Journal of Antimicrobial Chemotherapy

J Antimicrob Chemother 2013; **68**: 1455–1464
doi:10.1093/jac/dkt072 Advance Access publication 13 March 2013

Antibiotic activity against small-colony variants of *Staphylococcus aureus*: review of *in vitro*, animal and clinical data

Laetitia G. Garcia[1], Sandrine Lemaire[1], Barbara C. Kahl[2], Karsten Becker[2], Richard A. Proctor[3], Olivier Denis[4], Paul M. Tulkens[1] and Françoise Van Bambeke[1]*

[1]*Pharmacologie cellulaire et moléculaire & Louvain Drug Research Institute, Université catholique de Louvain, Brussels, Belgium;* [2]*Medical Microbiology, University Hospital Münster, Münster, Germany;* [3]*University of Wisconsin, School of Medicine and Public Health, Madison, WI, USA;* [4]*Laboratoire de Référence MRSA-Staphylocoques, Department of Microbiology, Hôpital Erasme, Université libre de Bruxelles, Brussels, Belgium*

*Corresponding author. Pharmacologie cellulaire et moléculaire, Louvain Drug Research Institute, avenue E. Mounier 73 B1.73.05, B-1200 Bruxelles, Belgium. Tel: +32-2-764-73-78; Fax: +32-2-764-73-63; E-mail: francoise.vanbambeke@uclouvain.be

The pathogen *Staphylococcus aureus* uses various strategies for persisting in the host, among which switching to a small-colony variant (SCV) phenotype is of particular biological and therapeutic significance. Phenotypically, SCVs are characterized by a slow growth rate, atypical colony morphology and unusual biochemical features, constituting a real challenge for identification by the clinical microbiology laboratory. Their metabolic defects also alter their susceptibility to antibiotics, which, combined with the ability to survive intracellularly and, for some strains, to form biofilms, largely contributes to therapeutic failures. This paper reviews the available literature on antibiotic activity against SCVs of *S. aureus in vitro*, in animal models and in clinics. *In vitro*, aminoglycosides and antifolate agents show high MICs for electron-transport-defective and thymidine-dependent SCVs, respectively. The other antibiotic classes usually show MICs comparable to those measured for the parental strains, but they are less bactericidal. Intracellularly, auxotrophs for thymidine, haemin or menadione show contrasting behaviours with respect to their response to antibiotics, resulting from differences in their intracellular fate. In animal models, SCVs often persist in various locations, including metastatic ones, in spite of the administration of active antibiotics. In healthcare, several case reports mention the selection of SCVs after prolonged administration of not only aminoglycosides and antifolate agents, but also several other antibiotic classes. Apparent eradication requires several weeks or even months of aggressive polytherapy combined, whenever possible, with surgical intervention. Further research is thus warranted for optimizing the treatment of infections caused by SCVs.

Keywords: intracellular infections, biofilms, persistence, haemin, menadione, thymidine

Introduction

Small-colony variants (SCVs) of *Staphylococcus aureus* are found in antibiotic-refractory infections such as osteomyelitis, chronic airway infections in patients with cystic fibrosis and device-related infections (see Proctor *et al.*[1] for a review). These naturally occurring variants gain a survival advantage by their ability to persist within eukaryotic cells, which protects them from host defences and antibiotics.[2–4] SCVs are characterized by non-pigmented, non-haemolytic colonies ~10 times smaller than those of the normal phenotype. This tiny size is often due to auxotrophy for distinct growth factors such as menadione, haemin and/or thymidine.[1–4] Worryingly, SCVs often escape detection in routine laboratory investigations because these uncommon morphological and physiological features make their recovery and identification often difficult. As specific nutritional supplementation and prolonged culture are required for their isolation,[1,5] their prevalence may be largely underestimated in clinical specimens.

Two major types of SCV are found in clinical isolates, namely electron-transport-defective strains that are auxotrophs for menadione or haemin, and thymidine auxotrophs (Figure 1 illustrates how these auxotrophisms may affect susceptibility to antibiotics). Auxotrophism for menadione or haemin makes the bacteria unable to synthesize menaquinone and cytochromes, respectively.[6,7] This most probably results from mutations in genes coding for enzymes involved in the biosynthesis of these two molecules.[5,8] Thiamine auxotrophs can be considered as a subtype of menadione-dependent strains because thiamine-pyrophosphate is a cofactor in menadione synthesis. Yet these strains have rarely been identified in human infections,[9] and will therefore not be considered as such in this review.

The decrease in transmembrane potential observed in electron-transport-defective mutants impairs the penetration

