In Vitro Susceptibility of Clinical *Staphylococcus aureus* Small-Colony Variants to β -Lactam and Non- β -Lactam Antibiotics

Evgeny A. Idelevich,^a André Kriegeskorte,^{a*} Nina Schleimer,^a Georg Peters,^a Christof von Eiff,^{a*} Karsten Becker^a

^aInstitute of Medical Microbiology, University Hospital Münster, Münster, Germany

ABSTRACT The *Staphylococcus aureus* small-colony variant (SCV) phenotype has been associated with relapsing and antibiotic-refractory infections. However, little is known about the activities of antibiotics on clinical SCVs. Here, we demonstrated that SCVs without detectable auxotrophies were at least as susceptible to most β -lactam and non- β -lactam antibiotics *in vitro* as their corresponding clonally identical strains with a normal phenotype. After prolonged incubation, a regrowth phenomenon has been observed in gradient diffusion inhibition zones irrespective of the strains' phenotype.

KEYWORDS *Staphylococcus aureus*, small-colony variants, β -lactam antibiotics, susceptibility, resistance, aminoglycosides, antibiotic resistance, daptomycin, lincosamides, linezolid, trimethoprim

Staphylococcus aureus represents one of the major invasive pathogens causing a wide range of clinically important and life-threatening infections worldwide (1). Small-colony variants (SCVs) represent a naturally occurring slow-growing subpopulation of *S. aureus* associated with chronic and recurrent courses of infection (2). The SCV phenotype is characterized by an altered metabolism as an adaptation to their intracellular lifestyle (3–5). β -Lactam antibiotics (BLAs), especially penicillinase-stable penicillins or first- and second-generation cephalosporins, are antimicrobials of choice for the treatment of severe *S. aureus* infections unless methicillin-resistant *S. aureus* (MRSA) is involved (6). Most of the data on the susceptibility of *S. aureus* SCVs toward β -lactams and other antibiotics have been derived from studies performed with deletion mutants mimicking the SCV phenotype or SCV isolates colonizing the airways of cystic fibrosis patients (7–9). However, only little is known about the effects of BLAs on *S. aureus* SCVs recovered from clinical samples. In this study, we investigated the susceptibilities of stable clinical SCVs and their corresponding clonally identical clinical strains with a normal phenotype (NP) toward a broad set of BLAs and non-BLAs.

(This work was presented in part at the 52nd Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, CA, 9 to 12 September 2012 [10].)

Ten methicillin-susceptible *S. aureus* (MSSA) strain pairs recovered from clinical samples (skin and soft tissue infections, bone and joint infections, wound infections, and intra-abdominal infections) at the University Hospital Münster, Germany, were included. Isolates from cystic fibrosis patients were excluded. Each strain pair consisted of a stable SCV and its corresponding NP for which clonality was confirmed by pulsed-field gel electrophoresis (11, 12). Since spontaneous switching of SCVs into the NP is possible (13), stable phenotypes were confirmed by observation of colony morphology during the experiments. Auxotrophy testing was performed as described elsewhere (9) using 10 μ l of hemin (1 mg/ml), menadione (160 μ g/ml), and thymidine (100 μ g/ml; Sigma-Aldrich, Inc., St. Louis, MO, USA) and Mueller-Hinton agar (MHA; Merck KGaA, Darmstadt, Germany). None of the SCVs exhibited auxotrophy for the tested substances. In addition to clinical strain pairs, genetically defined stable *hemB*- and *thyA*-knockout mutants of MSSA labora-

Received 12 December 2017 Returned for
modification 4 January 2018 Accepted 19
January 2018

Accepted manuscript posted online 29
January 2018

Citation Idelevich EA, Kriegeskorte A,
Schleimer N, Peters G, von Eiff C, Becker K.
2018. *In vitro* susceptibility of clinical
Staphylococcus aureus small-colony variants to
 β -lactam and non- β -lactam antibiotics.
Antimicrob Agents Chemother 62:e02532-17.
<https://doi.org/10.1128/AAC.02532-17>.

Copyright © 2018 American Society for
Microbiology. All Rights Reserved.

Address correspondence to Karsten Becker,
kbecker@uni-muenster.de.

