Emerging issues on *Staphylococcus aureus* endocarditis and the role in therapy of daptomycin plus fosfomycin

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Abstract

Introduction: Methicillin-resistant and -susceptible *Staphylococcus aureus* (MRSA /MSSA) infections are a major global healthcare problem. Bacteremia with *S. aureus*, exhibits high rates of morbidity and mortality and can cause complicated infections such as infective endocarditis (IE). The emerging resistance profile of *S. aureus* is worrisome, and several international agencies have appealed for new treatment approaches to be developed.

Areas covered: Daptomycin presents a rapid bactericidal effect against MRSA and has been considered at least as effective as vancomycin in treating MRSA bacteremia. However, therapy failure is often related to deep-seated infections, e.g., endocarditis, with high bacterial inocula and daptomycin regimens <10 mg/kg/day. Current antibiotic options for treating invasive *S. aureus* infections have limitations in monotherapy. Daptomycin in combination with other antibiotics, e.g., fosfomycin, may be effective in improving clinical outcomes in patients with MRSA IE.

Expert opinion: Exploring therapeutic combinations has shown fosfomycin to have a unique mechanism of action and to be the most effective option in preventing the onset of resistance to and optimizing the efficacy of daptomycin, suggesting the synergistic combination of fosfomycin with daptomycin is a useful alternative treatment option for MSSA or MRSA IE.

Keywords: methicillin-sensitive *Staphylococcus aureus*, methicillin-resistent *Staphylococcus aureus*, daptomycin, fosfomycin, synergy, combined therapy, bactericidal activity, infective endocarditis**.**

Article highlights:

- *S. aureus* IE is a relatively rare but serious infection that, despite medical and surgical improvements, continues with an overall mortality of around 20% of the cases. This rate can increase up to 40% in the case of prosthetic valve IE.
- Choosing the best antibiotics for its combined treatment continues to be a challenge for clinicians, especially in the initial phase of the disease.
- Following the guidelines, beta-lactams and vancomycin traditionally constitute the cornerstone of antibiotic therapy recommended for methicillin susceptible and resistant *S. aureus* respectively. In the last decade, daptomycin combined therapies have emerged as a new wave option. It is generally accepted to use beta-lactams as coupled with daptomycin, and nowadays there is strong evidence that confirms it.
- Nevertheless in those cases of patients allergic to penicillin, the choice is very limited and in this scenario fosfomycin can be a good option for replacing beta-lactams antibiotics.
- This review includes the works published to date, and although it still includes a few preclinical studies, all of them highlight the synergistic and bactericidal activity of the combination, as well as the prevention of the appearance of resistant subpopulations.
- As regards the clinical studies carried out to date and reported in this review, the non-inferiority of the combination with respect to its comparators and its safety should be noted, although some side effects associated with the high salt load of fosfomycin have been described.
- Although reports are still scarce in number, the evidence suggests the synergistic combination of fosfomycin with daptomycin may be a useful treatment option in both methicillin-susceptible (MSSA) and methicillin-resistant *Staphylococcus aureus* (MRSA) IE.
- Additional studies reporting the pre-clinical and clinical efficacy and safety of this combination in the current era of high antimicrobial resistance are much needed to know the role of this combined treatment.

1. Introduction:

Infective endocarditis (IE) was described for the first time in 1646 by Lazare Riviere, a French medical doctor [1]. Since then it has been defined as an entity that affects multiple organ systems as a result of an infection caused by septic vegetations [2, 3] and inflammatory foci in the endocardium of the heart or equivalent prosthetic surfaces [4]. IE is a rare disease, so only few studies have addressed temporal trends in endocarditis incidence, that is considered low, with an estimated range from 1.5 to 11.6 cases per 100,000 persons [5, 6]. However, IE is still associated with a high morbidity and a mortality rate around 25% with rates varying considerably across different patient subgroups [7]. Usually the etiologic agents are bacteria although in a few cases it can also be fungi [8,9] and even viruses [10]. It is important to highlight that until the second half of the twentieth century, IE was most often caused by oral streptococci as a complication of rheumatic heart disease [11, 12] and also that, before antibiotics were available, mortality was uniform [2]. Nevertheless, over the last decades, there has been a switch, mainly in high-income countries [7, 13]. It has been detected an increase of risk factors such as intravenous drug use, degenerative valvular heart disease, prosthetic valve replacement, intracardiac electronic devices and congenital heart disease. Nowadays about 30% of the endocarditis cases are caused by staphylococci [10,11,14]. Table 1 shows the global epidemiology of the main pathogens involved in IE. (Data obtained from Murdoch et al, 2009 [14]). In developed countries, *S. aureus* is the first etiological agent causing up to 28-43% of all cases varying among epidemiological settings [7,14-17]. *S. aureus* IE presents high mortality rates, up to 40 % or more among patients with prosthetic valve IE [11,18] and worse prognosis [15]. It has also been demonstrated [19] that most cases of health care-associated native valve endocarditis, acquired inside or outside the hospital, were caused by *S. aureus*. Moreover, as *S. aureus* causes more destructive valve infections, the overall burden of this disease is certainly increasing [16]. In addition, almost one third of cases can be caused by methicillin-resistant (MRSA) strains [15].

S. aureus is a commensal bacterium colonizing roughly 30% of the human population [20] but *S. aureus* is part of the normal human flora (it can be found in different sites e.g., the skin, rectum, vagina, gastrointestinal tract and axilla) and does not usually cause infection. Human-beings are also often colonized with *S. aureus* in their noses (with the anterior nares and vestibules as the main reservoir). It has been estimated that previous nasal colonization in 30% of the cases of bacteremia was due to *S. aureus* [21].