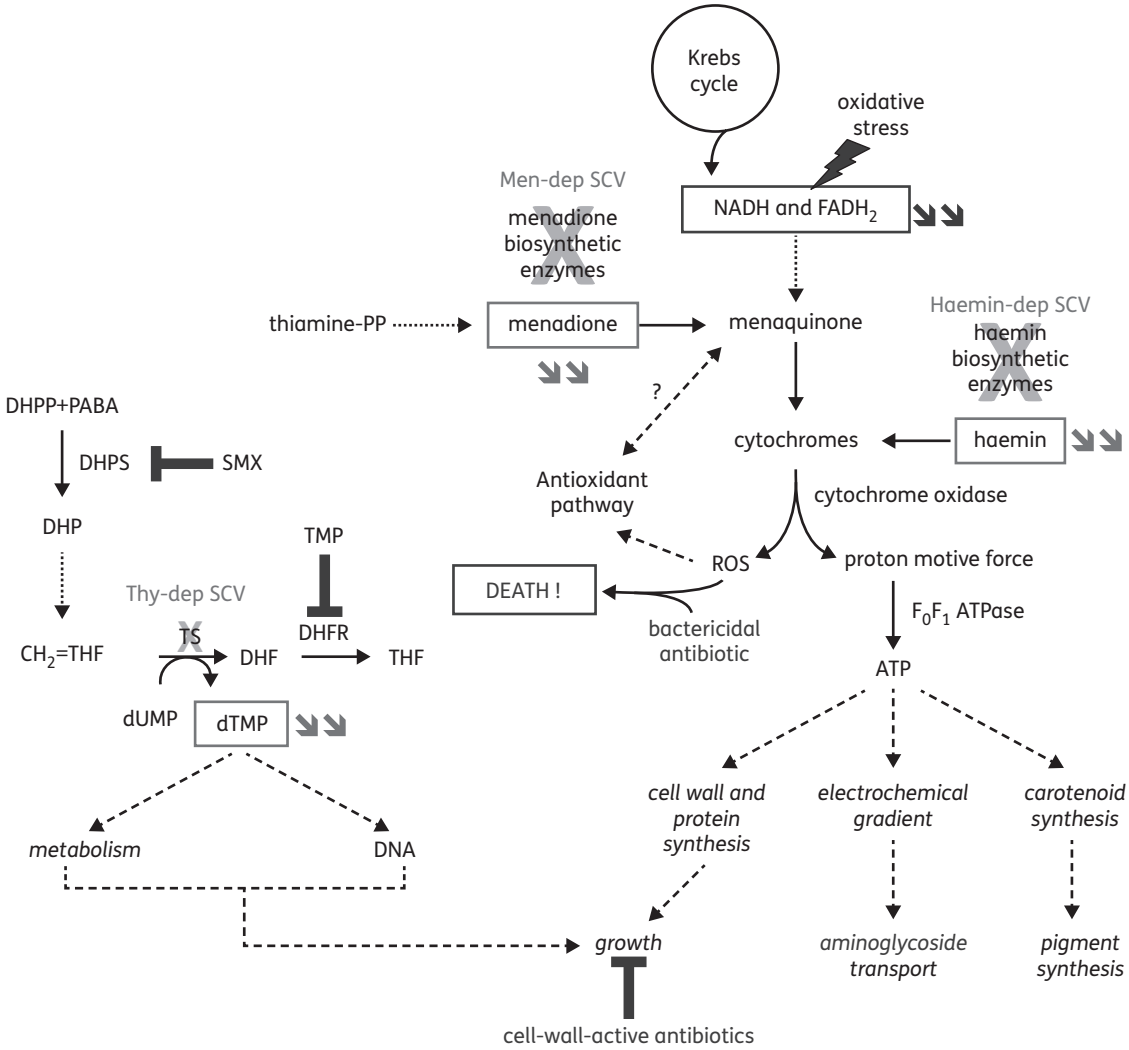

Figure 1. Illustration of the mechanisms leading to the SCV phenotype in *Staphylococcus aureus* and of their link to reduction in susceptibility to specific antibiotic classes (adapted from Proctor *et al.*[1] and McNamara and Proctor[6]). Double arrows refer to metabolites whose concentrations are reduced in the corresponding SCVs. Electron-transport-deficient SCVs show alterations in the pathways leading to the synthesis of menadione or haemin (subsequent to mutations in biosynthetic enzymes), which causes a reduction in the amount of ATP produced. This leads to a reduced growth rate, which may affect the efficacy of antibiotics active against dividing bacteria, such as cell-wall-active agents, and to a reduction in transmembrane potential, which impairs aminoglycoside uptake. Menadione-dependent SCVs are hypersusceptible to oxidant species, possibly because of reduced electron transport and alteration of the induction of antioxidant pathways (shown to be regulated by menaquinone in Gram-negative bacteria[54]). Thymidine-dependent SCVs are unable to convert dUMP into dTMP [using dihydrofolate (DHF) as a cofactor] due to mutations in thymidylate synthase (TS), leading to dTMP depletion. These strains are non-susceptible to antifolate agents that act on successive steps in this pathway, namely to sulphonamides such as sulfamethoxazole (SMX) [inhibitors of dihydropteroate synthase (DHPS) producing dihydropteroate (DHP) from dihydropteridine pyrophosphate (DHPP) and *para*-aminobenzoic acid (PABA)], and to diaminopyridines such as trimethoprim (TMP) [inhibitors of dihydrofolate reductase (DHFR), which catalyses the reduction of DHF to tetrahydrofolate (THF)]. They also show a reduced growth rate. Globally, antibiotics may also be less bactericidal towards electron-transport-deficient SCVs due to a reduced production of reactive oxygen species (ROS).[55] Haemin-dep, haemin dependent; Men-dep, menadione dependent; Thy-dep, thymidine dependent.

of cationic antimicrobial compounds,[6,10–12] as well as the activity of aminoglycosides and antifolate antibiotics.[2,13] Gentamicin treatment can select for these SCVs,[13] which can show associated resistance to fusidic acid due to combined mutations in the *rplF* gene encoding the ribosomal protein L6 and in genes required for haemin or menadione biosynthesis.[14]

Thymidine dependence relies on mutations in thymidylate synthase (*thyA*), the enzyme responsible for the conversion of dUMP to dTMP.[15] As sulphonamides and diaminopyridines act upon the biosynthetic pathway of tetrahydrofolic acid, a by-product of the reaction, thymidine-dependent SCVs often emerge after long-term treatment with trimethoprim/sulfamethoxazole in cystic fibrosis or other patients, and are resistant to these agents.[2]

Worryingly also, exposure to antiseptic agents used in healthcare such as the biguanide triclosan can select for an SCV

phenotype that does not show any particular auxotrophism, but is resistant to this biocide.[16]

Finally, SCVs may appear in the absence of any selective pressure through a constitutive process depending on bacterial replication.[17] Conversely, they can spontaneously revert to a normal phenotype, depending on the basal mutation rate of the strain and/or of the type of mutation conferring the SCV phenotype, with point mutations being presumably more easily reversible than base deletions.[17]

Like normal phenotypes, SCVs can also acquire and express all classical mechanisms of resistance to antimicrobial agents. Poor intrinsic susceptibility to specific antibiotics combined with such acquired resistance creates a real challenge for effective treatment. This paper reviews the current literature describing antibiotic activity against *S. aureus* SCVs, from *in vitro* and animal models to clinical data.

In vitro studies

Susceptibility to antibiotics

Routine *in vitro* susceptibility methods have been developed and approved for testing rapidly growing bacteria. Because SCVs fail to meet this first key property, MIC data need to be interpreted with caution.[18] No large epidemiological survey is as yet available. Anecdotal reports for specific strains suggest, however, that MICs are globally similar for SCVs and their normal phenotype counterparts for most antibiotics (see Table S1, available as Supplementary data at *JAC* Online). Because of the mechanism leading to auxotrophism, however, aminoglycosides and antifolate agents show an almost systematic loss of activity in menadione- or haemin-dependent-, and in thymidine-dependent, SCVs, respectively (Table 1). One clinical isolate with an SCV phenotype was described with high-level resistance to rifampicin, but this was due to a mutation in *rpoB* and was therefore unrelated to its SCV character.[19] Another study also reported increased MICs of tigecycline for a collection of 48 SCVs isolated from patients with cystic fibrosis (Table S1).[20] Although MICs may not be affected, pharmacodynamic studies suggest that the bactericidal activity of several antibiotics against SCVs may be markedly reduced. This has been shown for daptomycin (for which a bactericidal effect was only obtained after prolonged exposure at high concentrations)[21] and for vancomycin and β-lactams (decreased efficacy against menadione- or haemin-dependent SCVs).[18,22] For cell-wall-active agents, this may result from the slow multiplication rate of SCVs.

In comparative studies examining several antibiotics against SCVs with different auxotrophisms, fluoroquinolones (e.g. moxifloxacin) appeared consistently highly effective against thymidine-, menadione- or haemin-dependent SCVs. Gentamicin was very active against the thymidine-dependent strain only, and rifampicin and daptomycin against the menadione- and haemin-dependent ones.[22,23] Another study showed that ciprofloxacin MICs were higher for SCVs than for isolates with normal phenotype, while no marked difference was observed for other fluoroquinolones (moxifloxacin, levofloxacin and finafloxacin).[24] Of particular interest, the enhanced activity of finafloxacin at low pH might facilitate SCV eradication in acidic environments such as in foci of osteomyelitis, skin infections, abscesses, and lung infections in patients with cystic fibrosis.[24]

Among other investigational agents, two membrane-active drugs, the lipoglycopeptide oritavancin[22,23] and the dicationic porphyrin XF-70,[25] have proved as bactericidal against SCVs as against their parental normal phenotype strain. At a still earlier stage in discovery, tomatidine, the aglycon form of the tomato secondary metabolite tomatine described as an antimicrobial saponin, shows lower MICs for menadione- and haemin-dependent SCVs than for normal-phenotype strains (Table 1). While tomatidine is only bacteriostatic, its activity seems to be linked to the dysfunction of the electron transport system in SCVs.[26] Tomatidine has therefore been reported as synergistic with aminoglycosides against electron-defective SCVs.[27]

Antimicrobial peptides are an integral part of the host defence against invading microorganisms. Unfortunately, haemin- and menadione-dependent SCVs can emerge upon exposure to sub-MIC concentrations of protamine,[10] and both types of SCV are resistant to lactoferrin B.[28] In addition, higher MICs of host cationic peptides such as thrombin-induced platelet microbicidal protein (tPMP) were observed.[12,29]

Based on these *in vitro* studies, it remains difficult to define optimal therapy for infections due to *S. aureus* SCVs. Reversion to the normal phenotype has been observed in several *in vivo* and *in vitro* models of persistent infection.[30] Revertants might also occur upon *in vitro* testing, making the organisms apparently susceptible to antibiotics and thereby misrepresenting the actual values. In the case of menadione auxotrophs, this reversal can also be obtained *in vivo* by administering vitamin K to patients.[31]

Activity against intracellular bacteria

SCVs easily persist intracellularly[3,4] and can even be selected in the intracellular milieu.[32] Studying antibiotic activity against intracellular SCVs is therefore particularly relevant. Several *in vitro* models using human or animal cells have been developed to test intracellular activity.

