

Cell wall-affecting antibiotics modulate natural transformation in SigH-expressing *Staphylococcus aureus*

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1 **NOTE**

2 **Cell wall-affecting antibiotics modulate natural transformation**
3 **in SigH-expressing *Staphylococcus aureus***

4 **Le Thuy Nguyen Thi ¹, Veronica Medrano Romero ² and Kazuya Morikawa ^{3,*}**

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6 ¹ Ph.D. Program in Human Biology, School of Integrative and Global Majors, University of Tsukuba,
7 1-1-1 Tennodai, Tsukuba, 305-8575, Japan; E-Mail: lenacns2005@gmail.com (L.T.N.T.)

8 ² Graduate School of Comprehensive Human Sciences, University of Tsukuba, 1-1-1 Tennodai,
9 Tsukuba, 305-8575, Japan; E-Mail: veromedrom@gmail.com (V.M.R.)

10 ³ Faculty of Medicine, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, 305-8575, Japan; E-Mail:
11 morikawa.kazuya.ga@u.tsukuba.ac.jp (K.M.)

12 * Author to whom correspondence should be addressed;

13 E-Mail:morikawa.kazuya.ga@u.tsukuba.ac.jp (K.M.); Tel.: +81-29-853-3928; Fax: +81-29-853-3928.

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15 Running title: antibiotics affecting transformation in *Staphylococcus aureus*

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21 *Staphylococcus aureus* is a major opportunistic human pathogen causing a broad spectrum of
22 infections¹. This bacterium has an extraordinary ability to rapidly acquire resistance to antibiotics, and
23 methicillin-resistant strains (MRSA), the most common cause of nosocomial infections, are now
24 spreading into the community^{2,3}. In *S. aureus* genome, many virulence and antibiotic resistance genes
25 are found in mobile genetic elements^{4,5}, indicating that horizontal gene transfer (HGT) must play a
26 critical role in the evolution. In general, Gram-positive bacteria have three HGT mechanisms:
27 conjugation, transduction, and transformation⁶. Conjugation occurs in *S. aureus*, but it requires a series
28 of *tra* genes or conjugative plasmids, which are not widespread among *S. aureus* strains^{7,8}.
29 Transduction is thought to be more predominant, because most *S. aureus* isolates have prophages in
30 their genome. The head size of phages limits the DNA length that can be transferred (c.a. 45kbp)⁴, but
31 a giant phage in the environment can transfer larger DNA fragments⁹.

32 We recently demonstrated for the first time that *S. aureus* can develop natural genetic competence
33 for DNA transformation in a manner dependent on SigH¹⁰. SigH is one of the alternative sigma factors.
34 It associates with the core RNA polymerase, and renders the resultant holoenzyme the ability to
35 recognize the promoter sequence and initiate the transcription of competence operons (*comG* and
36 *comE* operons) that encode the machinery for DNA incorporation¹¹. SigH expresses in a minor
37 subpopulation by two distinct mechanisms¹⁰. One is short-junction duplication generating a new *sigH*

38 fusion gene. SigH expression by short-junction duplication is spontaneous and its frequency is less
39 than 10^{-5} . Alternatively, SigH is expressed by post-transcriptional regulation, and SigH-expressing
40 cells increase up to $\sim 10^{-2}$ in complete synthetic medium (CS2). Thus, SigH expression is limited to a
41 small subpopulation, which was one of the reasons for the difficulty in experimental detection of
42 transformation. The transformation frequency in SigH-expressing cells is experimentally detectable,
43 but the efficiency varies depending on the culture conditions, suggesting that unknown factor(s) affect
44 the transformation of SigH-expressing cells.

45 Drug resistance is initially manifested in the settings where antibiotics constitute a selective
46 pressure³. However, the effects of antibiotics on *S. aureus* transformation have not been explored yet.
47 Here, we describe the effects of antibiotics on the efficiency of transformation in SigH-expressing cells.

48 The SigH-expressing strain (N315ex w/o ϕ h,¹⁰) was used as the recipient. In this strain, the
49 prophage was eliminated to exclude the possibility of “pseudo-competence” DNA transfer with the
50 help of phage components, which is distinct from real competence. SigH is expressed by a plasmid,
51 pRIT-sigH¹¹. Transformation assay was carried out as previously described with some modifications¹⁰.
52 Briefly, log-phase cells were suspended in fresh tryptic soy broth (TSB) with or without the antibiotics
53 to be tested. After 5h incubation, cells were washed and replaced with fresh medium. Ten μg of
54 unmethylated pHY300 (Tet^R) purified from *E. coli* HST04 (*dam-/dcm-*) was added; methylation status
55 does not affect transformation frequency (data not shown). Following 2.5 h incubation at 37°C with
56 shaking, transformants were selected in brain heart infusion (BHI)-agar medium supplemented with 5
57 $\mu\text{g ml}^{-1}$ tetracycline. Some transformants were tested for the presence of the *tet^R* gene by colony PCR.
58 In line with our previous study¹⁰, no spontaneous Tet^R mutant was detected throughout this study. The
59 transformation frequency was calculated as the ratio of total number of transformants to the total viable
60 cells after the antibiotics treatment and incubation with DNA.