But *S. aureus* is also a major human pathogen, and a leading cause of life threatening human infections [22]. It is included in the ESKAPE pathogens (this group encompasses six pathogens; *Enterococcus faecium*, *S. aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter spp*.). They are identified as a significant cause of human bacterial infections (mainly nosocomial infections) and are frequently multidrug-resistant. The emerging resistance profile of these organisms prompted calls from different international agencies to develop new approaches to treat infections caused by ESKAPE pathogens [23]. In the case of *S. aureus,* its importance as a human pathogen is due to the many different virulence-associated genes yielding expansive options to harm the human body. This is due to the great genomic plasticity of *S. aureus*, given by its ability to incorporate phages and other mobile genetic elements as plasmids [24,25]. The existing literature describes a vast array of cell wall-bound adhesins, toxins and different virulence factors (cell-wall anchored or secreted) that mediate host colonization, invasion of damaged skin and mucosa, dissemination through the body, and evasion of host defense mechanisms [26-28]. Binding to fibrinogen is of particular significance in pathogenesis. Fibrinogen-binding proteins such as clumping factors A and B (ClfA and ClfB, respectively) allow *S. aureus* to attach to extracellular matrix proteins, fibrin, and platelets. Other cell wall-associated factors are FnBPA and FnBPB (fibronectin-binding proteins) that facilitate binding to both fibrinogen and fibronectin and also play a role in subsequent endothelial cell invasion and inflammation [29, 30]. Expression of most virulence factors in *S. aureus* is under the control of the *agr* (accessory gene regulator) locus, which encodes a two-component signalling pathway and its activating ligand, a bacterial-density-sensing peptide called the auto inducing peptide [31].

Although this substantial evidence suggests that clinical manifestations of *S. aureus* are influenced by the genetic characteristics of the infecting strain, the association between *S. aureus* genes and severity of illness is incompletely understood. There are studies that have demonstrated a significant association between specific *S. aureus* isolates genotypes and infection severity [32]. One study has showed that IE isolates are more likely to belong to CC30 and to contain specific virulence genes [33]. On the other hand, a study using whole genome sequencing found that no specific genetic characteristics were able to distinguish between *S. aureus* strains from bacteremia and IE patients [34]. In a longitudinal, prospective and observational study in 15 Spanish hospitals were analyzed the *S. aureus* isolates from IE patients [35]. The authors

concluded that phenotype and genotype provided no additional predictive value beyond conventional clinical characteristics.

In addition to presenting great versatility in terms of pathogenicity factors, *S. aureus* also has a great capacity to adapt to the selective pressure of antibiotics. The appearance and spread of isolates of methicillin-resistant *S. aureus* (MRSA) in a short period of time, exemplifies this great capacity already mentioned. MRSA is one of the most important nosocomial pathogens and accounts for up to 30% of *S. aureus* IE [14] and remains a key pathogen in both community and hospital settings [36,37].

Antibiotics are the mainstay of endocarditis treatment. The high bacterial density, biofilms properties and in general low microorganism metabolic activity are the typical features of bacteria within IE vegetations. Bactericidal regimens are recommended over bacteriostatic drugs and treatment should be sufficiently long (2-6 weeks of native valve endocarditis and at least 6 weeks for prosthetic valve infections) to eradicate the infection in the vegetations [4, 38]. The presence or absence of antibiotic resistance, especially methicillin is decisive for the different treatment recommendations. The European Society of Cardiology and the American Heart Association issue the most important international guidelines with great impact on clinical practice. Moreover, their guidelines represent the official position of both societies and are regularly updated [17, 39]. According to these guidelines, cloxacillin (or oxacillin) continues to be the drug of choice to treat *S. aureus* IE whereas the addition of gentamicin is no longer recommended [17, 39]. For penicillin-allergic patients or when treating methicillin-resistant staphylococci, vancomycin is the drug of choice for parenteral therapy. However, vancomycin presents a well-known suboptimal efficacy even when its pharmacodynamics parameter is adjusted to pharmacokinetic index ABC/CMI>400. Further disadvantages of vancomycin treatments include varying tissue penetration, slow bacterial killing, and the emergence of drug-resistant strains [40-42]. Instead, daptomycin presents a rapid bactericidal effect against MRSA and has been considered at least as effective as vancomycin in treating MRSA bacteremia [43]. Based on this evidence, the American Heart Association guidelines [39] included daptomycin at monotherapy, just for the treatment of MRSA native valve endocarditis (NVE) at 8 mg/Kg/day dose and await additional study data to define optimal dosing. Also as a recommendation, they suggested that daptomycin is a reasonable alternative to vancomycin for native valve endocarditis caused by MSSA. However, account should be taken of the possible risk of exacerbating daptomycin drug resistance in use of

daptomycin in monotherapy for MSSA, given the fact that daptomycin has much broader spectrum than necessary for MSSA treatment.

Instead, the European guidelines, recommend administering daptomycin at high doses when treating staphylococcal endocarditis and to combine it with other antibiotics such as beta-lactams or fosfomycin for NVE, and with gentamicin or rifampicin, for prosthetic valve endocarditis [39] to overcome the aforementioned disadvantages and improve the clinical outcome of patients. Despite these recommendations, it is important to point out that daptomycin and fosfomycin are not available in some European countries. In the US, intravenous fosfomycin is not yet approved by the FDA. In these cases, the combination of daptomycin plus beta-lactam antibiotics e.g., cloxacillin or oxacillin or ceftaroline, could be a good option. Thus, there is a continuing need to develop novel more effective antimicrobial agents and to explore strategies that may enhance the potency of existing agents. These include performing pharmacokinetic/pharmacodynamic (PK/PD) modelling studies and the use of combined therapy. In general terms, the treatment of infections caused by *S. aureus* represents a growing challenge for the health system. Up to now, strategies to manage antibiotic resistance have focused on the use of higher doses, combination treatment or the development of new antimicrobials agents. Broadly, the rationale of using combination therapy is as follows: provision of wide-spectrum benefits (especially in empiric therapy), acquisition of the synergistic effect, and low risk of emergence of drug-resistant strains (mainly targeted therapy).

2. Objective.

This article aims to review all the available published studies that used daptomycin plus fosfomycin combined therapy for the treatment of methicillin-susceptible (MSSA) or resistant *S. aureus* (MRSA) infective endocarditis. We will discuss the rationale of combination antibiotics and the mechanism that enhances the activity of daptomycin focused on the role of fosfomycin.

