PNAS

**Supporting Information for**

# Precise spatial structure impacts antimicrobial susceptibility of *S. aureus* in polymicrobial wound infection

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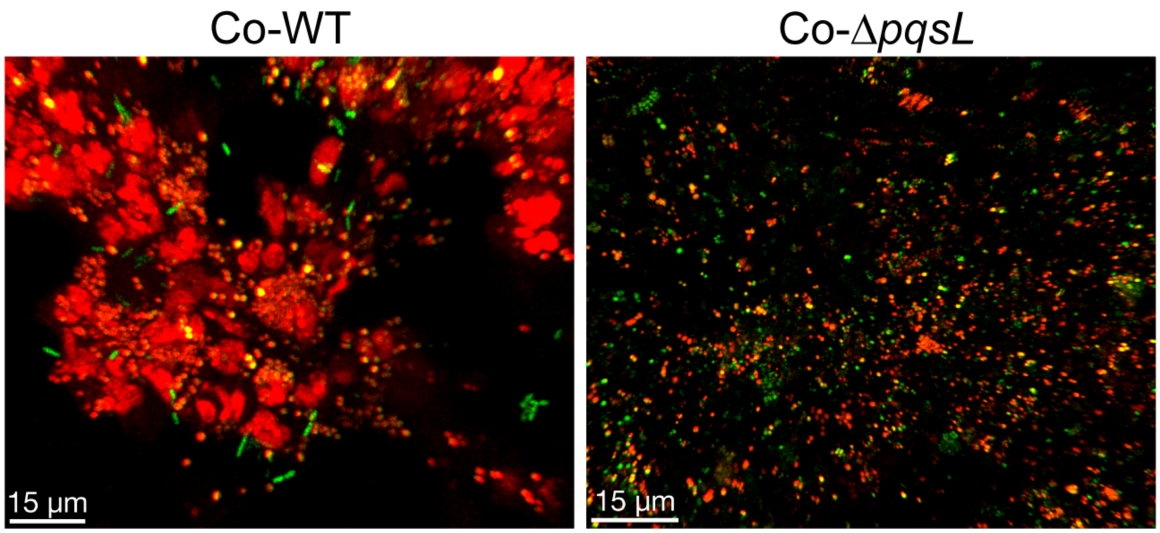



**Fig. S1. Images of co-infected murine wounds.** Confocal images of murine wounds co-infected with *S. aureus* and *P. aeruginosa* PA14 WT (Co-WT) or the isogenic *ΔpqsL* mutant (Co-*ΔpqsL*) at 4 days post-infection. *S. aureus* constitutively expresses DsRed and *P. aeruginosa* constitutively express GFP. Host cells (blue) were stained with NucBlue in the mounting medium.

