

## In vitro antibiotic activity against intraosteoblastic *Staphylococcus aureus*: a narrative review of the literature

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*Staphylococcus aureus* – a major aetiological agent of bone and joint infection (BJI) – is associated with a high risk of relapse and chronicity, in part due to its ability to invade and persist in non-professional phagocytic bone cells such as osteoblasts. This intracellular reservoir protects *S. aureus* from the action of the immune system and most antibiotics. To date, the choice of antimicrobial strategies for BJI treatment mostly relies on standard susceptibility testing, bone penetration of antibiotics and their ‘antibiofilm’ activity. Despite the role of intracellular persistent *S. aureus* in the development of chronic infection, the ability of antibiotics to target the *S. aureus* intraosteoblastic reservoir is not considered in therapeutic choices but might represent a key determinant of treatment outcome. This review provides an overview of the intracellular pharmacokinetics of antistaphylococcal drugs used in the treatment of BJI and of their ability to target intraosteoblastic *S. aureus*. Thirteen studies focusing on the intraosteoblastic activity of antibiotics against *S. aureus* were reviewed, all relying on *in vitro* models of osteoblast infection. Despite varying incubation times, multiplicities of infection, bacterial strains, and the types of infected cell lines, rifamycins and fluoroquinolones remain the two most potent antimicrobial classes for intraosteoblastic *S. aureus* eradication, consistent with clinical data showing a superiority of this combination therapy in *S. aureus* orthopaedic device-related infections.

### Introduction

Bone joint infections (BJI) are polymorphic infections, ranging from native joint septic arthritis to difficult-to-treat chronic osteomyelitis and orthopaedic device-related infections. Apart from the risk of functional sequelae, a major concern are chronic and/or device-associated infections, resulting in a rate of relapse approaching 20% despite complex and costly medical and surgical management.<sup>1–3</sup> Implicated in up to 30% of cases,<sup>4,5</sup> *Staphylococcus aureus* is associated with particularly difficult-to-treat BJI, due to persistent phenotypes facilitating chronic infection and relapse.<sup>6</sup> Among these, internalization and persistence in non-professional phagocytic cells such as osteoblasts represent a well-described interaction of *S. aureus* with its human host.<sup>6,7</sup> After an active cellular process controlled by the actin cytoskeleton, bacteria are found in an endosome from which they can escape before lysosomal fusion or survive within the phagolysosome,<sup>8–14</sup> and multiply in the

cytoplasm or vacuoles.<sup>12,14,15</sup> *S. aureus* infection can also induce osteoblast death, due to intracellular expression of virulence factors such phenol-soluble modulins (PSMs) or protein A.<sup>16–18</sup> In addition to participating in bone destruction, cell lysis allows bacterial release into the extracellular medium, and thus the infection of new host cells and persistence of the infection.<sup>10,13</sup> Additionally, *S. aureus* phenotype switching to small colony variants (SCVs) and persists, morphotypes associated with better intracellular persistence due to their reduced virulence, have been observed intracellularly.<sup>9,19,20</sup>

This intracellular reservoir allows bacteria to escape the action of the host’s immune system and most antibiotics. Consequently, the intrinsic antistaphylococcal activity, antibiofilm efficacy and bone/joint penetration of antimicrobials might not be sufficient to predict their efficacy in BJI: their ability to eradicate the intracellular bacterial reservoirs could represent an essential determinant of treatment outcome. After the description of intracellular

pharmacokinetics parameters, we present a review of the *in vitro* intraosteoblastic activities of antibiotics against *S. aureus*.

