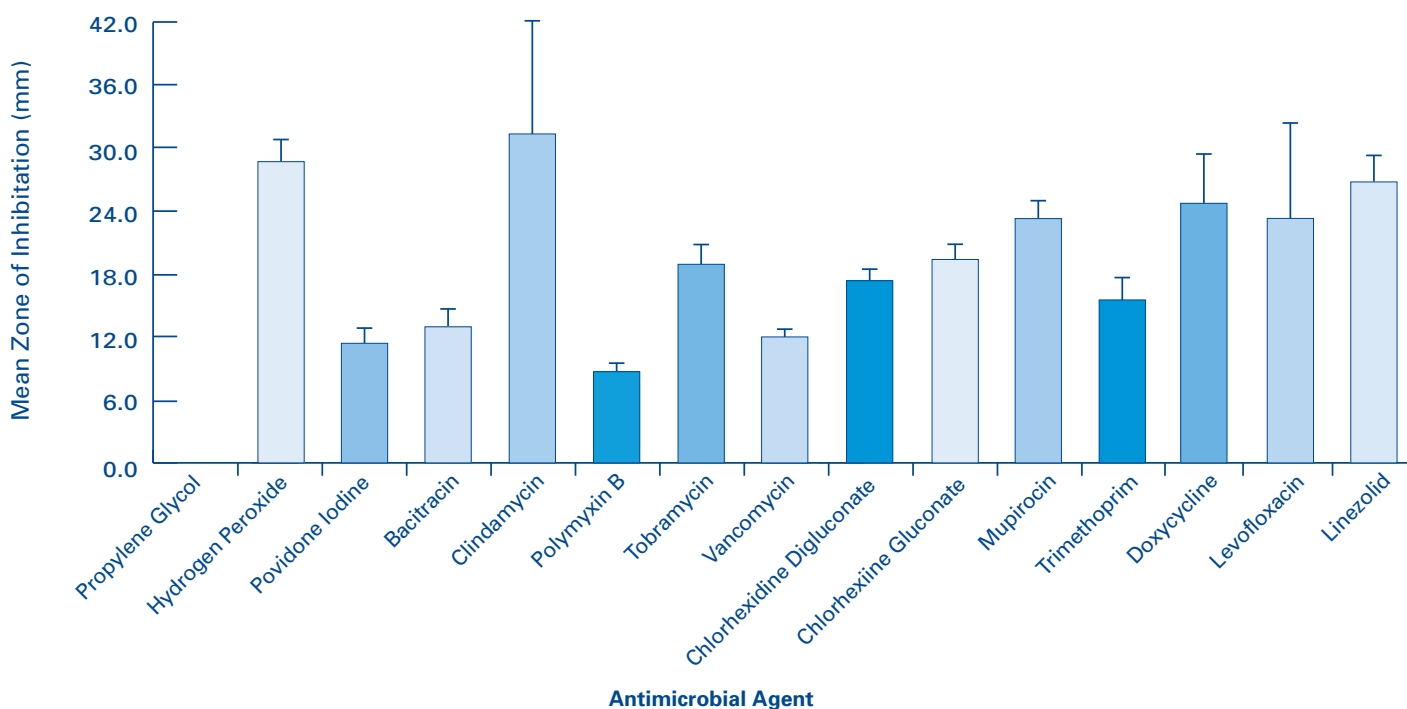
## MRSA

Testing of antimicrobial-containing TheraGauze™ for 10 community-associated MRSA isolates showed mean zones of inhibition greater than 10.0 mm for: hydrogen peroxide, povidone iodine, clindamycin, tobramycin,

**Figure 4: Community-associated MRSA (n=10)**



**Figure 5: Hospital-associated MRSA (n=5)**



The majority of antimicrobial agents tested produced mean zones of inhibition greater than 10.0 mm for both groups of MRSA (FIGURES 4-5). Clindamycin, levofloxacin, doxycycline, linezolid, and hydrogen peroxide produced the largest zones of inhibition for both community-associated and hospital-associated MRSA. Bacitracin produced a mean zone of inhibition greater than 10.0 mm for hospital-associated MRSA only. Polymyxin B did not show significant antimicrobial activity against either group of MRSA.