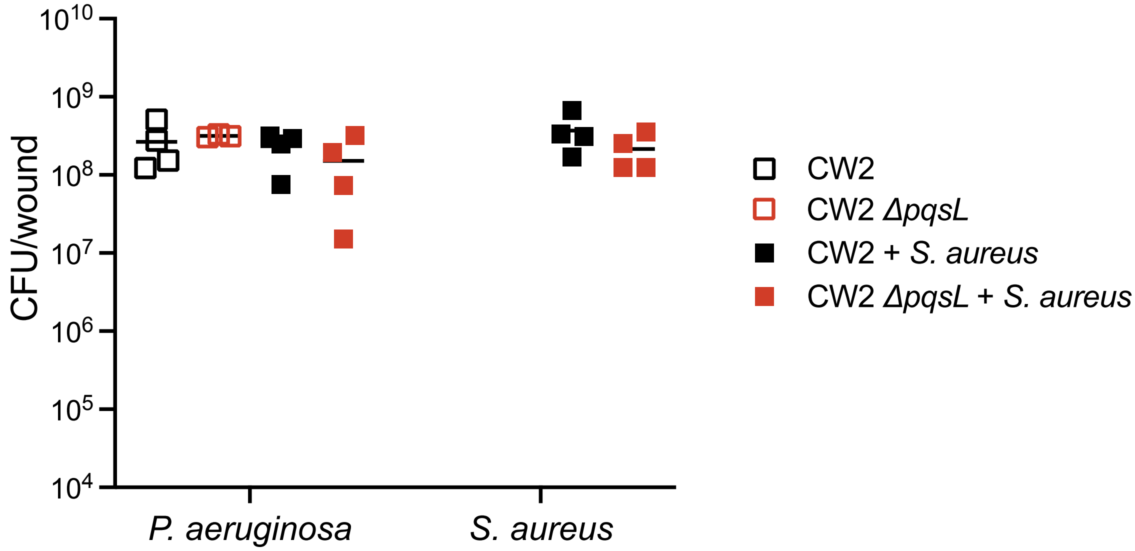
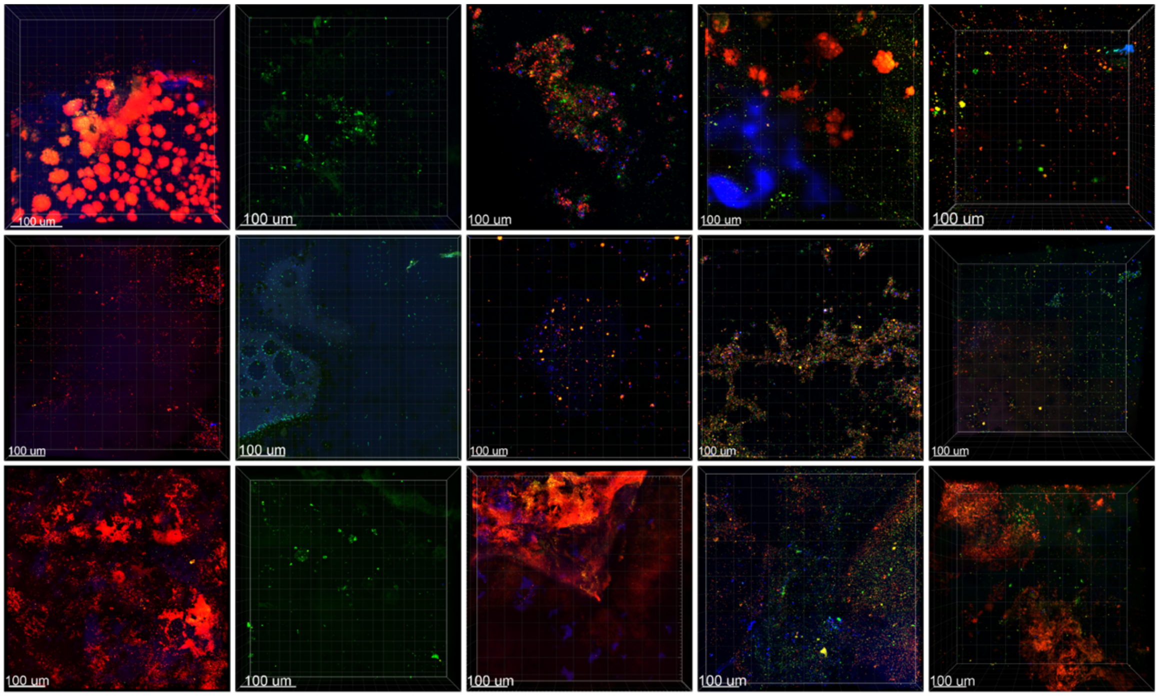



**Fig. S2. *S. aureus* and a *P. aeruginosa* wound clinical isolate co-exist in a murine surgical wound model.** Colony forming units of *P. aeruginosa* CW2-B1 or *S. aureus* in mono- (open squares) and co-infection (closed squares) with wildtype (black), or CW2-B1 *ΔpqsL* (red) in the murine wound model. Each symbol indicates a single animal infection.




*S. aureus* Mono-infection | *P. aeruginosa* Mono-infection | *S. aureus - P. aeruginosa* WT Co-infection | *S. aureus - P. aeruginosa* Δ*pqsL* Co-infection | *S. aureus - P. aeruginosa* Δ*phz1/2* Co-infection

**Fig S3. Patchy distribution of *S. aureus* and *P. aeruginosa* within wounds.** *S. aureus* (red), *P. aeruginosa* (green), and host cells (blue) are shown. Images are representative of the range of observed spatial organization.