### The *in vitro* model of osteoblast infection

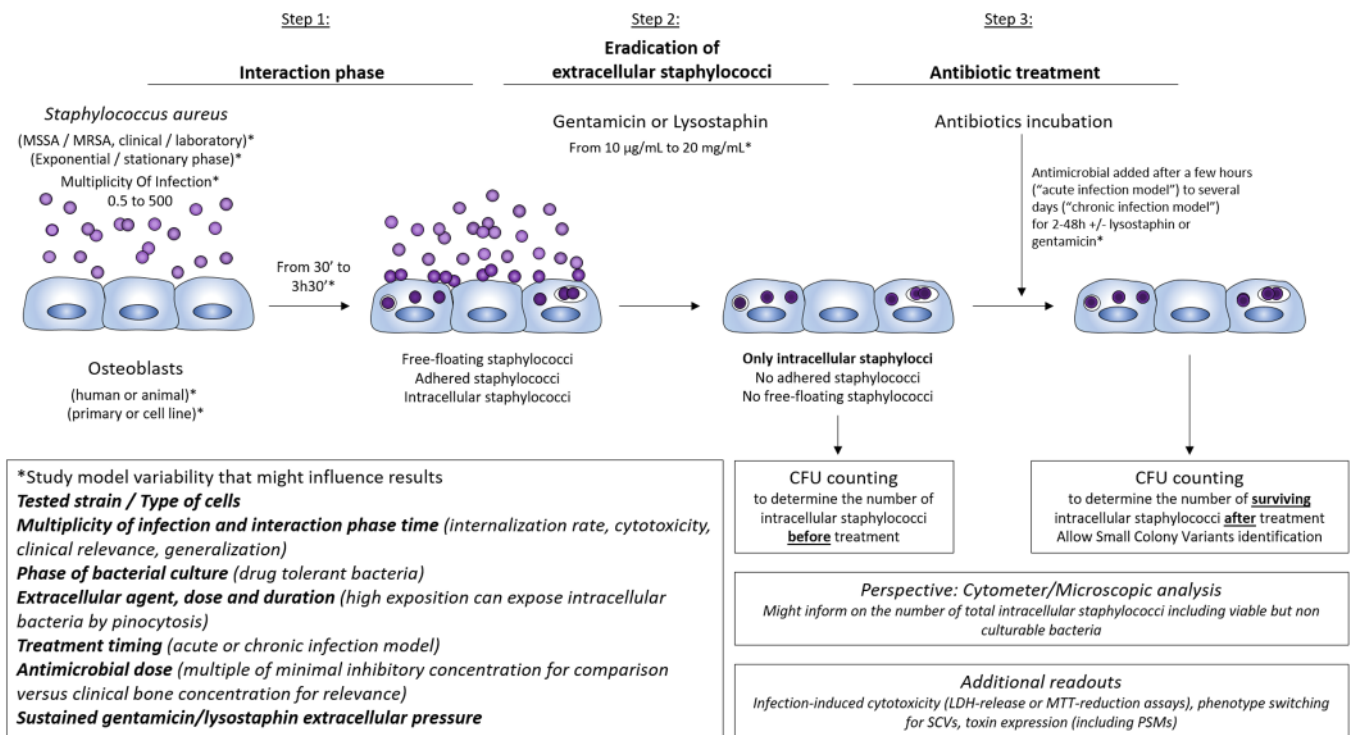
Antibiotic activity against intraosteoblastic *S. aureus* has mostly been studied using a conserved lysostaphin/gentamicin protection assay presented in Figure 1. The specific details of the co-culture model design vary slightly among the different studies reviewed. Hence, each step is subject to adaptations that might influence the recorded results (Figure 1, Table 1). Briefly, confluent eukaryotic cells (mostly human osteoblastic primary cells or osteoblastic cell lines) are infected with *S. aureus* (mostly MSSA laboratory strains) at a defined inoculum [multiplicity of infection (moi) ranging from 0.5–500 bacteria per cell, 100:1 being the most frequent]. After a variable co-incubation time allowing bacterial adhesion and internalization, a non-permeant drug—either gentamicin or lysostaphin—is added to the culture medium to exclusively kill the remaining extracellular bacteria. Extracellular pressure with lysostaphin or gentamicin can then be maintained to prevent bacteria released by cell lysis infecting neighbouring cells.<sup>10</sup> Tested antibiotics are then added into the culture medium for a determined period. Time before treatment—defining ‘acute’ (early treatment after cell infection) or ‘chronic’ (treatment delayed for several days) infection models—is an important parameter, as bacterial wall modifications after long-term intracellular persistence can affect antimicrobial efficacy.<sup>21</sup> Importantly, the tested doses are mostly multiples of the MIC to allow comparisons, or the clinical bone concentration, but cellular pharmacokinetic/

pharmacodynamic (PK/PD) assessments are lacking (except in one study<sup>22</sup>), limiting interpretation of results. Finally, remaining intracellular bacteria are numerated by plating cell lysates, and compared with untreated infected cells. Of note, this technique cannot account for viable but non-growing bacteria, despite the phenotypical heterogeneity of the surviving intracellular bacterial population.<sup>23,24</sup> To overcome this limitation, flow cytometry or microscopy assays have been proposed. Furthermore, monitoring the delay in appearance and the size of colonies helps to evaluate the ratio of SCV in the surviving population.<sup>25–27</sup> Infection-induced cytotoxicity can also be recorded by MTT reduction, propidium iodide incorporation or lactate dehydrogenase (LDH)-release assays.<sup>26,28–31</sup> One study additionally investigated the impact of antimicrobials on intraosteoblastic PSM secretion.<sup>31</sup>

### Pharmacokinetics and intraosteoblastic activity of antistaphylococcal antibiotics

Knowing both the intraosseous and intracellular PK parameters of antibiotics is a prerequisite to understanding their potential activity against *S. aureus* subcellular reservoirs. All PK parameters relevant for the interpretation of intraosteoblastic activity of antimicrobials are reported in Table 2.

Bone concentrations of antimicrobials achieved in clinical practice are unclear, due to the absence of standardized conditions for PK parameter measurement (dose, number of administrations before sampling), the heterogeneity of antimicrobial penetration between cancellous and cortical bone, and impact of local



**Figure 1.** Principles of the gentamicin/lysostaphin assays evaluating the efficacy of antimicrobials against intraosteoblastic *S. aureus* eradication, focusing on experimental variable conditions. This figure appears in colour in the online version of JAC and in black and white in the print version of JAC.

**Table 1.** Summary of the reviewed studies focusing on the experimental parameters influencing the assessment of intraosteoblastic activity of antimicrobials