In models using human monocytes, a haemin-dependent SCV showed an intracellular growth similar to that of a normal phenotype strain, suggesting that it finds inside cells the haem-like compounds required for growth. Conversely, a thymidine-dependent SCV was reported to grow more slowly, and a menadione-dependent strain not to grow over a 24 h incubation time,[22,23] which is supposed to decrease their response to antibiotics.

Systematic comparisons of anti-staphylococcal agents have therefore been performed in this model using a pharmacodynamic approach allowing characterizing antibiotic potency and efficacy. The three types of SCV displayed contrasting behaviours, which rely, at least in part, on their respective capacity to grow inside the cells. Against the stable thymidine-dependent SCV isolated from a patient with cystic fibrosis, vancomycin, oxacillin, fusidic acid, clindamycin, linezolid and daptomycin were much less active than quinupristin/dalfopristin, moxifloxacin, rifampicin, and oritavancin. Yet, for all drugs, the maximal efficacy was markedly reduced against the thymidine-dependent SCV when compared with the normal-phenotype and revertant isogenic strains, probably due to its slower growth.[23] Against the haemin-dependent SCV derived from the COL methicillin-resistant *S. aureus* (MRSA) strain, oritavancin and moxifloxacin were also much more effective than vancomycin, gentamicin,

Table 1. MICs of antibiotics for *Staphylococcus aureus* with a normal or SCV phenotype[a]

		MICs (mg/L) of antibiotics according to phenotype[b]												
		strains												
					SCV and auxotrophism[c]									
		wild-type			not specified		MSSA				MRSA			
Class	Antibiotic	not specified	MSSA	MRSA	not specified	men	not specified	men	haem	thy	not specified	men	haem	Reference(s)
Aminoglycosides	amikacin	2	<0.031–2	1	**64**			**64**	0.5–2					39,44
	gentamicin	<0.125–4	<0.031–8	0.25–1	**<0.125 to >128**	**16**	**8 to >8**	**8; 12**	**0.5–16**	0.125; 4	0.3	0.5; **32**	**1; 32**	2,4,8,18–20,22,23,26,34,39,44,45,56,57
	kanamycin		0.25	2–4					**2**			**64–128**	**64–128**	4,56
	tobramycin			0.5–1								**32**	**32**	56
Antifolates	trimethoprim		2				1–**8**		**8**					57
	trimethoprim/sulfamethoxazole	0.004–32	0.06; 0.064		0.023 to **>32**		0.06–**0.5**		**0.5**	**>32**				2,20,57,58
Other agents	tomatidine		>16				*0.12*		*0.12*					26
	lactoferrin B		16–64	256	**>256**			**>256**	**>256**			**>256**	**>256**	28

[a]Only studies comparing normal phenotype and SCV strains have been included in this table.
[b]The table only shows antimicrobial agents for which MICs are systematically different between the normal phenotype and SCVs (values in bold correspond to MICs that are at least two dilutions higher than those for the corresponding parental strain with a normal phenotype, and values in italics correspond to MICs that are at least two dilutions lower than those for the corresponding parental strain with a normal phenotype). See Table S1, available as Supplementary data at *JAC* Online for a comprehensive table showing MIC data for all antimicrobial agents investigated so far.
[c]men, menadione dependent; haem, haemin dependent; thy, thymidine dependent.

daptomycin or rifampicin, and their activity was indistinguishable from that observed against the parental strain, in line with the restored intracellular growth of this SCV.[22] Against the menadione-dependent SCV also derived from the COL MRSA strain, the maximal efficacy of antibiotics remained unaffected, which is surprising in view of its slow intracellular growth. Yet the affected pharmacodynamic parameters were rather the amplitude of the dose–response curve (which was reduced) and the potency of antibiotics (which was increased).

Among other agents that are highly active extracellularly, XF-70 showed a rapid bactericidal effect at low concentrations against a methicillin-susceptible *S. aureus* (MSSA) and its haemin-dependent SCV phagocytized by human polymorphonuclear neutrophils.[25] Conversely, the bacteriostatic tomatidine only inhibited the intracellular replication of SCVs within polarized cystic fibrosis-like epithelial cells, but did not decrease their number.[26]

Because of the difficulty of eradicating intracellular SCVs, two strategies have been evaluated to improve antibiotic activity. The first consists of combining antibiotics with different modes of action. Several studies indeed suggest that combinations allow for an improved intracellular killing of SCVs, especially when they include rifampicin or a highly bactericidal agent such as oritavancin.[33,34] The second strategy aims to reinforce the cell defence mechanisms. Thus, when human monocytes were activated into macrophages by phorbol 12-myristate 13-acetate, the intracellular growth of menadione- or haemin-dependent SCVs and of their normal parental strains was reduced. This did not affect the maximal efficacy of the antibiotics, but rather increased their potency (a lower concentration being needed to reach a static effect intracellularly).

Interestingly, this effect was not, however, systematic. First, an increase in potency was observed only for certain antibiotics such as gentamicin or moxifloxacin, the MICs of which were reduced in the presence of H_2O_2, but not for oritavancin or vancomycin, the MICs of which were not affected by H_2O_2. This suggests that a synergy between reactive oxygen species and certain antibiotics may actually require functional oxidative host defences for optimal activity. Conversely, antibiotics for which cell activation has a minimal effect on intracellular activity should remain as effective when host defences are weakened. Second, this higher potency was seen for the haemin-dependent mutant and its parental strain, but not for the menadione-dependent mutant.[35,36] The latter may not have been influenced by cell activation simply because the antibiotics already showed a higher potency towards this strain in non-activated cells.[36] Thus, these data suggest that the menadione-dependent strain is hypersusceptible to oxidant species (see Figure 1 for an illustration of the potential link between menadione dependence and susceptibility to oxidant species).

This is further corroborated by the strain's unanticipated susceptibility to β-lactams. β-Lactams have been shown to regain activity against normal-phenotype MRSA intracellularly, due to a conformational change of PBP2a occurring at a pH (~5.5) similar to that prevailing in phagolysosomes, which allows its acylation at a much faster rate than at neutral pH.[37] Interestingly enough, this effect was exacerbated for the menadione-dependent SCV of the COL MRSA strain, which was 100- to 900-fold more susceptible to β-lactams than its parental strain when inside the cells.[35] The same trend was also observed for an MSSA strain, but the shift in potency was less marked. *In vitro* studies have suggested that this high potency is due to a cooperation between an acidic pH and oxidant species because it can be reproduced when measuring MICs at acidic pH after pre-exposure to H_2O_2.[35]

Activity against biofilms

Biofilms are another form of persistent infection presenting major difficulties for eradication.[18] A recent study has suggested that menadione-dependent SCVs are more prone to form biofilms *in vitro* than are thymidine-auxotrophic ones[38] (due to an enhanced production of polysaccharide intercellular adhesin).[39] This is consistent with the fact that menadione-dependent strains are mainly recovered from foci of osteomyelitis or device-associated infections, which are often biofilm-related. The situation may, however, be different *in vivo*, since biofilms are also frequent in patients with cystic fibrosis, who are more frequently infected by thymidine-dependent SCVs. In this case, the switch to a high biofilm producer SCV phenotype could be induced by the presence of quorum-sensing molecules produced by *Pseudomonas aeruginosa*, which is also present in the respiratory tract of these patients.[40]

Exposure to antibiotics may actually induce the formation of a biofilm. Thus, subinhibitory concentrations of gentamicin have been shown to trigger not only the emergence of SCVs, but also the development of *S. aureus* biofilms owing to activation of the alternative transcription of sigma factor B.[41] Conversely, non-auxotrophic SCVs selected by triclosan were reported to be weak biofilm producers.[42]

Very few studies have examined antibiotic activity against SCVs growing in biofilms. Biofilms of the reference MSSA strain ATCC 29213 are much more resistant to the action of oxacillin, cefotaxime, amikacin, ciprofloxacin or vancomycin, with none of these drugs being able to reduce bacterial counts even at high multiples of their respective MICs.[43] Thus, surviving bacteria within the biofilm seem to harbour a persister phenotype, but only ciprofloxacin also selected for SCVs within the biofilm. These SCVs did not seem to be associated with increased resistance within the biofilm as they easily reverted to a normal phenotype upon subculture. A few studies also examined stable menadione-dependent mutants, demonstrating (i) a higher propensity to biofilm formation[39] and (ii) a profound decrease in antibiotic activity against bacteria growing on fibronectin-coated surfaces compared with the planktonic forms.[18] These studies were, however, carried out with single reference strains and need to be extended to more strains, including clinical isolates.