* Present address: André Kriegeskorte, GSK
Vaccines GmbH Marburg, Marburg, Germany;
Christof von Eiff, Pfizer Pharma GmbH, Berlin,
Germany.

E.A.I. and A.K. contributed equally to this work.

TABLE 1 MICs of 10 clinical *S. aureus* strain pairs consisting of SCVs and their clonally identical parental isolates with NP

Antimicrobial agent	MIC data (μg/ml) ^a						No. of resistant strains ^b		No. of strains with RCs in inhibition zone ^c	
	NP			SCV						
	MIC ₅₀	MIC ₉₀	Range	MIC ₅₀	MIC ₉₀	Range	NP	SCV	NP	SCV
β-Lactams										
Benzylpenicillin	0.094	2	0.023–24	0.125	1.5	0.016–4	5	4	4	5
Ampicillin	0.25	3	0.094–12	0.25	1.5 (2)	0.023–4 (0.023–6)	NA	NA	3	2
Ampicillin-sulbactam	0.19 (0.25)	1	0.094–1.5	0.19	0.75	0.016–0.75 (0.016–1)	NA	NA		1
Piperacillin	1.5	4	0.75–24 (0.75–32)	1.5	3	0.25–4 (0.25–12)	NA	NA	2	3
Piperacillin-tazobactam	1	2	0.75–3	0.5	1	0.064–2	NA	NA		
Oxacillin	0.38	0.75	0.19–1	0.125	0.5	0.023–0.5			1	1
Cefoxitin	2	3	1–3	1	3	0.25–4				2
Cephalothin	0.38	0.5	0.19–0.75	0.125	0.38	0.016–0.5	NA	NA		2
Cefuroxime	0.75	0.75	0.38–1.5	0.25	0.75	0.064–0.75	NA	NA		
Ceftriaxone	3	4	2–6	2	4	0.38–4	NA	NA		3
Cefepime	3	3	1.5–3 (1.5–4)	1.5	3	0.38–3	NA	NA	5	3
Imipenem	0.064	0.094	0.047–0.094	0.023	0.064	0.012–0.064	NA	NA	6	3
Non-β-lactams										
Vancomycin	1	1.5	0.75–1.5	1	1.5	0.75–2				1
Daptomycin	0.19	0.25	0.094–0.38	0.094 (0.19)	0.38	0.094–0.5				
Moxifloxacin	0.064	0.064	0.032–0.064	0.047	0.19	0.023–0.19				
Clarithromycin	0.38	0.75	0.094–256	0.19	1.5	0.023–256	1	2		
Clindamycin	0.094	0.125	0.064–0.125	0.064	0.19	0.016–0.38		1		1
Linezolid	0.5 (0.75)	1 (1.5)	0.25–1 (0.25–1.5)	0.38	1.5	0.094–1.5				
Rifampin	0.012	0.016	0.006–0.016	0.008	0.012	0.003–0.016			1	
Fosfomycin	4	12	1.5–16	4	12	0.064–16			2	
Quinupristin-dalfopristin	0.5	0.5 (0.75)	0.25–0.75	0.38	0.5	0.047–0.75			6	3
Tigecycline	0.38	0.5	0.19–0.75	0.38	0.5 (0.75)	0.094–0.75	1	1 (2)		
Trimethoprim-sulfamethoxazole	0.032	0.047	0.023–0.19	0.032	0.094	0.003–0.125				
Gentamicin	0.38	24	0.125–256	0.125 (0.25)	4	0.064–256	2	4	1	1
Mupirocin	0.094	0.19 (0.38)	0.094–0.19 (0.094–0.38)	0.094	0.19	0.064–0.19	NA	NA		

^aChanges in MIC after 48 h are given in parentheses.^bAccording to EUCAST breakpoints 2017 (24); intermediate and resistant strains were combined into the resistant category. Changes in the number of resistant strains after 48 h are given in parentheses. NA, not applicable.^cRCs, regrown colonies.

tory strains 8325-4 and SH1000, respectively, and their corresponding parental and complemented mutant (CM) strains were tested (14, 15). Furthermore, MSSA reference strain ATCC 29213 was included as a quality control.