3. Search strategy and selection criteria.

This is a narrative overview to assess what is already published and to present the currently available evidence about the topic. To do this, we conducted a systematic literature search of all the English-language peer-reviewed articles about daptomycin plus fosfomycin activity against *S. aureus* from the PubMed database from 2008 to

March 2022. The following terms and connectors were included in the search: "*Staphylococcus aureus*" or "*S. aureus*" or "methicillin-resistant" or "MRSA" or "daptomycin and fosfomycin" or "endocarditis" or "experimental endocarditis". The selection included *in vitro* studies, *in vivo* experimental studies, clinical trials, observational studies, review articles and society guidelines.

4. Fosfomycin.

Fosfomycin (also termed phosphomycin and phosphonomycin), is a natural bactericidal antibiotic compound produced by several *Streptomyces* and *Pseudomonas* species (44,45) that was discovered in Spain in 1969. The fosfomycin molecule has a very low molecular weight, and comprises its own class of antibiotics unrelated to any other antibiotic family. Fosfomycin presents broad-spectrum bactericidal activity against both Gram-negative and Gram-positive bacteria.

4.1 Mechanism of action.

Fosfomycin has a unique mechanism of action (it acts as a phosphoenolpyruvate analogue) and inhibits the initial step in peptidoglycan biosynthesis binding irreversibly to MurA an essential enzyme for peptidoglycan biosynthesis and as a consequence leading to bacterial cell lysis and death [46]. It is also described that fosfomycin acts by lowering the Penicillin Binding Protein (PBP) formation, specifically PBP 2 (essential for the synthesis of murein by *S. aureus*) and PBP 4 [47]. In addition, there is some evidence that fosfomycin also exerts immunomodulatory effects enhancing the bactericidal activity of macrophages and human neutrophils against *S. aureus* [48,49].

4.2 Mechanism of resistance.

Although fosfomycin presents a highly bactericidal activity, it is well described that it cannot be used as monotherapy to treat systemic infections due to easy development of fosfomycin resistance in bacteria [50,51]. Three different mechanisms leading to fosfomycin resistance have been described; reduced permeability of fosfomycin, modification of the antibiotic target MurA (it is one of the most common mechanisms to acquire antibiotic resistance in bacteria), and antibiotic modification. In clinical isolates, the main mechanism for the development of fosfomycin resistance is a reduced permeability of the cell membrane [45]. A recent review [50] updates the frequency and clinical consequences of fosfomycin resistance.

Regarding the fosfomycin *in vitro* susceptibility testing for *S. aureus*, it is worth mentioning that routine testing in diagnostic microbiology laboratories may present some difficulties (52). The reference standard is agar dilution with addition of 25 mg/L glucose-6-phosphate according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (53). But this method is laborious and is not routinely used. Instead, disk diffusion method is used more frequently, although it has been shown give inaccurate results for fosfomycin. For this reason, simpler, rapid, and less time-consuming tests are being developed (54).

4.3 Pharmacokinetics and pharmacodynamics (*pK/pD).*

Regarding its pK/pD properties, fosfomycin is a hydrophilic drug with negligible protein binding that is highly distributed throughout body tissues. In the treatment against *S. aureus*, it exhibits a short post-antibiotic effect (among 0.5 and 1.4 hours) and a time-dependent bactericidal activity [55]. Traditionally fosfomycin at monotherapy as a single oral dose has been used mainly for the treatment of uncomplicated urinary tract infections [56]. The intravenous drug form, administered in combination with other antibiotics, has been used for infections caused by gram-negative and gram-positive bacteria [57]. Some adverse events, mainly sodium overload and hypokalaemia have been described when fosfomycin is administered intravenously [58,59]. Thus, potassium supplements should be administered and its levels monitored regularly in patients receiving fosfomycin intravenously.

In summary, fosfomycin can be used in a broad range of tissues and targets and does not present cross-resistance with other antibiotics [60]. In addition, it is well described that fosfomycin shows synergy when combined with a wide variety of other antibiotics against MRSA such as cefotaxime, cefamandole, cephazolin, ceftriaxone, ciprofloxacin, imipenem, and rifampicin [61-64]. Therefore, fosfomycin in combination with other antimicrobials can serve as a suitable candidate for the treatment of infections caused by multidrug-resistant pathogens as an extensive systematic review has shown [65].

5. Daptomycin

Daptomycin is an antibiotic belonging to the cyclic lipopeptide class that is included in the peptide antimicrobial family. The development of daptomycin began in 1984 when the compound LY 146032 was described as a new molecule belonging to the complex of acid lipopeptide antibiotics which was designated A21978C, a fermentation product

of *Streptomyces roseosporus* [66,67]. First *in vitro* and *in vivo* studies published [68] confirmed the bactericidal activity of LY 146032 against a variety of gram-positive bacteria, many of which resistant to available antimicrobial agents. It showed to be effective against many clinically important gram-positive pathogens, including vancomycin-resistant enterococci, MRSA, and penicillin-resistant *Streptococcus pneumoniae*.

Like other members of the A21978C group of lipopeptides, the daptomycin structure contains a thirteen amino acid hydrophilic peptide core with a lipophilic fatty acid "tail" which acrylates the N-terminus of the exocyclic side chain [66,69]. Daptomycin is an amphiphilic molecule that needs physiologic levels of calcium to exert its bactericidal activity. After Ca2+ binds to daptomycin, the molecule becomes more hydrophobic due to charge neutralization and is solubilized to form micelles that act as vehicles to deliver daptomycin to the bacterial cell membranes in high local concentrations. Once in contact with the membrane, the daptomycin micelle would then dissociate, allowing monomeric daptomycin to insert into the cytoplasmic membrane and thereby disruption of the target membrane [69-72]. A second conformational change occurs when the interaction of the complex daptomycin/calcium with the negatively charged headgroup of phosphatidylglycerol induces oligomerization and deeper membrane insertion that results essential for the bactericidal activity [71,72].

5.1 Mechanism of action.

The binding between daptomycin and the phosphatidylglycerol cell membrane confers a fast bactericidal activity to the compound, although the process has not yet been fully defined [74]. This mechanism is also observed in several antimicrobial peptides, including the pore-forming antibiotic nisin [75]. The insertion of several daptomycin molecules into the cell membrane forms channels and causes membrane depolarization with the loss of intracellular components like K^+, Mg^{2+}, and ATP. The synthesis of nucleic acids is disrupted and protein synthesis is probably also affected [74]. Several studies also suggest that membrane depolarization is requiered to facilitate antibiotic entry into the bacteria [76-78] and that bactericidal activity of the drug is a result of cytoplasmic membrane potential dissipation [70,79,80].

