# Antimicrobial pharmacodynamics against MRSA in an *in vitro* infection model: Comparing monotherapy to combinations under standard and altered conditions

By

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**ABSTRACT**

Methicillin-resistant *S.aureus* (MRSA) is a highly virulent pathogen associated with serious healthcare-associated (HCA-MRSA) and community-associated (CA-MRSA) infections. MRSA is an increasingly important cause of skin and skin-structure, bloodstream and other invasive infections including pneumonia and endocarditis. The pharmacodynamics of existing treatments and anovel cephalosporin with activity against MRSA were studied in an *in vitro* infection model comparing the antibacterial effects of monotherapy and combination therapy under standard and altered environmental conditions.

The activity of monotherapy with vancomycin, daptomycin, linezolid and ceftobiprole against clinical MRSA isolates were tested along with combinations of vancomycin-ceftobiprole, daptomycin-ceftobiprole and linezolid-ceftobiprole. Antibacterial response under standard conditions supporting optimal bacterial growth were compared to altered conditions with acidic pH 5.5, diluted nutrient broth (1:2) and increased temperature 40°C. Two clinical isolates including one HCA-MRSA (#81655) and one CA-MRSA (#79002) were studied in an *in vitro* pharmacodynamic model (IPDM) over 24 hours. Clinical dosing regimens equivalent to vancomycin 1500 mg intravenously every 12 hours (peak =24.4 mg/L, trough =7.4 mg/L), daptomycin 6 mg/kg (420 mg) intravenously every 24 hours (peak =8.2 mg/L, trough =0.8 mg/L) and linezolid 600 mg intravenously every 12 hours (peak =9.2 mg/L, trough =2.8 mg/L) were tested. Ceftobiprole was administrated as a bolus dose followed by constant infusion of 10 mg/L. Antibacterial effects were quantified as initial bacterial kill rate over 4 hours (KR4) and absolute bacterial kill at 24 hours (BK24). Minimum inhibitory concentrations

(MIC) were measured via E-test® methods using initial isolates and those recovered after 24 hours of therapy.

The KR4 with daptomycin and vancomycin were equivalent (*P=0.14*), yet daptomycin was more rapid than ceftobiprole *(P=0.03)* and linezolid *(P<0.0001)*. The BK24 was greatest with ceftobiprole and vancomycin which were superior to linezolid (*P<0.0001*, *P<0.0001*, respectively) and daptomycin (*P=0.0001*, *P=0.0001*, respectively). Daptomycin was associated with bacterial re-growth and increasing MICs from 0.25 mg/L to 2-4 mg/L during therapy for isolate #79002 under standard conditions. Furthermore, daptomycin activity against both isolates was significantly reduced under altered conditions (KR4, *P=0.0001*; BK24, *P=0.04*). Combination therapy with vancomycin-ceftobiprole was indifferent compared with either agent alone. Although daptomycin-ceftobiprole prevented daptomycin non-susceptibility during therapy and resulted in significantly greater BK24 compared with daptomycin alone (BK24 difference of 4.07 $\log_{10}$ cfu/mL, *P=0.0001*), the combination was indifferent from ceftobiprole alone. Finally, linezolid-ceftobiprole was similar to linezolid but significantly less active than ceftobiprole alone (BK24 difference of 1.39 $\log_{10}$ cfu/mL, *P=0.005*) raising concerns of potential antagonism with this combination. In conclusion, this study provides important data regarding antimicrobial pharmacodynamics against MRSA. Overall, monotherapy with either ceftobiprole or vancomycin was most active. Combination therapy with ceftobiprole prevented the emergence of daptomycin non-susceptibility during therapy, but demonstrated potential antagonism with linezolid.

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# TABLE OF CONTENT

**Page**

Abstract…….ii

Acknowledgments……..iv

Table of content……..……..v

List of tables…….....ix

List of figures...…….x

List of abbreviations……xi

Introduction…….....1

MRSA associated disease and mechanism of resistance…….....1

Antimicrobials……..4

Vancomycin…….5

Spectrum of activity and mechanism of action……5

Susceptibility breakpoints and mechanism of resistance……..5

Indication and adverse effects……....6

Pharmacokinetics (PK) and standard dose……..7

Pharmacodynamics (PD)…….....7

PD-In vitro and IPDM studies……..8

PD-In vivo animal and clinical studies……10

Daptomycin…….12

Spectrum of activity and mechanism of action……....12

Susceptibility breakpoints and mechanism of resistance…….12

Indication and adverse effects……...13

Pharmacokinetics (PK) and standard dose…….13

Pharmacodynamics (PD)……14

PD-In vitro and IPDM studies…….14

PD-In vivo animal and clinical studies…….17

Linezolid……..19

Spectrum of activity and mechanism of action……...19

Susceptibility breakpoints and mechanism of resistance…….20

Indication and adverse effects……...20

Pharmacokinetics (PK) and standard dose…….....20

Pharmacodynamics (PD)……....21

PD-In vitro and IPDM studies……..21

PD-In vivo animal and clinical studies……..23

Ceftobiprole…….....24

Spectrum of activity and mechanism of action……...24

Susceptibility breakpoints and mechanism of resistance…….25

Indication and adverse effects……...25

Pharmacokinetics (PK) and standard dose…….26

Pharmacodynamics (PD)……27

PD-In vitro and IPDM studies…….27

PD-In vivo animal and clinical studies…….28

*In vitro* Pharmacodynamic Model (IPDM)…….....29

GOALS and OBJECTIVES……...33

Material and Methods……....34

Bacterial strains…….34

Antimicrobials……34

Media and Reagents……35

Susceptibility testing…….36

*In vitro* Pharmacodynamic Model…… …….....36

Data analysis……..39

Results……..40

Vancomycin……...40

Monotherapy…….40

Combination……..41

MIC……..41

Daptomycin……41

Monotherapy…….41

Combination……..41

MIC……..42

Linezolid…….42

Monotherapy……..42

Combination……..42

MIC……..42

Ceftobiprole……...43

Monotherapy……..43

Combination……...43

MIC……...43

Effects of Environmental conditions……..43

Effect of Isolates…….44

Discussion…….....45

Limitations and Future Research……....52

Conclusion……..53

References……..66

## LIST OF TABLES

**Table……Page**

**Table 1:** Summary of antimicrobial characteristics…….....54

**Table 2:** Clinical isolates…….55

**Table 3:** Antimicrobial concentration profiles *in vivo* and dosing determinations in the *in vitro* pharmacodynamic model…….....56

**Table 4:** Initial kill rate over 4 h (KR4) and bacterial kill at 24 h (BK24) for all antimicrobial regimens…….57

**Table 5:** Comparisons of initial kill rate over 4 h (KR4) for (A) antimicrobial monotherapy and (B) combinations……..58

**Table 6:** Comparisons of bacterial kill at 24 h (BK24) for (A) antimicrobial monotherapy and (B) combinations…….....59

**Table 7:** Comparisons of standard versus altered environmental conditions on (A) initial kill rate over 4 h (KR4) and (B) bacterial kill at 24 h (BK24) for all antimicrobial regimens……60

**Table 8:** Inter-isolate comparisons of (A) initial kill rate over 4 h (KR4) and (B) bacterial kill at 24 h (BK24) for all antimicrobial regimens……..…..61

## LIST OF FIGURES

**Figures……Page**

**Figure 1:** *In Vitro* Pharmacodynamic Model (IPDM)……62

**Figure 2:** Comparisons of growth controls over 24 hours of two clinical MRSA isolates under standard and altered conditions……63

**Figure 3:** Comparisons of (A) initial kill rate over 4 h (KR4) and (B) bacterial kill at 24 h (BK24) for all antimicrobial regimens……64

## LIST OF ABBREVIATION

**Abbreviation……Explanation**

agr……. accessory gene regulator

ANOVA……. Analysis Of Variance

AUC/MIC…….. Area under the curve/minimum inhibitory concentration

BK24……. Bacteria Kill at 24 Hours

BSI……Blood Stream Infection

CaCl…….. Calcium chloride

CA-MRSA……Community associated methicillin resistant *S.aureus*

CDC…….. Centers for Disease Control and Prevention

cfu…… Colony Forming Unit

CI……... Continuous Infusion

CLSI…….. Clinical and Laboratory Standards Institute

CPK…….. Creatine Phosphokinase

CSMHB…….Cation Supplemented Mueller-Hinton Broth

cSSTI…..……Complicated Soft Skin and Tissue Infection

cSSSI…….Complicated Skin and Skin Structure infection

$DAP^r$…….. Daptomycin resistant gene

t½ ……..Half-life

HA-MRSA……Healthcare associated methicillin resistant *S.aureus*

HCL…….. Hydrochloric acid

hVISA……Heterogeneous vancomycin intermediate-resistant *S.aureus*

ICU…….Intensive Care Unit

IPDM…… *In Vitro* Pharmacodynamic Model

KR4……. Initial Kill Rate over 4 Hours

LPG……. lysyl-phosphotidylglycerol

MBC…… Minimum bactericidal concentration

MgCl…… Magnesium chloride

MHA…… Mueller-Hinton Agar

MHB…… Mueller-Hinton Broth

MIC……. Minimum Inhibitory Concentration

MRSA……. Methicillin Resistant *Staphylococcus aureus*

MRSE……. .Methicillin-Resistant *Staphylococcus epidermidis*

MSSA…….. Methicillin-Susceptible *S.aureus*

NaCl…… Normal saline (sodium chloride)

PBP2A…… .Penicillin-Binding Protein 2a

PD…… Pharmacodynamics

PK….....Pharmacokinetics

PVL…….…….. Panton-Valentine Leukocidin

T>MIC……Time above the MIC

VISA……...Vancomycin Intermediate-Resistant *S.aureus*

VRE……Vancomycin Resistant Enterococci

VRSA…….Vancomycin Resistant *S.aureus*

## INTRODUCTION

### Methicillin-resistant *Staphylococcus aureus* (MRSA)

First isolated in the 1960s, methicillin-resistant *staphylococcus aureus* (MRSA) is a highly virulent pathogen associated with serious healthcare and community-associated infections. MRSA can be acquired through direct contact with infected patients or contaminated surfaces or equipment (e.g. razors) (1), and can become colonized increasing the risk of subsequent MRSA infection. MRSA is an increasingly important cause of skin and skin-structure, bloodstream and other invasive infections including pneumonia and endocarditis (2-5). According to data from *Canadian Nosocomial Infection Surveillance Program*, MRSA infection rates in Canadian hospitals rose from 7.75 per 1,000 patients in 2006 to 8.62 per 1,000 patients in 2007 (6). A study of MRSA infections by the Centers of Disease Control and Prevention (CDC) reported approximately 94,000 cases and 18,000 deaths associated with MRSA infections in U.S. hospitals during 2005 (7-9). The global prevalence of MRSA varies significantly by geographical location. In 2003, the *National Nosocomial Infection Surveillance* (NNIS) system found that MRSA was associated with more than 59% of intensive care units (ICU) infections in the U.S. which was similar to some European countries like Italy (58%) and Portugal (54%) but higher than Southern India (31%) and Quebec (25%) and much higher than the Netherlands (2%) (10-12).

MRSA is an important pathogen in hospitalized patients especially in ICU settings where antimicrobial use is high. Other factors such as infection control practice and patients' health status also contribute to MRSA emergence. Patient

factors including multiple-comorbidities, human immunodeficiency virus infection, diabetes, hemodialysis, catheterization, surgery and prolonged hospitalization are associated with higher risk of *S.aureus* including MRSA colonization and infection (12,13). The clinical and economic impacts of MRSA infections are significant including delays in appropriate therapy, prolonged treatment durations (1.5 fold increase), extended hospital stays and increased healthcare cost (2 fold increase) (14-17). Moreover, MRSA infections are associated with significant patient morbidity and mortality related to persistent infection and treatment failure (14-17).

The penicillinases-resistant penicillins such as cloxacillin and nafcillin are important therapies of choice for *S.aureus* infections. However, treatment options have been limited by the rapid and continuous emergence of MRSA which confers resistance to β-lactam antimicrobials (3,18,19). The resistance is associated with the *mecA* gene which encodes for an altered penicillin-binding protein 2a (PBP2a) with low affinity for β-lactam-related agents (4). PBP2a is responsible for continuing cell wall synthesis as well as acylating and inactivating regular PBPs in the presence of β-lactams (4).

For over 50 years, the glycopeptide, vancomycin, has largely remained the treatment of choice for MRSA infections. However, vancomycin is slowly bactericidal, and has long been considered less effective than penicillins like cloxacillin for treating serious methicillin-susceptible *S.aureus* (MSSA) infections such as endocarditis (15,16,18-20). Furthermore, MRSA has recently shown a propensity for vancomycin tolerance, “MIC creep” and emerging resistance

especially vancomycin intermediate-resistant *S.aureus* (VISA) (15,16,18-20). MRSA biofilm has also been described in antimicrobial tolerance and resistance, especially in catheter or other device-related infections (2,3). Biofilm plays a major role in microorganism protection against antimicrobials like vancomycin which is a large molecule with low biofilm penetration compared to smaller molecules like daptomycin. MRSA in biofilm have demonstrated increases in MIC up to 1,000 fold due to factors such as the protective matrix, growth heterogeneity and limited drug penetration (21,22).

There is concern that vancomycin dosing regimens currently used in clinical practice are inadequate for treating serious MRSA infections (23). In response in 2006, the Clinical Laboratory Standards Institute (CLSI) lowered the “susceptible” vancomycin breakpoint from 4 mg/L to 2 mg/L (23,24). Furthermore, consensus guidelines on vancomycin therapeutic drug monitoring published in 2009 recommended more aggressive vancomycin dosing to achieve serum trough concentration of 10 to 20 mg/L or 15 to 20 mg/L for complicated infections (23,25).

Several new antimicrobials active against MRSA including linezolid and daptomycin have become available during the past decade. Most recently, new generation cephalosporins with MRSA activity including ceftobiprole and ceftaroline have been introduced. Unfortunately, none has demonstrated consistent superiority over vancomycin. Treatment failure rates for serious, invasive MRSA infections remain high in the range of 40 to 60% (15,26). Such observations continue to drive investigations for more effective therapeutic

strategies against MRSA which optimize antibacterial effects and minimize the impact of existing and future resistance (15,17). Antimicrobial combination therapy is an important approach with potential benefits in eradicating tolerant or resistant pathogens or managing otherwise difficult-to-treat infections. The study of antimicrobial combinations against MRSA to date has produce variable results of synergy, antagonism and indifference (27-31). Some important confounding variables in the study of antimicrobial combinations, however, include antimicrobial and isolate selection, experimental design, *in vitro* versus *in vivo* testing and static versus dynamic concentration exposure. For example, synergy or additivity of single, high dose gentamicin and vancomycin were shown in a simulated-endocardial-vegetation in IPDM, but not observed in time-kill studies (27-29). Combinations of β-lactams such as oxacillin, nafcillin and imipenem with vancomycin have shown the potential for synergy in both time-kill and checkerboard experiments (32,33). The addition of rifampicin, gentamicin or β-lactams like oxacillin or ampicillin/sublactam to daptomycin has also demonstrated better responses in time-kill testing, biofilm models and animal studies (28,34-36). On the other hand, no additive effects were observed in time-kill or animal models with the addition of rifampicin or gentamicin to linezolid (30,37), whereas, antagonism was found in vancomycin-linezolid time-kill studies (31).