Reference	Prokaryotic strain				Bacterial state	Extracellular bacterial killing agent/ concentration and time	Time of co-incubation and extracellular killing before antibiotics addition (37°C)	Antibiotic MIC (mg/L)	Concentration (clinically mimicking)	Time of antibiotic incubation (h)
	Eukaryotic model	MSSA	MRSA	Multiplicity of infection <sup>a</sup>						
Ellington 2006 <sup>21</sup>	Clonetics normal human NHOst and primary mouse osteoblasts	UAMS-1 (ATCC 49230, CDC 587)		125	Stationary phase	Gentamicin 25 mg/L (Sustained)	3.75 h or 15.75 h	Erythromycin (8) Rifampicin (20) Clindamycin (4)	1 × MIC	12, 24 and 48
Lemaire 2010 <sup>22</sup>	Clonetics normal human osteoblasts NHOst		SA238 SA238L	0.5	Exponential phase	Gentamicin (Not specified)	2 h	Linezolid (2 and 16) Radezolid (0.5–1 and 2)	0.01 to 100 mg/L	24
Kreis 2013 <sup>54</sup>	Primary human osteoblast	Cowan I (ATCC 12598, NCTC 8530) Septic arthritis isolate UAMS-1 (ATCC 49230, CDC 587) Chronic osteomyelitis isolate		100	Stationary phase	Lysostaphin 20 mg/L (Not specified)	30 min at RT then 3 h	Tigecycline (ND) Gentamicin (ND) Rifampicin (ND)	10 mg/L 10 mg/L 7 mg/L (serum and tissue)	20 and 40
Noore 2013 <sup>44</sup>	Rat osteoblastic cell line UMR-106 (CRL-1661)	One Clinical isolate (ampicillin, ceftioxin and penicillin resistant)		500	Exponential phase	Lysostaphin 50 mg/L (Not sustained, 2 h)	4 h	Clindamycin (ND) Cefazolin (ND)	45 mg/L for both (not specified)	2
Mohamed 2014 <sup>74</sup>	SAOS-2 osteoblast-like cell line	EDCC5055 Wound infection isolate Cowan I (ATCC 12598, NCTC 8530)		30	Exponential phase	Lysostaphin 2 × 10 <sup>6</sup> mg/L (Not sustained, time not specified)	30 min	Gentamicin (ND) Rifampicin (ND)	30, 100 and 200 mg/L 7.5 mg/L (serum and tissue)	4 and 24
Sanchez 2015	Primary human osteoblast		SAMMC-58 Clinical isolate (Pulse field type BAMC type 15)	50	Not specified	Gentamicin 200 mg/L (Not sustained, 1 h)	30 min at 4°C then 2 h	Gentamicin (ND) Vancomycin (ND) Rifampicin (ND) Rifabutin (ND) Rifapentine (ND) Rifaximin (ND)	200 mg/L 128 mg/L 32 mg/L 32 mg/L 32 mg/L 32 mg/L (not specified)	4 and 24
Valour 2015 <sup>26</sup>	Human osteoblastic cell line MG63 (CRL-1427)	HG001 (NCTC8325, rsbU+) Sepsis isolate		100	Stationary phase	Lysostaphin 10 mg/L (Sustained)	30 min at 4°C then 3 h	Oxacillin (0.094) Ceftaroline (0.19) Clindamycin (0.032) Fosfomycin (2) Vancomycin (1.5) Teicoplanin (1.5) Daptomycin (0.19) Linezolid (1) Ofloxacin (0.5) Rifampicin (0.004) Tigecycline (0.125)	10, 4, 4, 6, 3, 5, 8, 2, 6 and 0.3 mg/L respectively. Concentrations 3 times higher and lower were also investigated. (intraosseous)	24
Tuchscher 2015 <sup>25</sup>	Primary human osteoblast	6850 SH1000 Two clinical osteomyelitis isolates		50	Stationary phase	Lysostaphin 20 mg/L (Sustained)	3.5 h or 7 days	Cefuroxime (ND) Vancomycin (ND) Rifampicin (ND) Moxifloxacin (ND) Fluorocloxacillin (ND)	4.0 mg/L 50 mg/L 20 mg/L 10 mg/L 30 mg/L	48

Continued

**Table 1.** Continued

Reference	Eukaryotic model	Prokaryotic strain		Multiplicity of infection <sup>a</sup>	Bacterial state	Extracellular bacterial killing agent/ concentration and time	Time of co-incubation and extracellular killing before antibiotics addition (37°C)	Antibiotic MIC (mg/L)	Concentration (clinically mimicking)	Time of antibiotic incubation (h)
		MSSA	MRSA							
Dupieux 2017 <sup>40</sup>	Human osteoblastic cell line MG63 (CRL-1427)	<i>mechA::kana</i> LUG359 (COL)	LUG359 (COL)	100	Stationary phase	Lysostaphin 10 mg/L (Sustained)	3 h	Linezolid (ND) Daptomycin (ND) Clindamycin (ND) Fosfomycin (ND) Gentamicin (ND)	20 mg/L 60 mg/L 20 mg/L 250 and 500 mg/L 20 and 140 mg/L (serum)	24
Abad 2018 <sup>30</sup>	Human osteoblastic cell line MG63 (CRL-1427)	6850 Two clinical isolates	/	100	Stationary phase	Lysostaphin 10 mg/L (Sustained)	3 h	Daptomycin (0.064 and 0.19 at pH 7) Oxacillin (0.25 and >256 at pH 7) Ceftaroline (0.125 and 0.25 at pH 7) Linezolid (1.5) Tedizolid (0.25)	5 mg/L 10 mg/L 4 mg/L (intraosseous)	24
Mendez 2019 <sup>27</sup>	Human osteoblastic cell line MG63 (CRL-1427)	Ten clinical isolate (117-1, 116-1, 804-1, 221-1, 111-1, 806-1, 201-1, 401-1, 203-1, 104-1) Seattle 1945 (ATCC 25923)		100		Lysostaphin 10 mg/L (Sustained)	3 h	Rifampicin (0.016, 0.012, 0.008, 0.008, 0.008, 0.012, 0.008, (cortical and cancellous respectively) Levofloxacin (0.19, 0.19, 0.13, 0.19, 0.25, 0.13, 0.19, 0.13, 0.19, 0.25, respectively)	2.5 and 5 mg/L 2.5 and 6 mg/L cellous intraosseous	24
Abad 2020 <sup>31</sup>	Human osteoblastic cell line MG63 (CRL-1427)	6850 Two clinical isolates		100	Stationary phase	Lysostaphin 10 mg/L (Sustained)	3 h or 7 days	Rifabutin (0.031, 0.031, 0.062 respectively) Rifapentin (0.062, 0.031, 0.062) Rifampicin (0.016, 0.008, 0.031)	0.1, 1, 10 and 100× MIC for all (intraosseous)	24
Yu 2020 <sup>28</sup>	RFP-labelled mouse osteoblast cell line MC3T3-E1		GFP-labelled USA300 (ATCC BAA-171)	10	Exponential phase	Not treated	3 h	Vancomycin (ND) Cefazolin (ND) Cephalexin (ND) Gentamicin (ND) Rifampicin (ND)	10 mg/L 100 mg/L 100 mg/L 50 mg/L 4 mg/L (arbitrary from studies investigating concentration range)	1 to 14