Animal models

A few animal models have been developed to study the fate of SCVs as well as their response to antibiotics. Interestingly enough, the data obtained in these models are coherent with those obtained *in vitro*, including for the intracellular forms. These studies are summarized below. They suggest in many cases, but not systematically, that SCVs can not only persist and spread in the body, but also be more difficult to eradicate than their normal-phenotype counterparts, thereby contributing to the chronic character of the infection.

In rabbit endocarditis models, both haemin- and menadione-dependent mutants of the 8325-4 strain were equally able to establish the infection, but only the haemin-dependent mutant achieved the same bacterial density in the spleen or kidneys as its parental strain, which was not the case for the corresponding menadione-dependent mutant.[11] In agreement with observations made in infected cells, this suggests that the target organs may have been replete with haemin during the course of endocarditis as a consequence of haemorrhagic necrosis, restoring the wild-type phenotype. In these studies, oxacillin reduced bacterial counts in all target tissues for animals infected with the parent strain or the haemin-dependent mutant, but only in vegetations and not in kidneys and spleen for animals infected by the menadione-dependent mutant, probably due to its low multiplication rate.[11]

In another study, gentamicin treatment easily selected for SCVs which, although being less virulent, were themselves able to re-establish the infection and to colonize blood, heart valve vegetations, spleen, kidney and liver as efficiently as the parental strain.[44] β-Lactams were effective in this model, and combination with an aminoglycoside was useful against the normal-phenotype strain, but not against the SCV.

In a mouse mastitis model, the cephalosporin cefapirin showed a reduced ability to control the infection caused by the haemin-dependent mutant of the strain Newbould 305 compared with its isogenic parent.[45] This occurred despite the fact that both strains displayed similar MICs and that the SCV mutant showed a lower propensity to colonize the mammary glands.

In a rabbit model of chronic osteomyelitis, vancomycin loaded in a hydroxyapatite cement proved highly effective to treat the infection caused by *S. aureus* SCVs isolated from patients with osteomyelitis, none of the infected animals that were treated showing signs of infection after 42 days thanks to the slow release of high concentrations of antibiotic.[46]

In a mouse peritonitis model allowing for simultaneous testing of activity against both extracellular and intracellular bacteria, colonization of both the extracellular and intracellular compartments was lower for a menadione-dependent SCV than for its parental counterpart, leading to fewer signs of sickness. However, metastatic spread to the kidneys and persistence at 96 h were observed for the SCV.[47] Linezolid and dicloxacillin were able to control both intra- and extracellular infections caused by either phenotype, but not to clear SCVs from the kidney after a single dose. Parallel experiments performed in the THP-1 *in vitro* model showed, as described above, reduced intracellular growth for the menadione-dependent mutant, an increased potency for antibiotics against this strain, but no change in maximal efficacy, which reached about 1 log reduction from the initial inoculum, as also observed *in vivo*.

Clinical data

There are no large clinical trials examining therapeutic options for SCV infections, but only case reports or studies of small series describing successful or unsuccessful approaches. Table 2 summarises these studies and describes the antibiotics used prior to SCV identification and for their subsequent treatment. Globally, SCVs have been isolated after long and/or unsuccessful antibiotic exposure. They have all needed aggressive and prolonged polytherapy for their eradication. Effective regimens have often included rifampicin or a fluoroquinolone, as well as quinupristin/dalfopristin in one specific case,[38] which is consistent with their high intrinsic activity *in vitro*. β-Lactams (for MSSA) or glycopeptides (for MRSA) are also often administered, although they are considered to be less active against SCVs based on *in vitro* testing.[18,35] When applicable, surgical debridement and removal of infected devices are probably key determinants in clinical success. Two studies mention the administration of vitamin K aimed at reversing the SCV phenotype (see Table 2). Globally, however, antibiotic choices remain largely empirical. At the present time, no guideline has been proposed for treating infections associated with this particular phenotype. In spite of apparent favourable clinical and microbiological responses, careful patient follow-up remains essential because SCV infections have been associated with recurrence after intervals as long as 54 years.[31]

Notably, prolonged treatment may also lead to the selection of resistance, further complicating treatment. Thus, a remarkable adaptive response of *S. aureus* to antimicrobial challenge during chronic infection was demonstrated for an SCV isolated from a patient with persistent and recurrent MRSA bacteraemia who received apparently extensive and appropriate antimicrobial therapy combining rifampicin, ciprofloxacin, and vancomycin (thereafter replaced by linezolid)[19] (see Table 2). The isolated SCV showed resistance to linezolid (23S RNA ribosomal methylation), rifampicin (a mutation in *rpoB*), fluoroquinolones (a mutation in *parC*) and β-lactams (plasmid-encoded β-lactamase). Likewise, thymidine auxotrophs of *S. aureus* have been shown to be hypermutable and might therefore be more likely to acquire mutational antimicrobial resistance than normal colony phenotypes.[48] This hypermutability may explain the emergence of resistance to rifampicin and daptomycin during treatment in a clinical case report.[38] Yet emergence of resistance during treatment is not systematically associated with selection of SCVs. No correlation was found, for example, between the treatment-related selection of macrolide-resistant *S. aureus* in cystic fibrosis patients receiving long-term azithromycin and SCV isolation.[49]

Conclusions

Although clearly challenging for both the microbiologist and the clinician, SCVs of *S. aureus* remain an ill-explored field, at least with respect to the more appropriate therapeutic options to prevent their emergence on the one hand and to eradicate them when present on the other. Although long-term therapy with gentamicin and antifolate agents is clearly associated with their selection, clinical reports suggest that other drugs may also be incriminated. *In vitro* susceptibility testing should also be performed in conditions that allow SCV susceptibility to be examined (48 h incubation). Clinical investigations specifically targeting SCV-related infections are probably difficult to perform because their diagnosis escapes routine procedures. The present review suggests that *in vitro* or animal pharmacodynamic models may be of great help (i) to determine the conditions of antibiotic exposure selecting for SCVs, and (ii) to define antibiotic regimens or drug combinations most likely to act upon these

Table 2. Summary of case reports of infections by SCVs of *Staphylococcus aureus*, with descriptions of antibiotics received before and after isolation of the SCV and of treatment outcome