**Vancomycin**

*Spectrum of activity and mechanism of action:*

Vancomycin is a glycopeptide with slow and variable bactericidal activity against Gram positive bacteria including MRSA and methicillin-resistant *Staphylococcus epidermidis* (MRSE) (18,23,38-40). Vancomycin binds to the terminal amino acid residue, D-alanyl--D-alanyl, in the peptidoglycan precursor disrupting cell wall synthesis and causing cell lysis (18,41).

*Susceptibility breakpoints and mechanism of resistance:*

As a result of growing concerns regarding vancomycin resistance and reduced efficacy against *S.aureus*, the CLSI lowered the MIC breakpoints in 2006 from ≥32 mg/L to ≥16 mg/L for "resistant", from 8-16 mg/L to 4-8 mg/L for "intermediate" and from 4 mg/L to 2 mg/L for "susceptible" (23,24).

The mechanism of resistance in MRSA involves the *mecA* gene which encodes for PBP2a or PBP2' which has low affinity for β-lactam antibiotics (42). More recent resistance concerns especially in MRSA, involve the emergence of reduced susceptibility to vancomycin. Heterogeneous vancomycin-intermediate *S.aureus* (hVISA) was first isolated in the Mu3 strain from Japan. hVISA isolates are vancomycin susceptible (MIC ≤2 mg/L) according to standard MIC determinations but contain resistant subpopulations that can be selected with exposure to vancomycin (43). Mu3 possessed a dysfunctional accessory gene regulator (*agr*) believed to contribute to reduced vancomycin susceptibility (43). Estimates of hVISA vary considerably from 0 to 74% leaving the actual prevalence largely unknown (10). VISA isolates have been less frequently

isolated and vancomycin resistant *S.aureus* (VRSA) rarely identified (10). Resistance in hVISA and VISA is predominantly conferred by a thickening of the cell wall with excess D-alanyl-D-alanine residues which bind vancomycin and prevents it from reaching the target site. In contrast, VRSA involves the transfer of the *vanA* or *vanB* genes from VRE, for example, which leads to D-alanyl-D-lactate in the peptidoglycan precursor and reduced affinity for vancomycin (10,44). *VanB* mutant genes encode for transposons which also lead to low affinity for vancomycin (e.g. Tn1547 or Tn5382) (45).

*Indications and adverse effects:*

Vancomycin is used to treat for various infections associated with Gram positive pathogens such as *S.aureus* including MRSA, enterococci and streptococci including penicillin-resistant *Streptococcus pneumoniae*. Vancomycin can be used to treat infections involving the skin or skin structure, blood stream, respiratory tract, bone and cardiac valves (46).

The adverse effects of vancomycin include red man syndrome (>10%), neutropenia, and rarely nephrotoxicity and ototoxicity (<1%) (23,46,47). The use of more aggressive vancomycin dosing and targeting higher trough concentrations has revived the debate regarding nephrotoxicity (23,25,39). Currently however, data supporting the relationship between vancomycin trough concentrations and nephrotoxicity are limited. Existing studies are retrospective, observational designs with selection biases and confounding variables which prevent the determination of causality (48). Previous data has suggested relatively low incidences of vancomycin-associated nephrotoxicity. Pestotink

*et.al*. found 1.4% nephrotoxicity among 1750 patients receiving vancomycin, and studies by Rybak and Farber reported rates <5%. The recent consensus guidelines on vancomycin therapeutic monitoring also concluded that available evidence support a relatively low risk for vancomycin-associated nephrotoxicity (23,39,49,50).

*Pharmacokinetics (PK) and standard doses:*

The volume of distribution (Vd) of vancomycin ranges from 0.4 to 1.0 L/kg. Protein binding to serum albumin is variable with mean estimates ranging from 30 to 70% (47). Vancomycin clearance occurs via renal (75-90%) and non-renal routes. The average elimination half-life (t½) is 6 hours in patients with "normal" renal function, and increases as renal function declines with age or disease (18,23,38-40,47).

Traditional standard vancomycin doses are 1 g every 12 hours in patients with average body weight and normal renal function (23,46). Vancomycin administration via intermittent or continuous infusion have undergone limited study and shown indifferent outcomes (23,51-54). Traditional dosing monograms were not developed for the new target trough concentrations of 10–20 mg/L. It is suggested that maintenance doses of 10–20 mg/kg every 8-12 hours are required to achieve targets in most patients. Reduced doses and/or extended intervals would be required in those with reduced renal function (23,25).

*Pharmacodynamics (PD):*

Recent vancomycin therapeutic guidelines recommend pharmacodynamic targets of area under the curve/MICs (AUC/MIC) ≥400 as the most reliable

pharmacodynamic parameter for determining vancomycin efficacy (23,25). The suggestions also include using vancomycin serum trough concentrations of 10-20 mg/L as surrogates for target attainment which should be achieved against *S.aureus* isolates with vancomycin MIC ≤1 mg/L. Vancomycin pharmacodynamics against hVISA has not been fully characterized. Concentration-dependent yet variable bactericidal activity was observed by Turner *et.al.* who found that high vancomycin peaks (15, 30 mg/L every 12 hours) were associated with greater bacterial reduction in comparison to lower levels (0.75, 3 mg/L) against hVISA even with 100% time above the MIC (T>MIC) (55). More data are needed to determine the efficacy and safety of vancomycin against MRSA, hVISA and VISA strains (25,55-57).

*PD-in vitro and IPDM studies:*

Vancomycin pharmacodynamics has been characterized in several time-kill and IPDM studies. Rybak *et al*. compared vancomycin standard (1 g every 12 hours) and high dose (2 g every 12 hours) in an simulated-endocardial-vegetation IPDM against four hVISA and two MRSA isolates. After 72 hours, standard doses revealed slow bactericidal activity against MRSA with activity against hVISA. Moreover, high doses were not superior against six of the isolates tested (5). A study by Bowker *et.al*. evaluated vancomycin against five MRSA and one VRSA isolate using low and high inocula of $10^6$ and $10^8$ colony forming unit (cfu)/mL, respectively. Although vancomycin (1 g every 12 hours) had a slow bactericidal activity against both isolate tested, the effects were significantly less with the high inocula (58). In contrast, vancomycin (1 g every

12 hours) demonstrated minimal bactericidal activity against high MRSA and MSSA inocula in a simulated-endocardial-vegetation IPDM where both isolates showed four folds increase in MIC (27).

IPDM studies have also investigated vancomycin in combination with other antibiotics against *S.aureus* with variable results. Data regarding synergy for vancomycin-gentamicin have been conflicting. Several studies found no difference in bacterial kill with the addition of gentamicin to vancomycin (27,56,59). However, synergism has been demonstrated by others. For example, Houlihan *et.al.* studied various dosing regimens involving intermittent and continuous vancomycin administration in a fibrin-clot (endocarditis) *in vitro* infection model and found that vancomycin intermittent dose of 2 g every 24 hours plus gentamicin achieved 99.9% killing against MRSA (60). Shelburne *et.al.* evaluated the addition of gentamicin or rifampin to vancomycin against CA-MRSA, HA-MRSA and MSSA in an *in vitro* time-kill model. Vancomycin-gentamicin combination was superior to either drug alone or vancomycin-rifampin. Adding gentamicin to vancomycin was synergistic against 92% of CA-MRSA, 50% of HA-MRSA and 73% of MSSA. In contrast, vancomycin-rifampin varies between synergy and antagonism (61). In the study of another combination, vancomycin-imipenem, Totsuka *et.al.* found rapid bactericidal activity against 35 MRSA clinical isolates (62). Ribes *et.al.* also observed synergy between vancomycin (60 mg/kg every 5 hours) and imipenem (30 mg/kg every 5 hours) against hVISA and VISA in time-kill experiments. However the same investigators reported that vancomycin-linezolid (60 mg/kg and 35 mg/kg

every 5 hours, respectively) was more effective against VISA but that linezolid reduced the activity of vancomycin against hVISA (63).

The emergence of vancomycin resistance has also been studied in the IPDM. A recent vancomycin IPDM study correlated increasing MICs and cell wall thickening in hVISA isolates. Regardless of the vancomycin regimen used (250, 500, 1000, 1500 or 2000 mg every 12 hours), hVISA MICs increased to ≥3 mg/L due to increased cell wall thickening ($P$ <0.0001) (43). Another study by Rose *et.al*. evaluated vancomycin activity in an *in vitro* model against two hVISA and one non-hVISA using low and high inocula. Again, vancomycin activity was reduced against hVISA at low and high inocula regardless of the regimen used (0.750-5 g every 12 hours). Yet vancomycin retained bactericidal against low inocula of non-hVISA (64). Moreover, vancomycin demonstrated reduced efficacy against MRSA embedded in biofilms (21,22,65). In summary, there are concerns regarding the efficacy of vancomycin for treating MRSA infections and failures related to prolonged treatment durations that provoke cell wall thickening, increased MIC and resistance.

*PD-in vivo animal and clinical studies:*

Pharmacokinetic and pharmacodynamic parameters are used to correlate optimal antibiotic dosing with antibacterial activity and clinical outcomes. A recent consensus report from the American Society of Health System Pharmacists, Infectious Disease Society of American and Society of Infectious Diseases Pharmacists suggested an increase to standard vancomycin dosing in order to maintain trough concentrations >10 mg/L or 15-20 mg/L for serious or

complicated infections (23,25). These recommendations were based on work by Moise-Broder *et.al.* which characterized vancomycin pharmacodynamics in patients with *S.aureus* infection. The investigators found that an AUC/MIC ≥400 was associated with improved clinical and microbiological outcomes in patients with MRSA or MSSA infections. On the other hand, %T>MIC was not associated with treatment response (66).

Resistance to vancomycin has been a concern since the isolation of Mu3 in Japan. Subsequent studies linking decreased susceptibility and treatment failures to vancomycin exposure especially to low concentrations have also raised concerns. In response, the CSLI lowered the MIC breakpoints, and consensus guidelines were published recommending higher vancomycin doses to maintain troughs above 10 mg/L (38,39,67). Further reports have suggested increased treatment failures with vancomycin against *S.aureus* pathogens with MICs above 1 mg/L (23,51-54). In response, some have proposed aggressive dosing to achieve troughs at the upper limit of 20 mg/L or combining vancomycin with agents such as gentamicin (68,69). In one case report by Schairer *et.al*. vancomycin troughs of 16.5 mg/L were not successful against MRSA endocarditis and bacteremia. With an initial vancomycin MIC of 2 mg/L which rose to 4 mg/L (intermediate resistance), the patient was switched to daptomycin and achieved complete eradication (19). Several similar cases have reported reduced efficacy of vancomycin against MRSA especially with increased MICs (42,70).

**Daptomycin**

*Spectrum of activity and mechanism of action:*

Daptomycin is a lipopeptide produced by *Streptomyces roseosporus*, with rapid bactericidal activity against MRSA, VRE and Streptococci. Daptomycin was approved by Food and Drug Administration as well as European Medicines Evaluation Agency in 2003 and by Health Canada in 2007 (5,38,71-76). Daptomycin binds to bacterial cell membranes in a calcium-dependent manner causing rapid membrane depolarization and release of intra-cellular magnesium and potassium. As a result RNA, DNA and protein synthesis are inhibited and cell death occurs (5,38,71-73,77,78).

*Susceptibility breakpoints and mechanism of resistance:*

Daptomycin MIC breakpoints according to the CSLI are: susceptible at ≤1 mg/L and non-susceptible at >1 mg/L (79,80). Some reports have indicated reduced susceptibility with MICs exceeding 1 mg/L leading to prolonged treatments and in some cases clinical failure. Several theories in regards to daptomycin resistance have been postulated. *S.aureus* harboring an uncommon $DAP^r$ gene may synthesize large amounts of lysyl-phosphotidylglycerol (LPG) which is translocated on the outer membrane leaflet. An increase in positive charge on the membrane may disrupt membrane fluidity and reduce daptomycin binding. Another mechanism of resistance may involve cell-wall thickening which is associated with prolonged exposure to vancomycin and reduced susceptibility to glycopeptides. Finally, mutations in *mprF* and *yycG* genes have been observed in *S.aureus* with reduced susceptibility to daptomycin (36,81,82).

*Indications and adverse effects:*

Daptomycin is used for treating complicated soft skin and tissue infections (cSSTI), endocarditis and blood stream infections (BSI). It is not recommended for pneumonia due to its high affinity for and inactivation by pulmonary surfactant (5,72,73,76,78,79,83,84).

Daptomycin is associated with constipation (6.2%) and nausea (5.8%), diarrhea (5.2%) and vomiting (3.2%). Only 0.2% of patients experience muscle pain (myalgia, myopathy) in conjunction with elevated creatine phosphokinase (CPK) (2.8%). CPK levels should be monitored every two days and therapy should be terminated in patients with unexplained muscle pain associated with CPK levels >1000 U/L (85,86).

*Pharmacokinetics (PK) and standard doses:*

The volume of distribution (Vd) of daptomycin is 0.9 L/kg and protein binding to serum albumin is approximately 90%. Daptomycin clearance occurs via renal (78%) and non-renal routes (5.7%). The elimination half-life ($t_{½}$) is 8 hours in patients with “normal” renal function (38). Daptomycin safety and efficacy has not been determined in patients with creatinine clearance <30 mL/min (79). In general, daptomycin had shown linear pharmacokinetics with standard (4 or 6 mg/kg) and higher (10-12 mg/kg) doses (82).

Daptomycin is administered intravenously with 0.9% normal saline (NaCl) in two standard doses of 4 or 6 mg/kg every 24 hours (79). Higher doses of 8, 10 and 12 mg/kg every 24 hours have been used for difficult-to-treat infections,

however the toxicity profile of these doses has not been fully characterize (38,73,79,87,88).

*Pharmacodynamics (PD):*

Daptomycin is a concentration-dependent antibiotic which has rapid bactericidal activity against MRSA. The primary pharmacodynamic indices related to microbiological and clinical outcome are $C_{max}$/MIC and AUC/MIC. Targets of 60-100X for $C_{max}$/MIC and 400-550 for AUC/MIC have been proposed (82). Some studies have shown benefits to combination therapy especially in treating refractory infections (82).