Prior studies have shown that agents with minimal tissue toxicity (0-1+) include: bacitracin, clindamycin, polymyxin B, tobramycin, chlorhexidine digluconate, mupirocin, linezolid, and silver. Prior studies have shown that agents with minimal tissue absorption (0-1+) include: hydrogen peroxide, povidone iodine, bacitracin, clindamycin, polymyxin B, tobramycin, vancomycin, chlorhexidine digluconate, chlorhexidine gluconate, mupirocin, trimethoprim, linezolid, and silver. The results from these experiments and previous studies suggest that tobramycin and chlorhexidine digluconate in TheraGauze™ are broadly antimicrobial and are predicted to have minimal tissue toxicity and tissue absorption.

### Characteristics of Antimicrobial Agents

Antimicrobial agents showing broad spectrum activity against the Gram-negative rods and MRSA include: hydrogen peroxide, tobramycin, chlorhexidine digluconate, chlorhexidine gluconate, and levofloxacin

(TABLE 2).

**Table 2: Characteristics of Antimicrobial Agents**

	Concentration	Activity <sup>a</sup>					Tissue Toxicity <sup>b</sup>	Tissue Absorption <sup>b</sup>
		<i>Acinetobacter spp.</i>	<i>Klebsiella spp.</i>	<i>P. aeruginosa</i>	Community-associated MRSA	Hospital-associated MRSA		
Propylene Glycol	control	0	0	0	0	0	0	0
Hydrogen Peroxide	0.03 mL/mL	+++	++	++	+++	+++	+++	0
Povidone Iodine	0.10 mL/mL	+	+	0	++	++	++++	0
Bacitracin	9.09 x10 <sup>2</sup> U/mL	+	0	0	+	++	0	0
Clindamycin	10.0 mg/mL	++	++	0	+++	+++	0	+
Polymyxin B	2.73 x 10 <sup>4</sup> U/mL	++	++	++	+	+	0	0
Tobramycin	0.91 mg/mL	++	++	+++	++	++	0	0
Vancomycin	0.45 mg/mL	0	0	0	++	++	++	+
Chlorhexidine Digluconate	0.02 mL/mL	++	++	++	++	++	+	+
Chlorhexidine Gluconate	0.04 mL/mL	++	++	++	+++	++	++	+
Mupirocin	0.45 mg/mL	0	0	0	+++	+++	+	0
Trimethoprim	0.45 mg/mL	0	++	0	++	++	++	+
Doxycycline	2.72 mg/mL	+++	++	+	+++	+++	+++	++
Levofloxacin	15.0 mg/mL	++++	++++	++++	+++	+++	+++	++
Linezolid	2.72 mg/mL	0	0	0	+++	+++	0	0
Ag-TheraGauze	0.83 mg/mL	ND	++	++	+	+	+	+
SilvaSorb Gel	as sold	ND	+	+	+	+	+	+

a. +: 6.1-9.9 mm, ++: 10.0-19.9 mm. +++: 20.0-29.9 mm. ++++: 30.0+ mm ND: Not Done