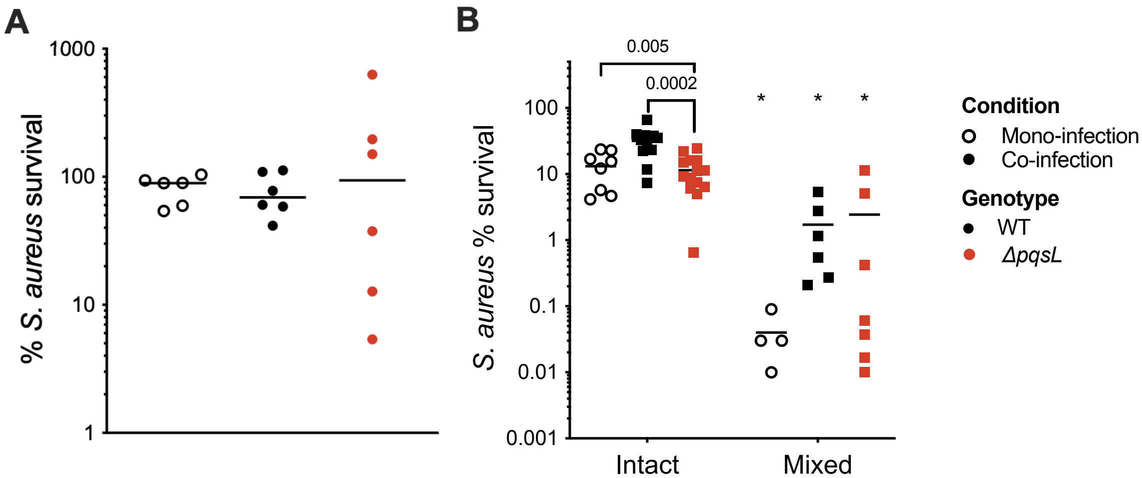



**Fig. S4. *S. aureus* vancomycin and aminoglycoside tolerance in intact and mixed wounds. A)** Percent survival of *S. aureus* to vancomycin in intact (structured) wounds compared to untreated portions of the same wound. Mono-infection is shown with open circles, co-infection with WT *P. aeruginosa* with closed circles, and co-infection with *P. aeruginosa ΔpqsL* with red circles. **B)** Percent survival of *S. aureus* to gentamicin in intact (structured) or mixed (homogenized) wounds compared to untreated portions of the same wound. Mono-infection is shown with open circles, co-infection with WT *P. aeruginosa* CW2-B1 with black squares, and co-infection with *P. aeruginosa* CW2-B1 *ΔpqsL* with red squares. All P-values were determined by a Mann-Whitney test, asterisks indicate $P < 0.01$ comparing Intact and Mixed wounds of each condition. Each symbol indicates a single animal infection.

