In vitro and animal studies of antibiotic synergy for newer antistaphylococcal agents for _Staphylococcus aureus_

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Invasive *Staphylococcus aureus* infections are often refractory to antibiotic monotherapy (especially vancomycin). So, combination therapy is often employed. Moreover, many experienced clinicians use combination therapy from outset in severely-ill patients for invasive *S. aureus* infection.

We performed a literature review of the newer antistaphylococcal antibiotics in which synergy was assessed in vitro or in animal studies (Table). Methicillin-sensitive *Staphylococcus aureus* (MSSA), methicillin-resistant *Staphylococcus aureus* (MRSA) and heterogeneous glycopeptide-intermediate *Staphylococcus aureus* (hGISA) were studied. For *in vitro* studies, time-kill methods, checkerboard methods and E-tests were used. The definition of synergy was the fractional inhibitory concentration index (FICI) ≤ 0.5 in checkerboard method, and reduction >2 $\log_{10}$ CFU/ml in time-kill studies.

Three different combinations of antimicrobials showed synergistic interaction against *Staphylococcus aureus* by two different investigative groups:

- Daptomycin plus gentamicin (4,21)
- Quinupristin/dalfopristin plus vancomycin (12, 22)
- Quinupristin/dalfopristin plus rifampin (7, 20)

In one study, the triple combination of daptomycin, gentamicin and rifampin was superior to monotherapy ($p<0.01$) for MRSA in a cell model as well as agar dilution testing (2).

Several regimen employing linezolid showed evidence of synergy in vitro: Linezolid plus fosfomycin (18), linezolid plus ertapenem (8), and linezolid plus imipenem (10) also showed synergistic interaction *in vitro* against *S. aureus*. Linezolid plus rifampin (9), and levofloxacin plus rifampin (13) showed synergistic interaction; however, in other studies, these same combinations were found to be indifferent (5).

Rifampin is an antibiotic that may play a special role for *S. aureus* infections. It has superb intracellular penetration and is also highly active against *S. aureus*. Rifampin was synergistic *in vitro* with quinupristin as mentioned above with linezolid in one study (9), with levofloxacin in one study (13) and as part of a triple combination of daptomycin plus gentamicin (2).

Tigecycline is unique among MRSA antibiotics in that it possesses activity against the enteric gram-negative bacilli and anaerobes. Synergy was found for tigecycline plus gentamicin, and indifference for tigecycline plus rifampin and tigecycline plus vancomycin.

Whether synergistic interactions *in vitro* or in animal models translate into clinical success remains to be determined. Nevertheless, these *in vitro* results may be of interest for clinicians treating selected patients who have failed standard antibiotic therapy.

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Table: *In vitro*/animal Synergy Studies for *Staphylococcus aureus*

Highlighted: synergistic interaction

Reference Number	Combinations	Pathogen	Test Method	Interaction	Definition
Tsuji, 2006	Dapto+Vanco	hGISA	E-test	Additive	FIC index > 0.5 ≤
Credito, 2007	Dapto + Gent	MSSA MRSA(CA, HA, PVL+/-) VISA VRSA	Time kill	Synergistic	≥2 $\log_{10}$ decrease in CFU/ml
Tsuji, 2006		hGISA	E-test Time kill	Additive Additive	FIC index >0.5 to ≤1 1to 2-$\log_{10}$ CFU/ml at 24h
Snydman, 2005		MRSA	Checkerboard Time-kill	Synergistic	FIC index ≤ 0.5 Reduction >2 $\log_{10}$ CFU/ml
Tsuji, 2005		GISA MSSA MRSA	E-test Time-kill Time-kill	Additive Enhance 24h Enhance 4-24h	FIC index >0.5 to ≤1 ≥ 20-log2- $\log_{10}$ CFU/ml ≥ 20-log2- $\log_{10}$ CFU/ml
LaPlante, 2004		MSSA/MRSA	Time-kill	Increased bacteriocidal activity	The time to 99% bacteriocidal kill by 3.8h to 5.2h (statistically not significant)
Sakoulas, 2003	Dapto+Rif	MRSA	Animal study (vegetation bacterial density)	Superior to monotherapy	The difference of mean bacterial densities between dapto + rif and dapto monotherapy was statistically significant (p=0.006)
Baltch, 2007	Dapto+Gent+Rif	MRSA	Time-kill using Monocyte-derived Macrophages (MDM),	Superior to monotherapy	Greater activitiy than double or single (p<0.01)
Tsuji, 2006	Linez + Vanco	hGISA	E-test	Additive	FIC index > 0.5 to ≤ 1
Grohs, 2003		MSSA/MRSA	Time-kill	Antagonistic	Decrease 100-hold at 24/48h
Chiang, 2003		MRSA	Checkerboard	Indifferent	

