chemotherapeutic agents. Furthermore, in the original case description [2], the disease relapsed, despite a 12-month course of antimicrobials. It was not until after bilateral upper lung lobectomies and additional chemotherapy that cure was achieved.

We describe the first case of peritoneal infection with *M. heckeshornense*, in the setting of a patient with end-stage renal disease on PD. The peritonitis resolved without the use of specific antimicrobial medications, and this can probably be attributed to the source control involving peritoneal catheter removal and fluid drainage. Adequate source control alone has been found to be sufficient to clear *M. heckeshornense* infections in multiple reports, and our case of *M. heckeshornense* peritonitis is consistent with these findings.

Transparency Declaration

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Differences in daptomycin and vancomycin *ex vivo* behaviour can lead to false interpretation of negative blood cultures

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Abstract

In clinical studies on bacteraemia, the negativity of blood cultures is an important endpoint for comparing the efficacy of different therapeutic regimens. In FAN® anaerobic blood culture medium (BacT/ALERT system), daptomycin displayed increased MIC against *Staphylococcus aureus* and improved abolishment of its carryover effect in charcoal when compared with vancomycin. Differences between these two drugs can lead to a false interpretation of negative blood cultures. To compare different antibiotic regimens for the treatment of bacteraemia, preliminary studies are mandatory to ensure that *ex vivo* antibiotic behaviour is similar in the blood-culture system used.

Keywords: Bacteraemia, blood cultures, daptomycin, *Staphylococcus aureus*, vancomycin

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Vancomycin remains the backbone of treatment for serious infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) strains, in particular for bacteraemia and endocarditis [1,2]. However, emergence of *S. aureus* strains with intermediate susceptibility [3] or that are even resistant to vancomycin is worrying [4]. Furthermore, the pharmacokinetic features of vancomycin, including low bactericidal activity, weak post-antibiotic effect [5] and poor tissue distribution [6], may explain its slow clinical response, notably when the MIC is >1 mg/L [7].

Daptomycin, a cyclic lipopeptide that is active on Gram-positive bacteria including MRSA [8], displays rapid concentration-dependant bactericidal activity, which can reduce, *in vitro*, the bacterial inoculum faster than vancomycin [5,9]. Daptomycin is approved for the treatment of complicated skin and soft-tissue infections [10] and for the treatment of bacteraemia and right-side endocarditis [11,12].

In clinical studies on bacteraemia, the negativity of blood cultures is an important endpoint for comparing the efficacy of different therapeutic regimens [12,13]. However, bacterial growth in blood cultures is affected by the presence of residual concentrations of antibiotics: the so-called 'carryover effect'. To resolve this issue, blood-culture medium containing resin or charcoal is often used to adsorb antibiotics [14,15]. However, the influence of the medium on the *ex vivo* efficacy of different drugs, particularly for new antibiotics, has not been fully studied. Herein, using the BacT/ALERT system (BioMerieux, Lyon, France), we have studied the influence of a blood-culture medium that contained charcoal (FAN® anaerobic BacT/ALERT FN) on the bacterial viability of *S. aureus* in the presence of various concentrations of daptomycin or vancomycin.

Taking into account that daptomycin binds to the cytoplasmic membrane via a Ca^{2+}-dependant process, the MICs of *S. aureus* ATCC29213 were assessed in Muller Hinton (MH) broth medium with an inoculum of 10^4 CFU/mL supplemented or not with 50 mg/L of Ca^{2+} [16,17]. Because the FAN® anaerobic medium is supplemented with Brain Heart Infusion (BHI), the MICs were also performed in the BHI broth. As previously described [5,17], in the absence of Ca^{2+}supplementation, daptomycin MICs were one to three $\log_2$ dilutions higher: 2 mg/L vs. 1 mg/L in MH, and 16 mg/L vs. 2 mg/L in BHI (Table 1). In the absence of Ca^{2+} supplementation, the differences observed for MIC values obtained in BHI and MH are explained by the composition of the media, in particular the basic concentration of Ca^{2+} (3 mg/L vs. 37 mg/L for BHI and MH, respectively). As expected, the FAN® anaerobic medium that contained charcoal led to a significant increase in the MICs, from 2 to 16 mg/L for vancomycin and 16 to >256 mg/L for daptomycin, compared with the medium without charcoal (Table 1). However, adsorption by charcoal seemed to be more efficient for daptomycin than for vancomycin.

To reproduce *in vitro* the clinical conditions of blood culture collected from patients with staphylococcal bacteraemia and treated with vancomycin or daptomycin, bacterial-growth experiments were performed using an *ex vivo* blood culture (EVBC). The EVBC mixture was prepared as follows: horse blood, containing 10–60 mg/L of daptomycin or vancomycin, corresponding to concentrations close to those expected in human serum, was inoculated with 1, 10 and 100 CFU/mL, corresponding to the bacterial loads encoun-

TABLE 1. MICs of vancomycin and daptomycin for the *Staphylococcus aureus* ATCC29213 strain according to the medium used

Medium used	Daptomycin MIC (mg/L)		Vancomycin MIC (mg/L)	
	Without Ca^{2+} supplementation	Ca^{2+} supplementation (50 mg/L)	Without Ca^{2+} supplementation	Ca^{2+} supplementation (50 mg/L)
MH broth	2	1	1	1
BHI broth	16	2	2	2
Standard anaerobic BacT/ALERT SN (without charcoal)	16	ND	2	ND
FAN® anaerobic BacT/ALERT FN (with charcoal)	>256	ND	16	ND

ND, not done.