5.2 Mechanism of resistance.

Daptomycin received U.S. Food and Drug Administration (FDA) approval for the treatment of Gram-positive soft-tissue infections in 2003 and for the use in the treatment of *S. aureus* bacteremia, and right-sided native valve endocarditis in 2006.

Shortly thereafter, the development of daptomycin resistance during therapy was widely described [43,81-83] and daptomycin non-susceptible *S. aureus* (DNS) strains were isolated from patients failing daptomycin treatment. Phenotypically, DNS strains often have a thickened cell wall, altered cell surface charge and membrane phospholipid composition, and abnormal membrane fluidity properties [84-86]. The mechanism of daptomycin resistance has yet to be completely understood but it seems to be linked to a charged repulsion of the daptomycin molecule from the cell surface and changes in *MprF* gene [74, 87] and others as *cls* gene involved in phospholipid metabolism as cardiolipin synthases [88]. Additionally, in the setting of decreased susceptibility to vancomycin VISA/hVISA isolates have been shown to concomitantly exhibit decreased daptomycin susceptibility [89-92].

5.3 Pharmacodynamics (*pK/pD).*

Daptomycin is rapidly bactericidal against *S. aureus* in a concentration-dependent manner. The area under the curve/minimum inhibitory concentration ratio (AUC/MIC) is the pharmacokinetic/pharmacodynamic (PK/PD) index that best correlates with daptomycin activity, whereas toxicity correlates well with daptomycin plasma trough concentrations [93-94]. To achieve an acceptable probability of bactericidal target attainment against *S. aureus* strains with a MIC < 2 mg/L, formal benefit-to-risk analyses favoured daptomycin doses of 10 mg/kg daily [95]. Higher doses up to 12 mg/kg daily over 14 days have been successfully evaluated without evidence of musculoskeletal toxicity [96,97]. But achieving the necessary AUC/MIC exposures against DNS strains, patients can be subjected to greater risk of toxicity (e.g. musculoskeletal) due to the higher daptomycin doses required. Creatine kinase should be monitored routinely, especially if the patient concurrently takes an HMG-CoA reductase inhibitor [98]. Daptomycin is highly bound to serum proteins (90%) so it has a long half-life (about 8 hours) and is distributed primarily in the extracellular fluid with penetration to vascular tissues. Moreover, daptomycin has shown to reach adequate concentrations in a homogenous distribution in vegetations and cardiac valves [99,100].

6. Daptomycin in the treatment of *S. aureus* endocarditis. The advantages of combined therapy.

Despite its demonstrated bactericidal activity, from the first data reported and the approval of daptomycin for clinical use in 2004 until now, several cases of therapeutic failure with the emergence of resistance have been described, especially associated with deep-seated infections as endocarditis, with high bacterial inoculum and daptomycin regimens below 10 mg/kg/day dose [80, 101]. Current guidelines [17, 39] for treating MRSA IE recommend administering daptomycin at high doses because daptomycin activity is concentration-dependent; therefore higher doses are expected to provide increased activity and prevent the development of resistance. Only the European guidelines [39] included in their recommendations to administered daptomycin combined with other antibiotics [17] to overcome the aforementioned disadvantages and improve the clinical outcome of patients. When exploring therapeutic combinations, the addition of the second agent is usually aimed at addressing different important items: to broaden the spectrum of activity, to increase the bactericidal activity, and to prevent the appearance of resistant strains [62,102-105]. Most data come from *in vitro* (time-kill curves, checkerboard testing or simulated pharmacodynamics models mainly) and *in vivo* studies based on animal infection models [106].

7. Rationale of daptomycin plus fosfomycin as treatment for *S. aureus* endocarditis.

Many studies have demonstrated that daptomycin is a potent bactericidal antistaphylococcal antibiotic. In fact, a recent meta-analysis comparing daptomycin and vancomycin for the treatment of MRSA bloodstream infection with or without IE published by Maraolo et al [107] concludes that daptomycin seems to be associated with a lower risk of clinical failure and it is better tolerated than vancomycin.

But it is also well documented, that shortly after their approval by the FDA, daptomycin non-susceptible *S. aureus* strains were being isolated from patients failing daptomycin treatment [43,79-81]. Combined therapy is the best option to prevent the onset of resistance and optimize the efficacy of daptomycin [108]. Many different families of antibiotics including beta-lactams, rifampicin, gentamicin and fosfomycin have been studied in combination with daptomycin. However, fosfomycin has a unique mechanism of action and therefore cross-resistance is very rare [109]. This fact makes

intravenous fosfomycin a very versatile and good candidate that can be combined with different antibiotic groups to enhance their activity [84]. Both antibiotics act as cell wall agents. Although the exact mechanism of synergy is not known yet, it could involve a decrease in the positive charge of the membrane that may increase daptomycin binding because of alteration in the electrical charge of the outer bacterial membrane, as it is described for other cell wall agents as beta-lactams [110-112]. Also PBP production could be involved as it has been shown in the combined therapy of fosfomycin plus imipenem [63]. In this study after incubation of two MRSA strains with fosfomycin, the production of PBP1 and PBP2 was drastically reduced. In addition, the generation of PBP3 also decreased with the addition of imipenem (Fig. 1). A recent study [113] has provided new data on the role of fosfomycin in the synergistic activity with daptomycin. It seems that fosfomycin could contribute to decreasing the positive charge in the membrane. Fosfomycin can also alter the rate and distribution of the membrane cardiolipin phospholipids and, with that, the fluidity of the cellular membrane.

7.1 In vitro and in vivo studies (*pre-clinical studies*).

The synergy between fosfomycin and daptomycin has been studied in *in vitro* and *in vivo* models. In an *in vitro* study, Miro et al [114] investigated seven MSSA and seven MRSA strains (two of them were glycopeptide intermediate resistant *S. aureus* [GISA] strains) with time-kill curves method. The combination of daptomycin and fosfomycin showed to be synergistic and bactericidal in five of the seven MSSA strains and in five of the seven MRSA (including the two GISA strains).