Infection	Previous treatment	SCV characteristics	Treatment	Clinical outcome (time after diagnosis of SCV)	Reference
Bacteraemia	ciprofloxacin, rifampicin, vancomycin, linezolid	MRSA, resistant to fluoroquinolones, rifampicin, linezolid; not haemin or menadione dependent	linezolid + SXT	unknown	19
Osteomyelitis	cefotaxime, gentamicin beads		surgical debridement coupled with antibiotic combination (cefotaxime + ciprofloxacin)	cure (2 years)	59
Osteomyelitis (four cases)	gentamicin beads	haemin or menadione dependent		failure	13
Sternoclavicular arthritis		haemin or menadione dependent; resistant to gentamicin	intravenous nafcillin followed by oral rifampicin; cloxacillin; oral vitamin K	cure (1 month)	50
Prosthetic joint infection (five cases)	rifampicin + levofloxacin in 5/5 after flucloxacillin (2/5) or vancomycin + cefepime (1/5)	1/85 resistant to SXT; 1/5 resistant to rifampicin	flucloxacillin + rifampicin (1/5); levofloxacin + rifampicin (1/5); flucloxacillin (1/5); flucloxacillin followed by levofloxacin + rifampicin (1/5); penicillin + levofloxacin (1/5)	cure or probable cure (3–23 months)	60
Recurrent abscess in AIDS patient	clindamycin	thymidine dependent, MRSA, resistant to erythromycin, clindamycin, ciprofloxacin, gentamicin, SXT	vancomycin	failure	61
Abscess	clindamycin	haemin or menadione dependent; resistant to penicillin, ampicillin and tetracycline	flucloxacillin + rifampicin	cure (4 weeks)	62
Infection of peritoneal dialysis exit site	ciprofloxacin, vancomycin, amoxicillin/clavulanic acid, cefalexin	MRSA			63
Endocarditis related to pacemaker lead infection	aminoglycoside and vancomycin	haemin dependent; resistant to rifampicin	flucloxacillin	cure (7 months)	64
Endocarditis in a patient with a pacemaker on haemodialysis	cefuroxime, rifampicin, fusidic acid, dicloxacillin, vancomycin		intravenous cefuroxime and vancomycin for 4 weeks followed by prophylactic oral cefuroxime	cure	65
Left ventricular assist device infection and prosthetic valve and pacemaker endocarditis	vancomycin, rifampicin, gentamicin, daptomycin, and SXT	thymidine dependent; MRSA; resistant to tobramycin, amikacin, kanamycin, rifampicin, daptomycin, erythromycin, levofloxacin and SXT	replacement of infected prosthetic tricuspid valve and left ventricular assist device + vancomycin, gentamicin, quinupristin/dalfopristin, rifampicin, SXT	cure (>7 months)	38
Brain abscess	meropenem, clindamycin and gentamicin; intrathecal gentamicin	haemin dependent; MRSA	combination of vancomycin and rifampicin followed by prolonged treatment with teicoplanin	cure (3 months)	66
Meningitis and ventriculoperitoneal shunt infection	ciprofloxacin, vancomycin	MRSA	combination of ciprofloxacin, vancomycin and rifampicin	cure (4 months)	67
Multiorgan infection	amoxicillin/clavulanate, ampicillin, gentamicin, cefotaxime, doxycycline, vancomycin	MSSA, gentamicin resistant	combination of oxacillin, rifampicin, SXT and vitamin K	cure (3 months)	51

SXT, trimethoprim/sulfamethoxazole.

slow-growing, metabolically defective strains. Strategies aimed at favouring reversion may also be worth investigating in the future. Vitamin K supplementation to restore growth and, subsequently, susceptibility to antibiotics, has attracted interest in anecdotal situations of infection by menadione-dependent mutants.[50,51] Taking advantage of spontaneous reversion may be more dangerous, because it may be associated with hypermutators[17,48] that are notably more prone to acquiring resistance to antibiotics.[48,52,53]

Acknowledgements

L. G. G. was *Boursière* of the Belgian *Fonds pour la Formation à la Recherche dans l'Industrie et dans l'Agriculture* (FRIA). S. L. was *Chargé de recherches* and F. V. B. is *Maître de recherches* of the Belgian *Fonds de la Recherche Scientifique* (F.R.S.-FNRS). This review was written in the frame of research programs financed by the *Fonds National de la Recherche Médicale* (F.R.S.M. grants 3.4639.09 and 3.4530.12).

Transparency declarations

None to declare.

Supplementary data

Table S1 is available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

References

1 Proctor RA, von Eiff C, Kahl BC *et al*. Small colony variants: a pathogenic form of bacteria that facilitates persistent and recurrent infections. *Nat Rev Microbiol* 2006; **4**: 295–305.

2 Kahl B, Herrmann M, Everding AS *et al*. Persistent infection with small colony variant strains of *Staphylococcus aureus* in patients with cystic fibrosis. *J Infect Dis* 1998; **177**: 1023–9.

3 von Eiff C, Becker K, Metze D *et al*. Intracellular persistence of *Staphylococcus aureus* small-colony variants within keratinocytes: a cause for antibiotic treatment failure in a patient with Darier's disease. *Clin Infect Dis* 2001; **32**: 1643–7.

4 von Eiff C, Heilmann C, Proctor RA *et al*. A site-directed *Staphylococcus aureus* hemB mutant is a small-colony variant which persists intracellularly. *J Bacteriol* 1997; **179**: 4706–12.

5 von Eiff C. *Staphylococcus aureus* small colony variants: a challenge to microbiologists and clinicians. *Int J Antimicrob Agents* 2008; **31**: 507–10.

6 McNamara PJ, Proctor RA. *Staphylococcus aureus* small colony variants, electron transport and persistent infections. *Int J Antimicrob Agents* 2000; **14**: 117–22.

7 Kriegeskorte A, Konig S, Sander G *et al*. Small colony variants of *Staphylococcus aureus* reveal distinct protein profiles. *Proteomics* 2011; **11**: 2476–90.

8 Lannergard J, von Eiff C, Sander G *et al*. Identification of the genetic basis for clinical menadione-auxotrophic small-colony variant isolates of *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2008; **52**: 4017–22.

9 Acar JF, Goldstein FW, Lagrange P. Human infections caused by thiamine- or menadione-requiring *Staphylococcus aureus*. *J Clin Microbiol* 1978; **8**: 142–7.

10 Sadowska B, Bonar A, von Eiff C *et al*. Characteristics of *Staphylococcus aureus*, isolated from airways of cystic fibrosis patients, and their small colony variants. *FEMS Immunol Med Microbiol* 2002; **32**: 191–7.

11 Bates DM, von Eiff C, McNamara PJ *et al*. *Staphylococcus aureus menD* and *hemB* mutants are as infective as the parent strains, but the menadione biosynthetic mutant persists within the kidney. *J Infect Dis* 2003; **187**: 1654–1.

12 Baumert N, von Eiff C, Schaaff F *et al*. Physiology and antibiotic susceptibility of *Staphylococcus aureus* small colony variants. *Microb Drug Resist* 2002; **8**: 253–60.

13 von Eiff C, Bettin D, Proctor RA *et al*. Recovery of small colony variants of *Staphylococcus aureus* following gentamicin bead placement for osteomyelitis. *Clin Infect Dis* 1997; **25**, 1250–1.

14 Lannergard J, Cao S, Norstrom T *et al*. Genetic complexity of fusidic acid-resistant small colony variants (SCV) in *Staphylococcus aureus*. *PLoS One* 2011; **6**: e28366.

15 Chatterjee I, Kriegeskorte A, Fischer A *et al*. In vivo mutations of thymidylate synthase (encoded by *thyA*) are responsible for thymidine dependency in clinical small-colony variants of *Staphylococcus aureus*. *J Bacteriol* 2008; **190**: 834–42.

16 Bayston R, Ashraf W, Smith T. Triclosan resistance in methicillin-resistant *Staphylococcus aureus* expressed as small colony variants: a novel mode of evasion of susceptibility to antiseptics. *J Antimicrob Chemother* 2007; **59**: 848–53.

17 Edwards AM. Phenotype switching is a natural consequence of *Staphylococcus aureus* replication. *J Bacteriol* 2012; **194**: 5404–12.

18 Chuard C, Vaudaux PE, Proctor RA *et al*. Decreased susceptibility to antibiotic killing of a stable small colony variant of *Staphylococcus aureus* in fluid phase and on fibronectin-coated surfaces. *J Antimicrob Chemother* 1997; **39**: 603–8.

19 Gao W, Chua K, Davies JK *et al*. Two novel point mutations in clinical *Staphylococcus aureus* reduce linezolid susceptibility and switch on the stringent response to promote persistent infection. *PLoS Pathog* 2010; **6**: e1000944.

20 Yagci S, Hascelik G, Dogru D *et al*. Prevalence and genetic diversity of *Staphylococcus aureus* small-colony variants in cystic fibrosis patients. *Clin Microbiol Infect* 2013; **19**: 77–84.