*PD-in vitro and IPDM studies:*

Daptomycin activity against four MRSA and one MSSA was evaluated by Rose *et.al.* in a simulated-endocardial-vegetation IPDM over 8 days. MRSA isolates demonstrated susceptibility towards daptomycin with no MIC change regardless of prior vancomycin administration. However, some daptomycin reduced susceptibility was observed in MSSA following vancomycin exposure (89). A study by Rybak *et.al,* daptomycin standard (6 mg/kg every 24 hours) and high dose (10 and 12 mg/kg every 24 hours) were evaluated against four hVISA and two MRSA isolates. Consistent with previous reports, high doses of daptomycin (10 and 12 mg/kg every 24 hours) were rapidly bactericidal even against multidrug resistant isolates (5). Daptomycin doses of 6 and 8 mg/kg every 24 hours were tested in a simulated-endocardial-vegetation IPDM against *S.aureus* (including MRSA and VISA). Daptomycin showed rapid bactericidal activity at 8 hours (99.9% kill rate) in comparison to vancomycin. No MIC

increase was observed among tested isolates, however MRSA re-growth was observed with doses of 6 mg/kg (90). A recent study using a foreign-body infection model evaluated high doses of daptomycin compared with vancomycin, linezolid and rifampicin against MRSA in stationary and log growth phase with daptomycin showing superiority over all other agents tested (91).

Bowker *et.al.* used an IPDM to evaluate daptomycin (6 mg/kg every 24 hours) against MRSA and VRSA at low ($10^6$ cfu/mL) and high ($10^8$ cfu/mL) inocula. Bactericidal activity was observed against both isolates at low inoculum, yet efficacy was reduced against high inocula of VRSA (58). Huang *et.al.* designed an IPDM to study several daptomycin regimens with varying half-lives (*t½* 8 and 30 hours) against MRSA and MSSA. First, regimens of 4, 6 and 8 mg/kg every 24 hours (t½ 8 hours) and every 48 hours (t½ 30 hours) were tested followed by regimes of 2, 3 and 4 mg/kg every 24 hours (t½ 30 hours). Doses of 4, 6 and 8 mg/kg were superior to 2, 3 and 4 mg/kg regardless of the variability in half-lives used. Daptomycin pharmacodynamics was determined for both isolates. AUC/MICs were 188-581 for MRSA and 94-392 for MSSA (92). Akins *et.al.* supported the use of daptomycin at 6 mg/kg every 24 hours or 3 mg/kg every 12 hours over 4 mg/kg against VISA in an IPDM. Daptomycin was rapidly bactericidal and superior to vancomycin ($P$ <0.03) at 100% and 50% T>MIC yet regrowth were observed with 4 mg/kg. Daptomycin (3 an 6 mg/kg) pharmacodynamics were also determined in which $AUC_{0-24}$/MICs were 370-461 for MRSA versus 80-116 for VISA and for daptomycin dose of 4 mg/kg was 320 for MRSA and 80 for VISA(93).

As for combinations, an *in vitro* time-kill study by Credito *et al*. evaluated daptomycin monotherapy compared to combinations with gentamicin or rifampicin against MRSA, MSSA, VRSA and VISA. Over all monotherapy was less bactericidal than combination therapy. Daptomycin-rifampicin showed synergy against VISA isolates whereas daptomycin-gentamicin were synergistic against 64% of tested isolates (35,35). Rose *et al.* evaluated daptomycin at standard doses (6 mg/kg every 24 hours) and high doses (10 mg/kg every 24 hours) alone and in combination with gentamicin 5 mg/kg per day or rifampicin 300 mg every 8 hours against daptomycin susceptible and non-susceptible *S.aureus* in a simulated-endocardial-vegetation IPDM. Daptomycin (standard and high dose) monotherapy and combinations were both bactericidal against daptomycin susceptible isolates. However, daptomycin had minimal bactericidal activity against two non-susceptible isolates with high doses, versus no activity with standard doses. Daptomycin (6 or 10 mg/kg) in combination with gentamicin or rifampicin against the non-susceptible isolates demonstrated better bactericidal activity than daptomycin alone (94). In another simulated-endocardial-vegetation IPDM study by LaPlante *et.al,* daptomycin (6 mg/kg every 24 hours) alone and in combination with gentamicin was bactericidal against high inocula ($10^9$ cfu/mL) of MRSA and MSSA. High inocula and albumin had noticeable effects on both isolates with 32 fold increases in MIC and decreased $AUC/MIC_{(albumin)}$ from 1,511 to 94.4 and 47.2 (MSSA and MRSA, respectively) (27). Tsuji and Rybak used the simulated-endocardial-vegetation IPDM to investigate the efficacy of daptomycin and vancomycin in combination with single

high dose gentamicin at 5 mg/kg or three daily doses of 1 mg/kg against MRSA and MSSA. Increased bactericidal activity was observed with daptomycin-gentamicin when used with single high-dose gentamicin (29,72,94).

Daptomycin resistance or reduced susceptibility is relatively uncommon but has been isolated *in vitro*, in animal models and in the clinical setting (82). Mishra *et.al.* evaluated MRSA ($DAP^r$ isolate) selected for daptomycin resistance by serial passage and exposure to "sublethal concentrations of daptomycin" for 20 days. They found remarkable increases in MIC from 1 to 32 mg/L along with increased vancomycin MIC into the VISA range (4 mg/L). Daptomycin resistance in their study was correlated to cell wall thickening, increased LPG production as well as reduced cell membrane fluidity (95). Rose *et.al.* evaluated daptomycin doses against susceptible and non-susceptible *S.aureus* in a simulated-endocardial-vegetation IPDM (48 hours). Doses were 1.5, 3 mg/kg every 12 hours and 6, 10 mg/kg every 24 and 48 hours. With the lowest dose, there was minimal activity against both isolates along with four fold increase in MICs. Optimal bactericidal activity was achieved with 10 mg/kg against both isolates without MIC change (96).

*PD-in vivo animal and clinical studies:*

Daptomycin is rapidly bactericidal and has shown superiority over vancomycin in both *in vitro* and *in vivo* models. AUC/MIC (189-520) and $C_{max}$/MIC are the best parameters to evaluate daptomycin *in vivo* (82).

Mortin *et.al.* evaluated daptomycin activity (50 mg/kg) in healthy and neutropenic peritonitis mice. Daptomycin was bactericidal against MRSA and

MSSA in both groups with 100% survival rate in healthy and 40% in neutropenic mice (91). Murillio *et.al.* studied 50 MRSA infected rats with treatment initiation after 72 hours. Human doses equivalent to 6, 8 and 10 mg/kg every 24 hours were simulated, and tissue cage fluid samples were drawn prior and post treatment to determine bacterial counts and biofilm formation. Results were similar to those found *in vitro* in which daptomycin and rifampicin alone were superior to vancomycin and linezolid alone ($P < 0.05$). Resistance was not observed (91). Sakoulas *et al*. evaluated daptomycin at 25 and 40 mg/kg every 24 hours (equivalent to 4 and 6 mg/kg every 24 hours in humans) compared to vancomycin continuous infusion at 150 mg/kg over 24 hours alone and in combination with rifampicin at 25 mg/kg every 24 hours in an endocarditis (aortic valve) rat model. Increased reductions in MRSA vegetations were observed with combinations in general and with daptomycin-rifampicin in particular ($P=0.006$) (97). Similar findings were observed in a guinea pig foreign body infection model conducted by John *et.al.* (71). In contrast, Miro *et.al.* found that daptomycin alone (6 mg/kg) and daptomycin-gentamicin were equivalent against MRSA rabbit endocarditis ($P=0.83$) yet daptomycin-rifampicin failed to eradicate MRSA ($P=0.01$) (98).

Despite promising results in both *in vitro* and *in vivo* studies, daptomycin has not demonstrated superiority in clinical studies with failure in some (99). Daptomycin doses of 6, 8, 10 and 12 mg/kg every 24 hours were evaluated for tolerability and safety in 36 healthy volunteers and showed no significant toxicity including CPK elevation (88). On the other hand, several studies have correlated

daptomycin treatment failure due to resistance especially in individuals with endocarditis or bacteremia. A randomized trial (2002-2005) evaluated daptomycin at 6 mg/kg every 24 hours to vancomycin-gentamicin (1 g every 12 hours and 2 g every 4 hours, respectively) against MRSA in patients for 42 days. There were indifferent outcomes with increasing MICs over time in both groups. Daptomycin MICs rose to 2-4 mg/L and vancomycin MICs increased to 2 mg/L. The elevations in MICs were associated with potentially inadequate dosing, prolonged treatments and complicated infections including osteomyelitis and those involving indwelling devices (78).

**Linezolid**

*Spectrum of activity and mechanism of action:*

Linezolid was first discovered in 1990s and was approved by Food and Drug Administration in 2000. Linezolid ("Zyvoxam™), is an oxazolidinone with bacteriostatic activity against Gram positive bacteria including MRSA, VISA and VRE (38,100-102). Linezolid has not shown superiority to vancomycin in clinical trials, and some cases of resistance have been reported ( 12 cases in Spain, 8 in U.S, 2 in Germany, 1 case in united kingdom, brazil and Colombia) (103).

Linezolid binds to the 23S ribosomal RNA of the 50S subunit disrupting translation and protein synthesis, and inhibiting bacterial growth and reproduction (38,102).

*Susceptibility breakpoints and mechanism of resistance:*

The susceptible MIC breakpoints for linezolid according to the CSLI are: susceptible ≤4 mg/L and resistant >4 mg/L (103,104).

Linezolid resistance was first reported in 1999. Several researches correlated resistance in *S.aureus* to a single nucleotide mutation where adenine was substituted by guanine G2576T or T2500A (105). More recently, the involvement of *cfr* gene mutation has been identified (103).

*Indication and adverse effects:*

Linezolid is used in the treatment of various infections including pneumonia, skin and soft tissue infections and bone and joint infections associated with susceptible Gram positive pathogens. However linezolid is not recommended for catheter-related blood stream infections or infective endocarditis due to high mortality rates and co-infections with Gram negative pathogens (7,38,106,107).

Linezolid-associated adverse effects were studied in several phase III clinical trial (more than 2000 patient). Diarrhea (2.8-11%), headaches (0.5-11.3%) and nausea (3.4-9.6%) were most common (104). Less common but significant adverse effects include, lactic acidosis, thrombocytopenia, neuropathy and optic neuropathy (38,104,108-111).

*Pharmacokinetics (PK) and standard doses:*

Linezolid has rapid oral absorption (1 to 2 hours) and 100% bioavailability. Linezolid has a volume of distribution of 10-50 L and average protein binding to serum albumin of 31%. Linezolid clearance occurs via renal (30%) and non-

renal routes (65%). The average elimination half-life (t½) is 5 hours in patients with “normal” renal function (40,104,112-115).

Standard dosing of linezolid is 600 mg every 12 hours administrated either orally or intravenously (104). Dose adjustments are not required in patients with creatinine clearance ≤40 mL/min. However, dosing reductions (or supplemental dose) are necessary in end-stage renal disease (116,117). Researchers did not find significant difference in linezolid volume of distribution, $C_{max}$ and AUC between critically ill patients with sepsis and healthy individuals (118). However, to achieve optimal and less variable linezolid concentrations, Adembri *et.al.* studied linezolid administration via continuous infusion rather than intermittent doses in patients with septic shock (119).

*Pharmacodynamics (PD):*

Linezolid is a time dependent antibiotic with clinical efficacy best determined by %T>MIC and AUC/MIC. Low concentrations have been associated with reduced susceptibility in MRSA and hVISA. A T>MIC of 100% and AUC/MIC of at least 100 are the suggested targets for optimizing linezolid pharmacodynamics in the treatment of patients with Gram positive infections (116).

*PD-in vitro and IPDM studies:*

IPDM studies have generally concluded that linezolid is bacteriostatic against MRSA isolates. A recent *in vitro* time-kill study by Baldoni *et.al*. tested three linezolid regimens equivalent to 25, 50 and 75 mg/kg every 12 hours against two MRSA isolates with MICs of 2.5 mg/L. Similar bacteriostatic effects were observed for all regimens (2). A study by LaPlante *et.al.* using a simulated-

endocardial-vegetation IPDM also showed bacteriostasis with linezolid (600 mg every 12 hours) at 100% T>MIC against high and moderate inocula of MRSA and MSSA (27,120). In another IPDM study, linezolid had more reduction against *S.aureus* biofilm compared to vancomycin (121), but another investigation by Raad *et.al.* using an antimicrobial catheter-lock for 24 hours demonstrated little linezolid activity against MRSA biofilm (34).

Numerous studies have evaluated the ability of linezolid co-administration with bactericidal agents such as gentamicin or rifampin to improve bacterial response and outcomes. Linezolid-rifampin demonstrated superiority to linezolid alone and prevented MRSA rifampin resistance in an *in vitro* time-kill studies (2,31). Arce *et.al*. found indifference with linezolid-rifampin and linezolid-vancomycin in an *in vitro* model using the checkerboard method (122). In *in vitro* time-kill studies, antagonism was observed with linezolid-vancomycin and linezolid-gentamicin against MRSA (30,31,123). LaPlante *et.al,* tested linezolid-gentamicin combination against MRSA and MSSA for 72 hours in the simulated-endocardial-vegetation IPDM. This combination was equivalent in activity to linezolid alone against both high and moderate *S.aureus* inocula (27). In *in vitro* time-kill and animal studies, Singh *et.al.* reported antagonism with linezolid-vancomycin combination against MRSA and cautioned against the use of this combination in patients (124).

Bacterial persistence due to bacteremia or indwelling devices has been associated with antimicrobial treatment failure in patients. Sabath described the “antibiotic tolerance” phenomena with wide ranges between MIC and MBC (125).

Linezolid resistance in MRSA is rare *in vitro* and *in vivo* with few cases reported in the clinical setting (103). Boak *et.al.* simulated different linezolid doses for resistance development in an IPDM including 600 mg and 120 mg every 12 hours, and 30 mg and 120 mg as constant infusions over 24 hours against MRSA, hVISA, VISA and VRE. Doses of 120 mg every 12 hours and 30 mg every 24 hours had activity against VISA while 600 mg every 12 hours was effective among all tested isolates (>3 $\log^{10}$ cfu/mL kill). Whereas 120 mg every 24 hours (continuous infusion) had some effects against hVISA and VISA, re-growth and MIC increases to ≥8 mg/L occurred in two MRSA and one hVISA. Thirty mg every 24 hours was not effective and resistance developed with continuous infusion administration (126).