In a later study Aktas et al [115] selected twenty-five clinical MRSA strains for a synergy study combining daptomycin with different antibiotics using the micro broth checkerboard technique. They found that the most active combination was daptomycin plus fosfomycin with synergistic effect in all strains (25 of 25 strains) even though only 44% of the strains showed to be fosfomycin susceptible. The addition of fosfomycin to daptomycin also showed to be effective in delaying the emergence of daptomycin resistance in another *in vitro* study [110] although the combination were ineffective in maintaining the suppression over the entire study (4 weeks). Lee et al. [116] conducted a multicentre study to determine the synergistic effects of daptomycin combined with fosfomycin among other antibacterial agents. They tested a total of 100 MRSA isolates with a daptomycin MIC of 1 mg/L using the checkerboard method. The authors concluded that daptomycin in combination with fosfomycin had the highest synergistic

and additive effect against the strains studied out of all the different combinations studied [116].

Recently, two different groups have published results showing the efficacy and bactericidal activity of daptomycin plus fosfomycin in the *in vivo* model of experimental endocarditis. Garcia de la Maria et al [117] studied the *in vitro* activity of daptomycin plus fosfomycin or cloxacillin against five different MRSA strains by time-kill curves methodology. Afterwards one of the strains was selected for the *in vivo* study. Results showed the bactericidal activity of both combinations *in vitro* and *in vivo* and in no case daptomycin-resistant strains were recovered from the valve vegetations so the authors concluded that both regimens presented bactericidal activity. The same group obtained very similar results when they aimed to study the activity of daptomycin plus fosfomycin or cloxacillin compared with cloxacillin and cloxacillin plus gentamicin against MSSA strains [118]. Authors found that the addition of fosfomycin or cloxacillin to daptomycin was synergistic, bactericidal and enhancing the activity of daptomycin and showing better activity than cloxacillin plus gentamicin treatment against MSSA in the experimental endocarditis model. Moreover, no daptomycin resistant strains were found in vegetations during the study. Reed et al. [119] published an *in vitro* and *in vivo* experimental endocarditis study. In their *in vitro* results, authors showed that the combination of daptomycin and fosfomycin was effective against both daptomycin susceptible and their variant non susceptible strains. In the *in vivo* model, the combination significantly reduced bacterial densities in the target tissues (vegetations, kidney, and spleen) but it was unable to prevent the *in vitro* emergence of daptomycin non-susceptible strains. More recently, Mishra et al [113] have published the first study focused on figuring out the synergy mechanisms of daptomycin plus fosfomycin in combination using an isogenic daptomycin susceptible and resistant clinical MRSA strain pair. The authors assess the interrelatedness of bacterial synergy, resistance prevention, and resensitization with the combination for the resistant phenotype. Their results were obtained from: a) *in vitro* studies by time-kill curve, b) *ex vivo* simulated endocardial vegetation model, and c) *in vivo* IE model and the synergy mechanism study. The results obtained allowed the authors to provide some insights into the synergy mechanism. Fosfomycin's ability to significantly increase overall content of cardiolipin could explain the less positively charged surface, which implies an associated enhancement in daptomycin binding. In addition, fosfomycin also induced

a mislocalitzation of cardiolipin which might improve daptomycin insertion and cellular membrane pore formation, increasing its bactericidal activity.

7.2 Clinical studies.

Although the experience is limited, there are some reports of cases of MRSA endocarditis treated with the combination of daptomycin and fosfomycin. Chen et al [120] reported a case of an implantable cardiac defibrillator device-related endocarditis complicated with osteomyelitis caused by daptomycin non susceptible MRSA that was successfully treated with high-dose daptomycin (i.v. at 10 mg/kg/day) in combination with fosfomycin (i.v. at 2 g/6h/day) and surgical intervention. Miro J et al [114] described three patients with *S. aureus* endocarditis (one MSSA and two MRSA cases) who were successfully treated with the intravenous combination of daptomycin (10 mg/kg/d) and fosfomycin (2g/6h). In a recent study by Coronado-Álvarez et al [121], the authors reviewed their clinical experience and performed a retrospective review of all patients with severe gram-positive infections who received fosfomycin as part of their treatment from 2011 to 2017. A total of 67 patients with persistent bacteremia caused by *S. aureus* were included. Fosfomycin was prescribed as add-on therapy in 45 patients and the other 22 patients received fosfomycin-based combination as initial therapy. In the first group, patients were initially treated with oxacillin (15 cases with MSSA), daptomycin (20 cases with MSSA or MRSA) or vancomycin (10 cases with MRSA) and after 72h of persistent bacteremia fosfomycin was added. Regarding the second group, it included patients treated with daptomycin (10 cases with MRSA), oxacillin (7 cases with MSSA) and vancomycin (5 cases with MRSA) plus fosfomycin. Patients were considered successfully treated if blood cultures sterilization was achieved after fosfomycin therapy. Globally, the combination of daptomycin plus fosfomycin was the most effective, with 95% success rate for the first group and 90% for the second group. On the other hand, the combination with vancomycin was the least effective combination with rates of blood culture sterilization of 47% and 40%, respectively. Regarding the side effects of the treatments, the authors reported that the fosfomycin combinations were safe, with minor side effects described (phlebitis or minor hypernatremia in 9 patients). Only in one case a severe side effect was described (acute cardiogenic pulmonary edema) that was resolved after combination therapy was discontinued. In addition, the authors also tested *in vitro* three *S. aureus* strains (two MSSA and two MRSA) to investigate the presence of synergism in different fosfomycin

combinations. Globally the combination of daptomycin plus fosfomycin was the most effective one [121].