The MICs of 12 BLAs and 13 non-BLAs (Table 1) were determined by the gradient diffusion method (Etest; bioMérieux, Marcy-l'Étoile, France), according to the manufacturer's recommendations. Briefly, a bacterial suspension was prepared from colonies growing overnight on solid medium and adjusted to a turbidity of 0.5 McFarland. This inoculum was streaked onto MHA (Merck KGaA) using a plate rotator (type EPA; Robin SA, Beaucazé, France). Etest strips were applied within 15 min after the excess moisture dried. For clinical SCVs not growing on MHA as well as for the *thyA*-knockout mutant, MHA with 5% sheep blood (Merck KGaA, Darmstadt, Germany) was used. The MICs were read and documented after 18 h of incubation at 36°C and for oxacillin and vancomycin after 24 h, according to the recommendations of the manufacturer. Since SCVs exhibit deficient growth, MICs were also read after 48 h of incubation. The results were read by three investigators, and median MICs were calculated for analysis. Since the appearance of single colonies in the inhibition zone was observed after prolonged incubation in the preliminary experiments, the plates were further incubated and read every 24 h for a total period of 7 days to check for appearance of colonies in the inhibition zone, indicating surviving cells.

For most tested BLAs, the MIC₅₀ and MIC₉₀ were either equal to or slightly lower for clinical SCVs than for the corresponding NPs (Table 1). The same applied to the MICs for *hemB* and *thyA* mutants compared to their wild types (Table 2). Since incubation was

TABLE 2 MICs of β -lactam and non- β -lactam antibiotics determined with Etest for two *S. aureus* triplets consisting of isolates with NP, their isogenic knockout mutants with an SCV phenotype, and their CMs

Antimicrobial agent	MIC ($\mu\text{g/ml}$) ^a					
	8325-4			SH1000		
	NP (25)	<i>hemB</i> mutant (14)	CM (14)	NP (26)	<i>thyA</i> mutant (15)	CM (15)
β-Lactams						
Benzylpenicillin	0.023	0.016	0.032	0.047	0.032	0.064
Ampicillin	0.125	0.19	0.19	0.25	0.094	0.19
Ampicillin-sulbactam	0.094	0.023	0.125	0.19	0.032	0.125
Piperacillin	0.75	0.25	1	1.5 ^b	0.5	1.5 ^b
Piperacillin-tazobactam	0.75	0.19	0.75 ^b	1.5	0.5	1.5
Oxacillin	0.38	0.064	0.38	0.75	0.19	0.5
Cefoxitin	3	3	2	4	2	3
Cephalothin	0.25	0.094	0.38	0.38	0.25	0.38
Cefuroxime	0.38	0.19	0.5	0.75	0.38	0.75
Ceftriaxone	3	1.5	3	4 ^b	3	3
Cefepime	2	2	2 ^b	4 ^b	2	3 ^b
Imipenem	0.047	0.032	0.064 ^b	0.064 ^b	0.047	0.047 ^b
Non-β-lactams						
Vancomycin	1	0.75	1	1.5	1.5	1.5
Daptomycin	0.25	0.25	0.25	0.38	0.19	0.38
Moxifloxacin	0.094	0.023 (0.032)	0.064	0.094	0.032	0.047
Clarithromycin	0.38	>256 ^c	>256 ^c	0.75	>256 ^c	>256 ^c
Clindamycin	0.094	>256 ^c	>25 ^c	0.125	>256 ^c	>256 ^c
Linezolid	0.5 (0.75)	0.5	0.5	1.5	0.75	1
Rifampin	0.008	0.008	0.012 (0.016)	0.016 (0.023)	0.008	0.012 (0.016)
Fosfomycin	6 (8)	0.75	8	24	6	12 ^b
Quinupristin-dalfopristin	0.38 ^a	0.25	0.5 (0.75)	1 ^b	1.5 (2) ^b	1.5 ^b
Tigecycline	0.19	0.19	0.19 (0.25)	0.75	1 ^b	0.5
Trimethoprim-sulfamethoxazole	0.032	0.064 ^b	0.032	0.047	>32	0.016
Gentamicin	0.19	3	0.125	0.19	0.25	0.125 (0.25)
Mupirocin	0.064 (0.125)	0.064	0.19	0.125	0.094	0.125

^aChanges in MICs after 48 h are given in parentheses. NP, wild type displaying the normal phenotype. References for the respective strain/mutant are given in parentheses.

