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Short Communication

# Mutant prevention concentrations of daptomycin for *Enterococcus faecium* clinical isolates

Clara Sinel [a], Clara Jaussaud [a], Michel Auzou [b,c], Jean-Christophe Giard [a], Vincent Cattoir [a,b,c,*]

[a] Université de Caen Normandie, EA4655 (Équipe 'Antibio-résistance'), F-14032 Caen, France
[b] CHU de Caen, Service de Microbiologie, F-14033 Caen, France
[c] Centre National de Référence (CNR) sur la résistance aux antibiotiques (Laboratoire associé 'Entérocoques'), F-14033 Caen, France

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ABSTRACT

Owing to the emergence of vancomycin-resistant *Enterococcus faecium*, treatment of enterococcal infections has become challenging. Although spontaneous in vitro resistance frequencies are low, the emergence of resistance is increasingly reported during daptomycin therapy. The mutant selection window (MSW), comprised between the minimum inhibitory concentration (MIC) and the mutant prevention concentration (MPC), corresponds to the concentration range within which resistant mutants may be selected. Since no data are available for enterococci, the aim of this study was to determine MPCs and MSWs for 12 representative *E. faecium* clinical isolates. MICs and MPCs were determined by broth microdilution and agar dilution methods, respectively. A basic MSW-derived pharmacodynamic analysis was also performed using mean maximum plasma concentration ($C_{max}$) values obtained with dosages from 4 to 12 mg/kg. MICs and MPCs of daptomycin ranged from 0.5 to 4 mg/L and from 2 to 32 mg/L, respectively, with no correlation between them. The wideness of MSWs ranged from 2× to 32× MIC. Mean plasma $C_{max}$ values of daptomycin were calculated from 55 to 174.5 mg/L when using a dosage from 4 to 12 mg/kg. All $C_{max}$ values were above the MPCs whatever the dosage. Taking into account the protein binding of daptomycin (ca. 90%), the unbound fraction $C_{max}$ was just within the MSW in 67–92% of strains at recommended dosages (4–6 mg/kg) and was above the MPC for the majority of strains only with the highest dosage (12 mg/kg). This study shows that free daptomycin $C_{max}$ values usually fell into MSWs when using lower dosages (<10 mg/kg).



## 1. Introduction

Although considered harmless members of the human intestinal microbiota, enterococci have become a leading cause of a wide range of hospital-acquired infections [1]. Notably, there has been an emergence of vancomycin-resistant enterococci (VRE) clinical isolates, especially within the species *Enterococcus faecium*, with the worldwide spread of a specific lineage of hospital-adapted clonal complex 17 (CC17) clones [2].

Daptomycin (DAP) is the first licensed cyclic lipopeptide antibiotic that is active against a broad spectrum of Gram-positive pathogens, including VRE [3]. DAP acts by altering bacterial cell envelope homeostasis through an irreversible calcium-dependent interaction with phospholipids of the cell membrane, leading to the formation of pores with subsequent intracellular potassium ion release, membrane depolarisation and cell death [4]. DAP exerts an in vitro bactericidal activity that is concentration-dependent [pharmacodynamic parameter, ratio of maximum concentration ($C_{max}$) to minimum inhibitory concentration (MIC)] and has a long half-life (ca. 8–9 h) [3].

Despite lacking approval by regulatory agencies, DAP constitutes an alternative therapeutic option commonly used in the treatment of VRE infections [5]. Whereas doses of 4 mg/kg and 6 mg/kg are approved by the US Food and Drug Administration (FDA), numerous in vitro and animal data suggest that higher doses (up to 12 mg/kg) may improve DAP bactericidal activity and reduce the risk of emergence of resistance whilst drug tolerance is well conserved [3,6]. Although the development of DAP resistance has so far remained uncommon in enterococci (spontaneous in vitro resistance frequencies of ca. $10^{-9}$), failures of DAP therapy are now increasingly reported with the emergence of high-level resistance [7].