During the last years two multicenter open-label clinical trials have been conducted at 18 Spanish hospitals evaluating the efficacy of two different combined fosfomycin therapies against *S. aureus* bacteremia. The BACSARM (NCT01898338), a randomized, open-label and parallel-group multicentre phase 3 study [59,122] that evaluated the efficacy of daptomycin plus fosfomycin against MRSA bacteremia (including among 10-12% of patients with IE in each treatment arm). It was completed in January 2018 (patients were recruiting between 2013 and 2017). Patients aged from 18 years with MRSA bacteremia indicated by one or more positive blood cultures within the last 72 hours before randomization and with symptoms and signs of infection were eligible for the study. The primary endpoint was treatment success at the test of cure visit 6 weeks after the end of therapy. It is important to highlight that in this trial, the criteria for treatment failure were relatively strict. For example, if the patient failed to have blood cultures at the test of cure, it was not counted as treatment success regardless of the patient's clinical outcomes which could have contributed to obscuring statistical significance in treatment success. Finally, 74 patients were included in the daptomycin plus fosfomycin group, and 81 patients in the daptomycin group (Table 2). The results reported [59,122] showed that daptomycin plus fosfomycin provided a 12% higher rate of treatment success than daptomycin alone although this difference did not reach statistical significance (fig 2). The antibiotic combination (table 2) prevented microbiological failure and complicated bacteremia. Also, a significantly lower number of positive blood cultures in the combination therapy group were observed. Nevertheless the antibiotic combination was more often associated with adverse events (cardiac failure, hypokalemia and hypocalcemia), particularly in elderly patients receiving a median of 10 days of therapy [59]. The authors point out that these fosfomycin side effects appeared after a median of 10 days of therapy and the high microbiological efficacy was achieved at three and seven days of the combination therapy suggesting that fosfomycin should essentially be administered during the first week of treatment.

The SAFO study (NCT03959345), still on recruitment status, aims to evaluate the efficacy of cloxacillin and fosfomycin combination versus cloxacillin monotherapy in patients with MSSA bacteremia. In this study the primary endpoint is the treatment success measured at one week of treatment.

At present, in the clinical trial registers of the U.S. National Library of Medicine (ClinicalTrials.gov) [123], there is only registered from 2016, one *ongoing* clinical trial related to fosfomycin and endocarditis. As it is described in the record, this is an European, multicentre, non-comparative, observational, non-interventional prospective clinical registry (FORTRESS with NCT02979951). Its purpose is to document and evaluate the clinical outcome and safety of the treatment of severely infected patients with intravenously administered fosfomycin, including patients with osteomyelitis, complicated urinary tract infection, nosocomial lower respiratory tract infection, bacterial meningitis/central nervous system infection, bacteremia/sepsis, skin and soft tissue infection, endocarditis or other infections, each as far as covered by the respective nationally relevant SmPC. The primary outcome measure was defined as the percentage of patients with clinical success defined as clinical cure (defined as resolution of signs and symptoms and microbiological cure or no additional antibiotic therapy for the targeted infection necessary) or clinical improvement (defined as a partial resolution of signs and symptoms and microbiological cure or no additional antibiotic therapy for the targeted infection necessary).

Outcomes should be analyzed at the end of fosfomycin treatment, up to six months after start of fosfomycin treatment. It is expected to end by 2023 and, as far as we have been able to find out, no previous data or report has been documented.

8. Expert opinion

Fosfomycin is unique among bactericidal antibiotics given its peculiar mechanism of action. It acts as an analog of phosphoenolpyruvate, i.e., by inhibiting the initial step in peptidoglycan biosynthesis by irreversibly binding to the essential enzyme MurA. It also has the ability to produce a synergistic effect against staphylococci in combination with a wide variety of other antibiotics. In particular, the synergistic and bactericidal combination of fosfomycin with daptomycin has been shown to be extraordinarily active against endocarditis-producing isolates of MSSA and MRSA in both *in vitro* studies and *in vivo* models of experimental endocarditis. The combination has also been shown to prevent the development of intrinsic resistance to daptomycin during treatment, a feared and well-described complication in high-inocula infections, as epitomized by endocarditis.

Only the BACSARM clinical trial has compared this combination with the standard of care. The patients with MRSA bacteremia showed markedly better microbiological results, although a clear benefit in terms of mortality remains undemonstrated. From the point of view of safety, account must be taken of the risk of some adverse events in patients treated with intravenous fosfomycin, mainly sodium overload and hypokalemia. It is interesting to mention, however, that these adverse events are preventable by a timely potassium supplement and/or pretreatment with loop diuretics in low doses. Furthermore, they were described more frequently in patients, whose treatment was longer than 10 days, suggesting they would be quite unusual in shorter therapeutic regimens. Accordingly, fosfomycin should essentially be administered during the first week of treatment.

Although reported clinical experience using the combination to treat endocarditis is still scarce and focused mostly on episodes caused by MRSA, it has nevertheless proved to be successful and suggests its potential usefulness in treating staphylococcal endocarditis, whether caused by methicillin-sensitive or methicillin-resistant isolates.

When assessing the therapeutic alternatives available for a disease like IE, it is necessary to emphasize that patients develop local damage of such magnitude that up to one third may present clinically with acute heart failure (caused mainly by valve destruction) and up to 50% require valve surgery at some point of the disease course. In this context, it seems to be both reasonable and desirable to optimize the initial bactericidal action of antibiotic treatment with the aim of accelerating the sterilization of vegetations and stopping local endo-myocardial damage. In this sense, the addition of fosfomycin during the first days or weeks – taking the appropriate precautions above mentioned – could benefit to patients with endocarditis. Given the enormous difficulty of performing randomized controlled trials in the specific subgroup of patients with staphylococcal IE, it may only be feasible to rely on the limited clinical evidence available. With the robust evidence issuing from the pharmacodynamic studies, as summarized in this manuscript, it seems reasonable to administer this powerful combination to patients with this deadly disease.

9. Conclusions. Current antibiotic options for treating invasive *S. aureus* infections have limitations when used in monotherapy, and therefore combination therapy should frequently be considered. Although reports are still only few in number, the evidence

suggests the synergistic combination of fosfomycin with daptomycin may be a useful treatment option in both methicillin-susceptible (MSSA) and methicillin-resistant *Staphylococcus aureus* (MRSA) IE. Additional studies reporting the clinical efficacy of this combination in the current era of high antimicrobial resistance are much needed.

Authors' contribution statement: All the authors contributed to the conception and design, drafting of the review, critical revision, and final approval of the manuscript.