^bAppearance of regrown colonies in inhibition zone.

^cResistance is due to allelic replacement of *hemB* or *thyA* by erythromycin resistance cassette *ermB*.

performed for 48 h, considerable MIC reading bias due to slower growth of SCVs is unlikely. As expected for MSSA, all strain pairs were susceptible to cefoxitin and oxacillin, with SCVs showing MICs slightly lower than those for NPs. The *hemB* and *thyA* mutants also exhibited oxacillin MICs lower than those of corresponding NPs and CMs. This is in accordance with earlier reports assessing the MICs of defined *hemB* mutant strains (16, 17). The cefoxitin MICs of the tested mutants were comparable to those from corresponding NPs and CMs. Interestingly, intracellular forms of *menD* mutant SCVs were shown to be hypersusceptible to BLAs in a recent study (18).

The MIC₅₀s of most non-BLAs were identical to or slightly lower for clinical SCVs than for NPs, whereas the MIC₉₀ values for most non-BLAs were identical or slightly higher for SCVs than for NPs. In seven of 10 pairs, the SCV strains had slightly or considerably lower MICs for gentamicin than their corresponding NP strains after 18 h of incubation (see Table S1 in the supplemental material). This finding is in contrast to those of prior reports about the reduced susceptibility of SCVs to aminoglycosides (9, 11, 19, 20) and may suggest that most clinical SCVs tested here exhibited an intact electron transport, which implies functional aminoglycoside uptake (21). However, we also found SCVs with higher gentamicin MICs than those of their NPs in two strain pairs, resulting in categorization as resistant, while the corresponding NPs were susceptible (Table S1). The *hemB* mutant exhibited a higher MIC for gentamicin than for their corresponding NP and CM (Table 2). This is in accordance with previous findings (14, 16, 17, 22) and is explained by electron transport deficiencies due to the *hemB* knockout. Interestingly, Garcia et al. postulated that gentamicin used in cell culture-based experiments remained highly bactericidal against genetically defined electron transport-deficient SCVs in spite of its in-

FIG 1 Example of regrown *S. aureus* colonies (normal phenotype) in the inhibition zone of oxacillin gradient diffusion test appearing on day 6 (just becoming visible) and day 7. Inset, 3-fold magnification of regrown colonies on day 7.

creased MIC (7). For the *thyA* mutant, the gentamicin MICs were similar to those of corresponding NP and CM as described before for strains with mutations in *thyA* (23). For other tested non-BLAs, the SCV mutants exhibited no major differences from NPs and CMs irrespective of their knockouts, except for clarithromycin and clindamycin, toward which the mutants showed, as expected, resistance due to allelic replacement of *hemB* or *thyA* genes by erythromycin resistance cassette *ermB* (Table 2).

For some strains, we observed a regrowth phenomenon occurring as single colonies in the inhibition zone of several antibiotics. Typically, the regrown colonies appeared circularly in a concentration-dependent manner surrounding the Etest strip (Fig. 1) after prolonged incubation of up to 7 days. The regrowth was found more frequently with BLAs (19.2%) (Fig. 1 and Table 1). However, there were no marked differences between clinical NPs (17.5%) and SCVs (20.8%) regarding the occurrence of this phenomenon (Table 1). The regrowth colonies observed may result from survived persister cells. Further detailed investigations of this phenomenon are warranted, which should incorporate time-kill studies to investigate the impact of the slow growth of SCVs on killing kinetics.

In conclusion, BLAs and other antibiotics used in this study showed high *in vitro* activities against a diverse set of clinical MSSA-SCVs. Thus, the detection of SCVs in clinical samples should not curtail *a priori* the choice of appropriate antibiotics. Clinical studies on optimal treatment regimens for SCV-associated infections are warranted.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at <https://doi.org/10.1128/AAC.02532-17>.

SUPPLEMENTAL FILE 1, PDF file, 0.1 MB.

ACKNOWLEDGMENTS

We are grateful to B. Grünastel and M. Tigges for their expert technical assistance.

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

We declare no conflicts of interest.