21 Begic D, von Eiff C, Tsuji BT. Daptomycin pharmacodynamics against *Staphylococcus aureus hemB* mutants displaying the small colony variant phenotype. *J Antimicrob Chemother* 2009; **63**: 977–81.

22 Garcia LG, Lemaire S, Kahl BC *et al*. Pharmacodynamic evaluation of the activity of antibiotics against hemin- and menadione-dependent small-colony variants of *Staphylococcus aureus* in models of extracellular (broth) and intracellular (THP-1 monocytes) infections. *Antimicrob Agents Chemother* 2012; **56**: 3700–11.

23 Nguyen HA, Denis O, Vergison A *et al*. Intracellular activity of antibiotics in a model of human THP-1 macrophages infected by a *Staphylococcus aureus* small-colony variant strain isolated from a cystic fibrosis patient: pharmacodynamic evaluation and comparison with isogenic normal-phenotype and revertant strains. *Antimicrob Agents Chemother* 2009; **53**: 1434–2.

24 Idelevich EA, Kriegeskorte A, Stubbings W *et al*. Comparative in vitro activity of finafloxacin against staphylococci displaying normal and small colony variant phenotypes. *J Antimicrob Chemother* 2011; **66**: 2809–13.

25 Vaudaux P, Huggler E, Rhys-Williams W *et al*. Extracellular and intracellular bactericidal activities of XF-70 against small-colony variant *hemB* mutants of meticillin-susceptible and meticillin-resistant *Staphylococcus aureus*. *Int J Antimicrob Agents* 2011; **37**: 576–9.

26 Mitchell G, Gattuso M, Grondin G *et al*. Tomatidine inhibits replication of *Staphylococcus aureus* small-colony variants in cystic fibrosis airway epithelial cells. *Antimicrob Agents Chemother* 2011; **55**: 1937–45.

27 Mitchell G, Lafrance M, Boulanger S *et al*. Tomatidine acts in synergy with aminoglycoside antibiotics against multiresistant *Staphylococcus aureus* and prevents virulence gene expression. *J Antimicrob Chemother* 2012; **67**: 559–68.

28 Samuelsen O, Haukland HH, Kahl BC *et al*. *Staphylococcus aureus* small colony variants are resistant to the antimicrobial peptide lactoferricin B. *J Antimicrob Chemother* 2005; **56**: 1126–9.

29 Koo SP, Bayer AS, Sahl HG *et al*. Staphylocidal action of thrombin-induced platelet microbicidal protein is not solely dependent on transmembrane potential. *Infect Immun* 1996; **64**: 1070–4.

30 Tuchscherr L, Medina E, Hussain M *et al*. *Staphylococcus aureus* phenotype switching: an effective bacterial strategy to escape host immune response and establish a chronic infection. *EMBO Mol Med* 2011; **3**: 129–41.

31 Proctor RA, Peters G. Small colony variants in staphylococcal infections: diagnostic and therapeutic implications. *Clin Infect Dis* 1998; **27**: 419–22.

32 Vesga O, Groeschel MC, Otten MF *et al*. *Staphylococcus aureus* small colony variants are induced by the endothelial cell intracellular milieu. *J Infect Dis* 1996; **173**: 739–42.

33 Nguyen HA, Denis O, Vergison A *et al*. Intracellular activity of antibiotics in a model of human THP-1 macrophages infected by a *Staphylococcus aureus* small-colony variant strain isolated from a cystic fibrosis patient: study of antibiotic combinations. *Antimicrob Agents Chemother* 2009; **53**: 1443–9.

34 Baltch AL, Ritz WJ, Bopp LH *et al*. Activities of daptomycin and comparative antimicrobials, singly and in combination, against extracellular and intracellular *Staphylococcus aureus* and its stable small-colony variant in human monocyte-derived macrophages and in broth. *Antimicrob Agents Chemother* 2008; **52**: 1829–33.

35 Garcia LG, Lemaire S, Kahl BC *et al*. Intracellular forms of menadione-dependent small-colony variants of methicillin-resistant *Staphylococcus aureus* are hypersusceptible to β-lactams in a THP-1 cell model due to cooperation between vacuolar acidic pH and oxidant species. *J Antimicrob Chemother* 2012; **67**: 2873–1.

36 Garcia LG, Lemaire S, Kahl BC *et al*. Influence of the protein kinase C activator phorbol myristate acetate on the intracellular activity of antibiotics against hemin- and menadione-auxotrophic small-colony variant mutants of *Staphylococcus aureus* and their wild-type parental strain in human THP-1 cells. *Antimicrob Agents Chemother* 2012; **56**: 6166–74.

37 Lemaire S, Fuda C, Van Bambeke F *et al*. Restoration of susceptibility of methicillin-resistant *Staphylococcus aureus* to β-lactam antibiotics by acidic pH: role of penicillin-binding protein PBP 2a. *J Biol Chem* 2008; **283**: 12769–76.

38 Maduka-Ezeh A, Seville MT, Kusne S *et al*. Thymidine auxotrophic *Staphylococcus aureus* small-colony variant endocarditis and left ventricular assist device infection. *J Clin Microbiol* 2012; **50**: 1102–5.

39 Singh R, Ray P, Das A *et al*. Enhanced production of exopolysaccharide matrix and biofilm by a menadione-auxotrophic *Staphylococcus aureus* small-colony variant. *J Med Microbiol* 2010; **59**: 521–7.

40 Mitchell G, Seguin DL, Asselin AE *et al*. *Staphylococcus aureus* sigma B-dependent emergence of small-colony variants and biofilm production following exposure to *Pseudomonas aeruginosa* 4-hydroxy-2-heptylquinoline-*N*-oxide. *BMC Microbiol* 2010; **10**: 33.

41 Mitchell G, Brouillette E, Seguin DL *et al*. A role for sigma factor B in the emergence of *Staphylococcus aureus* small-colony variants and elevated biofilm production resulting from an exposure to aminoglycosides. *Microb Pathog* 2010; **48**: 18–27.

42 Latimer J, Forbes S, McBain AJ. Attenuated virulence and biofilm formation in *Staphylococcus aureus* following sublethal exposure to triclosan. *Antimicrob Agents Chemother* 2012; **56**: 3092–100.

43 Singh R, Ray P, Das A *et al*. Role of persisters and small-colony variants in antibiotic resistance of planktonic and biofilm-associated *Staphylococcus aureus*: an in vitro study. *J Med Microbiol* 2009; **58**: 1067–73.

44 Miller MH, Wexler MA, Steigbigel NH. Single and combination antibiotic therapy of *Staphylococcus aureus* experimental endocarditis: emergence of gentamicin-resistant mutants. *Antimicrob Agents Chemother* 1978; **14**: 336–43.

45 Brouillette E, Martinez A, Boyll BJ *et al*. Persistence of a *Staphylococcus aureus* small-colony variant under antibiotic pressure in vivo. *FEMS Immunol Med Microbiol* 2004; **41**: 35–41.

46 Joosten U, Joist A, Gosheger G *et al*. Effectiveness of hydroxyapatite-vancomycin bone cement in the treatment of *Staphylococcus aureus* induced chronic osteomyelitis. *Biomaterials* 2005; **26**: 5251–8.

47 Sandberg A, Lemaire S, Van Bambeke F *et al*. Intra- and extracellular activities of dicloxacillin and linezolid against a clinical *Staphylococcus aureus* strain with a small-colony-variant phenotype in an in vitro model of THP-1 macrophages and an in vivo mouse peritonitis model. *Antimicrob Agents Chemother* 2011; **55**: 1443–52.

48 Besier S, Zander J, Kahl BC *et al*. The thymidine-dependent small-colony-variant phenotype is associated with hypermutability and antibiotic resistance in clinical *Staphylococcus aureus* isolates. *Antimicrob Agents Chemother* 2008; **52**: 2183–9.