ND, not defined.

<sup>a</sup>Multiplicity expressed as number of bacteria per osteoblast.

**Table 2.** Pharmacokinetics parameters and intraosteoblastic activity of the main antistaphylococcal antimicrobials used in bone and joint infection

Class/ antibiotic	C <sub>bone</sub> /C <sub>plasma</sub>	C <sub>bone</sub> (mg/L)	C/E	Sub-cellular localization	Intraosteoblastic bacterial load decreased (C <sub>used</sub> )	Intraosteoblastic activity <sup>a</sup>	Intraosteoblastic activity in 'chronic' model	Small Colony Variant phenotype switching
<b>β-Lactams</b>								
Penicillins								
Oxacillin	0.17 <sup>26</sup>	4–10 <sup>26,33</sup>	<1 <sup>34,36,38,62</sup>	Cytosol <sup>83,84</sup>	24%–43% (10 mg/L)	Moderate	–	Decreased
Flucloxacillin	0.12–1.2 <sup>32</sup>	7.2–89.5 <sup>33,85,86</sup>			75% (30 mg/L)	Moderate	Lost	Not affected
<b>Cephalosporins</b>								
Cefaroline	0.19 <sup>26</sup>	4 <sup>26</sup>			30% (4 mg/L)	Moderate	–	Decreased
Cefazolin	0.18 <sup>32</sup>	75.4 <sup>33</sup>			2% (45 mg/L)	Low	–	–
Cefuroxime	0.04–0.08 <sup>32</sup>	–			50% (40 mg/L)	Moderate	Lost	Not affected
<b>Macrolides</b>								
Erythromycin	0.18–0.28 <sup>32</sup>	–	2–13, 38, 53, 59	2/3 Lysosomes	0% (8 mg/L)	None	Lost	–
Azithromycin	2.5–6.3 <sup>32</sup>	–	90–140 <sup>59,60</sup>	1/3 Cytosol <sup>57,87</sup>	–	–	–	–
<b>Fluoroquinolones</b>								
Levofloxacin	0.36–1.0 <sup>32</sup>	4.6–10 <sup>33</sup>	4–10 <sup>55,68–71</sup>	Cytosol <sup>70</sup>	>99% (3 mg/L)	Good	–	–
Moxifloxacin	0.33–1.05 <sup>32</sup>	2.8 <sup>33</sup>			75% (30 mg/L)	Moderate	Conserved	–
Ofloxacin	0.09–1.04 <sup>32</sup>	2 <sup>26</sup>			>99.9% (2 mg/L)	Good	–	Decreased
<b>Aminoglycosides</b>								
Gentamicin	0.17–33 <sup>85</sup>	1.6–4.6 <sup>85</sup>	<1–4 <sup>38,53</sup>	Lysosomes <sup>53</sup>	0%–99% (10–200 mg/L)	Moderate	Conserved	Increased
<b>Lincosamides</b>								
Clindamycin	0.21–0.45 <sup>32</sup>	6.9 <sup>33</sup>	5–20 <sup>35,38,61</sup>	Lysosomes Cytosol <sup>84,88</sup>	23%–99.9% (1.33–42 mg/L)	Good (variable de- pending on the studies)	Lost	Increased
<b>Cyclines</b>								
Tigecycline	0.35–1.95 <sup>32</sup>	0.3 <sup>26</sup>	64 <sup>66</sup>	–	99.9% (0.3 mg/L)	Good	–	Decreased
Rifamycins								
Rifampicin	0.08–0.56 <sup>32</sup>	1.3–6.5 <sup>33</sup>	2–10 <sup>67,72</sup>	Probably phagosomes <sup>89</sup>	60%–99.9% (0.8–32 mg/L)	Good	Conserved	Increased/Decreased (variable depending on the studies)
Rifapentine	–	–	60–87 <sup>73</sup>		45%–85% (3.1 × 10 <sup>-2</sup> –32 mg/L)	Moderate	Conserved	Increased
Rifabutin	–	–	–		55%–95% (3.1 × 10 <sup>-3</sup> –32 mg/L)	Good	Conserved	Increased
<b>Glycopeptides</b>								
Vancomycin	0.05–0.67 <sup>32</sup>	3.8–4.5 <sup>33</sup>	1.22–>10 <sup>49,50</sup>	Lysosomes <sup>45</sup>	0%–75% (6–128 mg/L)	Moderate	Conserved	Not affected
Teicoplanin	0.05–0.85 <sup>32</sup>	3 <sup>26</sup>	3.9–60 <sup>46,49,50</sup>		0% (3 mg/L)	None	–	–
<b>Oxazolidinones</b>								
Linezolid	0.4–0.51 <sup>32</sup>	6.3–9.1 <sup>90</sup>	1 <sup>67</sup>	Lysosomes, cytosol <sup>91</sup>	0%–99.9% (2.67–20 mg/L)	Good	Conserved	Decreased/Not affected (variable depending on the studies)
Tedizolid	–	–	–		0%–56% (0.625–10 mg/L)	Moderate	–	–
Radezolid	–	–	9.7 <sup>22</sup>		50% (1 mg/L)	Moderate	–	–