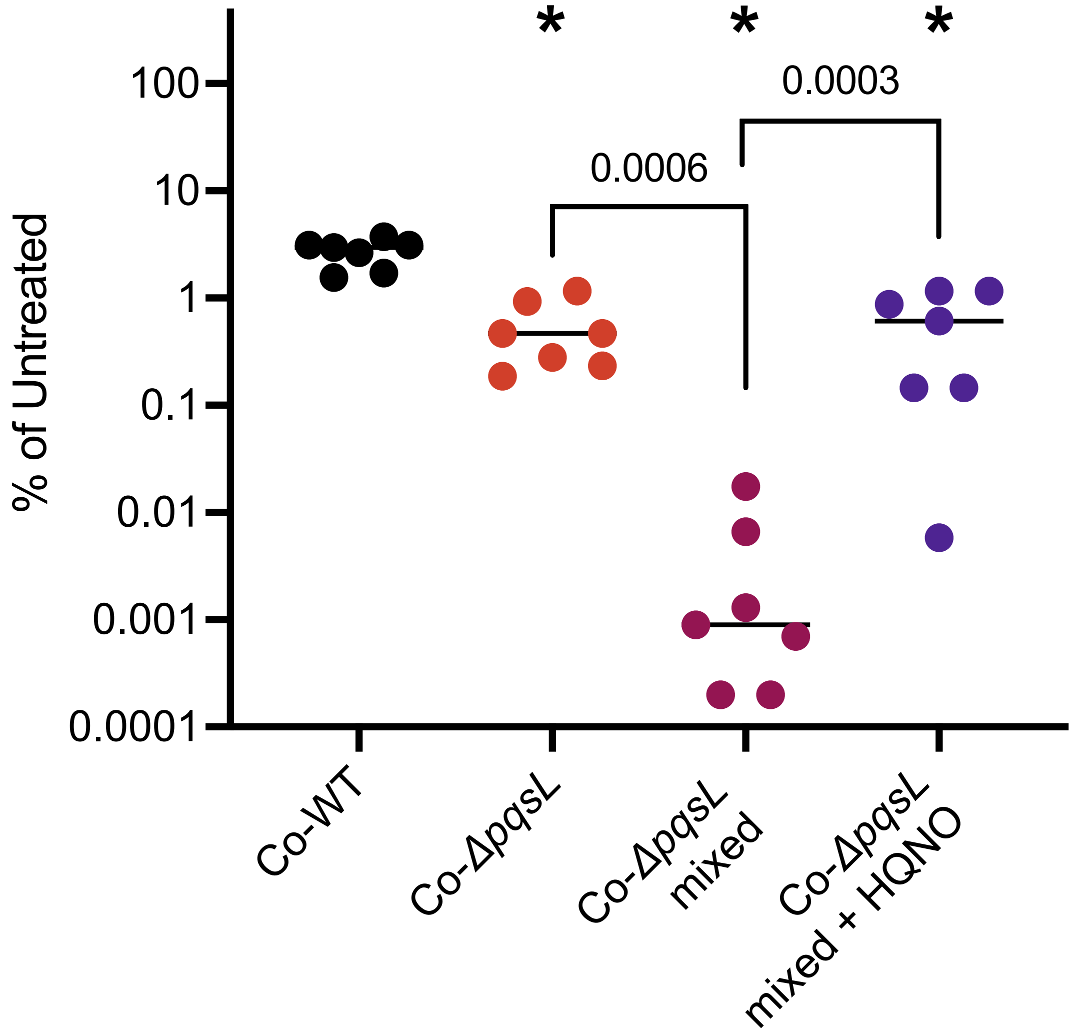



**Fig. S5. *S. aureus* tolerance to the aminoglycoside tobramycin during co-culture in an in vitro CF infection model.** Percent survival of *S. aureus* to tobramycin in vitro compared to untreated cultures. Co-infection with WT *P. aeruginosa* PA14 is shown with black circles (Co-WT), co-infection with *P. aeruginosa* PA14 *ΔpqsL* with red circles (Co-*ΔpqsL*), co-infection with *ΔpqsL* that has been vigorously mixed is shown in burgundy (Co-*ΔpqsL* mixed), and *ΔpqsL* with the addition of 5 μM HQNO prior to being vigorously mixed is shown in purple (Co-*ΔpqsL* mixed+HQNO). Statistical significance was determined with a Mann-Whitney test with a Benjamani, Krieger and Yeekutieli correction for multiple testing. Asterisks indicate an adjusted P-value ≦ 0.01 in comparison to Co-WT.