REFERENCES

1. Tong SYC, Davis JS, Eichenberger E, Holland TL, Fowler VG, Jr. 2015. *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. Clin Microbiol Rev 28:603–661. <https://doi.org/10.1128/CMR.00134-14>.
2. Proctor RA, von Eiff C, Kahl BC, Becker K, McNamara P, Herrmann M, Peters G. 2006. Small colony variants: a pathogenic form of bacteria that facilitates persistent and recurrent infections. Nat Rev Microbiol 4:295–305. <https://doi.org/10.1038/nrmicro1384>.
3. Kriegeskorte A, König S, Sander G, Pirkel A, Mahabir E, Proctor RA, von Eiff C, Peters G, Becker K. 2011. Small colony variants of *Staphylococcus aureus* reveal distinct protein profiles. Proteomics 11:2476–2490. <https://doi.org/10.1002/pmic.201000796>.
4. von Eiff C, McNamara P, Becker K, Bates D, Lei X-H, Ziman M, Bochner BR, Peters G, Proctor RA. 2006. Phenotype microarray profiling of *Staphylococcus aureus* *menD* and *hemB* mutants with the small-colony-variant phenotype. J Bacteriol 188:687–693. <https://doi.org/10.1128/JB.188.2.687-693.2006>.
5. Kriegeskorte A, Grubmüller S, Huber C, Kahl BC, von Eiff C, Proctor RA, Peters G, Eisenreich W, Becker K. 2014. *Staphylococcus aureus* small colony variants show common metabolic features in central metabolism irrespective of the underlying auxotrophism. Front Cell Infect Microbiol 4:141. <https://doi.org/10.3389/fcimb.2014.00141>.
6. Idelevich EA, Kreis C, Löffler B, Peters G. 2017. *Staphylococcus aureus*-associated musculoskeletal infections. Curr Top Microbiol Immunol 409: 229–261. https://doi.org/10.1007/82_2016_19.
7. Garcia LG, Lemaire S, Kahl BC, Becker K, Proctor RA, Denis O, Tulkens PM, Van Bambeke F. 2012. 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 56:3700–3711. <https://doi.org/10.1128/AAC.00285-12>.
8. Nguyen HA, Denis O, Vergison A, Theunis A, Tulkens PM, Struelens MJ, Van Bambeke F. 2009. 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 53:1434–1442. <https://doi.org/10.1128/AAC.01145-08>.
9. Kahl B, Herrmann M, Everding AS, Koch HG, Becker K, Harms E, Proctor RA, Peters G. 1998. Persistent infection with small colony variant strains of *Staphylococcus aureus* in patients with cystic fibrosis. J Infect Dis 177:1023–1029. <https://doi.org/10.1086/515238>.
10. Idelevich EA, Kriegeskorte A, von Eiff C, Peters G, Becker K. 2012. In vitro susceptibility and tolerance of *Staphylococcus aureus* small colony variants to beta-lactam antibiotics, abstr E-1483. 52nd Intersci Conf Antimicrob Agents Chemother, San Francisco, CA, 9 to 12 September 2012.
11. von Eiff C, Bettin D, Proctor RA, Rolaufts B, Lindner N, Winkelmann W, Peters G. 1997. Recovery of small colony variants of *Staphylococcus aureus* following gentamicin bead placement for osteomyelitis. Clin Infect Dis 25:1250–1251. <https://doi.org/10.1086/516962>.
12. Goering RV, Winters MA. 1992. Rapid method for epidemiological evaluation of Gram-positive cocci by field inversion gel electrophoresis. J Clin Microbiol 30:577–580.
13. von Eiff C, Becker K, Skov RL. 2015. *Staphylococcus*, *Micrococcus*, and other catalase-positive cocci, p 354–382. In Jorgensen J, Pfaller M, Carroll K, Funke G, Landry M, Richter S, Warnock D (ed), Manual of clinical microbiology, 11th ed. ASM Press, Washington, DC.
14. von Eiff C, Heilmann C, Proctor RA, Woltz C, Peters G, Götz F. 1997. A site-directed *Staphylococcus aureus* *hemB* mutant is a small-colony variant which persists intracellularly. J Bacteriol 179:4706–4712. <https://doi.org/10.1128/jb.179.15.4706-4712.1997>.
