Successful treatment with daptomycin and ceftaroline of multidrug resistant *Staphylococcus aureus* native valve endocarditis: a case report.

François-Régis DUSS MD[§*], Cristina GARCIA DE LA MÀRIA MD[§], Antony CROXATTO PhD, Stefano GIULIERI MD, Frédéric LAMOTH MD, Oriol MANUEL MD, José M MIRÓ MD PhD

Running title: DNS MRSA endocarditis

Word count:

- Abstract: 73
- Main body: …

[§]Equal contribution

[*]Corresponding author: Dr. François-Régis Duss. Infectious Diseases Service, University Hospital and University of Lausanne, Lausanne, Switzerland
Phone: +41 78 909 64 48
E-mail: francois-regis.duss@chuv.ch

Abstract

Methicillin-resistant *Staphylococcus aureus* is a major cause of infective endocarditis, and remains associated with a high mortality rate, particularly in case of high vancomycin MIC. We report here a case of daptomycin non–susceptible, ceftaroline and fosfomycin resistant MRSA community-acquired native left endocarditis that was successfully treated with valve repair, and a combination of high dose daptomycin and ceftaroline. *In vitro* studies confirmed the synergy and bactericidal activity of this combination.

Introduction

Methicillin-resistant *Staphylococcus aureus* community-acquired and nosocomial bacteremia and infective endocarditis are a major healthcare concern, associated with a high mortality rate.[1] Although vancomycin remains the drug of choice for the treatment of MRSA invasive infections,[2] its use is controversial notably in case of MIC ≥1.5 mg/L.[3] High dose of daptomycin has been shown to be efficacious for treating *S. aureus* bloodstream infection,[4] although the emergence of daptomycin non-susceptible (DNS) *S. aureus* strains is a concern in case of monotherapy with this agent.[5] Combination of daptomycin with new antibiotics, such as ceftaroline,[6] or older ones such as fosfomycin[7] or cloxacillin,[8] seems a good therapeutic option, but more evidence of their efficacy is needed.

We report here a case of a multidrug resistant (DNS, and ceftaroline and fosfomycin resistant) MRSA community-acquired native left-sided endocarditis successfully treated with valve repair and a combination of daptomycin and ceftaroline. We also provide *in vitro* evidence of the bactericidal activity of daptomycin in combination with ceftaroline.

Case-Report

A 90-year-old man presented at the emergency department with 72 hours duration of fever and asthenia. He had previous medical history of prostatic adenocarcinoma and a sigmoid adenocarcinoma three years before. He had an outpatient percutaneous suprapubic catheterization two months before this episode. The patient was known for MRSA colonization in the bladder. Empiric antimicrobial therapy of meropenem (2 g three times per day, IV) was administered. Blood cultures performed on day 1 grew *S. aureus* (2/2 pairs, 4/4 bottles), which was confirmed to be methicillin-resistant by Xpert® MRSA BC (Cepheid) and antibiotic susceptibility testing. Vancomycin 2000 mg (12.5 mg/kg twice a day, IV) was administered on day 2 and a transthoracic echocardiography performed on day 3 showed a mild mitral insufficiency. A pelvic CT scan revealed a pectineal muscle collection on a prostatic subcutaneous fistula tract and an ischiopubic symphysis lysis. A transoesophageal echocardiography performed on day 5 revealed severe mitral regurgitation and 1.3 cm mitral vegetation. Blood cultures remained positive for MRSA on day 5. Before the fourth dose of vancomycin, the serum through concentration was 18.9 mg/L. After three days of therapy, considering the MIC of 1.5 mg/L for vancomycin and the presence of a persistent bacteremia, vancomycin was discontinued and daptomycin 800 mg (10 mg/kg once a day, IV) plus fosfomycin 8 g (2 g four times per day, IV) was initiated. On day 10, blood cultures were still positive for *S. aureus*, and a mitral cardiac valve repair with autologous pericardial patch and

annuloplasty ring was performed. At this point, we received the results of the susceptibility testing, showing that the MIC of *S. aureus* by Etest® strips methods (see methods) were 2 mg/L for daptomycin, 1024 mg/L for fosfomycin and 1.5 mg/L for ceftaroline. Fosfomycin was switched to ceftaroline (600 mg three times a day, IV) whereas daptomycin was continued at the same dose. Culture of the mitral valve was negative (after 7 days of daptomycin plus fosfomycin), and FemA PCR assay was positive (quantification 2'810'000 copies/mL). Subsequent blood cultures from day 11 were sterile. The patient's condition subsequently improved. He received a total of six weeks of antibiotic therapy with ceftaroline and daptomycin post surgery. No relapse was observed at six months of follow-up (6 months blood cultures were sterile).