b. +: low, ++: moderately low, +++: moderately high, ++++: high

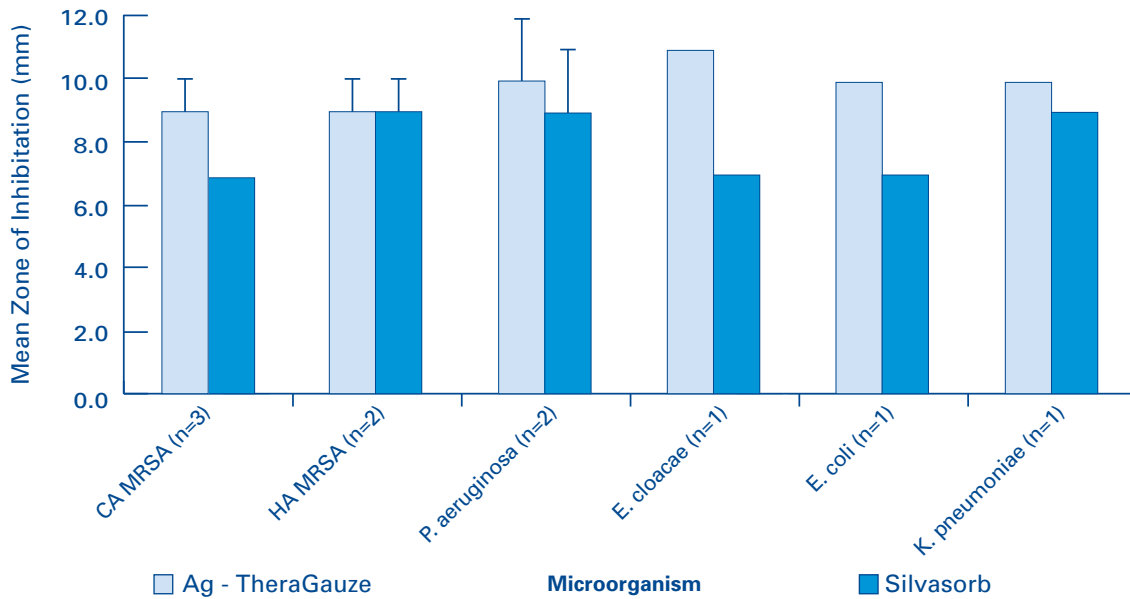
## Silver-impregnated TheraGauze™ vs. Silvasorb

Silver-impregnated TheraGauze™ (Ag-TheraGauze) and Silvasorb were tested for 3 isolates of community-associated MRSA, 2 isolates of hospital-associated MRSA, 2 isolates of *P. aeruginosa*, and 1 isolate each of *E. cloacae*, *E. coli*, and *K. pneumoniae* (FIGURE 6).

Ag-TheraGauze produced a larger zone of inhibition than Silvasorb for community-associated MRSA ( $P = 0.03$ ). Both agents showed similar zones of inhibition for hospital-

associated MRSA and *P. aeruginosa*. Ag-TheraGauze produced a larger zone of inhibition than Silvasorb for the enteric Gram-negative rods ( $P = 0.04$ ), combining the results for *E. cloacae*, *E. coli*, and *K. pneumoniae*.

**Figure 6: Ag-TheraGauze vs. Silvasorb**



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## Discussion

The current study tested 16 antimicrobial agents in a modern wound dressing with unique hydrodynamic properties, TheraGauze™, in order to evaluate for topical antimicrobial activity against a broad range of bacterial species that commonly cause wound infections, including MRSA. Thirty-seven clinical isolates were tested across 6 bacterial species that are associated with antibiotic resistance including *P. aeruginosa*, *Acinetobacter* species, and hospital- and community-associated MRSA. Five of the 16 antimicrobial agents in TheraGauze™ showed broad spectrum activity against all MRSA and Gram-negative rod isolates: hydrogen peroxide, tobramycin, chlorhexidine digluconate, chlorhexidine gluconate, and levofloxacin. Additionally, a silver-impregnated form of TheraGauze™ was found to have antimicrobial activity against hospital-associated MRSA, community-associated MRSA, *P. aeruginosa*, *E. cloacae*, *E. coli*, and *K. pneumoniae* isolates.

A review of prior studies suggests that, of the 6 agents found to possess broad-spectrum antimicrobial activity in TheraGauze™, three exhibited minimal tissue toxicity and absorption. Tobramycin, chlorhexidine digluconate, and silver in TheraGauze™ appear to have optimal antimicrobial properties *in vitro* and are likely to have excellent safety *in vivo*.

In the current era of increasing antibiotic resistance when systemic antibiotic treatment of wound infections is increasingly likely to fail, the ability to deliver high concentrations of antimicrobial agents locally to prevent wound infections is highly desirable.<sup>1</sup> Additionally, topical application greatly reduces the risk of antibiotic-associated systemic toxicities. Thus, a wound dressing with antimicrobial properties that decreases the risk of infection may have significant clinical benefit particularly in settings (e.g. contaminated traumatic wounds) and disease processes (e.g. diabetes mellitus) where there is a high likelihood of wound infection developing.

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