*PD-in vivo animal and clinical studies:*

Baldoni *et.al* studied linezolid monotherapy against MRSA in a guinea pig foreign-body infection model. Maximum bacterial killing was observed with doses of 50 and 75 mg/kg twice a day and bacterial re-growth was inhibited by 75 mg/kg. They also studied linezolid in combination with rifampin which prevented the development of rifampin resistance and was superior to linezolid alone ($P < 0.05$) (2). In a murine thigh infection model, linezolid demonstrated bacteriostasis against CA-MRSA (120). Dailey *et.al*. used an endocarditis rabbit model to show indifference in activity between linezolid alone (50 or 75 mg/kg) and in combination with rifampin against MSSA yet no antagonism was observed (37). Jacqueline *et.al.* tested linezolid (10 mg/kg in humans) plus gentamicin against MRSA endocarditis in rabbits. The combination was associated with

bactericidal activity against MRSA and was more effective than each drug alone (127). The same investigators tested linezolid in combination with ertapenem against MRSA endocarditis in rabbits which was highly bactericidal compared with each drug alone (128). However, Chiang *et.al.* demonstrated antagonism with linezolid-vancomycin (75 mg/kg and 30 mg/kg, respectively) against MRSA in rabbit a endocarditis model (129). In clinical trials, linezolid (600 mg every 12 hours) alone or in combination with carbapenems was successful against MRSA (nosocomial and community) and persistent bacteremia in 35 patients. Linezolid regimens remained superior to vancomycin in combination with gentamicin or rifampicin (*P*=0.006) (7).

Although linezolid resistance in *S.aureus* is rare, Sanchez-Garcia *et.al.* reported 12 cases of MRSA resistance in an ICU population in Spain, where linezolid MICs ranged from 16 to 32 mg/L. The resistance was associated with extensive usage of linezolid a relatively low doses for prolonged durations. In addition, genetic analysis of the isolates identified the contribution of the *cfr* gene in the outbreak (103). Cunha *et al*. published a single case report of a patient with MRSA infection who received linezolid at 600 mg every 12 hours for 5 days. Clinical failure at 5 days was associated with an increase in MIC to 4 mg/L (130).

**Ceftobiprole**

*Spectrum of activity and mechanism of action:*

Ceftobiprole is a new cephalosporin (5$^{th}$ generation) with activity against MRSA, currently marketed in Europe (38,131-133). Ceftobiprole also has activity

against Gram positive pathogens such as VISA, Enterococci and Gram negative organisms including *Pseudomonas aeruginosa* (38,131-133).

Ceftobiprole has high affinity for PBP2a or PBP2' forming an inhibitory complex and disrupting cell wall biosynthesis (38,133).

*Susceptibility breakpoints and mechanism of resistance:*

The proposed ceftobiprole MIC breakpoints are: susceptible ≤4 mg/L and resistance >4 mg/L (134,135).

Ceftobiprole resistance staphylococcus species is rare. It is not hydrolyzed by the TEM-2 and SHV-1 β-lactamases and maintains activity in the presence of the altered PBPs in penicillin-resistant *S.pneumoniae* and MRSA (133,134). Microorganisms carrying mutant genes resistant to particular group of cephalosporins could lead to cross-resistance and ceftobiprole is susceptible to hydrolysis by extended spectrum β-lactames (ESBLs) (134).

*Indications and adverse effect:*

Clinical studies have focused on the use of ceftobiprole in treating complicated skin and skin structure infections (cSSSI) including diabetic foot infections caused by Gram positive and Gram negative organisms (132,134,136). It remains under investigation for the treatment of pneumonia (137). Ceftobiprole is not recommended for patients with necrotizing fasciitis, renal failure (creatinine clearance <10 mL/min) or immunosupression due to a lack of safety evidence (134).

The adverse effects of ceftobiprole are considered mild to moderate and include nausea (9.1%), dysgeusia (5.6%), vomiting (4.8%) and hypersensitivity

(>0.9%) (132,134,136). Noel *el.al.* compared ceftobiprole to vancomycin-ceftazidime in a randomized double-blinded trail for cSSSI (2005-2006). Both antimicrobial groups showed similar cure rates and occurrences of adverse effects. Fifty six percent of the ceftobiprole group experienced nausea, dysgeusia, vomiting and headaches versus 57% of vancomycin-ceftazidime group (138). Another randomized double-blinded trail by the same investigators, compared ceftobiprole to vancomycin for the treatment of cSSSI. The incidence of all adverse effects for ceftobiprole was similar to vancomycin (52% versus 51%, respectively) (139).

*Pharmacokinetics (PK) and standard doses:*

Ceftobiprole has a volume of distribution of 0.3-0.4 L/kg (18 L) and protein binding to serum albumin of 16 to 38% (38,131-133). Ceftobiprole clearance occurs via renal (83%) and non-renal routes. The average elimination half-life (t½) is 3 hours in patients with “normal” renal function (134).

Ceftobiprole is intravenously administrated using standard doses of 500 mg every 8 or 12 hours. Prolonged infusion of 2 hours is recommended to enhance the pharmacodynamics of ceftobiprole when treating more serious infections. Dose adjustments are recommended for patients with creatinine clearance of ≤50 mL/min (134,138,139). Recent studies have used 250 mg every 12 hours (as 2 hour infusion) in patients with severe renal failure (creatinine clearance <30 mL/min) (134,140,141).

*Pharmacodynamics (PD):*

Ceftobiprole demonstrates time-dependent pharmacodynamics with %T>MIC as the key parameter used for dose determination in phase III trials (*fT*>MIC of ≤50%) against Gram positive and negative pathogens (134,140,141).

*PD-in vitro and IPDM studies:*

Zhanel *et al*. studied the activity of ceftobiprole versus vancomycin against MRSA, VISA, MSSA, and VRSA in an IPDM over 24 hours. Ceftobiprole at 500 mg every 8 hours was bactericidal against all tested isolates (MIC >2 mg/L, 100% T>MIC. Vancomycin at 1 g every 12 hours was slowly bactericidal to bacteriostatic against the majority of isolates except for VRSA (142). Similar findings were observed by Rouse *et al*. (143). Bogdanovich *et.al.* investigated ceftobiprole against 126 MRSA, 26 MSSA, five VISA and two VRSA with MICs ranging from 0.5 mg/L to 2 mg/L in an *in vitro* time-kill model. Ceftobiprole was bactericidal against all isolates except for VISA against which it was bacteriostatic (144). Leonard *et.al.* conducted an *in vitro* time-kill study and showed equivalent activity for ceftobiprole against four CA-MRSA and four HA-MRSA (MICs of 1-2 mg/L). However, ceftobiprole kill rate was less than daptomycin against CA-MRSA (145). Similar results were reported by Entenza *et.al.* using an *in vitro* time-kill model of MRSA (146).

For combinations, Leonard *et.al,* evaluated ceftobiprole-tobramycin against four CA-MRSA and four HA-MRSA in an *in vitro* time-kill study. The combination was indifferent in activity against both isolates in comparison to ceftobiprole alone (145). Ceftobiprole-gentamicin combination was also assessed by

Deshpande *et.al.* against MRSA, enterococci and streptococci. Time-kill experiments showed no difference between ceftobiprole monotherapy at eight times MIC compared with the combination against MRSA. The combination, however, was synergistic against the enterococci (147). Findings of indifference with ceftobiprole-vancomycin against MRSA were also found by Hilliard *et.al*. (148).

Ceftobiprole resistance in *S.aureus* is rare and difficult to select. One investigator described an isolate with potential for resistance development. Bogdanovich *et.al.* selected resistance *in vitro* in a single *S.aureus* isolate which demonstrated increasing MICs from 1 mg/L to 8 mg/L after prolonged serial passages with exposure to sub-inhibitory concentrations of ceftobiprole (144).

*PD-in vivo animal and clinical studies:*

Entenza *et al*. demonstrated bactericidal activity against two MRSA isolates in a rat endocarditis (aortic vegetation) model. Ceftobiprole dosed at 5, 10 and 20 mg/L was compared to vancomycin. After three days of therapy, ceftobiprole successfully eradicated and sterilized the infection site ($P<0.05$) (146). In a rabbit endocarditis model, ceftobiprole (25 mg/kg every 8 hours) showed superiority to vancomycin (30 mg/kg every 12 hours), daptomycin (18 mg/kg every 24 hours) and linezolid (75 mg/kg every 8 hours) $P<0.05$ against MRSA (MIC of 4 mg/L) (149). Another rabbit endocarditis model by Chambers, demonstrated bactericidal activity with ceftobiprole (30 mg/kg every 8 hours) against MRSA and VISA while vancomycin (30 mg/kg every 12 hours) was effective only against MRSA (150).

Only two randomized, double-blinded clinical by Noel *et al*. evaluated the efficacy of ceftobiprole for MRSA cSSSI. Both trials investigated the bactericidal and tolerability of ceftobiprole (500 mg every 8 hours) versus vancomycin regimens (1 g every 12 hours) in patients worldwide (America, Africa, Asia and Europe). There were no differences between groups with response rates exceeding 90% (138,139). Ceftobiprole efficacy was also evaluated in phase I, II and III clinical studies. In phase II trials, ceftobiprole 750 mg every12 hours was associated with 91% cure rates in 40 patients with cSSSI. Similarly, phase III trials demonstrated better ceftobiprole tolerability in infected individuals with 93% cure rate against MRSA (132,137). A recent trial by Kimko *et.al*. showed the correlation between T>MIC and cure rate for ceftobiprole against Gram positive pathogens causing cSSSI. T>MIC of ≥30% was associated with 90% cure rates and 94.6% fractional target attainment rate while no association was observed with AUC/MIC and $C_{max}$/MIC (151). In addition, *fT*>MIC of 14.1-27.8% found to be effective against *S.aureus* infections (134,140,141).

**In Vitro Pharmacodynamic Model (IPDM)**

Pharmacokinetics assesses the relationship between the body and the drug (i.e. the body's effect on the drug). Pharmacokinetics studies enable researchers to assess drug absorption, distribution, metabolism and excretion and important pharmacokinetic parameters including drug half-life, volume of distribution, clearance and elimination rate constant. An understanding of these parameters is required to determine appropriate antimicrobial dosing and achieve optimal

concentration profiles or exposure in patients (152,153). Pharmacodynamics assesses the relationship between an antimicrobial and pathogen and the ultimate effects on clinical response (i.e. the drug's effect on the body). Pharmacodynamic studies conducted *in vitro* and *in vivo* have contributed significantly to characterizing antimicrobial regimens with optimal microbiological and patient outcomes. Work over the past two decades have correlated pharmacodynamic indices such as $C_{max}$/MIC for aminoglycosides, %T>MIC for β-lactams and AUC/MIC for fluoroquinolones with clinical response allowing for the development of targeted dosing regimens (152,154,155).

*In vitro* pharmacodynamic models have been widely used in the study and characterization of antimicrobial pharmacodynamics including antibacterial activity and the emergence of resistance. Models are classified according to the working principles of (1) dilution and (2) diffusion or dialysis (156,157). Dilution IPDMs were introduced by O'Grady and Pennington (1966) who developed an *in vitro* "bladder" model. A glass vessel containing bacteria suspension was attached to a base where the culture was diluted using fresh broth. The *in vitro* "bladder" model was modified into an automated system by Greenwood and O'Grady (1978). Sanfilippo and Morvillo (1968) described a model which simulated plasma levels of antimicrobials *in vivo* whereas Rowe and Morozowich (1969) followed with an IPDM that simulated drug distribution processes more relevant to antibacterial activity. The addition of pumps was led by Otaya *et.al.*, Ozawa *et.al.* and Goto *et.al.* who incorporated air pumps (1976), Grasso *et.al.* who used peristaltic pumps (1978) and Bergan *et.al.*, Carlsen *et.al.* and

Fuglesang *et.al.* who combined double peristaltic pumps (1980). Murakawa *et.al.* introduced the concept of compartmental models to mimic the profiles of antimicrobial concentrations following intravenous administration (156,157). Dialysis or diffusion models were used by various researchers such as Drugeon *et.al*. (1979) and Zunner *et.al.,* (1981). Al-Asadi *et.al*, (1979), Shah (1980) and Greenwood and Tupper (1982) described the use of cellulose acetate membranes as a separation technique to prevent bacterial loss or dilution (156,157).

IPDM experiments are associated with some important limitations. Firstly, the environment lacks the immunological effects present *in vivo* and therefore are most indicative of worst-case scenarios such as infections in immunocompromized patients. IPDM studies are vary labour intensive compared with other *in vitro* testing such as determining MIC or minimum bactericidal concentration (MBC) or conducing time-kill or checkerboard synergy experiments. As a result, IPDM generally involve relatively small numbers of study isolates.

Some technical challenges of conducting IPDM experiments include maintaining a steady pump flow and constant temperature. The use of IPDM without filters also results in washing out or diluting bacterial counts. Some investigators incorporate cellulose membranes, while other compensate for bacterial loss by incorporating growth control data in the analysis. The IPDM apparatus can also be contaminated during the preparation, setup, antimicrobial administration or sampling procedures. Finally, biofilm formation can be

significant especially during prolonged experiments due to the favourable conditions in the infection flasks provided by the continuous infusion of fresh nutrient broth and constant stirring motion.

IPDM have been used extensively to characterize antimicrobial pharmacodynamics against bacteria, mycobacteria, fungi, yeast and viruses. IPDM have been successfully used to measure antimicrobial effects by quantifying bacterial colonies at different time points to determine rates or magnitude of bacterial kill over time. The experiments are also well suited to detect the development of resistance during antimicrobial therapy. IPDM can simulate clinical dosing regimens including those administered as intermittent or continuous infusions for agents given alone or in combination. IPDM can also be modified to mimic various types of infections such as bacteremia, endocarditis and cystitis (156,157).

Given the increasing prevalence and seriousness of MRSA infections and the limitations of currently available therapies, my study is important in its use of an established *in vitro* pharmacodynamic model to provide comparative data on the pharmacodynamics of existing and new clinical treatments alone and in combination. In addition to exploring potential advantages of combination therapy, my study tests antimicrobial activity under altered environmental conditions which may be more relevant at the site of infection. My ultimate goal is to contribute to the use of optimal treatments for patients with MRSA infections by investigating new and alternative therapeutic strategies.

## GOALS

The study was designed to test antimicrobial therapy with vancomycin, daptomycin and linezolid alone and in combination with a novel cephalosporin, ceftobiprole, against MRSA in an *in vitro* pharmacodynamic mode under standard and altered environmental conditions.

## OBJECTIVES

**1.** To characterize the pharmacodynamics of monotherapy with vancomycin, daptomycin, linezolid and ceftobiprole against clinical MRSA isolates (#81655 and #79002) in an *in vitro* infection model.

**2.** To compare monotherapy (above) to combinations of vancomycin plus ceftobiprole, daptomycin plus ceftobiprole and linezolid plus ceftobiprole.

**3.** To compare antimicrobial pharmacodynamics under standard conditions that support optimal bacterial growth with altered conditions including lower pH, reduced nutrients and increased temperature which may mimic the "infection site".

## MATERIAL AND METHODS

### Bacterial strains

Two clinical MRSA were studied (Table 2). Stock #81655 was a healthcare-associated isolate obtained from a blood sample collected at the Queen Elizabeth II Health Science Center, Halifax, NS, Canada in 2008. It was *mecA* gene positive and Panton-Valentine leukocidin (PVL cytotoxin) negative. In addition, the MIC for vancomycin, daptomycin, linezolid and ceftobiprole were 0.5, 0.25,1 and 1 mg/L, respectively. Stock #79002 was a community-associated isolate obtained from a wound sample collected at the Royal University Hospital, Saskatoon, SK, Canada in 2008. It was *mecA* gene positive and PVL positive. The MICs for vancomycin, daptomycin, linezolid and ceftobiprole were 1, 0.25, 2 and 1 mg/L, respectively.