Selective enrichment and amplification of a resistant subpopulation is proposed to arise when the antimicrobial concentration falls into a specific range, called the mutant selection window (MSW) [8]. The upper boundary of the MSW corresponds to the mutant prevention concentration (MPC), defined as the MIC of the least susceptible single-step mutant in a large bacterial population, whilst

* Corresponding author. CHU de Caen, Service de Microbiologie, Av. Côte de Nacre, 14033 Caen Cedex 9, France. Fax: +33 2 31 06 45 73.
*E-mail address:* cattoir-v@chu-caen.fr (V. Cattoir).

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the lower boundary is the actual MIC [8]. In practice, the MPC is approximated as the drug concentration at which no colony is recovered when a high inoculum (ca. $10^9$–$10^{10}$ cells) is applied onto drug-containing agar plates [8]. The MSW concept appears applicable for evaluation of antibiotics for which the primary resistance mechanism consists of chromosomal point mutations. This is the reason why most MPC studies have been conducted with fluoroquinolones [9]. Since DAP resistance solely results from chromosomal mutations, the MPC concept is likely to be relevant for this antibiotic and has been confirmed in three studies in *Staphylococcus aureus* [10–12]. In contrast, no evaluation is available in enterococci.

The aims of this study were therefore (i) to determine the MPCs and MSWs for a collection of representative *E. faecium* clinical isolates and (ii) to evaluate theoretically the risk of in vivo DAP resistance by comparing maximum plasma concentration ($C_{max}$) values of DAP (calculated depending on human dosages) and MSW values.

## 2. Materials and methods

### 2.1. Bacterial isolates

In addition to the *vanB*-positive reference strain *E. faecium* Aus0004 [13], 11 *E. faecium* clinical isolates received between 2006 and 2010 at the French National Reference Center for Enterococci (Caen, France) were included in the study. Species-level identification was determined using matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS) technology (Microflex; Bruker Daltonics, Bremen, Germany).

Determination of the presence of vancomycin resistance genes was carried out by multiplex and classical PCR assays as previously described [14]. Multilocus sequence typing (MLST) assays were performed as previously described [15] and different allelic profiles were assigned to sequence types (STs) based on the *E. faecium* MLST database (http://efaecium.mlst.net).

### 2.2. Antimicrobial susceptibility testing and determination of the mutant prevention concentration (MPC)

MICs of DAP were determined using the broth microdilution method according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (http://www.eucast.org/). *S. aureus* ATCC 25922, *S. aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212 were used as quality control strains.

MPCs of DAP were determined as described elsewhere for *S. aureus* with some minor modifications [10]. Briefly, the tested strains were cultured in brain–heart infusion broth and were incubated for 24 h. The suspension was then centrifuged at 4000× *g* for 10 min and the pellet was re-suspended in Mueller–Hinton broth to achieve a concentration of ca. $10^{11}$ CFU/mL. Agar plates containing DAP concentrations from 0 to 128 mg/L (with a concentration adjusted to 50 mg/L of calcium) were inoculated with ca. $10^{10}$ CFU of *E. faecium* bacterial cells. The plates were then incubated for 72 h at 37 °C and growth was visually screened each day. The MPC was recorded as the lowest DAP concentration that completely inhibited growth. All experiments were performed in triplicate.

### 2.3. Mutant prevention concentration (MPC)-derived pharmacodynamics analysis

The MSW was defined as the concentration range between the MIC and MPC values (MPC/MIC) and was expressed as fold MIC. Mean ± standard deviation values of DAP $C_{max}$ were calculated according to dosages ranging from 4 to 12 mg/kg [16–18]. Taking into account a protein binding of ca. 90%, the unbound fraction (i.e. 10%) of DAP $C_{max}$ was used to compare with each MPC value.

## 3. Results and discussion

The collection of 12 *E. faecium* strains was diverse in terms of STs (3 ST17, 1 ST18, 2 ST78, 1 ST192, 3 ST203, 1 ST280 and 1 ST323) and *van* genotypes (4 *vanA*, 6 *vanB* and 2 *van*-negative) (Table 1) and was representative of VRE clinical isolates recovered from human samples in France [14].