15. Kriegeskorte A, Block D, Drescher M, Windmüller N, Mellmann A, Baum C, Neumann C, Loré NI, Bragonzi A, Liebau E, Hertel P, Seggewiss J, Becker K, Proctor RA, Peters G, Kahl BC. 2014. Inactivation of *thyA* in *Staphylococcus aureus* attenuates virulence and has a strong impact on metabolism and virulence gene expression. mBio 5:e01447-14. <https://doi.org/10.1128/mBio.01447-14>.
16. Brouillette E, Martinez A, Boyll BJ, Allen NE, Malouin F. 2004. Persistence of a *Staphylococcus aureus* small-colony variant under antibiotic pressure in vivo. FEMS Immunol Med Microbiol 41:35–41. <https://doi.org/10.1016/j.femsim.2003.12.007>.
17. Moisan H, Brouillette E, Jacob CL, Langlois-Bégin P, Michaud S, Malouin F. 2006. Transcription of virulence factors in *Staphylococcus aureus* small-colony variants isolated from cystic fibrosis patients is influenced by SigB. J Bacteriol 188:64–76. <https://doi.org/10.1128/JB.188.1.64-76.2006>.
18. Garcia LG, Lemaire S, Kahl BC, Becker K, Proctor RA, Tulkens PM, Van Bambeke F. 2012. 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 67:2873–2881. <https://doi.org/10.1093/jac/dks325>.
19. Lewis LA, Li K, Bharosay M, Cannella M, Jorgenson V, Thomas R, Pena D, Velez M, Pereira B, Sassine A. 1990. Characterization of gentamicin-resistant respiratory-deficient (res-) variant strains of *Staphylococcus aureus*. Microbiol Immunol 34:587–605. <https://doi.org/10.1111/j.1348-0421.1990.tb01035.x>.
20. Baumert N, von Eiff C, Schaaff F, Peters G, Proctor RA, Sahl H-G. 2002. Physiology and antibiotic susceptibility of *Staphylococcus aureus* small colony variants. Microb Drug Resist 8:253–260. <https://doi.org/10.1089/10766290260469507>.
21. Mates SM, Eisenberg ES, Mandel LJ, Patel L, Kaback HR, Miller MH. 1982. Membrane potential and gentamicin uptake in *Staphylococcus aureus*. Proc Natl Acad Sci U S A 79:6693–6697. <https://doi.org/10.1073/pnas.79.21.6693>.
22. Mitchell G, Gattuso M, Grondin G, Marsault É, Bouarab K, Malouin F. 2011. Tomatidine inhibits replication of *Staphylococcus aureus* small-colony variants in cystic fibrosis airway epithelial cells. Antimicrob Agents Chemother 55:1937–1945. <https://doi.org/10.1128/AAC.01468-10>.
23. Besier S, Zander J, Siegel E, Saum SH, Hunfeld K-P, Ehrhart A, Brade V, Wichelhaus TA. 2008. Thymidine-dependent *Staphylococcus aureus* small-colony variants: human pathogens that are relevant not only in cases of cystic fibrosis lung disease. J Clin Microbiol 46:3829–3832. <https://doi.org/10.1128/JCM.01440-08>.
24. European Committee on Antimicrobial Susceptibility Testing. 2017. Breakpoint tables for interpretation of MICs and zone diameters. Version 7.1. European Committee on Antimicrobial Susceptibility Testing, Växjö, Sweden. http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_7.1_Breakpoint_Tables.pdf.
25. Novick R. 1967. Properties of a cryptic high-frequency transducing phage in *Staphylococcus aureus*. Virology 33:155–166. [https://doi.org/10.1016/0042-6822\(67\)90105-5](https://doi.org/10.1016/0042-6822(67)90105-5).
26. Horsburgh MJ, Aish JL, White IJ, Shaw L, Lithgow JK, Foster SJ. 2002. σ^B modulates virulence determinant expression and stress resistance: characterization of a functional *rsbU* strain derived from *Staphylococcus aureus* 8325-4. J Bacteriol 184:5457–5467. <https://doi.org/10.1128/JB.184.19.5457-5467.2002>.