Methods

Antibacterial susceptibility testing

MICs of the clinical strain were determined in real time by using the Etest method (Epsilometer-test® strips, BioMérieux) at the Institute of Microbiology, Lausanne, Switzerland following the manufacturer recommendations. The strain was then shipped to the research laboratory of the Infectious Diseases Service, Hospital Clinic, Barcelona, Spain. MIC was determined by microdilution broth method (MIC/MBC) according to standard recommendations.[9] *S. aureus* ATCC 29213 was used as the quality control strain in all experiments.

Synergy study

Time-kill and checkerboard methodologies were used to test the activity of combined antibiotics according to previously described criteria.[10]

Time-kill curves: Two final inocula of 5 x 10^5 and 1 x 10^8cfu/mL were used.[10,11] For synergy testing, concentration equal to 0.5 x MIC were chosen for daptomycin and ceftaroline. Due to resistance to cloxacillin and fosfomycin, high and low concentrations were used. A concentration equal to 64 mg/L (equivalent to 0.125 x MIC and 0.5 x MIC respectively) and 4 mg/L and 8 mg/L respectively, corresponding to Cmin serum level for each antibiotic (equivalent to 0.008 x MIC and 0.06 x MIC, respectively).[12] Synergy was defined as a 2-$\log_{10}$ decrease in the number of cfu/mL between the test tube with the combination and the test tube with the most active agent alone after 24 hours: the number of surviving organisms in the presence of the combination had to be 2 $\log_{10}$ cfu/mL below the starting inoculum. Bactericidal activity was defined as at least a 3-log reduction in cfu at 24h in comparison with the initial inoculum.

Checkerboard method: This was performed in customized 96-well plates to determine the MIC/FIC values in the combination well using the microdilution method as described previously.[10] All combinations were tested twice. In the checkerboards, the concentrations (in mg/L) used for the different antibiotics were as follows: daptomycin and ceftaroline started from 8 (4xMIC) to 0.12 (1/8xMIC) and a control (drug free) well; cloxacillin and fosfomycin started from 256 (1/2xMIC) to 1(1/512xMIC) and a control (drug free) well. The potential synergy of each combination was determined by the ΣFIC (sum of the fractional inhibitory concentration) method. ΣFIC is defined as the sum of the FIC (fractional inhibitory concentration) of the two combined antimicrobial agents, where FIC is the ratio of MIC of drug in combination (using the well with the maximal activity) to the MIC of drug alone. The estimated ΣFIC value indicates whether the combined effect is synergistic (ΣFIC <0.5), additive (0.5≤ ΣFIC ≤1), indifferent (1< ΣFIC ≤4) or antagonistic (ΣFIC >4).

Population analysis profile–area under the curve (PAPAUC) was performed by inoculating serial 10-fold dilutions of organisms onto increasing concentrations of vancomycin or daptomycin BHI agar (OXOID, Madrid, Spain), according to Wootton et al (ref). Colony growth at 48 h was counted and graphed as $\log_{10}$ CFU/ml versus vancomycin concentration. Control strain Mu3 (hVISA) was included in each batch. Area under the curve (AUC) was measured and ratio of tested isolate/Mu3 was calculated.

Results

In vitro *studies: Susceptibility testing*

By Etest, MIC for vancomycin was determined at 2 mg/L (EUCAST clinical breakpoint (CBP): 2 mg/L), for daptomycin at 2 mg/L (EUCAST CBP: 1 mg/L), fosfomycin at 1024 mg/L (EUCAST CBP: 32 mg/dL) and ceftaroline at 1.5 mg/L (EUCAST CBP: 1 mg/L). Screening for hetero VISA and VISA was negative using McFarland 0.5 inoculums on Mueller-Hinton and glycopeptide-containing agar plates mediums, and McFarland 2 inoculum on brain-heart infusion medium.

Using microdilution antimicrobial susceptibility testing for the determination of MIC and MBC, the *S. aureus* strain was resistant to cloxacillin (MIC/MBC >512 mg/L />512 mg/L), ceftaroline (2 mg/L /4 mg/L) and fosfomycin (128 mg/L /128 mg/L), but daptomycin susceptible (MIC/MBC = 1 mg/L /1 mg/L) according to the EUCAST standard MIC breakpoints. Vancomycin MIC was equal to 2 mg/L.