The MICs of DAP for the 12 *E. faecium* clinical isolates ranged from 0.5 mg/L to 4 mg/L ($MIC_{50}$ and $MIC_{90}$, 2 mg/L and 4 mg/L, respectively) (Table 1), which is consistent with previous epidemiological surveys ($MIC_{90}$, 2–4 mg/L) [3]. Using the Clinical and Laboratory Standards Institute (CLSI) susceptibility breakpoint (≤4 mg/L), 100% of strains were categorised as susceptible to DAP. It is important to note that some therapy failures have been reported in patients suffering from bloodstream infections caused by *E. faecium* isolates exhibiting DAP MICs at the higher end of the susceptibility range (3–4 mg/L). Indeed, it has been proposed that an MIC of 3–4 mg/L may be an indicator of possible treatment failure, likely corresponding to a first-step mutant predisposed to develop in vivo high-level resistance [19].

The range of MPC values for the 12 *E. faecium* clinical isolates was from 4 mg/L to 32 mg/L ($MPC_{50}$ and $MPC_{90}$, 4 mg/L and 16 mg/L, respectively), with a wide range of MSWs from 2× to 32× MIC (Table 1). These MPC and MSW values were similar to those reported for *S. aureus* in some studies: MPCs of 10–20 mg/L [11]; and $MPC_{50}$ of 32 mg/L, $MPC_{80}$ of 64 mg/L, and MSW >64× MIC [12]. Note that other authors estimated in *S. aureus* much lower MPCs and MSWs, at 1.1–5.5 mg/L and 3.1–5× MIC, respectively [10]. Also, the current results showed that there was a poor correlation between MIC and MPC values, as has been observed for a variety of fluoroquinolones with different bacterial species [8]. Altogether, using the MIC is likely to be inaccurate to predict the corresponding MPC, including for DAP in *E. faecium*. In addition, the MPC may also help explain the generation of resistant mutants and thus may represent a more reliable surrogate marker than MIC for the risk of treatment failure.

Mean plasma $C_{max}$ values of DAP ranged from 55 mg/L to 174.5 mg/L when using a dosage from 4 mg/kg to 12 mg/kg, with a linear dose-proportional relationship (Fig. 1), as previously shown over the 6–12 mg/kg dose range [18]. Interestingly, all $C_{max}$ values were above the MPCs for all tested isolates whatever the dosage. Even if simulation of free DAP concentrations is not well founded,

**Table 1**
Genotypic characteristics of 12 *Enterococcus faecium* strains and its corresponding minimum inhibitory concentrations (MICs) and mutant prevention concentrations (MPCs) for daptomycin.

| Strain | ST | *van* type | Daptomycin | | MPC/MIC (MSW[a]) |
|---|---|---|---|---|---|
| | | | MIC (mg/L) | MPC (mg/L) | |
| 08–807 | 17 | *vanA* | 2 | 8 | 4 |
| 09–001 | 17 | *vanB* | 2 | 16 | 8 |
| Aus0004 | 17 | *vanB* | 2 | 8 | 4 |
| 06–087 | 18 | – | 1 | 4 | 4 |
| 06–047 | 78 | *vanA* | 4 | 16 | 4 |
| 09–038 | 78 | *vanB* | 4 | 16 | 4 |
| 08–225 | 192 | *vanB* | 1 | 16 | 16 |
| 07–018 | 203 | *vanB* | 0.5 | 16 | 32 |
| 07–103 | 203 | *vanB* | 1 | 16 | 16 |
| 09–087 | 203 | *vanA* | 1 | 16 | 16 |
| 09–122 | 280 | *vanA* | 4 | 32 | 8 |
| 10–035 | 323 | – | 4 | 8 | 2 |

ST, sequence type.
[a] Mutant selection window (MSW) comprised between the MIC and MPC.