61 The effects of antibiotics on transformation were tested by at least 3 independent experiments
62 (Figure 1). Bacitracin reproducibly increased the transformation frequency at low concentrations, but
63 showed suppressive effect at higher concentrations. D-cycloserin showed no significant effect.
64 Transformants were rarely detected in 1 $\mu\text{g ml}^{-1}$ cefazolin treatment (frequency 0.8×10^{-11} , n = 1; none
65 detected, n =2). Oxacillin abolished transformation: no transformants were detected when cells were
66 treated at $\frac{1}{2}$ MIC of oxacillin. Mitomycin C suppressed transformation in a concentration-dependent
67 manner. Ciprofloxacin, norfloxacin and streptomycin had no significant effect.

68 Vancomycin and fosfomycin increased the transformation frequencies (Figure 2a, 2b), and the
69 effects were statistically significant ($p = 0.016$, n = 9 for vancomycin; $p = 0.012$, n = 10 for
70 fosfomycin) (Figure 2c). SigH-expressing cells lacking the competence genes (N315ex w/o ϕ ΔcomEh ,
71 N315ex w/o ϕ ΔcomGh) generated no transformant in the presence of these antibiotics (n = 2),
72 confirming that this is due to the transformation by natural genetic competence, rather than other
73 horizontal gene transfer mechanisms¹⁰. The transformation frequency in the presence of fosfomycin
74 was highly variable depending on the experiment (Figure 2c). This variation might be due to the killing
75 effect of fosfomycin (Figure 2b).

76 Bacitracin, vancomycin, and fosfomycin, are cell wall-affecting antibiotics with distinct modes of
77 action. Bacitracin binds to certain lipid carrier to block the supply of the cell wall components¹².
78 Vancomycin (glycopeptide antibiotics) binds to the peptidoglycan precursors, UDP-N-acetylmuramyl-
79 pentapeptides, and inhibit transglycosylation reactions¹³. Fosfomycin inhibits UDP-N-
80 acetylglucosamin enolpyruvoyl transferase (MurA) that is required for the first step in bacterial cell
81 wall biosynthesis¹⁴. This is the first report on the positive effect of cell wall-affecting antibiotics on
82 natural transformation among bacteria: e.g. in *Streptococcus pneumoniae*, mitomycin C and
83 norfloxacin (quinolone) have positive effect on transformation, but the cell wall-affecting antibiotics
84 such as vancomycin and ampicillin (β -lactam) have no effect¹⁵. We think that the staphylococcal
85 response to cell wall-affecting antibiotics to induce natural transformation has an important
86 significance with respect to *S. aureus* evolution. External physical damages to the cell wall (by silica
87 beads or lysostaphin, an enzyme that cleaves *S. aureus* cell walls¹⁶) did not facilitate the transformation
88 (data not shown). This suggests that effects of cell wall-affecting antibiotics on transformation involve
89 certain complex cellular activity.

90 Low concentrations of bacitracin are often combined with other antibiotics in triple-antibiotic
91 ointments used in the treatment of soft tissue infections¹⁷. The observed hormetic effect of bacitracin
92 on transformation suggests that this antibiotic might accelerate the horizontal gene transfer in clinical
93 settings. Vancomycin remains one of the effective resources for MRSA treatment, though we already
94 have reports on vancomycin resistant *S. aureus* (VRSA)¹⁸. Fosfomycin previously selected resistant
95 staphylococci (fosfomycin resistant *S. aureus* increased in Japanese hospitals in 1980's), but it is still
96 among the choices for treatment, often by combination with other antibiotics¹⁹. We emphasize that the
97 present study potentially raises a caution regarding medical prescription in the treatment of *S. aureus*
98 considering the induction of horizontal gene transfer. The results presented in this study are limited to
99 a single SigH-expressing strain: the effect of antibiotics on transformation efficiency may vary
100 depending on growth conditions and strains, and next studies must address these points.