Both isolates were stored in skim milk at -70ºC in cryo-vials. Isolates were retrieved from stock, plated on Mueller-Hinton agar (MHA, Fisher Scientific, Canada) and incubated at 35ºC for 24 hours. Isolates were re-plated three times prior to use. Stocks were sub-cultured prior to each experiment. Cultured plates were used a maximum of four times after which new stocks were obtained from the frozen stock.

### Antimicrobials

Antimicrobials were obtained from different manufactures. Vancomycin powder (Lot no. 23373/A) was purchased from Sigma Inc, Oakville, Ontario Canada. Daptomycin powder (Cubicin®, Lot no. 660903F) was obtained from Cubist pharmaceutical Inc., Lexington, Massachusetts, USA. Linezolid clinical

solution (Zyvoxam®, Lot no. 09F26Z59; Pfizer) was supplied by St. Boniface General Hospital Department of Pharmacy, Winnipeg, Manitoba, Canada. Ceftobiprole powder (Zeftera, Lot no. 08005R25C) was provided by Johnson & Johnson Pharmaceutical Research and Development, Raritan, New Jersey, USA. Vancomycin and linezolid were stored at room temperature, daptomycin was stored at 4°C and ceftobiprole was stored at -80°C. Antimicrobial solutions were prepared prior to each use according to manufacturer's instructions. Vancomycin was prepared in Mueller-Hinton Broth (MHB, lot no. 211443, Becton, Dickinson Company, Difco LABs, Sparks, Massachusetts, USA) and daptomycin was prepared in 0.9% NaCl using deionized water. Ceftobiprole powder (1.5 mg) was mixed with 99 uL of 99.5% dimethyl sulfoxide (DMSO, Sigma, Oakville, Ontario, Canada) and 10 uL of glacial acetic acid, vortexed for 15 minutes, and diluted in 891 uL of distilled water.

**Media and Reagents**

Pre-made MHA plates were supplied by Fisher Scientific, Canada. Mueller-Hinton broth (MHB) containing, beef extract (3.0 g), acid hydrolysate of casein (17.5 g) and starch (1.5 g) at a pH of 7.3± 0.1was prepared in 4 L batches as per the manufacturer's instructions (Lot no. 211443, Becton, Dickinson Company, Difco Labs, Sparks, Massachusetts, USA) and autoclaved at 121ºC for 10 minutes. Cation-supplemented Mueller-Hinton broth (CSMHB) with 25 mg/L of calcium chloride (CaCl) and 12.5 mg/L of magnesium chloride (MgCl) was used for vancomycin, linezolid and ceftobiprole. MHB supplemented with 50

mg/L of CaCl and 12.5 mg/L of MgCl was used for daptomycin as per the CLSI guidelines. CaCl and MgCl were prepared using sterile deionized water.

CSMHB was adjusted to the desired pH of 5.5 by adding hydrochloric acid 1N (HCl, 1N) when preparing broth for experiments conducted under the altered conditions. Broth pH was measured prior to and after autoclaving using a pH probe (Lot no. 1040101, VWR International, Beverly, Massachusetts, USA).

**Susceptibility testing**

MICs were tested using the E-test® method (AB Biodisk, Biomerieux, Solna, Sweden). A 0.5 McFarland standard of approximately $1x10^8$ cfu/mL was prepared using an overnight culture of 5 to 10 colonies suspended in 5 mL of sterile NaCl. A cotton swab was dipped into the suspension and used to swab an entire MHA plate. An E-test® strip was placed face-up in the center of the plate and incubated at 35°C for 24 hours.

The appropriate MIC reading scales were used as per the manufacturer's instructions (158). Vancomycin MIC was read at the end of the dip or slim ellipse, linezolid was read at the 90% growth inhibition and daptomycin and ceftobiprole were read at the inhibition ellipse.

***In vitro* Pharmacodynamic Model (IPDM):**

The IPDM was set up using aseptic technique and maintained under sealed conditions. Each IPDM (Figure 1) consisted of a 4 L reservoir flask containing fresh CSMHB, two 250 mL round, flat-bottom infection flasks and a 3 L waste flask. All flask connections were made using sterile silicon tubing. The infection

flasks were stirred with magnetic bars and maintained at a constant temperature in a heated water bath.

To allow the initial filling of the infection flasks, needle tips were inserted into the rubber septum and metal clamps were clipped to the silicon tubing. CSMHB was pumped through the infection flasks using a computerized pump (Masterflex®, L/S®; Cole-Parmer Company, Chicago, Illinois, USA) set at a flow rate of 4 mL/min. After the infection flasks were filled, the flow rate was calibrated to 0.41 mL/min.

Bacterial suspensions were adjusted to a 0.5 McFarland standard. Five to ten colonies from overnight culture were added to 5 mL of CSMHB, vortexed and incubated at 35$^{o}$C for 2 hours. A 2.5 mL volume of bacterial suspension was injected into each infection flask and left for 30 minutes to allow bacteria to enter logarihmic growth phase. Antimicrobials were injected into the infection flasks as required over 24 hours to simulate clinical doses and free concentration profiles observed in patients (Table 3). The computerized pump was programmed at a flow rate producing half-lives of 7 hours for vancomycin, daptomycin and linezolid.

Vancomycin was dosed at an equivalent clinical dose of 1500 mg every 12 hours (peak of 24.4 mg/L, trough of 7.4 mg/L) (46) , daptomycin at 6 mg/kg (420 mg/70 kg) every 24 hours (peak of 8.2 mg/L, trough of 0.8 mg/L) (79) and linezolid at 600 mg every 12 hours (peak of 9.2 mg/L, trough of 2.8 mg/L) (104). Ceftobiprole was dosed using a continuous infusion to accommodate the difference in half-life compared with the other agents (3 hours versus 7 hours).

The manufacture's recommended dose of 500 mg every 8 hours (2 hour infusion) and the pharmacodynamics of ceftobiprole were used to determine a comparable continuous infusion dose (134). Ceftobiprole was administered as a bolus dose followed by constant infusion of 10 mg/L from the reservoir flask.

For colony count determinations, 1.5 mL samples were collected from the infection flasks at 0, 2, 4, 12 and 24 hours. Samples (100 uL)were serially diluted ($10^{-1}$-$10^{-4}$) in 0.9 mL of NaCl at 4°C in 13x100 mm culture glass tubes (Lot no. 14609439, Fisher brand, USA), and aliquots of 10 uL were plated onto one-quarter sections of MHA plates. Plates were incubated at 35ºC for 24 hours and viable bacterial colonies between 10 and 100 were counted using a lower limit of detection of $1x10^2$ cfu/mL. Bacterial colony counts were determined at all time points, and MICs were measured at the end of the experiment using E-test methods.

IPDM experiments were conducted under standard and altered conditions. Standard conditions were performed using CSMHB prepared as per the manufacturer's instructions with 22 g of powder per 1 L of water, temperature of 37ºC and neutral pH of 7. Altered experimental conditions were performed using CSMHB diluted 1:2 by using 11 g of powder per 1 L of water (beef extract (1.5 g), acid hydrolysate of casein (8.75 g) and starch (0.75g)), elevated temperature of 40 ºC (±1) and acidic pH of 5.5 to 6.

All IPDM experiments were conducted with growth controls and at least in triplicate on separate occasions.

**Data analysis**

Antibacterial response was quantified as net bacterial kill or growth over 24 hours. The initial bacterial kill rate over 4 hours (KR4) was calculated as the difference between the initial inocula and colony count at 4 hours divided by 4 and reported in $\log_{10}$ cfu/mL/h. Bacterial kill at 24 hours (BK24) was calculated as the difference between the initial inocula and 24 hours colony count and was reported in $\log_{10}$ cfu/mL.

The activity of vancomycin, daptomycin, linezolid and ceftobiprole were characterized and tested for statistical differences. Monotherapies were compared with combinations, and the potential influence of environmental conditions and inter-isolate differences were assessed. The student's T-test or Analysis of Variance (ANOVA) with Tukey's post hoc test for multiple comparisons was used to compare mean KR4 and BK24 values. Statistical significance was determined using an $\alpha = 0.05$. In addition, standard definitions regarding combination therapy were applied including an increased in BK24 of ≥2 $\log_{10}$ cfu/mL with combination therapy compared with monotherapy for synergy, decreased in BK24 of >2 $\log_{10}$ cfu/mL with combination therapy compared with monotherapy for antagonism and <2 $\log_{10}$ cfu/mL difference between combination and monotherapy for indifference (72,159).

## RESULTS

Growth control experiments conducted over 24 hours for the two clinical MRSA isolates resulted in net growth of -1.28 ± 0.76 $\log_{10}$ cfu/mL. As demonstrated in Figure 2, the individual altered conditions (i.e., temperature, pH, diluted CSMHB) did not have significant effects on bacterial growth. However, bacterial growth under combined altered conditions resulted in 0.8 $\log_{10}$ cfu/mL less growth over 24 hours. Subsequent experiments testing antimicrobial pharmacodynamics were conducted under standard and combined altered conditions.

Antibacterial effects including KR4 and BK24 for vancomycin, daptomycin, linezolid and ceftobiprole are described below (table 5 and 6, figure 3). In addition, the results for vancomycin, daptomycin and linezolid in combination with ceftobiprole are presented (table 5 and 6, figure 3)

### Vancomycin

*Monotherapy:* The KR4 for vancomycin (0.32 $\log_{10}$ cfu/mL/h) was not significantly different from daptomycin (0.51 $\log_{10}$ cfu/mL/h, P=0.14) or ceftobiprole (0.26 $\log_{10}$ cfu/mL/h, P=0.99) but was more rapid than linezolid (0.04 $\log_{10}$ cfu/mL/h, **P=0.002**) (Table 4 and 5 A). The BK24 for vancomycin (2.89 $\log_{10}$ cfu/mL) was not significantly different than ceftobiprole (3.14 $\log_{10}$ cfu/mL, P=0.99) but was greater than linezolid (0.98 $\log_{10}$ cfu/mL, **P<0.0001**) and daptomycin (-0.22 $\log_{10}$ cfu/mL, **P=0.0001**) (Table 4 and 6 A).

*Combination therapy:* The KR4 for vancomycin-ceftobiprole (0.23 $log_{10}$ cfu/mL/h) was not significantly different from vancomycin (0.32 $log_{10}$ cfu/mL/h, P=0.92) and ceftobiprole (0.26 $log_{10}$ cfu/mL/h, P=0.99) alone (Table 4 and 5 B). The BK24 for vancomycin-ceftobiprole (4.03 $log_{10}$ cfu/mL) was not significantly different form vancomycin (2.89 $log_{10}$ cfu/mL, P=0.05) and ceftobiprole (3.14 $log_{10}$ cfu/mL, P=0.30) alone (Table 4 and 6 B).

*MICs:* No change in vancomycin MIC was found during monotherapy or combination experiments conducted under standard or altered conditions.

**Daptomycin**

*Monotherapy:* The KR4 for daptomycin (0.51 $log_{10}$ cfu/mL/h) was not significantly different from vancomycin (0.32 $log_{10}$ cfu/mL/h, P=0.14) but was more rapid than linezolid (0.04 $log_{10}$ cfu/mL/h, **P<0.0001**) and ceftobiprole (0.26 $log_{10}$ cfu/mL/h, **P=0.03**) (Table 4 and 5 A). The BK24 for daptomycin (-0.22 $log_{10}$ cfu/mL) was less than vancomycin (2.89 $log_{10}$ cfu/mL, **P=0.0001**), linezolid (0.98 $log_{10}$ cfu/mL, **P=0.014**) and ceftobiprole (3.14 $log_{10}$ cfu/mL, **P=0.0001**) (Table 4 and 6 A).

*Combination therapy:* The KR4 for daptomycin-ceftobiprole combination (0.47 $log_{10}$ cfu/mL/h) was not significantly different from daptomycin (0.51 $log_{10}$ cfu/mL/h, P=0.99) and ceftobiprole (0.26 $log_{10}$ cfu/mL/h, P=0.18) (Table 4 and 5 B). The BK24 for the daptomycin-ceftobiprole combination (3.85 $log_{10}$ cfu/mL) showed synergy and was greater than daptomycin alone (-0.22 $log_{10}$ cfu/mL, BK24 difference of= 4.07 $log_{10}$ cfu/mL, **P=0.0001**) but was indifferent from ceftobiprole alone (3.14 $log_{10}$ cfu/mL, P=0.59) (Table 4 and 6 B).

*MICs:* A significant increase in MIC from 0.38 mg/L initially to 2 to 4 mg/L for isolate #79002 was observed after 24 hours of monotherapy with daptomycin in all standard condition experiments (n =4). There was no increase in daptomycin MIC after 24 hours of combination therapy with ceftobiprole.

**Linezolid**

*Monotherapy:* The KR4 for linezolid (0.04 $\log_{10}$ cfu/mL/h) was significantly slower than daptomycin (0.51 $\log_{10}$ cfu/mL/h, **P<0.0001**) vancomycin (0.32 $\log_{10}$ cfu/mL/h, **P=0.002**) and ceftobiprole (0.26 $\log_{10}$ cfu/mL/h, **P=0.046**) (Table 4 and 5 A). The BK24 for linezolid (0.98 $\log_{10}$ cfu/mL/h) was greater than daptomycin (-0.22 $\log_{10}$ cfu/mL, **P=0.014**) but was less than vancomycin (2.89 $\log_{10}$ cfu/mL, **P<0.0001**) and ceftobiprole (3.14 $\log_{10}$ cfu/mL, **P<0.0001**) (Table 4 and 6 A).

*Combination therapy:* The KR4 for linezolid-ceftobiprole combination (0.03 $\log_{10}$ cfu/mL/h) was not different from linezolid (0.04 $\log_{10}$ cfu/mL, P=0.99) but was significantly slower than ceftobiprole alone (0.26 $\log_{10}$ cfu/mL/h, **P=0.046**) (Table 4 and 5 B). The BK24 for the linezolid-ceftobiprole combination (1.75 $\log_{10}$ cfu/mL) was not significantly different from linezolid alone (0.98 $\log_{10}$ cfu/mL, P=0.33) yet was less than ceftobiprole and potential antagonism was observed (3.14 $\log_{10}$ cfu/mL, BK24 difference of= 1.39 $\log_{10}$ cfu/mL, **P=0.005**) (Table 4 and 6 B).

*MICs:* No changes in linezolid MIC were found during monotherapy or combination experiments conducted under standard or altered conditions.