10. Declaration of Interest

All the authors listed meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship. They have substantially contributed to the conception and design of the review article and interpreting the relevant literature and have been involved in writing the review article or revised it for intellectual content. [Anonymized] has received consulting honoraria and/or research grants from Angelini, Bristol-Myers Squibb, Contrafect. Genentech, Gilead Sciences, Lysovant, MSD, Medtronic, Novartis, Pfizer, and ViiV. All other authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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Papers of special note have been highlighted as: * of interest and ** of considerable interest.

Table and Figures

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Figure 1. PBP profiles of MRSA and GISA strains incubated with fosfomycin (FOM) and imipenem (IPM) alone or in combination determined by polyacrylamide gel electrophoresis (SDS-PAGE) [64].

Figure 2. Primary endpoint [60].

The primary endpoint was treatment success at the test of cure (6 weeks after the end of therapy) and it was considered when patient was alive and had resolution of clinical manifestations of infection and negative blood cultures at the test of cure after completion of therapy.

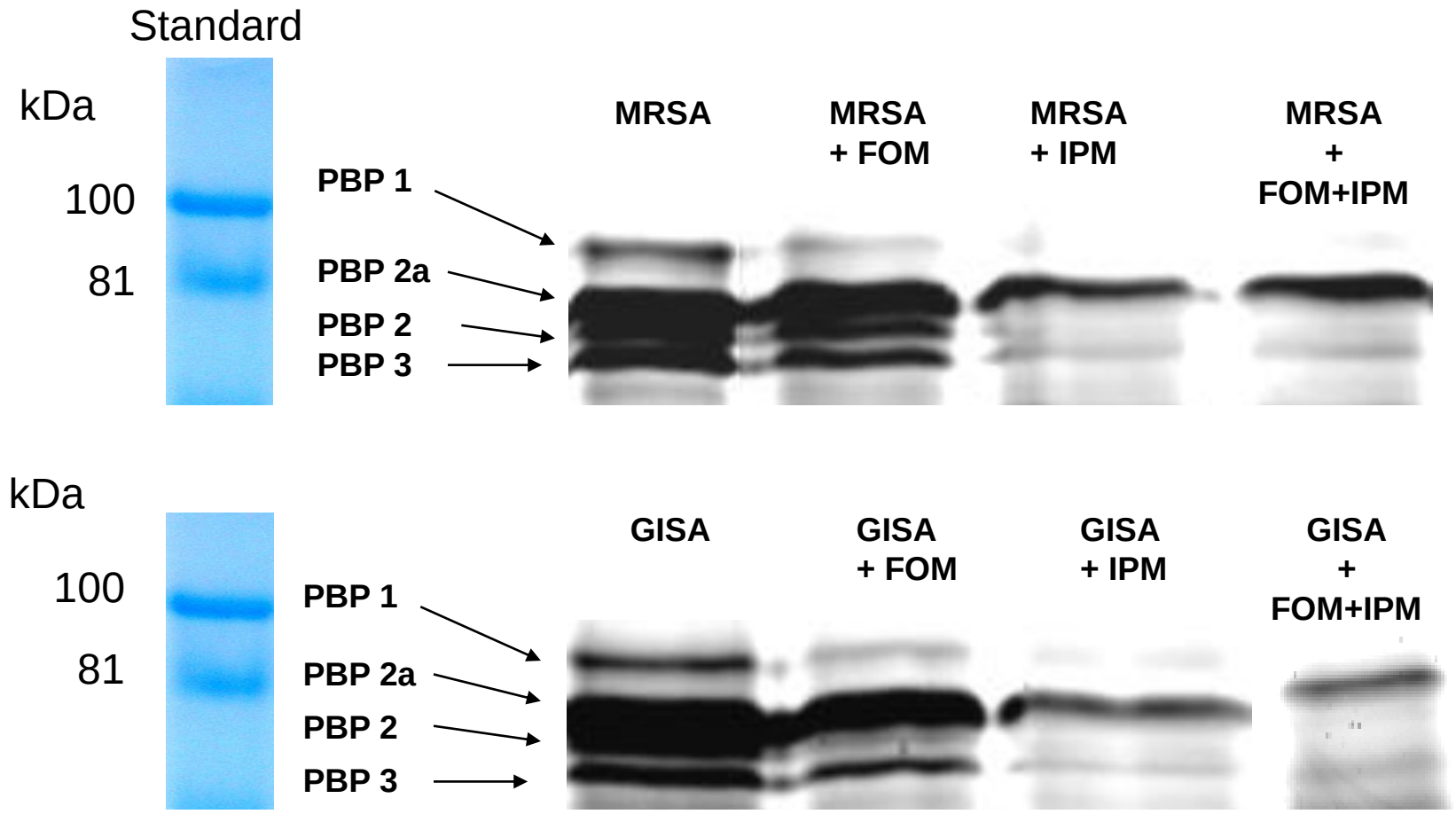

Fig 1. PBP profiles of MRSA and GISA strains incubated with fosfomycin (FOM) and imipenem (IPM) alone or in combination determined by polyacrylamide gel electrophoresis (SDS-PAGE).