**Table S1.** Strains and plasmids used in this study.

| Strain or plasmid | Description | Identifier | Source or Reference |
|---|---|---|---|
| RN4220 + pHC48 | *S. aureus* RN4220 containing pHC48 which constitutively expresses dsRed | AH3865 | (1) |
| LAC + pHC48 | *S. aureus* LAC* (AH1263) + pHC48 which constitutively expresses dsRed | CI31 | This study |
| LAC | *S. aureus* LAC* (AH1263) community-associated methicillin resistant USA300 isolate | CI3 | (2) |
| PA14 | *P. aeruginosa* strain PA14 | CI132 | (3) |
| PA14 *ΔpqsL* | PA14 with *pqsL* deleted | CI136 | (4) |
| PA14 *Δphz1/2* | PA14 with *phz1* and *phz2* deleted | CI138 | (5) |
| PA14 Tn7::GFP | PA14 with *gfp* at Tn7 site | CI143 | This study |
| PA14 *ΔpqsL* Tn7::GFP | PA14 *ΔpqsL* with *gfp* at Tn7 site | CI157 | This study |
| PA14 *Δphz1/2* Tn7::GFP | PA14 *Δphz1/2* with *gfp* at Tn7 site | CI154 | This study |
| CW2-B1 | *P. aeruginosa* strain CW2-B1 | CI202 | (6) |
| CW2-B1 *ΔpqsL* | CW2-B1 with pqsL deleted | CI203 | This study |
| pBK-miniTn7-gfp2 | *Escherichia coli* XL1-Blue + pBK-miniTn7-gfp2, constitutive GFP in miniTn7 transposon | AKN66 | (7) |
| puxBF13 | *E. coli* S17-1 + pUXBF13, transposase plasmid to allow for integration of miniTn7 | | (7) |
| pRK2013 | *E. coli* + pRK2013, conjugative helper plasmid | | (8) |
| pEXG2pq | Plasmid for allelic replacement of *pqsL* | | (4) |

**Table S2.** Differential expression by RNA-seq of operons adjacent to the *pqsL* locus in murine chronic wounds infected with *P. aeruginosa* wildtype or the *ΔpqsL mutant*.

| Gene ID | Log2 Fold-Change | Adjusted *P*-value |
|---|---:|---:|
| *bfiS* | -0.83 | 0.88 |
| *bfiR* | -0.19 | 1.00 |
| *PA14_09710* | 0.01 | 1.00 |
| *PA14_09730* | NA | NA |
| *PA14_09740* | -2.64 | 0.16 |
| *PA14_09750* | 0.05 | 1.00 |
| *PA14_09760* | -1.32 | 0.82 |

**SI References**


1. C. B. Ibberson, C. P. Parlet, J. Kwiecinski, H. A. Crosby, D. K. Meyerholz, A. R. Horswill, Hyaluronan modulation impacts *Staphylococcus aureus* biofilm infection. *Infect. Immun*. 84, 1917-1929 (2016).
2. B. R. Boles, M. Thoendel, A. J. Roth, A. R. Horswill, Identification of genes involved in polysaccharide-independent *Staphylococcus aureus* biofilm formation. *PLoS One* 5, e10146 (2010).
3. N. T. Liberati et al., An ordered, nonredundant library of Pseudomonas aeruginosa strain PA14 transposon insertion mutants. *Proc. Natl. Acad. Sci. U.S.A*. 103, 2833–2838 (2006).
4. J. P. Barraza, M. Whiteley, A *Pseudomonas aeruginosa* antimicrobial affects the biogeography but not fitness of *Staphylococcus aureus* during coculture. *mBio*. 12, e00047-21 (2021).
5. L. E. P. Dietrich, A. Price-Whelan, A. Petersen, M. Whiteley, D. K. Newman, The phenazine pyocyanin is a terminal signalling factor in the quorum sensing network of *Pseudomonas aeruginosa*. *Mol. Microbiol*. 61, 1308–1321 (2006).
6. A. da Silva, "*Understanding the diversity and evolution of complex wound infections*" (University of Nottingham, Nottingham, UK, 2019).
7. B. Koch, L.E. Jensen, O. Nybroe, A panel of Tn7-based vectors for insertion of the *gfp* marker gene or for delivery of cloned DNA into Gram-negative bacteria at a neutral chromosomal site. *J Microbiol Methods.* 45, 187-195 (2001).
8. D. H. Figurski, D. R. Helinski, Replication of an origin-containing derivative of plasmid RK2 dependent on a plasmid function provided in trans. *Proc. Natl. Acad. Sci. U.S.A*. 76, 1648–1652 (1979).