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Pronounced heterogeneity observed in high-level daptomycin-resistant viridans group streptococci☆

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ABSTRACT

Viridans group streptococci (VGS) have demonstrated high-level daptomycin resistance (HLDR) upon daptomycin exposure. This study evaluated the extent of heterogeneity and whether dose escalation or combination therapy could prevent resistance development. Five VGS strains (daptomycin MICs 0.25–2 mg/L) were evaluated. *In vitro* models utilised simulated daptomycin dosages of 4, 6, 8 and 12 mg/kg with estimated fC_{max} of 4.1, 6.6, 8.6 and 12.9 mg/L, respectively. Time–kill studies included fC_{max} simulations of daptomycin alone or combined with ceftriaxone, gentamicin, linezolid, rifampicin or vancomycin. Population analyses were performed on daptomycin-containing and non-containing agar plates. Extreme heterogeneity was observed in four strains with daptomycin population MICs 4–512-fold higher than broth microdilution. Whilst *Streptococcus gordonii* 1649 did not consistently develop HLDR, its population MIC was above the established daptomycin breakpoint. *In vitro* modelling demonstrated initial kill by daptomycin in all strains within 8 h, with substantial re-growth by 24 h despite increasing daptomycin. Daptomycin kill curves also displayed resistance development by 24 h. However, synergy or additivity was noted for most regimens and strains. Synergy was most notable with daptomycin plus linezolid or rifampicin. Overall, daptomycin plus ceftriaxone or gentamicin were the most potent regimens. Gentamicin or rifampicin with daptomycin were least additive. For combination regimens with colonies isolated at 24 h, HLDR was reduced 16–64-fold (MICs 4–16 mg/L). Daptomycin monotherapy for VGS led to rapid development of HLDR likely due to extreme heterogeneity. Combination therapy suppressed or minimised the degree of resistance although the mechanism remains unknown.

1. Introduction

Gram-positive bacteria comprise three of the top four pathogens associated with hospital-acquired infections [1]. In addition, the Infectious Diseases Society of America (IDSA) has promoted The 10 × '20 Initiative, which aims to produce 10 new drugs by the year 2020 to combat the 'ESKAPE' pathogens, two of which are Gram-positives (*Staphylococcus aureus* and enterococci) [2]. Whilst viridans group streptococci (VGS) are not included by the IDSA and have not historically demonstrated significant antimicrobial resistance, recent *in vitro* and *in vivo* resistance has been noted for antibiotics typically active against multidrug-resistant organisms. As resistance to antimicrobials continues to increase, the pipeline for new agents is scant; therefore, we are forced to rely upon older agents or combination therapies.

Historically, VGS were highly susceptible to antimicrobial agents, including β-lactams, macrolides and fluoroquinolones [3–7]. Although resistance in many patient populations remains low, an increased frequency of resistance to these agents, particularly penicillin, has been noted in neutropenic patients, often resulting in severe illness/bacteraemia [8]. Broad-spectrum

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agents, such as vancomycin, linezolid and daptomycin, have also had reports of resistance development in these organisms [9–15].

Daptomycin, a cyclic lipopeptide, displays potent activity against Gram-positive organisms and often retains activity despite-resistance or increased minimum inhibitory concentrations (MICs) to alternative therapies. In fact, the daptomycin MIC_{90} values for *S. aureus*,*Enterococcus faecium*, *Enterococcus faecalis* and *Streptococcus* spp. are 0.5, 4, 2 and 0.25 mg/L, respectively [16]. Despite incredibly low MICs for streptococci, case reports involving breakthrough bacteraemia or lack of eradication of organisms despite appropriate daptomycin concentrations have been described [17,18].

With clinical failures arising, *in vitro* studies have been conducted to determine the frequency and extent of resistance development. García-de-la-Mària et al. evaluated a selection of 92 *Streptococcus mitis* group strains to determine the extent of resistance including high-level daptomycin resistance (HLDR) [12]. Upon exposure to daptomycin, nearly two-thirds of the strains developed daptomycin resistance and 27% displayed HLDR (defined as increased daptomycin MICs to ≥256 mg/L). Similarly, our laboratory utilised *in vitro* pharmacokinetic/pharmacodynamic (PK/PD) modelling simulating human daptomycin exposures of 6 mg/kg and 8 mg/kg daily, which produced comparable results against four clinical VGS strains, with all isolates developing HLDR despite baseline MICs of 1 mg/L and 2 mg/L [15]. With HLDR in organisms that would normally be expected to remain fairly susceptible to daptomycin, it is crucial to determine potential mechanisms for the development of this resistance. Oftenorganisms display significant heterogeneity, with high-level-resistant subpopulations impacting antibiotic susceptibility. To date, population analysis of VGS, in particular to daptomycin, has not been conducted. In addition, as noted with previous studies with HLDR VGS, combination therapy with gentamicin and/or rifampicin may provide synergistic activity. However, the extent of kill or combination with other agents was not examined. Therefore, the objectives of this study were to evaluate the extent of heterogeneity of various VGS species, dose–response relationships (including low and high doses) and whether combination therapy prevents the emergence of daptomycin-non-susceptibility or minimises the level of resistance developed (i.e. only slight MIC increases).

