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IN VITRO CHECKERBOARD ANALYSIS OF COMBINED TAUROLIDINE AND CHLORHEXIDINE ACETATE AGAINST A PANEL OF GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA

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BACKGROUND

- Among patients requiring a central venous catheter (CVC) for hemodialysis (HD), the incidence of catheter-related bloodstream infections (CRBSI) is 1.1 to 5.5 episodes per 1000 catheter-days, and up to half of patients experience a CRBSI within 6 months of catheter placement.[1]
- Taurolidine is the antimicrobial component of DefenCath® (13,500 mg/L of taurolidine and 1,000 Units/mL of heparin) which in its pivotal Phase 3 trial demonstrated a 71% reduction of CRBSI risk compared to heparin alone. DefenCath® is the first FDA approved catheter lock solution indicated to reduce the risk of CRBSI in adult patients with kidney failure receiving chronic hemodialysis through a CVC.[2]
- Taurolidine is a broad-spectrum antimicrobial with a non-specific mechanism of action, exerting its effect through damage to microbial cell walls, preventing adherence of microorganisms to biological surfaces, and inhibiting select virulence factors (e.g., flagella and fimbriae).[3]
- Taurolidine/heparin catheter lock solution (DefenCath®) and a chlorhexidine acetate eluting catheter cap device (ClearGuard HD Antimicrobial Barrier Caps) are both available in the US to reduce CRBSIs in patients receiving HD through a CVC.
- Taurolidine and chlorhexidine both have activity against a wide variety of microbes including Gram-positive and Gram-negative pathogens.[3-4]
- Prior studies have shown that chlorhexidine may be synergistic with traditional antibiotics, but traditional antibiotic lock therapy is often avoided out of concerns for resistance and/or toxicity.[5] Taurolidine is not an antibiotic and may avoid these drawbacks.
- While the product information for the chlorhexidine cap device lists only saline, heparin, or sodium citrate as compatible catheter lock solutions,[6] there is a theoretical basis for which taurolidine/heparin locks could be utilized in clinical practice. Checkerboard synergy studies were undertaken given that no data exists about the combination of taurolidine and chlorhexidine.

METHODS

- Checkerboard synergy assays were completed using a range of taurolidine (max 2,048 mg/L) and chlorhexidine acetate (max 128 mg/L) concentrations for 46 bacterial isolates using methodology adapted from the American Society of Microbiology Clinical Microbiological Procedures Handbook.
- Bacteria included *Staphylococcus aureus* (n=11), Coagulase-negative *Staphylococcus* species (n=5), *Enterococcus faecalis* (n=5), *E. faecium* (n=5), *Escherichia coli* (n=5), *Klebsiella pneumoniae* (n=5), *Pseudomonas aeruginosa* (n=5), and *Acinetobacter baumannii* (n=5), with a range of phenotypic antibiotic resistance
- Synergy in checkerboard experiments were determined by the fractional inhibitory concentration index (FICI). The fractional inhibitory concentration (FIC) for each isolate was calculated for each agent using the following equations:

$$FIC\ of\ taurolidine\ (TLD) = \frac{\text{MIC of taurolidine in combination}}{\text{MIC of taurolidine alone}}$$

$$FIC\ of\ chlorhexidine\ acetate\ (CHA) = \frac{\text{MIC of CHA in combination}}{\text{MIC of CHA alone}}$$

$$\boldsymbol{FICI = \Sigma FIC = FIC_{TLD} + FIC_{CHA}}$$

- The sum of the FICs for each isolate (ΣFIC = FIC of taurolidine + FIC chlorhexidine acetate) is the FICI. The minimum, maximum, and mean FICI values were calculated for each isolate. FICI was interpreted, based on the minimum value, as follows:
  - Synergy, FICI ≤ 0.5
  - Additivity, 0.5 < FICI ≤ 1
  - Indifference, 1 < FICI ≤ 4
  - Antagonism, FICI > 4