49 Green N, Burns JL, Mayer-Hamblett N *et al*. Lack of association of small-colony-variant *Staphylococcus aureus* strains with long-term use of azithromycin in patients with cystic fibrosis. *J Clin Microbiol* 2011; **49**: 2772–3.

50 Spearman P, Lakey D, Jotte S *et al*. Sternoclavicular joint septic arthritis with small-colony variant *Staphylococcus aureus*. *Diagn Microbiol Infect Dis* 1996; **26**: 13–5.

51 Agarwal H, Verrall R, Singh SP *et al*. Small colony variant *Staphylococcus aureus* multiorgan infection. *Pediatr Infect Dis J* 2007; **26**: 269–71.

52 Prunier AL, Malbruny B, Laurans M *et al*. High rate of macrolide resistance in *Staphylococcus aureus* strains from patients with cystic fibrosis reveals high proportions of hypermutable strains. *J Infect Dis* 2003; **187**: 1709–16.

53 Jolivet-Gougeon A, Kovacs B, Gall-David S *et al*. Bacterial hypermutation: clinical implications. *J Med Microbiol* 2011; **60**: 563–73.

54 Yuan J, Wei B, Lipton MS *et al*. Impact of ArcA loss in *Shewanella oneidensis* revealed by comparative proteomics under aerobic and anaerobic conditions. *Proteomics* 2012; **12**: 1957–69.

55 Kohanski MA, Dwyer DJ, Hayete B *et al*. A common mechanism of cellular death induced by bactericidal antibiotics. *Cell* 2007; **130**: 797–810.

56 Gaupp R, Schlag S, Liebeke M *et al*. Advantage of upregulation of succinate dehydrogenase in *Staphylococcus aureus* biofilms. *J Bacteriol* 2010; **192**: 2385–94.

57 Moisan H, Brouillette E, Jacob CL *et al*. Transcription of virulence factors in *Staphylococcus aureus* small-colony variants isolated from cystic fibrosis patients is influenced by SigB. *J Bacteriol* 2006; **188**: 64–76.

58 Besier S, Ludwig A, Ohlsen K *et al*. Molecular analysis of the thymidine-auxotrophic small colony variant phenotype of *Staphylococcus aureus*. *Int J Med Microbiol* 2007; **297**: 217–25.

59 Rolauffs B, Bernhardt TM, von Eiff C *et al*. Osteopetrosis, femoral fracture, and chronic osteomyelitis caused by *Staphylococcus aureus*

small colony variants (SCV) treated by girdlestone resection-6-year follow-up. *Arch Orthop Trauma Surg* 2002; **122**: 547–50.

60 Sendi P, Rohrbach M, Graber P *et al*. *Staphylococcus aureus* small colony variants in prosthetic joint infection. *Clin Infect Dis* 2006; **43**: 961–7.

61 Seifert H, von Eiff C, Fatkenheuer G. Fatal case due to methicillin-resistant *Staphylococcus aureus* small colony variants in an AIDS patient. *Emerg Infect Dis* 1999; **5**: 450–3.

62 Goring H, Waldner H, Emmerling P *et al*. [Chronic fistulating wound infection after Lichtenstein repair of inguinal hernia, caused by a small colony variant of *Staphylococcus aureus*]. *Chirurg* 2001; **72**: 441–3.

63 Coman G, Mardare N, Copacianu B *et al*. [Persistent and recurrent skin infection with small-colony variant methicillin-resistant *Staphylococcus aureus*]. *Rev Med Chir Soc Med Nat Iasi* 2008; **112**: 104–7.

64 Seifert H, Wisplinghoff H, Schnabel P *et al*. Small colony variants of *Staphylococcus aureus* and pacemaker-related infection. *Emerg Infect Dis* 2003; **9**: 1316–8.

65 Nielsen XC, Nielsen FT, Kurtzhals JA *et al*. Management of recurrent pacemaker-related bacteraemia with small colony variant *Staphylococcus aureus* in a haemodialysis patient. *BMJ Case Rep* 2009; doi:10.1136/bcr.05.2009.1910.

66 Kipp F, Ziebuhr W, Becker K *et al*. Detection of *Staphylococcus aureus* by 16S rRNA directed in situ hybridisation in a patient with a brain abscess caused by small colony variants. *J Neurol Neurosurg Psychiatry* 2003; **74**: 1000–2.

67 Spanu T, Romano L, D'Inzeo T *et al*. Recurrent ventriculoperitoneal shunt infection caused by small-colony variants of *Staphylococcus aureus*. *Clin Infect Dis* 2005; **41**: e48–52.

Supplementary data

Table S1. MICs of antibiotics for *S. aureus* with a normal or SCV phenotype[a]

Class	Antibiotic	MICs (mg/L) of antibiotics according to phenotype[a]															Reference(s)
		strains															
		wild-type			SCV and auxotrophism[b]												
					not specified				MSSA				MRSA				
		not specified	MSSA	MRSA	not specified	men	haem	thy	not specified	men	haem	thy	not specified	men	haem	thy	
Aminoglycosides	amikacin	2	<0.031-2	1	**64**					**64**	0.5-2						1,2
	gentamicin	<0.125-4	<0.031-8	0.25 -1	**<0.125 to >128**	**16**			**8 to >8**	**8; 12**	**0.5-16** -	0.125; 4	0.3	0.5; **32**	**1; 32**		1-15
	kanamycin		0.25	2-4							**2**			**64-128**	**64-128**		6,13
	tobramycin			0.5-1										**32**	**32**		6
Antifolates	trimethoprim		2						1-**8**		**8**						10
	trimethoprim/ sulfamethoxazole	0.004-32	0.06; 0.064		0.023 to **>32**				0.06-**0.5**		**0.5**	**>32**					10,14-16
Penicillins	penicillin	0.016-24			0.016-32												14
	cloxacillin		0.25	128						0.125				128	128		17
	nafcillin	0.5			0.5												1
	oxacillin	<0.06-2	0.06-0.5		<0.06 to **>64**			0.125; 0.5	0.06-**1**	0.25-**2**	0.03-0.5						2,3,8-12,18
	piperacillin	0.064-32			**0.19**-32												14
Cephalosporins	cefuroxime		1-2	4 to >128					1-2				2 to >128				19
	cefotaxime		1							**4**							2
	ceftazidime	0.5-3			**3-8**												14
	ceftobiprole		0.13-0.5	0.5-2					0.13-0.25				0.5-4				19

	cefapirin		0.12					0.06						[12]
Carbapenems	doripenem		0.06	16			0.03					32	16	[17]
	meropenem		0.125	32			0.06					32	32	[17]
Fluoroquinolones	ciprofloxacin	0.032-32	0.12-0.5	0.5-32	0.03 to >32	0.12	0.5	0.06-0.5	0.5	2-32				[2,7,11,12,20]
	levofloxacin		0.25-0.38	3 to >32				0.25	0.25	4 to >32				[20]
	moxifloxacin		<0.03-0.25	0.03-4		<0.03-0.13		0.094	0.094; 0.125	1-8	0.125	0.125		[5,9,19,20]
	finafloxacin		0.19-0.38	1.5-4				0.5	0.25	1-6				[20]
Quinolones	fleroxacin		1				1							[3]
Glycopeptides	vancomycin	0.25-2	0.5-2	1-4	0.125-**2**	1;2	0.5-2	0.5-2	0.5-22	8	1	1		[2,3,7-12,17,21,22]
	teicoplanin			3						8				[7]
	telavancin		0.125						0.125					[9]
	oritavancin		0.03	0.25					0.015		0.125	0.03		[5,9]
Lipopeptides	daptomycin		0.125-1	0.19-1				1;2	0.125; 0.5	0.38	0.5	0.5; 2		[5,7-9,19,23]
Ansamycins	rifampicin		0.002-0.125	0.016-0.023		0.03-0.008		0.008-0.06	*0.0005*	**>256**	0.016	0.016		[5,7-10,12]
Macrolides	erythromycin		0.25-0.5			0.12		**1-2**						[11,12]
Lincosamides	clindamycin		0.060.125					0.25	0.125; 0.25					[8,9]
Oxazolidinones	linezolid	0.25-4	0.5-4	0.5-1	0.25-4	0.5-**4**			2	1-2				[7,9,15,19,19]
Other antibiotics	fusidic acid		0.125						0.03					[9]
	quinupristin/dalfopristin		0.5						0.5					[9]
	tigecycline	0.015-0.5	0.125	0.19	0.015-**2**				0.125	0.19				[7,9,15]
	XF-70		0.5-1	0.25-1				0.25-0.5				0.25-1		[24]
Other agents	tomatine		>16			>16		>16						[11]
	tomatidine		>16			*0.12*		*0.12*						[11]

	lysostaphin		2			1-4		1					[10,12]
	lactoferrin B		16-64	256	**>256**			**>256**	**>256**			**>256**	**>256**	[25]