**Ceftobiprole**

*Monotherapy:* The KR4 for ceftobiprole (0.26 $log_{10}$ cfu/mL/h) was less than daptomycin (0.51 $log_{10}$ cfu/mL/h, **P=0.03**), greater than linezolid (0.04 $log_{10}$ cfu/mL/h, **P=0.046**) and no different from vancomycin (0.32 $log_{10}$ cfu/mL/h, P=0.99) (Table 4 and 5 A). The BK24 for ceftobiprole (3.14 $log_{10}$ cfu/mL) was greater than for daptomycin (-0.22 $log_{10}$ cfu/mL, **P=0.0001**) and linezolid (0.98 $log_{10}$ cfu/mL, **P<0.0001**) but not significantly different from vancomycin (2.89 $log_{10}$ cfu/mL, P=0.99) (Table 4 and 6 A).

*Combination therapy:* Combination therapy with ceftobiprole did not improve KR4 for any agents, and in fact linezolid-ceftobiprole (0.03 $log_{10}$ cfu/mL/h) demonstrated significantly slower initial bacterial kill than ceftobiprole alone (0.26 $log_{10}$ cfu/mL/h, **P=0.046**) (Table 4 and 5 B). Although the combination of daptomycin-ceftobiprole (3.85 $log_{10}$ cfu/mL) was synergistic compared with daptomycin (-0.22 $log_{10}$ cfu/mL, BK24 difference of 4.07 $log_{10}$ cfu/mL, **P=0.0001**), it did not offer benefit over ceftobiprole alone (3.14 $log_{10}$ cfu/mL, P=0.59). Similar to the effects on KR4, linezolid-ceftobiprole showed less BK24 compared to ceftobiprole alone (1.75 versus 3.14 $log_{10}$ cfu/mL, **P=0.005**) (Table 4 and 6 B).

*MICs:* No changes in ceftobiprole MIC were found during monotherapy or combination experiments conducted under standard or altered conditions.

**Effect of Environmental Conditions**

For KR4, altered conditions had an inhibitory effect on daptomycin (0.22 versus 0.79 $log_{10}$ cfu/mL/h, **P=0.0001**), ceftobiprole (0.17 versus 0.36 $log_{10}$

cfu/mL/h, **P=0.014**), vancomycin-ceftobiprole (0.15 versus 0.31 $log_{10}$ cfu/mL/h, **P=0.02**) and daptomycin-ceftobiprole (0.19 versus 0.73 $log_{10}$ cfu/mL/h, **P=0.002**) (Table 7 A). The BK24 under altered conditions was significant reduced for daptomycin (-0.91 versus 0.48 $log_{10}$ cfu/mL, **P=0.04**) but not significantly different for any of the other antimicrobial regimens (Table 7 B).

**Effect of Isolates**

Isolates #81655 and #79002 were tested against each antimicrobial regimen. Isolate #81655 was more rapidly killed than isolate #79002 by vancomycin (0.412 versus 0.22 $log_{10}$ cfu/mL, **P=0.02**) and ceftobiprole (0.35 versus 0.18 $log_{10}$ cfu/mL, **P=0.031**) alone (Table 8 A). For BK24, no significant difference was observed between isolates (#81655 versus #79002) with vancomycin (2.78 versus 3 $log_{10}$ cfu/mL, P=0.72), daptomycin (0.44 versus -0.88 $log_{10}$ cfu/mL, P=0.06), linezolid (0.84 versus 1.12 $log_{10}$ cfu/mL, P=0.46), ceftobiprole (2.78 versus 3.5 $log_{10}$ cfu/mL, P=0.23), vancomycin-ceftobiprole (4.02 versus 4.05 $log_{10}$ cfu/mL, P=0.93), daptomycin-ceftobiprole (3.75 versus 3.95 $log_{10}$ cfu/mL, P=0.72) or linezolid-ceftobiprole (1.53 versus 1.98 $log_{10}$ cfu/mL, P=0.09) (Table 8 B).

## DISCUSSION

MRSA with reduced susceptibility to standard treatments is a growing concern which raises the need for new therapeutic options. My study explored the effectiveness of existing (vancomycin, daptomycin and linezolid) and new (ceftobiprole) antimicrobials against MRSA using an IPDM. It also evaluated monotherapies and combinations with ceftobiprole under standard and altered environmental conditions. There were several important findings in my study. First, ceftobiprole monotherapy was equivalent to vancomycin, and the ceftobiprole continuous infusion regimen showed similar outcomes to intermittent dosing studied by other investigators (142,144,145). Second, daptomycin was associated with re-growth during therapy with 10-20 fold increase in MIC for isolate #79002 under standard conditions. Notably, this resistance was not observed with the daptomycin-ceftobiprole combination. Daptomycin activity was also significantly reduced against both isolates under altered conditions. Finally, no combinations tested in IPDM were superior to ceftobiprole alone; however the combination of linezolid-ceftobiprole showed antagonism with reduced antibacterial response.

Vancomycin which is described as slowly bactericidal has been the standard therapy for MRSA infections for decades. However, tolerance towards vancomycin has been reported with the emergence of hVISA and VISA (15,20). Vancomycin troughs of 15-20 mg/L equivalent to AUC/MICs ≥400 are recommended in an attempt to increase penetration, reduce resistance and improve clinical outcomes for serious infections such as endocarditis,

osteomyelitis, bacteremia and pneumonia. These therapeutic targets are believed to be sufficient against isolates with MICs ≤1 mg/L, but questionable if vancomycin MICs are higher (23,55). In my study, vancomycin troughs of 7.4 mg/L equivalent to a total serum level of 14.8 mg/L were effective against two MRSA isolates with MICs of =1 mg/L via broth microdilution, or 1.5-2 mg/L via Etest®. The KR4 for vancomycin was not significantly different from daptomycin or ceftobiprole, but more rapid than linezolid. The BK24 for vancomycin was also equivalent to ceftobiprole yet superior to both daptomycin and linezolid (Table 5, Figure 3). Vancomycin had an average bacterial kill approaching 3 $\log_{10}$ cfu/ml, but did not achieve bacterial eradication in the IPDM. My findings were consistent with other IPDM studies of vancomycin 1-2 g every 12 hours which achieved slow bactericidal activity against MRSA (5,27,58).

My study of daptomycin (6 mg/kg every 24 hours) demonstrated good initial kill but diminished activity at 24 hours (Table 4). Daptomycin activity was reduced significantly against both isolates under altered environmental conditions, and was associated with increasing MICs during therapy for one isolate (#79002) under standard conditions. This is most likely explained by the fact that daptomycin activity is highly dependent on both cation concentration and pH. Studies conducted under altered conditions at a pH of 5.5 could significantly influence the pharmacodynamics of daptomycin as demonstrated by significant re-growth of both isolates. Lamp *et.al.* demonstrated reduced daptomycin as well as vancomycin activity in an acidic pH (6.4) versus a pH of 7.4 or 8 against MRSA and MSSA in an *in vitro* time-kill study (160).

Furthermore, in my study, daptomycin MIC increased from 0.38 mg/L to 2-4 mg/L under standard conditions for CA-MRSA (#79002) while vancomycin MIC remained the same (2 mg/L). This phenomenon has also been observed during daptomycin therapy in the clinical setting. In a randomized clinical trial (2002-2005), Fowler *et al*. found that daptomycin (6 mg/kg every 24 hours) was not superior to vancomycin (1 g every 12 hours) against MRSA and MSSA in 246 patients with endocarditis or bacteremia. Treatment failure was documented in 15.8% of those receiving daptomycin with 5.8% showing elevated MICs of 2-4 mg/L. Therapy was unsuccessful in 9.6% receiving vancomycin with 13.2% having increased MICs of 2 mg/L (78). In another prospective, randomized clinical trial by Rehm *et.al.* patients have received vancomycin prior to daptomycin administration. After vancomycin failure therapy, daptomycin was administrated demonstrating activity against MRSA even though MICs were increased (≥2 mg/L). This increase in MIC was not correlated to vancomycin initial therapy (161). However, daptomycin treatment failure against *S.aureus* with increasing MICs has also been reported. Resistance has been correlated to several mechanisms including enhanced *mprF* gene expression with point mutations (81), increased cell wall thickening as in VISA (162), reduced peptidoglycan cross-linking (163), and reduced daptomycin binding through changes in cell-membrane fluidity (164). However, *in vitro* and *in vivo* data in regards to the activity of daptomycin against MRSA are consistent. Daptomycin doses of 6, 10 and 12 mg/kg every 24 hours have been associated with greater

activity against MRSA, MSSA and hVISA than vancomycin (1 g every 12 hours) with doses of 4 mg/kg showing equivalence to vancomycin (91,165)(5).

Consistent with other literature (2,91), linezolid was the least effective against MRSA in my study. In clinical trial by Cunha *et.al*., linezolid 600 mg every 12 hours has been less effective in patients with MRSA bacteremia compared with daptomycin (6 and 12 mg/kg IV every 24 hours) (130). In my study, the BK24 for linezolid was lower than vancomycin and ceftobiprole yet greater than daptomycin due to bacterial re-growth as discussed previously (Figure 3 B). Linezolid monotherapy has not been associated with bacterial eradication unless used in combination. In time-kill and foreign-body animal models, linezolid at 75 mg/kg was able to reduce and inhibit MRSA re-growth whereas the addition of rifampin resulted in eradication (2).

Ceftobiprole showed no difference in activity when compared to vancomycin monotherapy (Figure 3). In clinical trials ceftobiprole was equivalent to vancomycin-ceftazidime against MRSA in CSSTI (>91% and >89%, respectively) (138,139). However, other *in vitro* studies by Zhanel *et.al*. and Rouse *et.al*. demonstrated superiority with ceftobiprole (500 mg every 8 hours, MIC 1 mg/L) over vancomycin (1 g every 12 hours, MIC 1 mg/L) against MRSA (142,143). Most notably, vancomycin was significantly less active in the study by Zhanel *et.al*. Such differences may be explained by experimental design, dosing regimens, duration of study or isolate variability. In specific, Zhanel *et.al.* reported similar results with ceftobiprole but significantly less activity with vancomycin compared with my findings (142). Their conclusion was superiority

for ceftobiprole, whereas my study showed similar antibacterial responses for ceftobiprole and vancomycin. In an endocarditis rat model, Entenza *et.al* showed ceftobiprole (MIC of 2 mg/L) to be better than vancomycin (MIC of 2 mg/L) (146). Similar findings were also shown in an osteomyelitis rabbit model conducted by Yin *et. al.* which had 100% MRSA eradication with ceftobiprole versus 73% with vancomycin or linezolid (MICs were 0.39 mg/L, 0.78mg/L and 1.56 mg/L, respectively) (166). Interestingly, Lemaire *et.al.* demonstrated greater activity with ceftobiprole against MRSA in macrophages and keratinocytes in both acidic and neutral pH compared to other cephalosporins by Lemaire *et.al.* (135). Furthermore, their study showed reduced ceftobiprole MICs in acidic pH (0.25-1 mg/L) compared to neutral pH (MICs 0.5-2 mg/L) (135). In my study we did not observe any changes in ceftobiprole pharmacodynamics in experiments conducted under neutral (MIC= 1.5 mg/L) compared with acidic (pH5.5, MICs 1-1.5 mg/L) conditions. However, ceftobiprole KR4 was significantly lower under altered condition **(P=0.014)**. Lemaire *et.al.* observed a slight decrease in ceftobiprole MICs for MRSA with increased intracellular activity in macrophages and keratinocytes cells.

Antimicrobial combinations of agents with different target sites aim to improve antibacterial effects particularly against more resistant microorganisms. The standard *in vitro* testing of ceftobiprole in combination with other agents such as checker board and time-kill studies are limited (145,147,148). To my knowledge, this is the first study of ceftobiprole combination therapy in an IPDM. Vancomycin-ceftobiprole combination was not different from vancomycin or

ceftobiprole alone (Table 5 B and 6 B, Figure 3). The few *in vitro* time-kill studies of ceftobiprole in combination with gentamicin, tobramycin or vancomycin against MRSA have observed indifference (145,147,148). On the other hand, vancomycin combination studies have shown synergy *in vitro* with β-lactams such cefepime, cefazoline and imipenem (62,167,168). Vancomycin-cefpirome was synergistic against gentamicin resistant and hVISA at 24 and 48 hours (vancomycin MIC were 1 and 4 mg/L, respectively) (168). Vancomycin (1-2 mg/kg) and imipenem (5 mg/kg) were also synergistic against MRSA *in vitro* (checkerboard) and in a neutropenic mouse model (62). Synergy was also observed with vancomycin-imipenem and vancomycin-cefazolin against MRSA in checkerboard and time-kill testing, whereas indifferent effects were found with vancomycin0netilmicin (169). However, in my study synergy was not observed in vancomycin-ceftobiprole combination thus it was similar in activity to each drug alone. Several factors played a role in our finding includes experiment duration, isolates tested, ceftobiprole continuous infusion of 10 mg/L, environmental conditions and the model. Majority of vancomycin combination synergy were observed with checkerboard and time-kill studies but not with IPDM.

Daptomycin-ceftobiprole in my study was more effective and synergistic compared with daptomycin alone (BK24 difference of= 4.07 $\log_{10}$ cfu/mL) (Figure 3 B) and was able to prevent the emergence of daptomycin reduced susceptibility during therapy. Daptomycin in combination with rifampin, gentamicin or β-lactams (e.g. oxacillin) has shown synergy and better cure rates in some *in vitro*, animal studies and human trials (27,34-36,71,97,170-172).

Snydman *et.al*. recommended daptomycin use in combination with β-lactams in which checkerboard and time-kill studies demonstrated variable synergism with daptomycin-imipenem, daptomycin-cefepime or daptomycin-oxacillin against MRSA, MSSA and VRE (173). However, few time-kill studies reported 94% indifference/additive in daptomycin combination with rifampin or gentamicin against *S.aureus* infections (35). Conversely, antagonism was reported by LaPlante *et.al*. with daptomycin-rifampin or daptomycin-gentamicin against MRSA biofilm in an IPDM (174).

Finally, potential antagonism was observed with linezolid-ceftobiprole against both isolates (#81655 and #79002). Significant decreases in KR4 and BK24 were observed with linezolid-ceftobiprole compared with ceftobiprole alone (BK24 difference of= 1.39 $\log_{10}$ cfu/mL, **P=0.005**) (Figure 3). This study, to our knowledge, is the first to report antagonism with ceftobiprole. In the case of linezolid combinations, a majority of studies have agreed with little to no benefit from the addition of another agent. For instance, antagonism has been reported with linezolid-vancomycin and linezolid-gentamicin against MRSA in time-kill studies (30,31,123,124). Indifference between linezolid-gentamicin and linezolid alone was reported in a simulated-endocardial-vegetation IPDM against MRSA (27). On the other hand, linezolid-rifampin has shown some benefit over linezolid alone (2,31). Regardless of the few studies reporting better outcomes with linezolid combinations, most of the evidence supports either indifference or antagonism against MRSA.