101

102 **Acknowledgments**

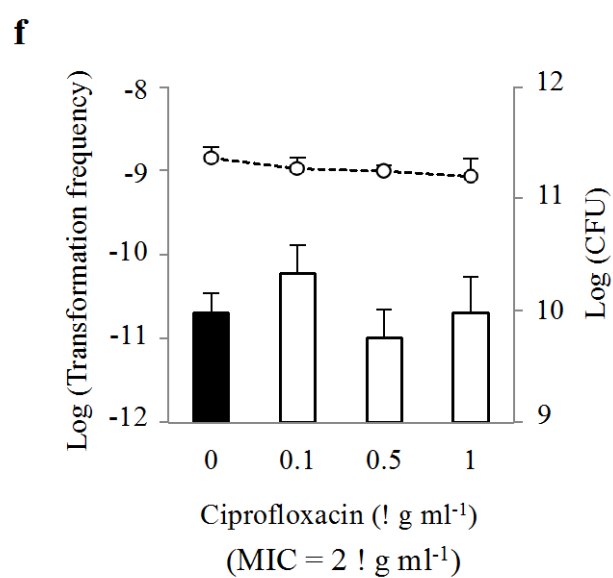
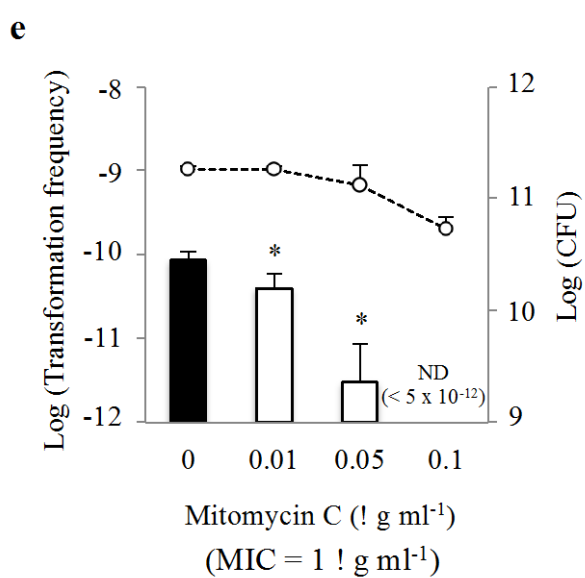
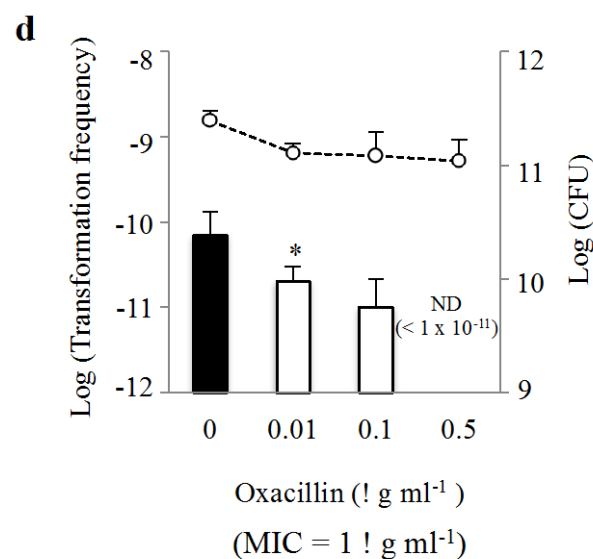
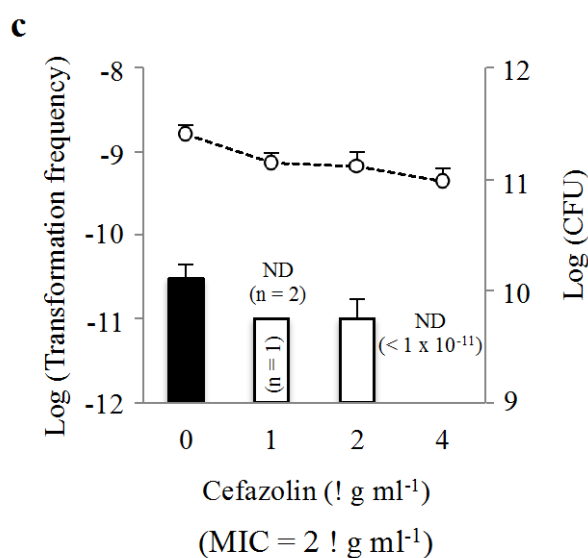
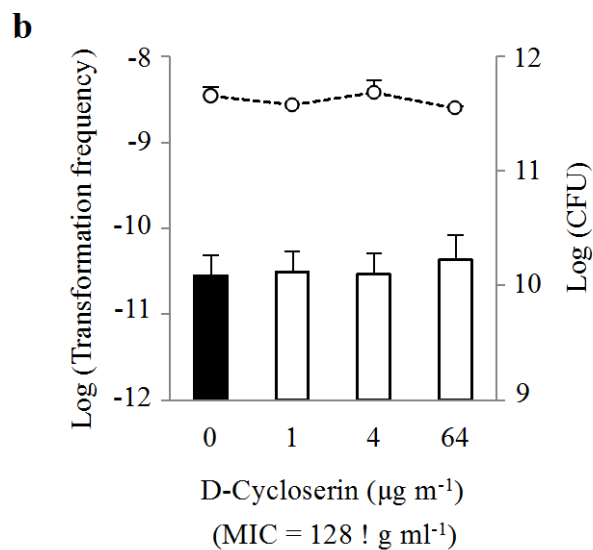
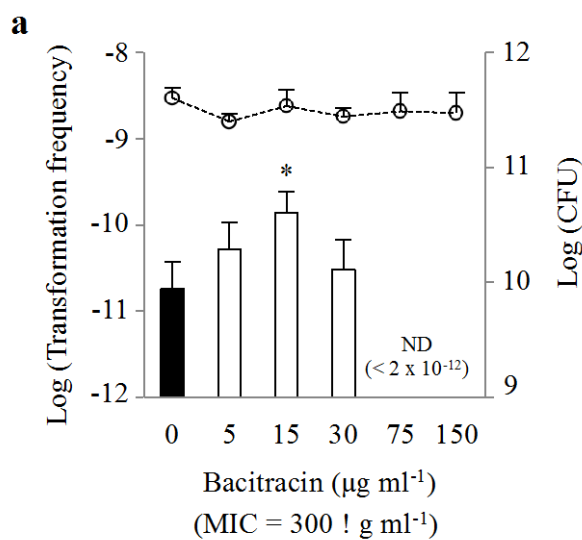
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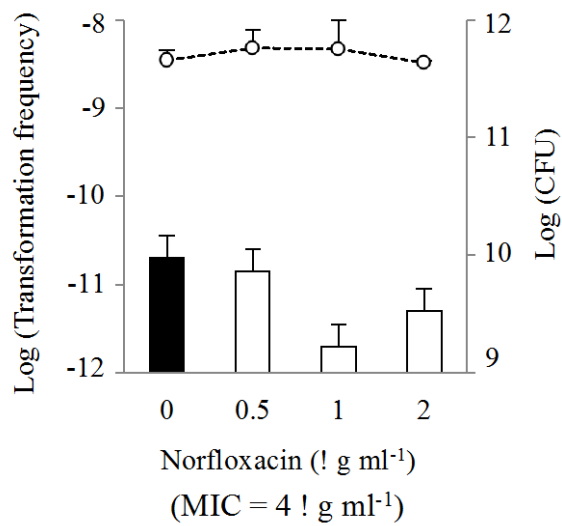
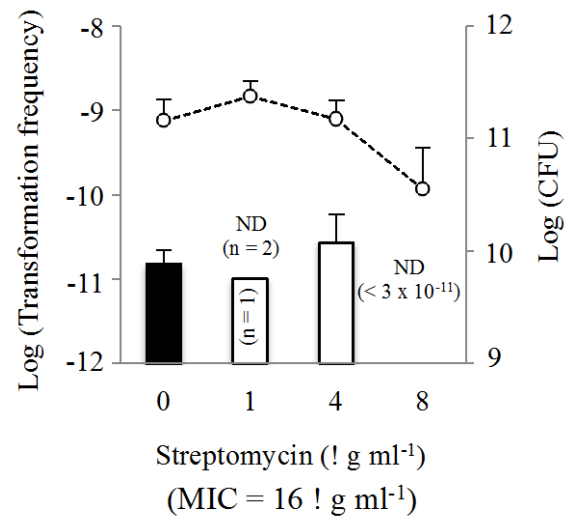
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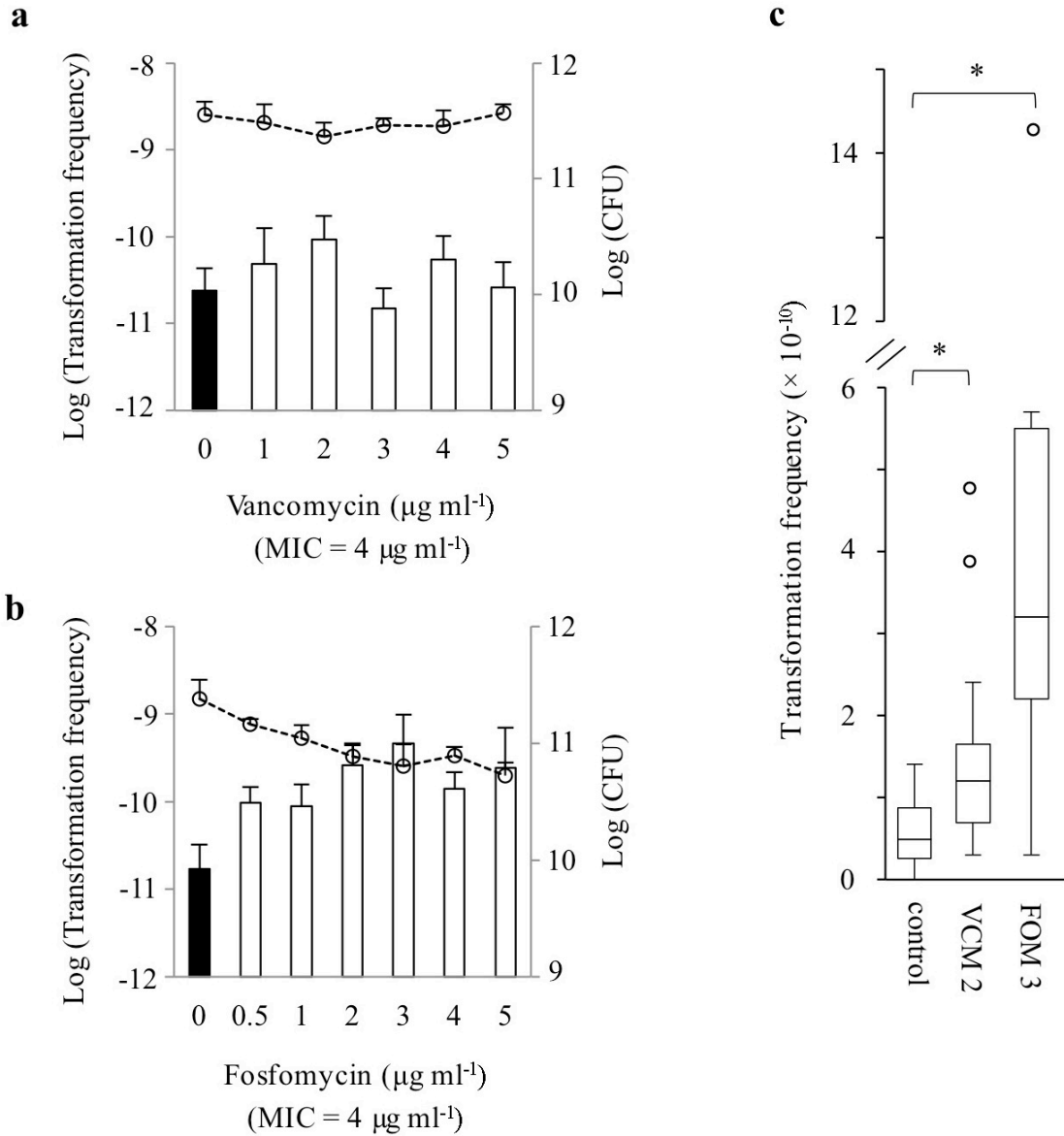
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g**h**

154 **Figure 1.** Effects of antibiotics on transformation. N315ex w/o ϕ h was exposed to different
 155 concentrations of antibiotics followed by the transformation with 10 μg of pHY300
 156 plasmid. Bars: Log_{10} (Transformation frequencies); dotted lines: Log_{10} (CFU). Average
 157 values of at least 3 independent experiments are shown with SD. ND: none detected. *:
 158 $p < 0.05$ by Student's T-test for log values of frequencies. MIC values of antibiotics were
 159 determined by microdilution method using TSB.

160



161 **Figure 2.** Effects of vancomycin (a) and fosfomycin (b) on transformation. (c) Repeated tests of the
 162 effects of $2 \mu\text{g ml}^{-1}$ vancomycin (VCM 2) and $3 \mu\text{g ml}^{-1}$ fosfomycin (FOM 3) on transformation
 163 frequencies are shown by box-plot. Boxes span the upper and lower quartile, lines inside the boxes
 164 indicate median, whiskers present the maximum and minimum values within the 1.5 interquartile range,
 165 empty circles represent data points that are outside of this range. control (no antibiotics) $n = 10$; VCM2
 166 $n = 9$; FOM3 $n = 10$; * : $p < 0.05$.
 167