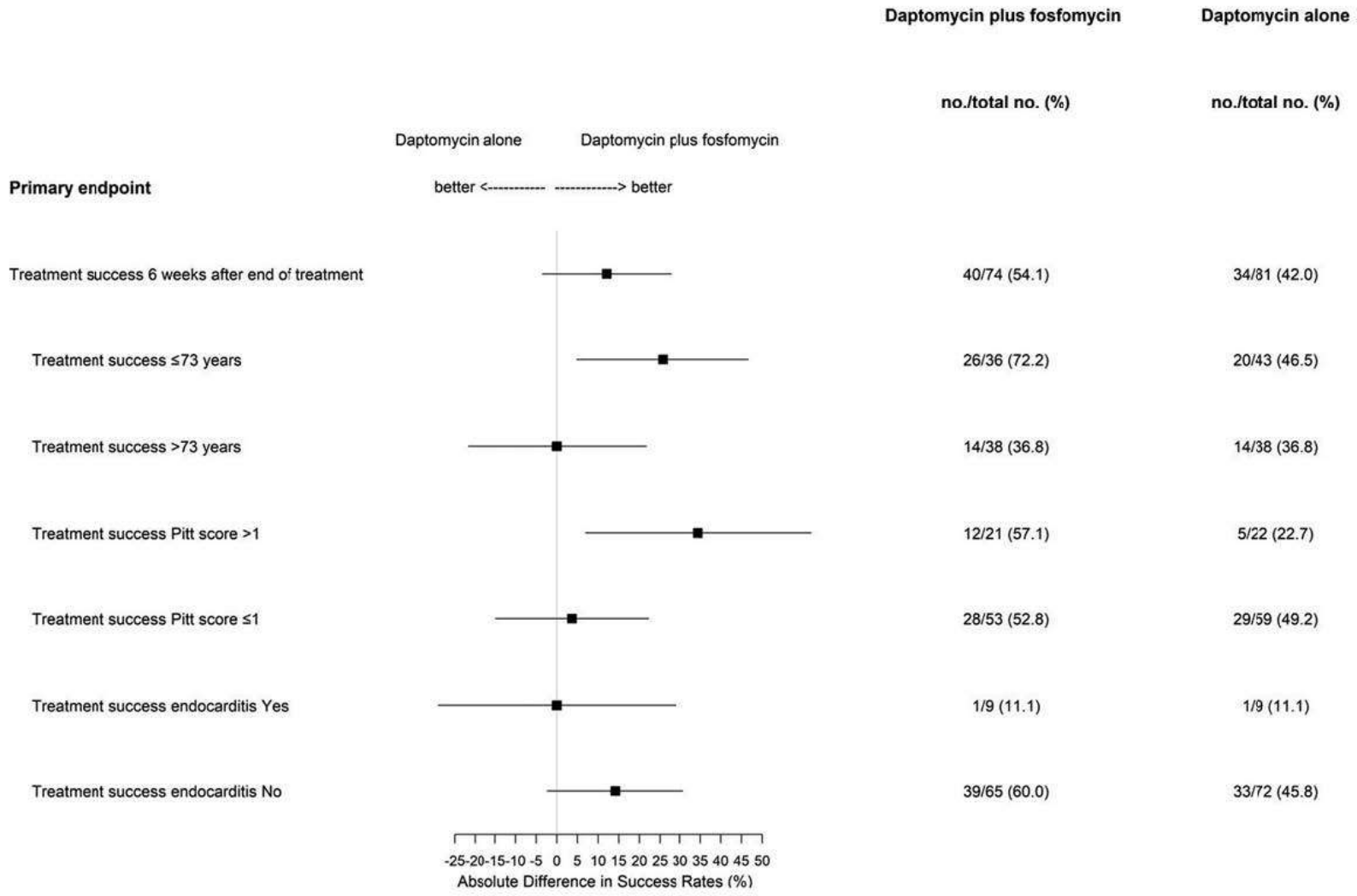
Daptomycin plus fosfomycin
Daptomycin alone
Daptomycin alone
Daptomycin plus fosfomycin
Primary endpoint
no./total no. (%)
no./total no. (%)
better <---------- ----------> better
Treatment success 6 weeks after end of treatment
40/74 (54.1)
34/81 (42.0)
Treatment success ≤73 years
26/36 (72.2)
20/43 (46.5)
Treatment success >73 years
14/38 (36.8)
14/38 (36.8)
Treatment success Pitt score >1
12/21 (57.1)
5/22 (22.7)
Treatment success Pitt score ≤1
28/53 (52.8)
29/59 (49.2)
Treatment success endocarditis Yes
1/9 (11.1)
1/9 (11.1)
Treatment success endocarditis No
39/65 (60.0)
33/72 (45.8)
-25 -20 -15 -10 -5 0 5 10 15 20 25 30 35 40 45 50
Absolute Difference in Success Rates (%)

Table 1. Microbiologic Etiology by Region in 2781 patients with Definitive Endocarditis.

	No. (%) of Patients*				
		Region			
Cause of Endocarditis	**Total Cohort (n=2781)**	**North America (n=597)**	**South America (n=254)**	**Europe (n=1213)**	**Other (n=717)**	**P Value for the Difference Between Regions**
Staphylococcus aureus	869 (31)	256 (43)	43 (17)	339 (28)	231 (32)	<.001
Coagulase-negative *Staphylococcus*	304 (11)	69 (12)	18 (7)	156 (13)	61 (9)	.005
Viridans group streptococci	483 (17)	54 (9)	66 (26)	198 (16)	165 (23)	<.001
Streptococcus bovis	165 (6)	9 (2)	17 (7)	116 (10)	23 (3)	<.001
Other streptococci	162 (6)	38 (6)	16 (6)	66 (5)	24 (6)	.86
Enterococcus species	283 (10)	78 (13)	21 (8)	111 (9)	73 (10)	.05
Negative culture findings	277 (10)	41 (7)	51 (20)	123 (10)	62 (9)	<.001
Other	238 (9)	52 (8.3)	22 (8.4)	104 (9)	60 (7.8)	ND

* Only percentages less than 1% are carried to the first decimal place. ND: not determinated

Table 2. Primary and Secondary Outcomes

Outcomes	Daptomycin Plus Fosfomycin, No. Of Patients/Total (%)	Daptomycin Alone, No. of Patients/Total (%)
Primary endpoint		
Treatment success at TOC	40/74 (54.1)	34/81 (42.0)
Secondary endpoints		
Positive blood cultures at day 3	2/74 (2.7)	15/81 (18.5)
Positive blood cultures at day 7	0/74 (0.0)	5/81 (6.2)
Positive blood cultures at TOC	0/74 (0.0)	4/81 (4.9)
Microbiological failure at TOC	0/74 (0.0)	9/81 (11.1)
No. of episodes of complicated bacteremia at TOC	12/74 (16.2)	26/81 (32.1)
Any AE leading to treatment discontinuation	13/74 (17.6)	4/81 (4.9)
Overall mortality at day 7	3/74 (4.1)	6/81 (7.4)
Overall mortality at TOC	18/74 (24.3)	22/81 (27.2)

Abbreviations: AE, adverse event; CI, confidence interval; TOC, test of cure.

Paients might have >1 reason for treatment failure (ie, mortality at TOC, clinical or microbiological failure, any AE leading to treatment discontinuation, additional antimicrobial therapy administered before TOC, lack of blood cultures before TOC).