Time-killing curve studies are depicted in **Figure 1**. At the standard inoculum (10^5cfu/mL), the three combinations of daptomycin plus ceftaroline (Fig 1A), fosfomycin (Fig 1B) or cloxacillin

(Fig 1C) at concentrations of 0.5 x MIC for daptomycin and fosfomycin and 0.125 x MIC for cloxacillin were synergistic and bactericidal. However, when these combinations (Fig 1D-F) were tested using a higher inoculum (10^8cfu/mL), all combinations were synergistic but only daptomycin plus ceftaroline had bactericidal activity. When cloxacillin and fosfomycin were tested at Cmin concentrations (4 and 8 mg/L respectively) combined with daptomycin at 0.5 x MIC using a standard inoculum, both combinations showed synergy but only daptomycin plus cloxacillin retained bactericidal activity. At high inocula, both combinations showed indifference. The checkerboard assay showed that for daptomycin plus ceftaroline or fosfomycin, synergistic activity (ΣFIC<0.5) was observed for the daptomycin range from 1/4xMIC to 1/16xMIC with the ceftaroline or fosfomycin range from 1/4xMIC to 1/32xMIC. For daptomycin plus cloxacillin, synergistic activity (ΣFIC<0.5) was observed at 1/4xMIC and 1/8xMIC daptomycin concentrations with cloxacillin range from 1/8xMIC to 1/128xMIC.

Population analysis profile (PAP) results are depicted in **Figure 2**. Vancomycin treatment was not selected for resistant subpopulations (**Figure 2A**) with an AUC ratio = 0.58. Similarly (**Figure 2B**), no resistant subpopulations were observed in the analysis of daptomycin (with the exception of a small subpopulation that grew at daptomycin concentrations equal to 4 mg/L).

Discussion

We present here a case of multidrug resistant MRSA community-acquired endocarditis with a vancomycin MIC of 2 mg/L and daptomycin MIC of 2 mg/L (DNS) by Etest and 1 mg/L by microdilution. Because the discrepancy between susceptibility tests (probably due to the limit of accuracy of the assays), we could not confirm that the strain was truly non-susceptible. However, the fact that the Etest showed a higher MIC suggests that the strain was not fully susceptible to daptomycin; for the clinical management of the patient, we assumed that the strain was DNS.

While daptomycin has emerged as a potential therapy for MRSA endocarditis in the context of high MICs for vancomycin,[13] there are scarce data on the best treatment in case of infection due to DNS strains. There is a risk of the potential emergence of DNS strains after treatment failure in patients with complicated bacteremia or endocarditis treated with monotherapy.[14] The best therapy for DNS strains is debated, with clinical experience only based on some series of cases treated with a salvage regimen of daptomycin in association with potential synergistic antibiotic, such as cloxacillin, ceftaroline or fosfomycin.[6,15,16]

The use of ceftaroline in monotherapy for treatment of MRSA bacteremia has been assessed in a case-control studies and case series, showing comparable clinical outcomes than

the use of vancomycin or daptomycin,[17] although data remain scarce. Non-susceptibility to ceftaroline has been described, with few reported cases of ceftaroline resistant MRSA endocarditis.[18] These patients were successfully treated with vancomycin, or combination therapy of daptomycin plus ceftaroline. However, to our knowledge, no cases of both daptomycin (Etest) and ceftaroline (Etest and microdilution) non-susceptibility has been described so far. In our case, because of the clinical and microbiological improvement observed despite increased MICs, we pursued the same combination therapy, with a successful outcome. This was later confirmed by the *in vitro* studies, showing synergy and bactericidal activity of the combination of daptomycin plus ceftaroline at 0.5 x MIC and 1 x MIC, respectively using standard and high inocula by time-kill curves and also by checkerboard. Conversely, the combinations of daptomycin plus either cloxacillin or fosfomycin at Cmin levels were indifferent when using high inocula. The fact that blood cultures became sterile concomitantly to the valve repair suggests that surgery participated in the cure of the infection by decreasing bacterial burden.

Enhancement of daptomycin activity against MRSA in case of combination therapy is explained by a penicillin-binding protein disruption with ceftaroline, allowing a better antibiotic membrane insertion of daptomycin.[19] Also, exposure to subinhibitory daptomycin concentration may result in increasing expression of the gene encoding for PBP1[20] and therefore in enhancing bacterial killing in combination with a β-lactam PBP1 specific targeting.

The limitations of this case report include an inability to assess a potential acquisition of resistance from the colonizing to the invasive strain, and a failure to perform genotyping of the strain.