Continued

Table 2. Continued

Class/antibiotic	$C_{\text{bone}}/C_{\text{plasma}}$	$C_{\text{bone}}$ (mg/L)	C/E	Sub-cellular localization	Intraosteoblastic bacterial load decreased ( $C_{\text{used}}$ )	Intraosteoblastic activity <sup>a</sup>	Intraosteoblastic activity in 'chronic' model	Small Colony Variant phenotype switching
Other								
Fosfomycin	0.13–0.45 <sup>32</sup>	4 <sup>26</sup>	1.8 <sup>48</sup>	-	>99% (4 mg/L)	Good	Lost	Increased/Decreased (Variable depending on the studies)
Daptomycin	0.08 <sup>92</sup>	3.3–3.4 <sup>92</sup>	<1 <sup>49,50</sup>	-	0%–75% (1.7–60 mg/L)	Moderate	Lost	Decreased/Not affected (Variable depending on the studies)

<sup>a</sup>The intraosteoblastic activity was categorized as follow depending on the intraosteoblastic bacterial load reduction: none (0%), low (<15%), moderate (15%–90%), good (>90%).

inflammation/infection on final tissue concentrations.<sup>32,33</sup> Values proposed here represent the best estimates presented to date.<sup>32,33</sup>

Almost all studies of cellular pharmacokinetics of antibiotics have been conducted on professional phagocytic cells in *in vitro* models, generally using fluorescently labelled molecules. Results are expressed by the ratio between cellular and extracellular drug concentration (C/E), which defines low (C/E < 1), intermediate (C/E = 1) and good (C/E > 1) cellular penetration. Again, values presented here constitute estimates, but actual cellular accumulation may vary according to the antimicrobial PK profile, including linearity of drug accumulation over time and according to extracellular concentration, protein binding, and possible active influx/efflux transporters.

### Antibiotics targeting the bacterial wall

#### $\beta$ -Lactams

Penicillins diffuse weakly into eukaryotic cells (C/E < 1),<sup>34–38</sup> with the exception of cloxacillin with a C/E of 4.7.<sup>39</sup> Oxacillin showed bactericidal activity and decreased the intracellular load of MSSA.<sup>40</sup> Surprisingly, it also reduced MRSA intraosteoblastic load, possibly due to local conditions. Indeed, low pH in intracellular organelles allows a conformational change of PBP2a, restoring the activity of  $\beta$ -lactams against MRSA.<sup>41–43</sup> Of note, a total loss of flucloxacillin activity was observed in a chronic infection model.<sup>25</sup> Similarly impeded by a low C/E ratio, cefazolin showed a weak intracellular activity,<sup>44</sup> and cefuroxime lost its activity on long-term intracellular-persisting bacteria.<sup>25</sup> Among new generation anti-MRSA cephalosporins, ceftaroline exhibited bacteriostatic intraosteoblastic activity and reduced the intracellular bacterial load by 30%.<sup>26,40</sup>

#### Glycopeptides and lipoglycopeptides

Vancomycin shows slow and modest accumulation in macrophages.<sup>45</sup> Teicoplanin, a more lipophilic compound, has a higher and faster accumulation (C/E = 60).<sup>46</sup> However, they were both ineffective or only bacteriostatic toward intraosteoblastic *S. aureus* at clinically relevant concentrations.<sup>26,28</sup> At higher concentrations, vancomycin was shown to be efficacious, including in a chronic infection model.<sup>25,29</sup> Dalbavancin decreases intraosteoblastic load by 50% at intraosseous concentrations, with no difference compared with vancomycin.<sup>47</sup>

#### Fosfomycin

Fosfomycin displays a moderate intracellular accumulation, with a reported C/E ratio of 1.8.<sup>48</sup> It exhibited bactericidal activity with a significant intracellular load decrease within osteoblasts but lost its activity in the chronic infection model, even at high concentrations.<sup>25,26</sup>

#### Daptomycin

Daptomycin penetrates weakly in eukaryotic cells (C/E = 0.7),<sup>49,50</sup> and was poorly effective against MSSA and MRSA intracellular load at clinical concentrations during short incubation periods.<sup>26,40</sup> Daptomycin was however more efficient when used at



higher concentration during 24 h or 48 h of incubation,<sup>26</sup> and lost its activity in the chronic infection model.<sup>25</sup>

### Antibiotics acting on bacterial protein synthesis

#### Aminoglycosides

Aminoglycosides have often been described as extracellular antimicrobials (C/E < 1),<sup>34,51,52</sup> However, studies on macrophages and fibroblasts have shown that prolonged cell exposure allowed an increase in the aminoglycosides' C/E ratio up to 2 to 4.<sup>53</sup> A time-dependent activity of gentamicin at 10 mg/L on *S. aureus* intracellular load was consequently highlighted,<sup>54</sup> sustained in the chronic model.<sup>25</sup> Because gentamicin is not able to diffuse into cells, its ability to target intracellular *S. aureus* could be due to infection-related pinocytosis allowing gentamicin to penetrate concomitantly in the same subcellular compartment as the bacteria. If aminoglycosides are not used in the long-term treatment of BJI, these results do not support the use of gentamicin for extracellular bacteria killing in these models—and especially not as sustained extracellular antibiotic pressure.