RESULTS

- Gram-positive activity
  - Taurolidine had $MIC_{50/90}$ of 512/1024 µg/mL (range 256 – 2048 µg/mL)
  - Chlorhexidine acetate had an $MIC_{50/90}$ of 1/2 µg/mL (range 0.25 – 4 µg/mL)
- Gram-negative activity
  - Taurolidine had $MIC_{50/90}$ of 512/1024 µg/mL (range 256 – 1024 µg/mL)
  - Chlorhexidine acetate had an $MIC_{50/90}$ of 4/16 µg/mL (range 0.25 – 16 µg/mL)

Table 1. Summary of Results by Bacterial Isolate

Organism	Identifier	Type[a]	MIC (µg/mL)		FICI		
			TLD	CHA	Min	Max	Mean
Staphylococcus aureus	ATCC 29213	Control; MSSA	512	0.25	**0.74**	1.25	1.04
	AR0568[b]	MSSA	512	0.5	**0.75**	1.25	1.05
	AR0569	MSSA	512	0.25	**0.75**	1.13	**0.92**
	AR0565	MRSA	512	0.25	**1**	1.25	1.14
	AR0566	MRSA	1024	0.5	**0.63**	1.12	**0.86**
	AR0561	MRSA (mecA), mupirocin resistant (mupA)	512	1	**0.56**	1.25	**0.91**
	AR0562	MRSA (mecA), mupirocin intermediate (mupA)	512	1	**1**	1.25	1.13
	AR0563	MRSA (mecA), mupirocin resistant (mupA)	256	0.5	**1**	1.25	1.15
	AR0564	MRSA (mecA), mupirocin resistant (mupA)	512	1	**1**	1.25	1.13
	AR0567	MRSA (mecA), mupirocin resistant (mupA)	512	1	**1**	1.25	1.13
	AR0570	MRSA (mecA), mupirocin resistant (mupA)	512	0.5	**1**	1.25	1.14
Staphylococcus epidermidis	AR0726	Methicillin resistant (mecA)	512	0.5	**1**	1.25	1.14
	AR0727	Methicillin (mecA), mupirocin resistant	2048	0.5	1.02	2.25	1.32
	AR0728	Methicillin resistant (mecA)	512	0.5	1.02	2.25	1.54
Staphylococcus haemolyticus	AR0729	Methicillin (mecA), mupirocin resistant	512	0.5	1.06	1.5	1.28
	AR0698	Methicillin resistant (mecA)	512	0.5	1.06	1.5	1.26
Enterococcus faecalis	ATCC 29212	Control; Vancomycin susceptible	512	2	1.03	1.5	1.21
	AR0577	Vancomycin susceptible	512	2	**1**	1.25	1.11
	AR0580	Vancomycin susceptible	512	2	1.03	1.5	1.21
	AMC-EFL27[c]	Vancomycin resistant	1024	4	**1**	1.25	1.1
	AMC-EFL42	Vancomycin resistant	512	2	1.03	1.5	1.21
Enterococcus faecium	AR0574	Vancomycin susceptible	512	2	**1**	1.25	1.11
	AR0578	Vancomycin susceptible	1024	2	**1**	1.25	1.1
	AR0579	Vancomycin susceptible	512	1	1.06	1.5	1.23
	AR0572	Vancomycin resistant (VanA)	1024	1	**0.75**	1.25	1.05
	AR0575	Vancomycin resistant (VanA)	512	1	1.06	1.5	1.23
Escherichia coli	ATCC 25922	Control; Pan susceptible	512	0.25	1.02	1.5	1.28
	AR0085	ampC (CMY-2)	512	0.5	1.06	1.5	1.28
	AR0086	Extended Spectrum β-lactamase (CTX-M-14)	512	1	**0.63**	1.25	**0.97**
	AR0061	Serine-carbapenemase (KPC-3)	512	1	**0.75**	2.25	1.54
	AR0055	Metallo-β-lactamase (NDM-1)	512	2	**0.75**	1.13	**0.98**
Klebsiella pneumoniae	AR0016	Narrow spectrum resistance (LEN-27)	512	16	**0.75**	1.25	**0.94**
	AR0010	ampC (CMY-94)	512	8	**0.75**	1.25	**0.92**
	AR0012	Extended Spectrum β-lactamase (SHV-12)	1024	8	**0.63**	1.13	1.05
	AR0004	Serine-carbapenemase (KPC-3)	1024	4	**0.75**	1.25	1.05
	AR0005	Serine-carbapenemase (KPC-2)	512	4	**0.75**	1.25	1.06
Pseudomonas aeruginosa	ATCC 27853	Control (ampC)	512	4	**0.63**	1.13	**0.92**
	AR0095	Serine-carbapenemase (OXA-50)	1024	4	**1**	1.25	1.11
	AR0064	Metallo-β-lactamase (SPM-1)	1024	8	**0.63**	1.25	**0.96**
	AR0103	Metallo-β-lactamase (IMP-1)	512	8	**0.63**	1.25	**0.99**
	AR0111	Metallo-β-lactamase (VIM-2)	1024	8	**0.75**	1.25	1.05
Acinetobacter baumannii	NCTC 13304	Control Serine-carbapenemase (OXA-23-like, OXA-51-like)	256	4	1.02	1.5	1.14
	AR0070	Serine-carbapenemase (OXA-51-like, OXA-58)	1024	4	**0.56**	2.13	1.3
	AR0102	Serine-carbapenemase (OXA-51-like) Chlorhexidine efflux (qacEdelta1)	256	16	1.02	1.5	1.21
	AR0309	Serine-carbapenemase (OXA-23, OXA-51-like)	512	16	**0.63**	1.13	**0.93**
	AR0083	Metallo-β-lactamase (NDM-1), Chlorhexidine efflux (AMVA, qacEdelta1)	256	8	1.03	1.5	1.23