[a]Only studies comparing normal phenotype and SCV strains have been included in this table.
[b]Values in bold correspond to MICs that are at least 2 dilutions higher than those for the corresponding parental strain with a normal phenotype and values in italics correspond to MICs that are at least 2 dilutions lower than those for the corresponding parental strain with a normal phenotype.
[c]men, menadione-dependent; hem, haemin dependent; thy, thymidine dependent.

References to Table S1

1. Miller MH, Wexler MA, Steigbigel NH. Single and combination antibiotic therapy of *Staphylococcus aureus* experimental endocarditis: emergence of gentamicin-resistant mutants. *Antimicrob Agents Chemother* 1978; **14**: 336-43.

2. Singh R, Ray P, Das A *et al.* Enhanced production of exopolysaccharide matrix and biofilm by a menadione-auxotrophic *Staphylococcus aureus* small-colony variant. *J Med Microbiol* 2010; **59**: 521-7.

3. Chuard C, Vaudaux PE, Proctor RA *et al.* Decreased susceptibility to antibiotic killing of a stable small colony variant of *Staphylococcus aureus* in fluid phase and on fibronectin-coated surfaces. *J Antimicrob Chemother* 1997; **39**: 603-8.

4. Lannergard J, von Eiff C, Sander G *et al.* Identification of the genetic basis for clinical menadione-auxotrophic small-colony variant isolates of *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2008; **52**: 4017-22.

5. Garcia LG, Lemaire S, Kahl BC *et al.* Pharmacodynamic evaluation of the activity of antibiotics against hemin- and menadione-dependent small-colony variants of *Staphylococcus aureus* in models of extracellular (broth) and intracellular (THP-1 monocytes) infections. *Antimicrob Agents Chemother* 2012; **56**: 3700-11.

6. Gaupp R, Schlag S, Liebeke M *et al.* Advantage of upregulation of succinate dehydrogenase in *Staphylococcus aureus* biofilms. *J Bacteriol* 2010; **192**: 2385-94.

7. Gao W, Chua K, Davies JK *et al.* Two novel point mutations in clinical *Staphylococcus aureus* reduce linezolid susceptibility and switch on the stringent response to promote persistent infection. *PLoS Pathog* 2010; **6**: e1000944.

8. Baltch AL, Ritz WJ, Bopp LH *et al.* Activities of daptomycin and comparative antimicrobials, singly and in combination, against extracellular and intracellular *Staphylococcus aureus* and its stable small-colony variant in human monocyte-derived macrophages and in broth. *Antimicrob Agents Chemother* 2008; **52**: 1829-33.

9. Nguyen HA, Denis O, Vergison A *et al.* Intracellular activity of antibiotics in a model of human THP-1 macrophages infected by a *Staphylococcus aureus* small-colony variant strain isolated from a cystic fibrosis patient: pharmacodynamic evaluation and comparison with isogenic normal-phenotype and revertant strains. *Antimicrob Agents Chemother* 2009; **53**: 1434-42.

10. Moisan H, Brouillette E, Jacob CL *et al.* Transcription of virulence factors in *Staphylococcus aureus* small-colony variants isolated from cystic fibrosis patients is influenced by SigB. *J Bacteriol* 2006; **188**: 64-76.

11. Mitchell G, Gattuso M, Grondin G *et al.* Tomatidine inhibits replication of *Staphylococcus aureus* small-colony variants in cystic fibrosis airway epithelial cells. *Antimicrob Agents Chemother* 2011; **55**: 1937-45.

12. Brouillette E, Martinez A, Boyll BJ *et al.* Persistence of a *Staphylococcus aureus* small-colony variant under antibiotic pressure in vivo. *FEMS Immunol Med Microbiol* 2004; **41**: 35-41.

13. von Eiff C, Heilmann C, Proctor RA *et al.* A site-directed *Staphylococcus aureus hemB* mutant is a small-colony variant which persists intracellularly. *J Bacteriol* 1997; **179**: 4706-12.

14. Kahl B, Herrmann M, Everding AS *et al.* Persistent infection with small colony variant strains of *Staphylococcus aureus* in patients with cystic fibrosis. *J Infect Dis* 1998; **177**: 1023-9.

15. Yagci S, Hascelik G, Dogru D *et al.* Prevalence and genetic diversity of *Staphylococcus aureus* small-colony variants in cystic fibrosis patients. *Clin Microbiol Infect* 2013; **19**: 77-84.

16. Besier S, Ludwig A, Ohlsen K *et al.* Molecular analysis of the thymidine-auxotrophic small colony variant phenotype of *Staphylococcus aureus*. *Int J Med Microbiol* 2007; **297**: 217-25.

17. Garcia LG, Lemaire S, Kahl BC *et al.* Intracellular forms of menadione-dependent small-colony variants of methicillin-resistant *Staphylococcus aureus* are hypersusceptible to β-lactams in a THP-1 cell model due to cooperation between vacuolar acidic pH and oxidant species. *J Antimicrob Chemother* 2012; **67**: 2873-81.

18. Bates DM, von Eiff C, McNamara PJ *et al. Staphylococcus aureus menD* and *hemB* mutants are as infective as the parent strains, but the menadione biosynthetic mutant persists within the kidney. *J Infect Dis* 2003; **187**: 1654-61.

19. von Eiff C, Friedrich AW, Becker K *et al.* Comparative in vitro activity of ceftobiprole against staphylococci displaying normal and small-colony variant phenotypes. *Antimicrob Agents Chemother* 2005; **49**: 4372-4.

20. Idelevich EA, Kriegeskorte A, Stubbings W *et al.* Comparative in vitro activity of finafloxacin against staphylococci displaying normal and small colony variant phenotypes. *J Antimicrob Chemother* 2011; **66**: 2809-13.

21. Yagci S, Hascelik G, Dogru D *et al.* Prevalence and genetic diversity of *Staphylococcus aureus* small-colony variants in cystic fibrosis patients. *Clin Microbiol Infect* 2011;

22. Tsuji BT, von Eiff C, Kelchlin PA *et al.* Attenuated vancomycin bactericidal activity against *Staphylococcus aureus hemB* mutants expressing the small-colony-variant phenotype. *Antimicrob Agents Chemother* 2008; **52**: 1533-7.

23. Begic D, von Eiff C, Tsuji BT. Daptomycin pharmacodynamics against *Staphylococcus aureus hemB* mutants displaying the small colony variant phenotype. *J Antimicrob Chemother* 2009; **63**: 977-81.

24. Vaudaux P, Francois P, Bisognano C *et al.* Increased expression of clumping factor and fibronectin-binding proteins by *hemB* mutants of *Staphylococcus aureus* expressing small colony variant phenotypes. *Infect Immun* 2002; **70**: 5428-37.

25. Samuelsen O, Haukland HH, Kahl BC *et al. Staphylococcus aureus* small colony variants are resistant to the antimicrobial peptide lactoferricin B. *J Antimicrob Chemother* 2005; **56**: 1126-9.