#### Macrolides

Macrolides show high C/E ratios in most cell types.<sup>55–58</sup> However, the ratio varies considerably depending on molecules, from 4 for erythromycin to 140 for azithromycin. The absorption and efflux of macrolides is generally rapid (3 to 15 min), with the notable exception of azithromycin.<sup>59,60</sup> Erythromycin showed a bacteriostatic activity in an acute infection model, but rapidly lost its activity against persisting bacteria.<sup>21</sup>

#### Lincosamides

Clindamycin accumulated well in eukaryotic cells, with C/E > 5.<sup>35,38,61</sup> Clindamycin used at relevant clinical concentrations impeded the growth of intraosteoblastic *S. aureus* when added early after infection, while no effect was recorded later.<sup>21</sup> However, conflicting results have been observed, probably due to differences between the models.<sup>25,26,44</sup>

#### Cyclines

The ability of tetracyclines to accumulate in eukaryotic cells is moderate (C/E = 1–7),<sup>62–65</sup> with a superiority of tigecycline and minocycline, with C/Es up to 64.<sup>66</sup> Tigecycline allowed a significant decrease of intraosteoblastic *S. aureus* load,<sup>26</sup> enhanced by prolonged exposure time.<sup>54</sup>

#### Oxazolidinones

Linezolid has been shown to reach poor intracellular concentrations (C/E ~ 1).<sup>22,67</sup> Used at bone or human serum concentration, linezolid significantly reduced the intraosteoblastic bacterial load and maintained its activity in 'chronic' infection models.<sup>25,26</sup> Additionally, linezolid and tedizolid displayed a strain-dependent activity against intraosteoblastic *S. aureus*, as highlighted in different clinical isolates despite similar MICs.<sup>30</sup> Of note, radezolid, a more lipophilic oxazolidinone, has better intracellular accumulation (C/E = 11) and was more potent than linezolid against intraosteoblastic bacteria, making it a promising alternative in BJI treatment.<sup>22</sup>

### Antibiotics acting on nucleic acid production

#### Fluoroquinolones

Cellular concentrations of fluoroquinolones are generally 4 to 10 times higher than in extracellular environment.<sup>55,68–71</sup> Used at clinical concentrations, ofloxacin and levofloxacin had bactericidal activity with a decrease of >99.5%, while moxifloxacin reduced the intracellular load of *S. aureus* by 75%.<sup>25–27</sup> Moxifloxacin remained active in chronic infection models, with about 90% decrease of the intraosteoblastic bacterial load.<sup>25</sup>

#### Rifamycins

They represent the most potent antimicrobials to eradicate intraosteoblastic *S. aureus*. Rifampicin accumulates intracellularly with a C/E ranging from 2 to 10, while rifapentine has higher C/E ratios from 60 to 80.<sup>62,72,73</sup> In the acute infection model, rifampicin killed more than 99.9% of intraosteoblastic bacteria in a time-dependent manner after 20–48 h,<sup>21,26,54,74</sup> even though less-impressive results have been reported in another study.<sup>27</sup> In the study by Ellington *et al.*,<sup>21</sup> efficacy of rifampicin rapidly decreased as the bacterial intracellular persistence time increased. However, maintained rifampicin efficacy in the chronic infection model has been reported.<sup>25</sup> Some studies compared rifampicin with the other members of the rifamycin family (rifabutin, rifapentine and rifaximin) showing similar excellent efficacy.<sup>29,31</sup> Further, rifabutin was the only rifamycin able to significantly decrease the intraosteoblastic bacterial load at low concentration (0.1 × MIC) in the 'acute' and 'chronic' models.<sup>31</sup> This higher activity is probably due to its 100-fold higher oil/water partition coefficient compared with rifampicin and it might be a clinical alternative to rifampicin in the BJI setting.

### Inter-class comparison

The main challenge when aiming to compare the intraosteoblastic activity of antibiotics from different classes against *S. aureus* lies in the choice of tested concentrations. Indeed, clinical concentrations (i.e. intraosseous or plasma concentrations for example) are sometimes very different. The use of multiple of MICs that include clinically relevant concentrations appears to be the more relevant for direct comparison. Additionally, the possible inter-strain variability and other variable experimental conditions discussed above make comparisons even more difficult. Nevertheless, a trend of antibiotic activity against intraosteoblastic *S. aureus* may be extrapolated (Figure 2, Table 2). Daptomycin and vancomycin appeared to be the least-potent drugs, having at best a bacteriostatic effect with concentrations similar to the clinical intraosseous one. Cefazolin, ceftaroline and teicoplanin have bacteriostatic activity at osseous concentrations. Drugs having significant bactericidal activity toward intraosteoblastic *S. aureus* at clinically relevant concentration are fosfomycin, oxazolidinones, tigecycline, fluoroquinolones, rifamycins and clindamycin. Among these, rifamycins, fluoroquinolones and clindamycin were the most efficient with activity recorded even at the minimal concentration tested, including against long-term persisting *S. aureus* for rifampicin and moxifloxacin.