TABLE 2. Bacterial growth of the *Staphylococcus aureus* ATCC29213 strain in an *ex vivo* blood culture (EVBC)[a]

Volume (mL)	Antibiotic concentration (mg/L)		Inoculum (CFU/mL blood)					
Ex vivo blood culture[a]	Blood	Bottle	Vancomycin			Daptomycin		
			1	10	100	1	10	100
10	10	2.0	+	+	+	+	+	+
10	15	3.0	–	+	+	+	+	+
10	20	4.0	–	+	+	+	+	+
10	25	5.0	–	–	–	+	+	+
10	30	6.0	–	–	–	+	+	+
10	40	8.0	–	–	–	+	+	+
10	60	12.0	–	–	–	+	+	+
5	10	1.1	+	+	+	+	+	+
5	15	1.7	+	+	+	+	+	+
5	20	2.2	+	+	+	+	+	+
5	25	2.8	–	+	+	+	+	+
5	30	3.3	–	–	–	+	+	+
5	40	4.4	–	–	–	+	+	+
5	60	6.7	–	–	–	+	+	+
2	10	0.5	+	+	+	+	+	+
2	15	0.7	+	+	+	+	+	+
2	20	1.0	+	+	+	+	+	+
2	25	1.2	+	+	+	+	+	+
2	30	1.4	+	+	+	+	+	+
2	40	1.9	+	+	+	+	+	+
2	60	2.8	+	+	+	+	+	+

[a]*Ex vivo* blood culture corresponded to a mixture of horse blood containing different concentrations of daptomycin or vancomycin, inoculated with three bacterial inocula and introduced into FAN® anaerobic medium with charcoal.
+, positive culture; –, negative culture.

tered during bacteraemia and endocarditis [18,19]. Aliquots of 2, 5 or 10 mL of the EVBC mixture were then quickly added to the bottle containing 40 mL of FAN® anaerobic medium, resulting in final antibiotic concentrations of 0.5–12 mg/L (Table 2). This ensured that the antibiotic did not have sufficient time to become active before introduction into the bottle. The bottles were then incubated for 6 days in a BacT/ALERT automate, and all experiments were performed in duplicate. For daptomycin, after the addition of 10 mL of EVBC mixture, corresponding to the volume of blood recommended by the manufacturer, EVBC became positive for growth whatever the antibiotic concentration and the bacterial inoculum tested (Table 2). In contrast, for vancomycin, whatever the bacterial inoculum used, no growth occurred when the final antibiotic concentration in the bottle was ≥5 mg/L, corresponding to vancomycin blood-concentrations of ≥25 mg/L.

The discrepancy between the concentration of vancomycin that inhibits growth in this experiment (5 mg/L) and the MIC value found in the FAN® anaerobic medium without charcoal (16 mg/L) may be because of the difference in the inoculum used in the two experiments (1–100 CFU/mL in the bottle vs. 10^4 CFU/mL for MIC determinations). In clinical practice, and according to the mode of administration and the susceptibility of the *S. aureus* strain, the concentration of vancomycin in serum is usually between 15 (target through concentrations for intermittent infusion) and 40 mg/L (for continuous administration) [2,20]. In these conditions, the inhibition of bacterial growth at a blood concentration of 25 mg/L, for a strain with a MIC of 1 mg/L in standard conditions (Table 2), could overestimate the *in vivo* efficacy of vancomycin due to the carryover effect. This effect is amplified when the bacterial load is very low (1 CFU/mL) because inhibition of bacterial growth is even observed at a concentration of 15 mg/L (Table 2). This effect became more pronounced using the FAN® aerobic BacT/ALERT FA medium due to the volume of medium present in the bottle (30 mL vs. 40 mL for FAN® anaerobic), which led to an increased antibiotic concentration in the FAN® aerobic medium (data not shown).

To overcome this issue, the volume of EVBC mixture introduced into the bottle was reduced to decrease antibiotic concentration. For daptomycin, as expected from the previous results, blood-volume reduction had no impact on bacterial growth (Table 2). In contrast, for vancomycin the cultures remained negative when an EVBC volume of 5 mL was inoculated, corresponding to a final concentration in the bottle of ≥3 mg/L (vancomycin blood concentrations ≥30 mg/L). Finally, when an EVBC volume of 2 mL was inoculated, all bottles became positive, even in the presence of a blood concentration of 60 mg/L of vancomycin (Table 2).

In conclusion, two important parameters were responsible for the differences observed between vancomycin and

daptomycin in the positivity of the EVBC. First, although the MICs of the two antibiotics were similar in standard conditions, they were very different in BHI and FAN® medium without charcoal, which underlines the essential role of BHI. Second, in the FAN® medium with charcoal, daptomycin was adsorbed more than vancomycin, which led to a stronger reduction in the carryover effect. In this context, for studies using negativity of blood cultures as the endpoint to compare different antibiotic regimens, *ex vivo* differences in antibiotic behaviour introduce an important bias. Our results also indicate that, for vancomycin, using the BacT/ALERT system, 10 mL of blood per bottle is probably not suitable for monitoring the negativity of blood culture. In this study, only 2 mL of blood allowed comparison between vancomycin and daptomycin whatever the therapeutic antibiotic concentration. Investigators should be aware that, for studies that compare different antibiotic regimens for the treatment of bacteraemia, preliminary studies are mandatory to ensure that *ex vivo* antibiotic behaviour is similar in the blood-culture system used.

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Transparency Declaration

The authors have no conflicts of interest to declare.

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