We described here a multidrug resistant MRSA community-acquired native left endocarditis successfully treated with a combination of ceftaroline and high dose daptomycin. *In vitro* data confirm the synergy between drugs and an increased bactericidal activity, suggesting that this may be an effective and safe association for the treatment of invasive MRSA infection. Our experience highlights the potential clinical use of synergism testing to guide difficult treatment decision in patients with multi-resistant invasive MRSA infections.

References:

1. van Hal SJ, Jensen SO, Vaska VL, *et al*. Predictors of mortality in Staphylococcus aureus Bacteremia. *Clin Microbiol Rev* 2012; **25**: 362–86.

2. Habib G, Lancellotti P, Iung B. 2015 ESC Guidelines on the management of infective endocarditis: a big step forward for an old disease. *Heart Br Card Soc* 2016; **102**: 992–4.

3. Aguado JM, San-Juan R, Lalueza A, *et al.* High vancomycin MIC and complicated methicillin-susceptible Staphylococcus aureus bacteremia. *Emerg Infect Dis* 2011; **17**: 1099–102.

4. Bassetti M, Nicco E, Ginocchio F, *et al.* High-dose daptomycin in documented Staphylococcus aureus infections. *Int J Antimicrob Agents* 2010; **36**: 459–61.

5. Moise PA, North D, Steenbergen JN, *et al.* Susceptibility relationship between vancomycin and daptomycin in Staphylococcus aureus: facts and assumptions. *Lancet Infect Dis* 2009; **9**: 617–24.

6. Shafiq I, Bulman ZP, Spitznogle SL, *et al.* A combination of ceftaroline and daptomycin has synergistic and bactericidal activity in vitro against daptomycin nonsusceptible methicillin-resistant Staphylococcus aureus (MRSA). *Infect Dis Lond Engl* 2017; **49**: 410–6.

7. Miró JM, Entenza JM, Del Río A, *et al.* High-dose daptomycin plus fosfomycin is safe and effective in treating methicillin-susceptible and methicillin-resistant Staphylococcus aureus endocarditis. *Antimicrob Agents Chemother* 2012; **56**: 4511–5.

8. Rand KH, Houck HJ. Synergy of daptomycin with oxacillin and other beta-lactams against methicillin-resistant Staphylococcus aureus. *Antimicrob Agents Chemother* 2004; **48**: 2871–5.

9. Clinical and Laboratory Standards Institute. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. CLSI, Wayne, PA, USA, 2018.

10. Eliopoulos GM, Moellering RC. Antibiotic combinations. In: *Antibiotics in laboratory medicine*. 4th ed. V. Lorian. Baltimore: The Williams & Wilkins Co, 1996; 330–96.

11. García-de-la-Mària C, Gasch O, García-Gonzalez J, *et al.* The Combination of Daptomycin and Fosfomycin Has Synergistic, Potent, and Rapid Bactericidal Activity against Methicillin-Resistant Staphylococcus aureus in a Rabbit Model of Experimental Endocarditis. *Antimicrob Agents Chemother* 2018; **62**: 2633–17.

12. Miró JM, García-de-la-Mària C, Armero Y, *et al.* Addition of gentamicin or rifampin does not enhance the effectiveness of daptomycin in treatment of experimental endocarditis due to methicillin-resistant Staphylococcus aureus. *Antimicrob Agents Chemother* 2009; **53**: 4172–7.

13. Cervera C, Castañeda X, Pericas JM, *et al.* Clinical utility of daptomycin in infective endocarditis caused by Gram-positive cocci. *Int J Antimicrob Agents* 2011; **38**: 365–70.

14. Marty FM, Yeh WW, Wennersten CB, *et al.* Emergence of a Clinical Daptomycin-Resistant Staphylococcus aureus Isolate during Treatment of Methicillin-Resistant Staphylococcus aureus Bacteremia and Osteomyelitis. *J Clin Microbiol* 2006; **44**: 595–7.

15. Baxi SM, Chan D, Jain V. Daptomycin non-susceptible, vancomycin-intermediate Staphylococcus aureus endocarditis treated with ceftaroline and daptomycin: case report and brief review of the literature. *Infection* 2015; **43**: 751–4.

16. Chen L-Y, Huang C-H, Kuo S-C, *et al.* High-dose daptomycin and fosfomycin treatment of a patient with endocarditis caused by daptomycin-nonsusceptible Staphylococcus aureus: case report. *BMC Infect Dis* 2011; **11**: 152.