### Antibiotic combinations

Given that combination therapies—and especially rifampicin-based ones<sup>75,76</sup>—have shown a clinical superiority in the treatment of *S. aureus* BJI, the evaluation of antimicrobials alone or in combination with regard to their intraosteoblastic activity against *S. aureus* appears relevant.

Rifampicin has been evaluated in combination with levofloxacin, tigecycline and vancomycin, with no clear advantages of combinations.<sup>27,28,54</sup> This observation might be due to the already optimal activity of rifampicin alone in this model.

Despite the unclear clinical application, gentamicin has been evaluated in combination with tigecycline, vancomycin and cefalexin, showing no clear benefits.<sup>28,54</sup>

Combination of daptomycin with oxacillin was significantly more potent than either antibiotic alone against MSSA and MRSA isolates. Moreover, the combination of daptomycin and ceftaroline was significantly more effective than daptomycin alone against MSSA (but not MRSA) to eradicate intraosteoblastic bacteria.<sup>40</sup>

### Antibiotics and osteoblast survival

Beyond the ability of antimicrobials to eradicate intracellular *S. aureus*, their capacity to prevent infection-induced cytotoxicity represents another important specific effect in BJI.

First, few studies assessed the toxicity of antibiotics themselves toward uninfected osteoblasts. Using an LDH release assay, Valour *et al.*<sup>26</sup> tested a large panel of antistaphylococcal molecules, and found no antibiotic-induced cytotoxicity at high concentration (3× the clinical bone concentration). Conversely, rifampicin, rifabutin,

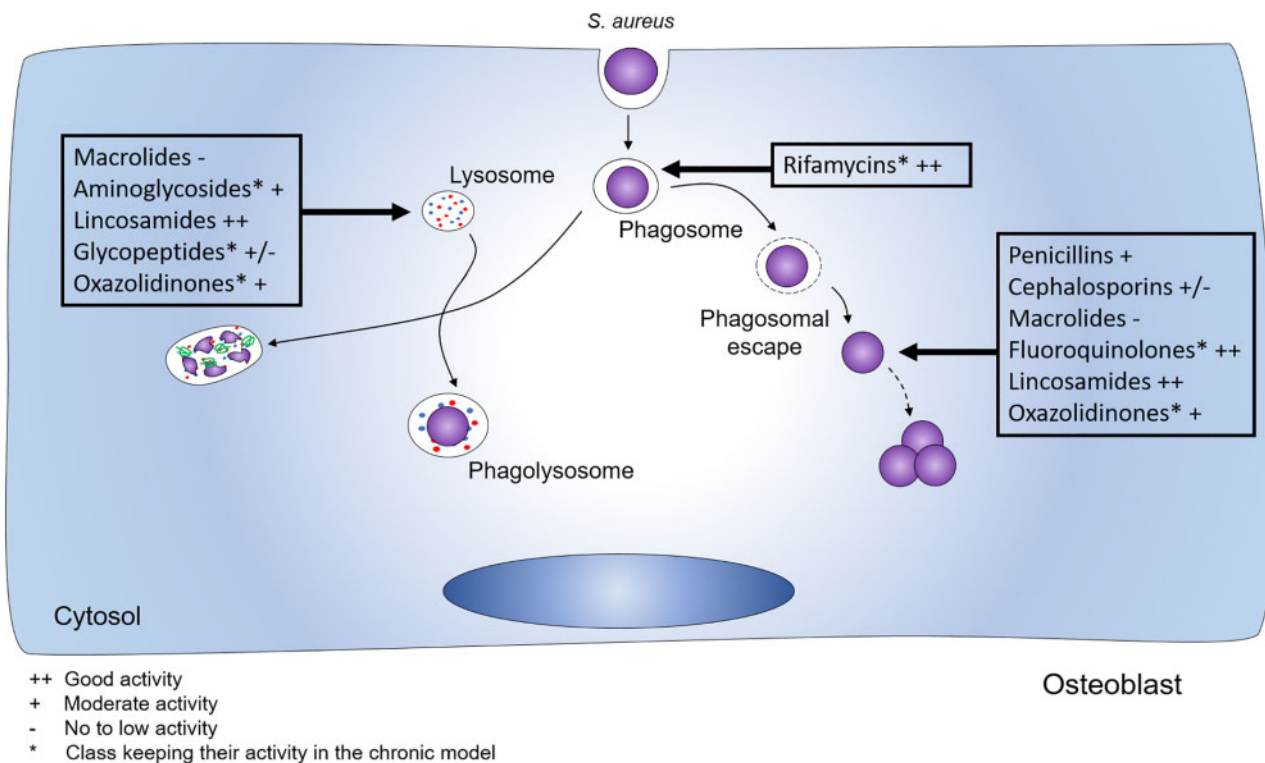
rifapentine and rifaximin induced >40% cell death after 24 h but used at doses largely above bone concentrations ( $\geq 64$  mg/L) using an MTT-reduction assay.<sup>29</sup> Also based on MTT cleavage, linezolid and tedizolid had no significant impact on osteoblast viability.<sup>30</sup>

Regarding the impact of antimicrobials on osteoblast infection-induced cytotoxicity, these studies showed that molecules reducing the intracellular *S. aureus* load consequently decreased infection-induced cellular damage.<sup>28,30</sup> The greatest reduction in cytotoxicity was observed with vancomycin and rifampicin in combination.