[a]Only the most relevant mechanism(s) for each description is shown. Most isolates have many resistance genes documented
[b]AR Isolates are from the CDC AR Bank with the listed isolate number
[c]AMC isolates are clinical bloodstream infection isolates from Albany Medical Center
TLD, taurolidine; CHA, chlorhexidine acetate; MIC, minimum inhibitory concentration; FICI, fractional inhibitory concentration index; MSSA, methicillin-susceptible *S. aureus*; MRSA, methicillin-resistant *S. aureus*; **Underlines** represent additivity by $FICI_{min} \leq 1$

- Additive activity by checkerboard was seen in 69.6% (32/46) of all isolates, with no overt synergy or antagonism.
- Additive activity with the combination of taurolidine with chlorhexidine acetate was seen at least once in each species **(Table 2)**, with *S. aureus* 100% (11/11), Coagulase-negative Staphylococcal species 20% (1/5), *E. faecalis* 40% (2/5), *E. faecium* 60% (3/5), *E. coli* 60% (3/5), *K. pneumoniae* 100% (5/5), *P. aeruginosa* 100% (5/5), and *A. baumannii* 40% (2/5).
- Genotypic and phenotypic resistance to traditional antibiotics did not appear to impact the activity of either taurolidine or chlorhexidine acetate alone or in combination

Table 2. Summary of FICI by species/group

Organism group	No strains	FICI			Additivity by $FICI_{min} \leq 1$
		Min	Max	Mean	
Staphylococcus aureus	11	0.86	1.23	1.05	100%
Coagulase-negative *Staphylococci*	5	1.03	1.75	1.31	20%
Enterococcus faecalis	5	1.02	1.4	1.17	40%
Enterococcus faecium	5	0.97	1.35	1.15	60%
Escherichia coli	5	0.84	1.53	1.21	60%
Klebsiella pneumoniae	5	0.73	1.23	1.00	100%
Pseudomonas aeruginosa	5	0.73	1.23	1.00	100%
Acinetobacter baumannii	5	0.85	1.55	1.16	40%

SUMMARY

- No overt synergy or antagonism was observed in this panel of 46 Gram-positive and Gram-negative bacterial isolates.
- In sum, additivity was seen in 69.6% of strains with additive activity being exhibited in all isolates of *S. aureus, K. pneumoniae*, and *P. aeruginosa*.
- Based on these findings, the combination of taurolidine and chlorhexidine acetate either modestly improved or did not impact the microbiological activity of the individual agents.
- Future studies are warranted to characterize potential clinical implications of these *in vitro* findings.

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DISCLOSURES

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