## LIMITATIONS AND FUTURE RESEARCH

This study using an IPDM only simulates the site of infection. A significant advantage of dynamic models is their ability to mimic *in vivo* dosing regimens and concentration profiles which can be tested against clinically relevant pathogens. However, important *in vivo* factors relevant to host immunity and the local site of infection are not present. This study modified the standard IPDM by altering the environmental conditions such as pH, nutrient concentrations and temperature and measuring the effects on antimicrobial activity. This was done in an effort to mimic the conditions of the septic environment; and thus test the robustness of our antimicrobial responses. Another limitation of this study is the testing of a small number of isolates which limits the characterization of inter-strain variability. As a result, studies of MRSA isolates including hVISA and VISA would be of interest. In addition, the potential effects of altered conditions on the activity of other antimicrobials against different pathogens would be worth investigation. My study only tested one dosing regimen for each agent which was represented common clinical doses. For example, my daptomycin dose of 6 mg/kg every 24 hours demonstrated rapid initial bactericidal activity with non-susceptibility emergence and re-growth at 24 hours. Higher doses up to 10-12 mg/kg every 24 hours have been tested and used experimentally in the clinical setting to improve the activity of daptomycin. The study of other dosing regimens and different bacterial inocula provide more opportunity to build on my initial work. Moreover, extending the study duration from 24 hours to 48-72 hours may increase the ability to detect the development of resistance during therapy.

## CONCLUSION

This study presented new information in regards to existing and new monotherapies and combinations against MRSA under standard and altered environmental conditions. Daptomycin demonstrated better initial kill rates with reduced activity and re-growth at 24 hours. Importantly, this phenomenon was associated with significant increasing MICs to 2-4 mg/L. The addition of ceftobiprole to daptomycin prevented non-susceptible emergence and enhanced the killing at 24 hours. Vancomycin and ceftobiprole monotherapy were most active and equivalent in activity. The combination was not significantly different from either agent alone (P=0.30). Consistent with the literature, potential antagonism was observed with linezolid-ceftobiprole against both isolates tested.

Given the increase and difficulty in treating MRSA infections, my study provides new and important information in an area with limited treatment options. Although the development of new antimicrobials is important, the optimal use of existing therapies is important to provide the best possible patient outcomes with the least risk of resistance in the future.

**Table 1:** Summary of antimicrobial characteristics

| | **Vancomycin** | **Daptomycin** | **Linezolid** | **Ceftobiprole** |
|---|---|---|---|---|
| **Spectrum of activity** | Gram positive - *S.aureus* including MRSA, Streptococci including penicillin-resistant *S.pneumoniae*, *vancomycin-susceptible Enterococci* | Gram positive - *S.aureus* including MRSA, Enterococci including vancomycin-resistant Enterococci | Gram positive - *S.aureus* including MRSA, Enterococci including vancomycin-resistant Enterococci | Gram positive - *S.aureus* including MRSA, Streptococci including penicillin-resistant *S.pneumoniae*, Enterococci including vancomycin-resistant Enterococci<br><br>Gram negative – Enterobacteriaceia, *Pseudomonas areuginosa* |
| **Mechanism of action** | Peptidoglycan<br><br>Binds to the terminal amino acid residue D-ALA -D-Ala disrupting cell-wall synthesis leading to cell lysis. | Lipopeptide<br><br>Inserts into bacterial cell membrane leading to ion (magnesium, potassium) release, membrane depolarization and cell death. Also interferes with proteins synthesis. | Oxazolidinone<br><br>Inhibits 23S ribosomes disrupting protein synthesis, and inhibiting bacterial growth and reproduction. | Cephalosporin<br><br>Binds with high affinity to penicillin-binding protein 2a (PBP2a) and PBP2x disrupting peptidoglycan layer and bacterial cell-wall synthesis. |
| **Clinical indications** | Serious infections due to Gram positive pathogens resistant to other classes (e.g. MRSA, penicillin-resistant *S.pneumoniae*); or in penicillin-allergic patients. | Skin and soft tissue infections, bacteremia and endocarditis but not recommended for pneumonia. | Skin and soft tissue infection, pneumonia and bone and joint infections. | Complicated skin and soft tissue infections |

**Table 2:** Clinical isolates

| **Isolate stock #** | **HCA-MRSA (#81655)** | **CA-MRSA (#79002)** |
|---|---|---|
| **Year of collection** | 2008 | 2008 |
| **Collection Site** | Queen Elizabeth II Health Sciences Centre, Halifax | Royal University Hospital, Saskatoon |
| **Collection Source** | Blood | Wound |
| ***mecA*** | Positive | Positive |
| ***agr* genotype** | Type II | Type I |
| **Panton-Valentine leukocidin** | Negative | Positive |
| **Phenotype classification** | Healthcare-associated | Community-associated |
| **MIC (mg/L) broth microdilution [Etest®]** | | |
| **Vancomycin** | 1 [ 2 ] | 1 [1.5 ] |
| **Daptomycin** | 0.25 [ 0.38 ] | 0.25 [ 0.38 ] |
| **Linezolid** | 1 [ 1 ] | 2 [1 ] |
| **Ceftobiprole** | 1 [ 1.5 ] | 1 [1 ] |

**Tables 3:** Antimicrobial concentration profiles *in vivo* and dosing determinations in the *in vitro* pharmacodynamic model.

[where t½ is half-life, t is time, N/A is not applicable]

**A)** *In vivo* steady-state concentrations.

| Antimicrobial | Dose (mg) | Interval (h) | t½ (h) | Mean peak (mg/L) | Mean trough (mg/L) | Free (*f*) fraction | *f* Peak (mg/L) | *f* Trough (mg/L) |
|---|---|---|---|---|---|---|---|---|
| **Vancomycin** | 1500 | 12 | 6 | 44 | 13.5 | 0.55 | 24.2 | 7.4 |
| **Daptomycin** | 6 /kg | 24 | 8 | 94 | 6.7 | 0.10 | 9.4 | 0.7 |
| **Linezolid** | 600 | 12 | 5 | 15 | 3.7 | 0.69 | 10.4 | 2.5 |
| **Ceftobiprole** | 500 | 8 | 3 | 35 | 9 | 0.85 | 29.8 | 7.8 |

**B)** Dosing in the *in vitro* pharmacodynamic model.

| Antimicrobial | Volume (mL) | Pump rate (ml/min) | t½ (h) | t = 0 dose (mg) | t = 12h dose (mg) | Target peak (mg/L) | Target trough (mg/L) |
|---|---|---|---|---|---|---|---|
| **Vancomycin** | 250 | 0.41 | 7 | 6.10 | 4.24 | 24.4 | 7.4 |
| **Daptomycin** | 250 | 0.41 | 7 | 2.05 | N/A | 8.2 | 0.8 |
| **Linezolid** | 250 | 0.41 | 7 | 2.30 | 1.60 | 9.2 | 2.8 |
| **Ceftobiprole** | 250 | 0.41 | 7 | 2.5 mg, 10 mg/L continuous | | 10 mg/L | |

**Table 4:** Initial kill rate over 4 h (KR4) and bacterial kill at 24 h (BK24) for all antimicrobial regimens.

[where cfu is colony forming unit, data are mean ± standard deviation]

| **Antimicrobial** | **KR4 ($log_{10}$ cfu/mL/h)** | **BK24 ($log_{10}$ cfu/mL)** |
|---|---|---|
| **Vancomycin** | 0.32 ± 0.18 | 2.89 ± 1.32 |
| **Daptomycin** | 0.51 ± 0.36 | -0.22 ± 1.39 |
| **Linezolid** | 0.04 ± 0.13 | 0.98 ± 0.82 |
| **Ceftobiprole** | 0.26 ± 0.16 | 3.14 ± 1.18 |
| **Vancomycin - Ceftobiprole** | 0.23 ± 0.12 | 4.03 ± 0.65 |
| **Daptomycin - Ceftobiprole** | 0.47 ± 0.35 | 3.85 ± 0.91 |
| **Linezolid - Ceftobiprole** | 0.03 ± 0.05 | 1.75 ± 0.53 |

**Table 5:** Comparisons of initial kill rate over 4 h (KR4) for (A) antimicrobial monotherapy and (B) combinations.

[where V is vancomycin, D is daptomycin, L is linezolid and C is ceftobiprole; values in brackets are means, and bolded P values are statistically significant differences]

**A)**

| **KR4 ($log_{10}$ cfu/mL/h)** | **Vancomycin** (0.32) | **Daptomycin** (0.51) | **Linezolid** (0.04) | **Ceftobiprole** (0.26) |
|---|---|---|---|---|
| **Vancomycin** (0.32) | | P = 0.14 | **P = 0.002 (V > L)** | P = 0.99 |
| **Daptomycin** (0.51) | P = 0.14 | | **P < 0.0001 (D > L)** | **P = 0.03 (D > C)** |
| **Linezolid** (0.04) | **P = 0.002 (V > L)** | **P < 0.0001 (D > L)** | | **P = 0.046 (C > L)** |
| **Ceftobiprole** (0.26) | P = 0.99 | **P = 0.03 (D > C)** | **P = 0.046 (C > L)** | |

**B)**

| **KR4 ($log_{10}$ cfu/mL/h)** | **Vancomycin** (0.32) | **Daptomycin** (0.51) | **Linezolid** (0.04) | **Ceftobiprole** (0.26) |
|---|---|---|---|---|
| **Vancomycin - Ceftobiprole** (0.23) | P = 0.92 | | | P = 0.99 |
| **Daptomycin - Ceftobiprole** (0.47) | | P = 0.99 | | P = 0.18 |
| **Linezolid - Ceftobiprole** (0.03) | | | P = 0.99 | **P = 0.046 (C > L-C)** |

**Table 6:** Comparisons of bacterial kill at 24 h (BK24) for (A) antimicrobial monotherapy and (C) combinations.

[where V is vancomycin, D is daptomycin, L is linezolid and C is ceftobiprole; values in brackets are means with positive values representing net bacterial kill and negative values representing net bacterial growth, and bolded P values are statistically significant differences]

**A)**

| **BK24 ($log_{10}$ cfu/mL)** | **Vancomycin** (2.89) | **Daptomycin** (-0.22) | **Linezolid** (0.98) | **Ceftobiprole** (3.14) |
|---|---|---|---|---|
| **Vancomycin** (2.89) | | **P = 0.0001 (V > D)** | **P < 0.0001 (V > L)** | P = 0.99 |
| **Daptomycin** (-0.22) | **P = 0.0001 (V > D)** | | **P = 0.014 (L > D)** | **P = 0.0001 (C > D)** |
| **Linezolid** (0.98) | **P < 0.0001 (V < L)** | **P = 0.014 (L > D)** | | **P < 0.0001 (C > L)** |
| **Ceftobiprole** (3.14) | P = 0.99 | **P = 0.0001 (C > D)** | **P < 0.0001 (C > L)** | |

**B)**

| **BK24 ($log_{10}$ cfu/mL)** | **Vancomycin** (2.89) | **Daptomycin** (-0.22) | **Linezolid** (0.98) | **Ceftobiprole** (3.14) |
|---|---|---|---|---|
| **Vancomycin -Ceftobiprole** (4.03) | P = 0.05 | | | P =0.30 |
| **Daptomycin - Ceftobiprole** (3.85) | | **P = 0.0001 (D-C > D)** | | P = 0.59 |
| **Linezolid - Ceftobiprole** (1.75) | | | P = 0.33 | **P = 0.005 (C > L-C)** |

**Table 7:** Comparisons of standard versus altered environmental conditions on (A) initial kill rate over 4 h (KR4) and (B) bacterial kill at 24 h (BK24) for all antimicrobial regimens.

**A)**

| KR4 ($log_{10}$ cfu/mL/h) | Standard conditions [Mean ± sd ($CI_{95\%}$)] | Altered conditions [Mean ± sd ($CI_{95\%}$)] | Mean difference | P value |
|---|---|---|---|---|
| **Vancomycin** | 0.27 ± 0.08 (0.21 - 0.33) | 0.37 ± 0.24 (0.19 - 0.54) | -0.09 | 0.24 |
| **Daptomycin** | 0.79 ± 0.19 (0.64 - 0.95) | 0.22 ± 0.22 (0.03 - 0.39) | 0.58 | **0.0001** |
| **Linezolid** | 0.04 ± 0.17 (-0.08 - 0.16) | 0.04 ± 0.09 (-0.02 - 0.11) | -0.002 | 0.97 |
| **Ceftobiprole** | 0.36 ± 0.15 (0.24 - 0.48) | 0.17 ± 0.13 (0.06 - 0.27) | 0.19 | **0.014** |
| **Vancomycin-Ceftobiprole** | 0.31 ± 0.10 (0.19 - 0.41) | 0.15 ± 0.09 (0.05 - 0.25) | 0.16 | **0.02** |
| **Daptomycin-Ceftobiprole** | 0.73 ± 0.31 (0.41 - 1.06) | 0.19 ± 0.08 (0.11 - 0.28) | 0.54 | **0.002** |
| **Linezolid-Ceftobiprole** | 0.05 ± 0.04 (0.01 - 0.09) | 0.01 ± 0.06 (-0.04 - 0.06) | 0.05 | 0.12 |

**B)**

| BK24 ($log_{10}$ cfu/mL) | Standard conditions [Mean ± sd ($CI_{95\%}$)] | Altered conditions [Mean ± sd ($CI_{95\%}$)] | Mean difference | P value |
|---|---|---|---|---|
| **Vancomycin** | 2.87 ± 1.42 (1.85 - 3.88) | 2.91 ± 1.29 (1.98 - 3.83) | -0.04 | 0.95 |
| **Daptomycin** | 0.48 ± 1.64 (-0.89 - 1.84) | -0.91 ± 0.59 (-1.41 - -0.41) | 1.39 | **0.04** |
| **Linezolid** | 1.01 ± 0.82 (0.43 - 1.59) | 0.95 ± 0 .85 (0.33 - 1.56) | 0.06 | 0.87 |
| **Ceftobiprole** | 3.33 ± 1.33 (2.21 - 4.43) | 2.95 ± 1.07 (2.06 - 3.84) | 0.37 | 0.54 |
| **Vancomycin-Ceftobiprole** | 3.82 ± 0.75 (3.02 - 4.61) | 4.25 ± 0.49 (3.74 - 4.76) | -0.43 | 0.27 |
| **Daptomycin-Ceftobiprole** | 3.86 ± 1.10 (2.71 - 5.02) | 3.83 ± 0.77 (3.02 - 4.65) | 0.03 | 0.95 |
| **Linezolid-Ceftobiprole** | 1.68 ± 0.55 (1.22 - 2.13) | 1.83 ± 0.54 (1.37 - 2.28) | -0.15 | 0.59 |

**Table 8:** Inter-isolate comparisons of (A) initial kill rate over 4 h (KR4) and (B) bacterial kill at 24 h (BK24) for all antimicrobial regimens.