Jacqueline, 2003		MRSA	Time-kill	Indifferent	
Allen, 2002		MSSA/MRSA/MRSE	In vitro pharmaco-dynamic model	Improvement	Increase in kill <2- $\log_{10}$ CFU/ml
Grohs, 2003	Linez+Gent	MSSA/MRSA	Time-kill	Indifferent	
Jacqueline, 2003		MRSA	Time-kill	Antagonistic	Increase C.C $\geq$2- $\log_{10}$ CFU/ml
LaPlante, 2004		MSSA/MRSA	Time-kill	Indifferent	
Grohs, 2003	Linez + Rif	MSSA/MRSA	Time-kill	Indifferent	
Jacqueline, 2003		MRSA	Time-kill	Synergistic	Decrease C.C $\geq$2- $\log_{10}$ CFU/ml
Dailey, 2003		MSSA	Experimental endocarditis model	Indifferent	
Allen, 2002	Linez + QD	MRSA	*In vitro* pharmacodynamic model	Enhance	Increase in kill $\geq$2- $\log_{10}$ CFU/ml
Sahuquillo Arce, 2006	Linez + fosfomycin	MSSA	Time-kill Checkerboard	Synergistic	Decrease $\geq$2- $\log_{10}$ CFU/ml FIC index $\leq$ 0.5
Jacqueline, 2006	Linez + Ertapenem	MRSA	Time-kill, Dynamic checkerboard (*in vivo* IE model)	Synergistic	Decrease $\geq$2- $\log_{10}$ CFU/ml
Jacqueline, 2005	Linez + Imipenem	hGISA	Time-kill, Dynamic checkerboard	Synergistic	Decrease C.C. $\geq$2- $\log_{10}$ CFU/ml
Tsuji, 2006	Q/D+Vanco	hGISA GISA	Time-kill E-test	Synergistic Additive	$\geq$2- $\log_{10}$ CFU/ml at 24h FIC index > 0.5 to $\leq$ 1
Kang, 1995		MRSA/MSSA	Time-kill	Additive	TR:>5-$\log_{10}$ CFU/ml
Allen, 2002		MRSA	*In vitro* pharmacodynamic model	Enhance	Increase in kill $\geq$2- $\log_{10}$ CFU/ml
Kang , 1997		MSSA/MRSA	Time-kill	Synergistic	Reduction $\geq$2- $\log_{10}$ CFU/ml at 24 h
Tsuji, 2006	Q/D + Gent	hGISA GISA	E-test Time-kill E-test Time-kill	Indifferent Indifferent Indifferent Indifferent	FIC index >0.5 to $\leq$ 1 1 to 2- $\log_{10}$ CFU/ml at 24h

Zarrouk, 2001	Q/D+Rif	S. aureus (HM1054, RP13, HM1054R)	Time kill Animal study (IE model)	Bactericidal Bactericidal	Decrease 3-$\log_{10}$ CFU/ml Decrease 3-$\log_{10}$ CFU/ml
Hamel, 2008	Q/D+Rif	MRSA	*In vivo*, rabbit arthritis model	Synergistic	No definition $\log_{10}$ CFU/ml reduction: -3.4 ±0.5 (p=0.0005 vs control)
Sambatakou, 1998		MRSA	Time-kill	Synergistic	Decrease ≥2- $\log_{10}$ CFU/ml
Petersen, 2006	Tige+Vanco	MRSA	Checkerboard Time-kill	Indifferent Indifferent	
Mercier, 2002	Tige+Gent	MRSA GISA	Time-kill Time-kill	Enhance Improve	> 100-hold or > in kill at 24h ≤ 100-hold
Petersen, 2006	Tige+Rif	MRSA	Checkerboard Time-kill	Indifferent Indifferent	
Palmer, 1996	Levo+Rif	MSSA MRRA	Time-kill Time-kill	Indifferent Indifferent	Mean CFU at 72h was ind. Mean CFU at 72h was ind.
Kang, 1994		MSSA MRSA	Checkerboard Checkerboard	Synergistic Synergistic	FIC index ≤ 0.5 FIX index ≤ 0.5

Dapto: Daptomycin Linez: Linezolid Q/D: Quinupristin/dalfopristin Tige:Tigecycline IE: Infectious endocarditis
Levo: Levofloxacin Vanco: Vancomycin Gent: Gentamicin Rif: rifampin
hGISA: heterogeneous glycopeptide-intermediate *Staphylococcus aureus* *HA: Hospital acquired* *CA: Community acquired* *PVL: Panton-Valentine leukocidin*
TR: Total reduction C.C: Colony count
FIC: Fractional inhibitory concentration
Enhance: Enhancement Improve: Improvement