17. Arshad S, Huang V, Hartman P, *et al.* Ceftaroline fosamil monotherapy for methicillin-resistant Staphylococcus aureus bacteremia: a comparative clinical outcomes study. *Int J Infect Dis IJID Off Publ Int Soc Infect Dis* 2017; **57**: 27–31.

18. Nigo M, Diaz L, Carvajal LP, *et al.* Ceftaroline-Resistant, Daptomycin-Tolerant, and Heterogeneous Vancomycin-Intermediate Methicillin-Resistant Staphylococcus aureus Causing Infective Endocarditis. *Antimicrob Agents Chemother* 2017; **61**: 1235–16.

19. Dhand A, Sakoulas G. Daptomycin in combination with other antibiotics for the treatment of complicated methicillin-resistant Staphylococcus aureus bacteremia. *Clin Ther* 2014; **36**: 1303–16.

20. Berti AD, Theisen E, Sauer J-D, *et al.* Penicillin Binding Protein 1 Is Important in the Compensatory Response of Staphylococcus aureus to Daptomycin-Induced Membrane Damage and Is a Potential Target for β-Lactam-Daptomycin Synergy. *Antimicrob Agents Chemother* 2015; **60**: 451–8.

REF. Wootton M, Howe RA, Hillman R, Walsh TR, Bennett PM, MacGowan AP. A modified population analysis profile (PAP) method to detect hetero-resistance to vancomycin in *Staphylococcus aureus* in a UK hospital. *J Antimicrob Chemother* 2001; **47**: 399–403

Comentado [FD1]: Is ref 9 correctly written? Yes. It is a more recent one, published in 2018.
Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing: Fifteen Informational Supplement M100-S15. CLSI, Wayne, PA, USA, 2005.

Figure legends

Figure 1: Time-kill curves of daptomycin (DAP) plus ceftaroline (CFT), fosfomycin (FOM) or cloxacillin (CLO) against MRSA-1081 strain (inoculum equal to 10^5 and 10^8 cfu/mL).

Figure 2: Population analysis profile (PAP) results for vancomycin (2A) and daptomycin (2B).

Supplementary table. Results of daptomycin (DAP) plus ceftaroline (CFT), fosfomycin (FOM) or cloxacillin (CLO) time-kill study against MRSA-1081 (inoculum equal to 10^5 and 10^8 cfu/mL).

Inoculum		**10^5 cfu/ml**		**10^8 cfu/ml**			**10^5 cfu/ml**		**10^8 cfu/ml**	
DAP+CFT	***[ATB] (mg/L)***	**4h**	**24h**	**4h**	**24h**	***[ATB] (mg/L)***	**4h**	**24h**	**4h**	**24h**
Control		+1	+3.4	+0.5	+0.7					
DAP	***0.5***	-0.9	+1.4	-0.5	-0.7					
CFT	***2***	+0.9	+2.1	-0.1	-0.3					
DAP+CFT		-2.5	-3.3	-1.4	-4					
DAP+CLO										
Control		+1.3	+3.1	+0.2	+0.5		+1.1	+3.3	+0.4	+0.6
DAP	***0.5***	-1.2	+2.1	-0.4	-0.1	***0.5***	-1.1	+1.6	-0.3	-0.4
CLO	***64***	+0.8	+2.7	+0.3	+0.2	***4***	+1	+2.8	+0.4	+0.4
DAP+CLO		-2.5	-3.8	-0.7	-1		-2.2	-3.5	-0.5	+0.1
DAP+FOM										
Control		+1.3	+2.9	+0.7	+1.1		+1.1	+3.1	+0.7	+1
DAP	***0.5***	-0.7	+2.1	-0.4	+0.3	***0.5***	-1.7	+1.5	-0.2	-0.1
FOM	***64***	+0.3	+1.8	-1.1	-0.2	***8***	+1.1	+1.3	0	+0.1
DAP+FOM		-3.1	-3.4	-2	-2.7		-2.8	-1.1	-1.2	0

In vivo study strain; Change in $\log_{10}$ cfu/mL;

Funding

JMM received a personal 80:20 research grant from the Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain, during 2017 to 2019.

Acknowledgements

The daptomycin and ceftaroline powder for in vitro studies were provided by MSD, Spain and Astra-Zeneca, Spain, respectively.

Transparency declarations

Potential financial conflicts of interest: JMM has received consulting honoraria and/or research grants from AbbVie, Angelini, Bristol-Myers Squibb, Contrafect, Jansen, Genentech, Gilead Sciences, Medtronic, Merck, Novartis, Pfizer, and ViiV Healthcare, outside the submitted work. All other authors: none to declare.