**A)**

| KR4 ($log_{10}$ cfu/mL/h) | # 81655 [Mean ± sd ($CI_{95\%}$)] | # 79002 [Mean ± sd ($CI_{95\%}$)] | Mean Difference | P value |
|---|---|---|---|---|
| **Vancomycin** | 0.412 ± 0.21 (0.26 - 0.56) | 0.22 ± 0.07 (0.17 – 0.27) | 0.19 | **0.02** |
| **Daptomycin** | 0.52 ± 0.42 (0.17 - 0.87) | 0.49 ± 0.32 (0.23 - 0.75) | 0.03 | 0.87 |
| **Linezolid** | 0.03 ± 0.07 (-0.02 - 0.07) | 0.06 ± 0.18 (-0.07 - 0.18) | -0.03 | 0.63 |
| **Ceftobiprole** | 0.35 ± 0.18 (0.19 - 0.50) | 0.18 ± 0.09 (0.09 - 0.36) | 0.18 | **0.031** |
| **Vancomycin-Ceftobiprole** | 0.28 ± 0.12 (0.15 - 0.41) | 0.18 ± 0.11 (0.06 - 0.29) | 0.1 | 0.17 |
| **Daptomycin-Ceftobiprole** | 0.59 ± 0.37 (0.20 - 0.98) | 0.34 ± 0.31 (0.01 - 0.67) | 0.26 | 0.23 |
| **Linezolid-Ceftobiprole** | 0.003 ± 0.06 (-0.04 - 0.05) | 0.06 ± 0.04 (0.02 - 0.09) | -0.06 | 0.053 |

**B)**

| BK24 ($log_{10}$ cfu/mL) | # 81655 [Mean ± sd ($CI_{95\%}$)] | # 79002 [Mean ± sd ($CI_{95\%}$)] | Mean Difference | P Value |
|---|---|---|---|---|
| **Vancomycin** | 2.78 ± 1.22 (1.91 - 3.65) | 3 ± 1.48 (1.93 - 4.06) | -0.22 | 0.72 |
| **Daptomycin** | 0.44 ± 1.59 (-0.89 - 1.77) | -0.88 ± 0.79 (-1.54 - -0.21) | 1.31 | 0.06 |
| **Linezolid** | 0.84 ± 0.92 (0.18 - 1.50) | 1.12 ± 0.71 (0.61 - 1.63) | -0.28 | 0.46 |
| **Ceftobiprole** | 2.78 ± 1.17 (1.79 - 3.75) | 3.5 ± 1.15 (2.54 - 4.46) | -0.73 | 0.23 |
| **Vancomycin-Ceftobiprole** | 4.02 ± 0.44 (3.56 - 4.47) | 4.05 ± 0.85 (3.15 - 4.95) | -0.03 | 0.93 |
| **Daptomycin-Ceftobiprole** | 3.75 ± 1.02 (2.68 - 4.82) | 3.95 ± 0.87 (3.03 - 4.87) | -0.19 | 0.72 |
| **Linezolid-Ceftobiprole** | 1.53 ± 0.35 (1.24 - 1.81) | 1.98 ± 0.61 (1.47 - 2.48) | -0.45 | 0.09 |

**Figure 1:** *In Vitro* Pharmacodynamic Model (IPDM).

[where A is a 4 liter flask containing fresh cation-supplemented Mueller Hinton broth (and 10 mg/L of ceftobiprole in select experiments), B is a computerized pump simulating antimicrobial half- life (*t*½) of 7 hours, C is an infection flask into which 2.5 mL of 0.5 McFarland standard of bacterial suspension is injected, and D is a waste flask.

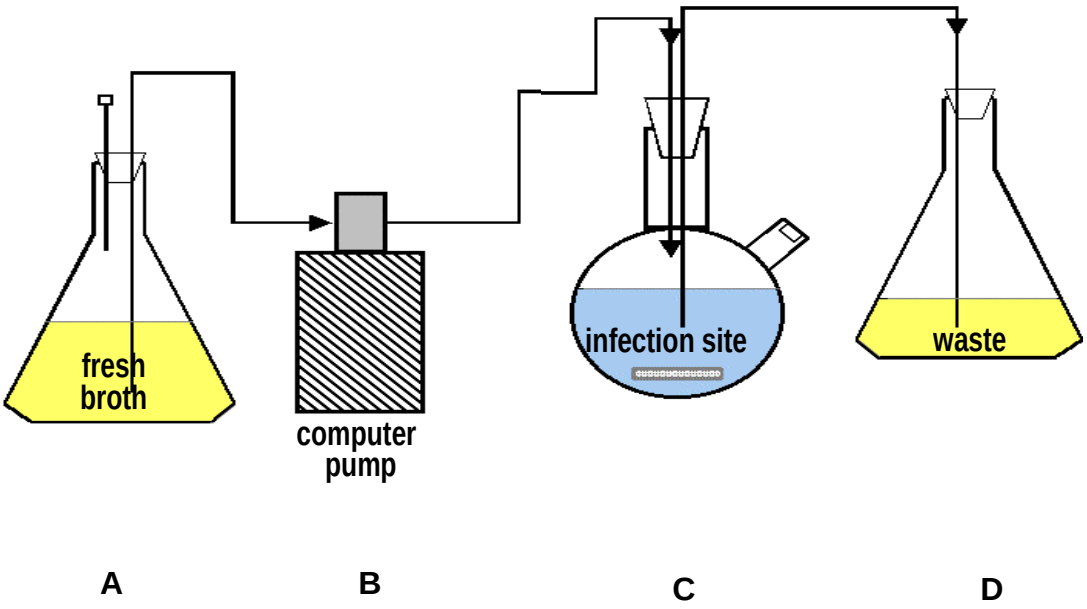


**Figure 2:** Comparisons of growth controls over 24 hours of two clinical MRSA isolates under standard and altered conditions.

[Where dark grey bars are HCA-MRSA (#81655) and light grey bars are CA-MRSA (#79002); Standard is temperature of 37°C, pH of 7 and undiluted cation-supplemented Mueller-Hinton Broth (CSMHB); and Altered is combined temperature of 40°C, pH of 5.5-6 and CMHB diluted 1:2)]

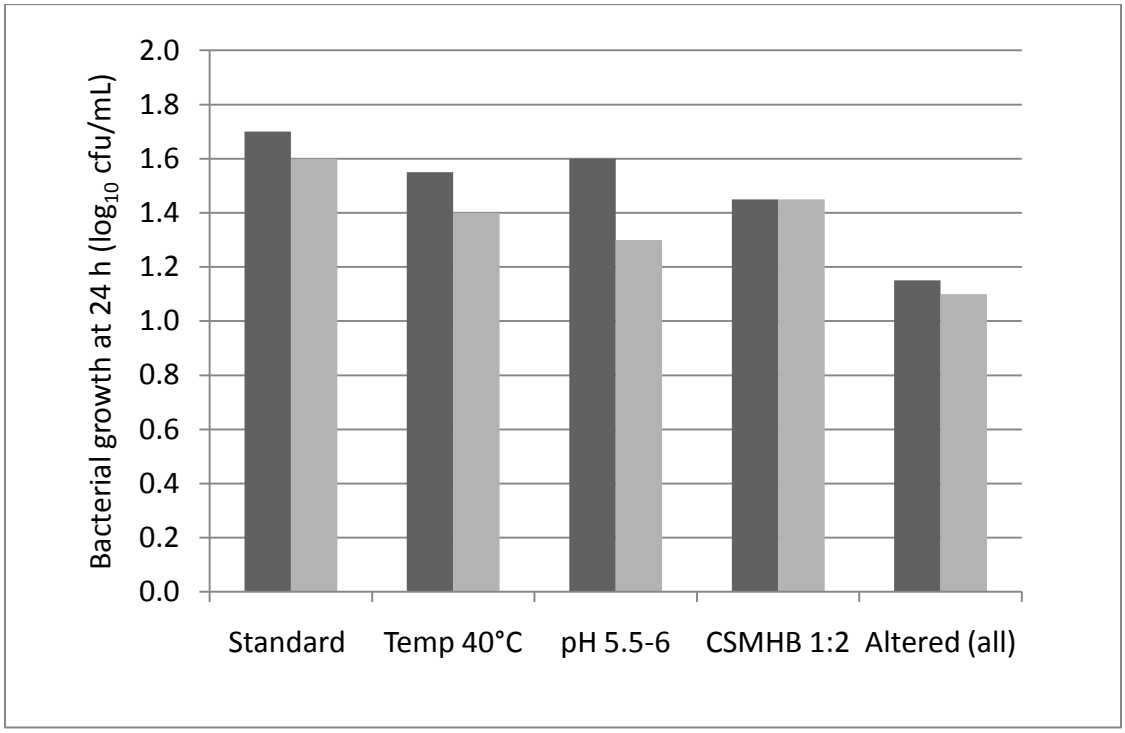
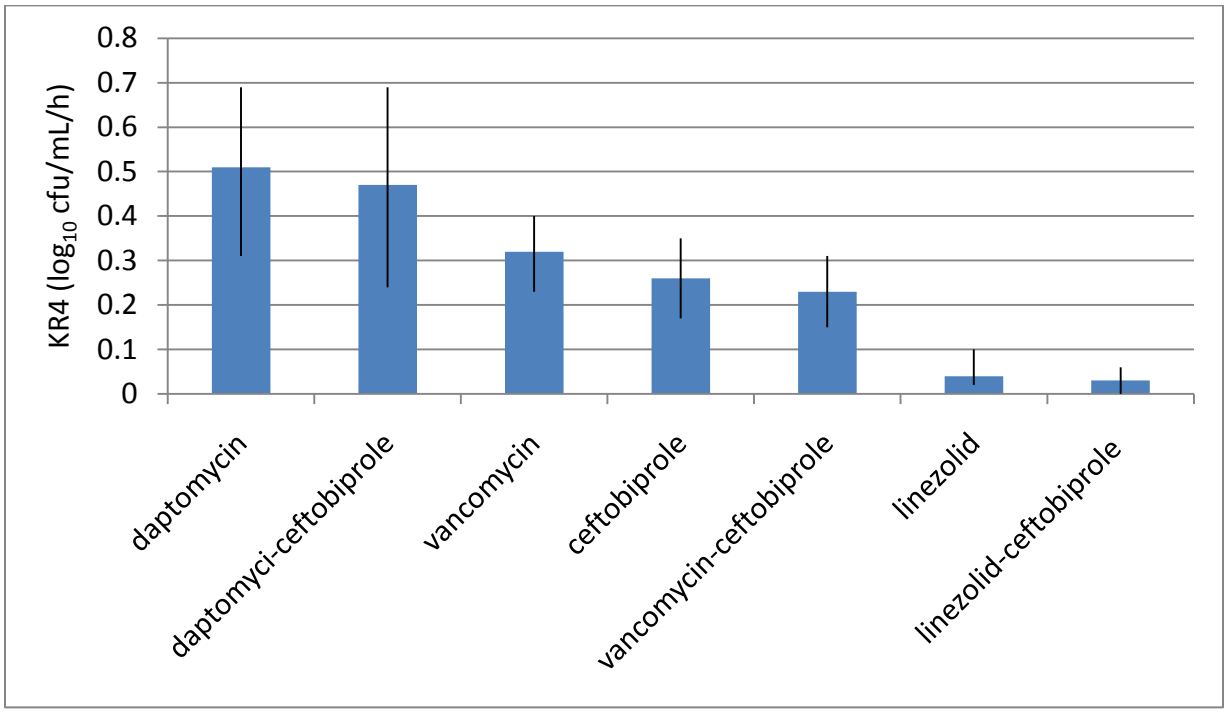


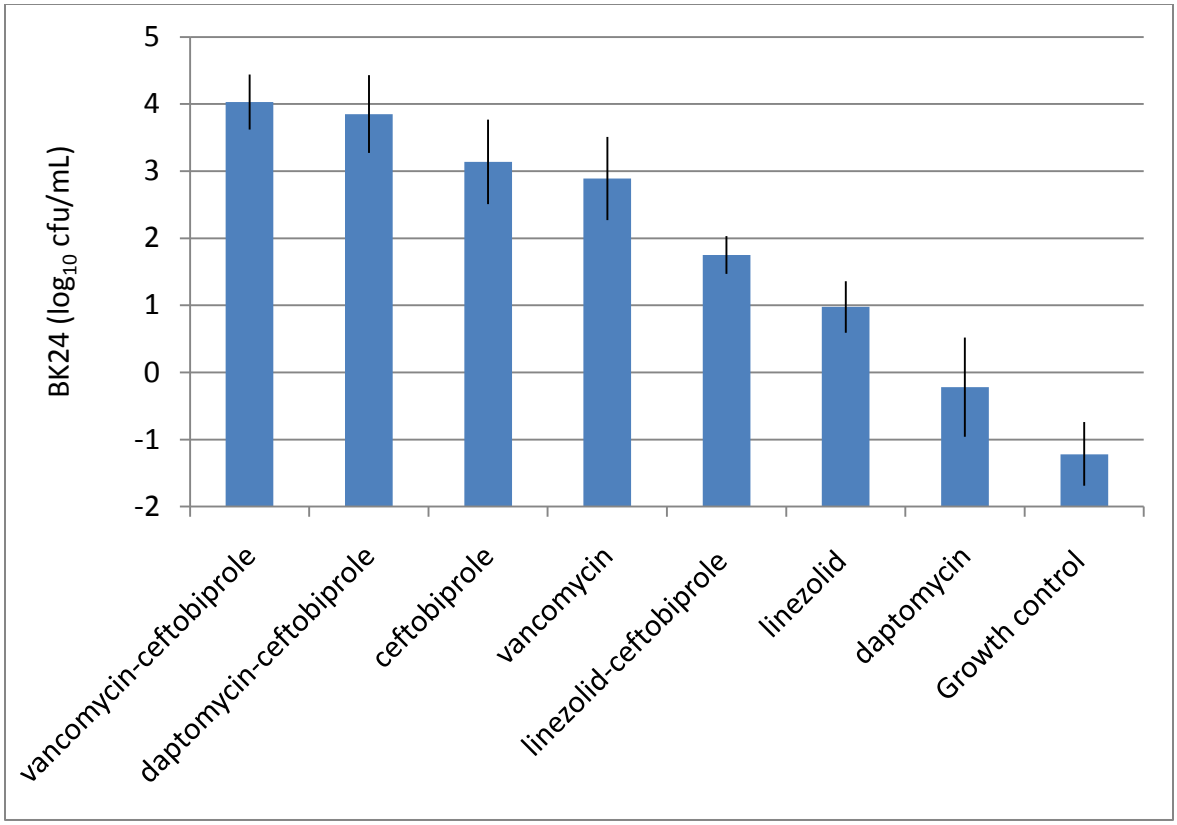
**Figure 3:** Comparisons of (A) initial kill rate over 4 h (KR4) and (B) bacterial kill at 24 h (BK24) for all antimicrobial regimens.

[Were values are means with 95% confidence intervals]

**A)**



**B)**


5
4
3
2
1
0
-1
-2
BK24 (log10 cfu/mL)
vancomycin-ceftobiprole
daptomycin-ceftobiprole
ceftobiprole
vancomycin
linezolid-ceftobiprole
linezolid
daptomycin
Growth control

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