Surprisingly, antimicrobial treatment was sometimes associated with an increase of viable cells even in conditions where no impact was observed on intracellular bacterial counts, as for rifapentine at  $0.1 \times \text{MIC}$  or both linezolid and tedizolid,<sup>30,31</sup> suggesting an impact on intracellular toxin secretion. This hypothesis was further investigated for rifamycins, highlighting an antibiotic-driven reduction of the intracellular expression of PSMs.<sup>31</sup> However, there is no doubt that such protein-synthesis-inhibiting antimicrobials impede other intracellular toxin secretions and thus prevent infection-induced cytotoxicity. Rifabutin had the highest preventing effect compared with both rifapentine and rifampicin and this effect was already maximal at  $0.1 \times \text{MIC}$ .<sup>31</sup>

### Antibiotics and intracellular formation of drug-tolerant bacteria

Prolonged intracellular persistence of *S. aureus* led to a decrease in efficacy of some antibiotics, as highlighted in the chronic infection models. The specific physiology of the bacteria at chronic stages



**Figure 2.** Intraosteoblastic activity of antistaphylococcal drugs. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.



might consequently impede the efficacy of antibiotics. Of note, none of the tested drugs eradicated the entire intracellular *S. aureus* population under any of the tested conditions within either the 'acute' or 'chronic' models.

While all the antibiotics tested using human serum concentrations were significantly effective in reducing the intracellular bacterial load using the 'acute' cell infection model, only rifamycins, gentamicin, moxifloxacin, linezolid and vancomycin retained this ability in the 'chronic' stage.<sup>25</sup> In contrast, cefuroxime, flucloxacillin, daptomycin, fosfomycin, erythromycin and clindamycin lost their ability to kill long-term intraosteoblastic-persisting *S. aureus* becoming antibiotic specifically drug-tolerant.<sup>25</sup> Using a transmission electron microscopy approach, Ellington *et al.*<sup>21</sup> showed that the cell surface of intraosteoblastic *S. aureus* was altered compared with extracellular bacteria. Actually, the thickness of the bacterial surface increased after intraosteoblastic passage, depending on intracellular life duration, since the proportion of the bacterial population possessing this altered capsular material increased with exposure time. This phenotypic change might explain the appearance of drug-tolerance.

A significant emergence of SCVs in the 'chronic' cell infection model was observed in the absence of treatment.<sup>25</sup> Challenging infected cells with ofloxacin, rifampicin and daptomycin at bone concentrations significantly reduced the percentage of SCVs. Likewise, oxacillin, ceftaroline, linezolid, fosfomycin, and tigecycline decreased the emergence of SCVs only at the highest concentration used.<sup>26</sup> In contrast, gentamicin, fosfomycin at 500 mg/L and clindamycin significantly enhanced the emergence of intraosteoblastic SCVs after 48 h of treatment.<sup>25</sup> Moreover, a significant increase of SCV formation after treatment with rifampentine, rifabutin and rifampicin alone or in combination with levofloxacin at cortical and cancellous bone concentrations was demonstrated.<sup>27,31</sup>

The increase of drug-tolerance during the 'chronic' stage appears to be drug-dependent and further investigations remain to be performed to elucidate its underlying mechanism.

## Conclusions

This review provides the first comparative analysis of the available literature regarding antimicrobial ability to eradicate the intraosteoblastic reservoir of *S. aureus*, which might represent a key determinant of BJI treatment outcome, given the importance of bacterial internalization within bone cells for BJI chronicity and relapse. The heterogeneity of experimental conditions—especially moi, duration of intracellular bacterial persistence before treatment and antimicrobial concentrations used—advocates for a more standardized approach, using clinically relevant settings. These results emphasize that beyond cellular penetration of antimicrobials, intraosteoblastic activity has more complex and multifactorial determinants, including their subcellular distribution and impact of local biochemical conditions (pH).

Among the antimicrobials used in the clinical setting, rifamycins (and especially rifabutin) and fluoroquinolones appear to be the more potent drugs for intraosteoblastic *S. aureus* reservoir eradication, reflecting the superiority of these combinations in the treatment of orthopaedic device-associated infections.<sup>76–78</sup> However, total eradication of intracellular bacteria is never achieved. Bacterial colonies are observed on agar plates when lysates from antibiotic-challenged osteoblasts are seeded, highlighting the

presence of drug-tolerant intracellular bacteria that could participate in infection relapse. Among those, SCVs and persisters can emerge, for which results are more controversial due to the absence of consensual definitions of these phenotypes, and to the balance between antimicrobial efficacy against these phenotypes and their emergence under antimicrobial pressure. Combination therapies might limit these potential sources of infection persistence, but rifampicin-based combination therapies failed to show superiority compared with rifampicin alone, probably due to the already optimal activity of rifampicin alone in the experimental conditions. On the contrary, the weak intracellular activity of daptomycin might be enhanced by its combination with a  $\beta$ -lactam antibiotic, especially oxacillin. Another striking aspect is the ability of antimicrobials to reduce infection-induced cytotoxicity. The reduction of osteoblast damage induced by protein synthesis inhibitors (and especially rifamycins and oxazolidinones), even in the absence of antibacterial activity, suggests their impact on intracellular bacterial toxin secretion.

Finally, some innovative approaches have been suggested to improve antibiotic efficacy intracellularly. For example, the use of bacterial efflux pump inhibitors can be considered for vancomycin.<sup>79</sup> Given the impact of the intracellular vacuole acidic pH on antibiotic activity, alkalinizing agents can also be promising, as shown for coxiellosis.<sup>80,81</sup> For example, an antibody-antibiotic conjugate allowing release of drugs after opsonized *S. aureus* internalization by intracellular proteases is also under investigation.<sup>82</sup>

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## Transparency declarations

None to declare.

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