2. Materials and methods

2.1. Bacterial strains

Five VGS isolates were analysed in this study. Four strains were clinical isolates obtained from archived cultures from the SENTRY Surveillance Program (JMI Laboratories, North Liberty, IA) collected from patients with infective endocarditis between 1999 and 2003, including *S. mitis* 1643, *Streptococcus oralis* 1647 and 1648, and *Streptococcus gordonii* 1649. These strains represented high wild-type MIC strains associated with invasive disease. In addition, the reference strain *S. oralis* ATCC 35037 was evaluated, which was included to provide a more daptomycin-susceptible comparative isolate.

2.2. Antimicrobials

Daptomycin analytical powder (lots CDCX01 and MCB2009) was provided by Cubist Pharmaceuticals, Inc. (Lexington, MA). Ceftriaxone, gentamicin, linezolid, penicillin, rifampicin and vancomycin were commercially purchased from each respective manufacturer.

2.3. Media

Cation-adjusted Mueller–Hinton broth (CA-MHB) (Difco, Detroit, MI) was used in all experiments and was supplemented to a calcium concentration of 50 mg/L and a magnesium concentration of 12.5 mg/L [15]. Colony counts were performed on tryptic soy agar with 5% sheep blood (TSA-SB) (Becton, Dickinson & Co., Sparks, MD).

2.4. Susceptibility testing

MICs were determined by broth microdilution of 5×10^5 CFU/mL as per Clinical and Laboratory Standards Institute guidelines (CLSI) [19] and Etest (bioMérieux, Inc., Durham, NC) according to the manufacturer's guidelines. All samples were incubated at 37 °C for 24 h.

2.5. Population analysis profiles (PAPs)

For PAPs, 100 μL of a 10^{9-10} CFU/mL suspension of each strain, both daptomycin selective pressured and non-selective pressured, were placed onto CA-MHB plus 5% horse blood (CA-MHB + HB) plates containing daptomycin (concentrations ranging from 0.25 to 2048 mg/L). A higher inoculum was selected to ensure diversity of organism subpopulations. Susceptibility profiles were determined after 1, 5 and 10 serial daily passages of each organism. Selective pressure (SP) was defined as and applied via serial passage of organisms grown on daptomycin-containing CA-MHB + HB plates of 0.125, 0.5 or 1 mg/L (0.5× the daptomycin MIC of each respective organism). Non-selective pressure (NSP) was defined as serially passage of organisms grown on CA-MHA + HB without any antibiotic incorporated into the medium.

2.6. In vitro *pharmacokinetic/pharmacodynamic models*

A well-described, *in vitro*, one-compartment PK/PD model with a 250 mL capacity and input and outflow ports was used [20,21]. Briefly, the apparatus was utilised over a 48-h time period. A starting inoculum of ca. $1 \times 10^{6-7}$ CFU/mL was targeted for each experiment with fresh medium continuously supplied and removed via a peristaltic pump (Masterflex; Cole-Parmer Instrument Co., Chicago, IL). The following daptomycin exposures, utilising free drug concentrations assuming 93% protein binding with a target half-life ($t_{1/2}$) of 8 h, were evaluated: 4 mg/kg [peak concentration of unbound daptomycin (fC_{max}), 4.1 mg/L]; 6 mg/kg (fC_{max}, 6.6 mg/L); 8 mg/kg (fC_{max}, 8.6 mg/L); 12 mg/kg (fC_{max}, 12.9 mg/L); and drug-free growth control. The models were performed in duplicate to ensure reproducibility.

2.7. Pharmacodynamic analysis

Samples were removed at 0, 4, 8, 24, 32 and 48 h and were serially diluted in cold 0.9% sodium chloride. Bacterial counts were determined with a lower limit of reliable detection of 2 $\log_{10}$ CFU/mL. Time–kill curves were constructed by plotting mean colony counts ($\log_{10}$ CFU/mL) versus time. Bactericidal activity was defined as a ≥3 $\log_{10}$ CFU/mL reduction from baseline. Emergence of resistance was evaluated at 48 h by plating 100 μL samples from the model on plates supplemented with daptomycin at a concentration 4× and 8× the daptomycin MIC. Plates were examined for growth after 48 h of incubation at 37 °C. Resistant colonies growing on screening plates were evaluated by Etest and broth microdilution methods to determine the mutant MIC.

2.8. Pharmacokinetic analysis

Pharmacokinetic samples were obtained through the injection port over the experimental time period for verification of target antibiotic concentrations. All samples were stored at −80 °C until analysis. Concentrations of daptomycin were determined by