Staphylococcal host adaptation compromises neutrophil killing by concealing bound opsonins with peptidoglycan

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Keywords: *Staphylococcus aureus* / neutrophil / antibody / peptidoglycan / opsonophagocytosis / immune evasion

Running title: Cell wall accumulation conceals opsonins

Summary

The bacterial pathogen *Staphylococcus aureus* adapts to the host environment by increasing the thickness of its cell wall. However, the impact of cell wall thickening on susceptibility to host defences is unclear. Here, we show that bacteria grown in culture medium were rapidly opsonised by antibody and complement upon incubation with human serum, resulting in efficient phagocytosis and killing by human neutrophils. However, as bacteria adapted to serum, the resulting increase in cell wall thickness led to the concealment of bound opsonins and a corresponding reduction in phagocytosis and killing by neutrophils. Partial digestion of accumulated peptidoglycan using the enzyme lysostaphin restored opsonin exposure, phagocytosis and killing, whilst the antibiotic fosfomycin blocked accumulation of peptidoglycan and maintained the susceptibility of *S. aureus* to opsonophagocytic killing by neutrophils. These findings reveal that the adaptation of *S. aureus* to the host environment significantly reduces the ability of the immune system to detect and kill this pathogen through concealment of bound opsonins via peptidoglycan accumulation.

Introduction

Staphylococcus aureus frequently infects wounds caused by surgery or insertion of intravenous access devices [1,2]. These infections can result in *S. aureus* seeding into the bloodstream, leading to bacteraemia and subsequent metastatic dissemination to sites including the heart, bones and joints [1,3,4,5]. Despite antibiotic therapy and a potent immune response, *S. aureus* infections have a high rate of relapse and frequently become chronic or recurrent [3,5].

Neutrophils are a key host defence against *S. aureus* infection and are recruited to the site of infection from the bloodstream [6,7,8,9,10,11]. The detection of *S. aureus* by neutrophils is largely dependent upon opsonisation of bacteria by bound antibody and complement, which is enabled in most people by the presence of antibodies against a range of different staphylococcal surface proteins [10,11,12,13]. The binding of neutrophils to opsonins on the surface of *S. aureus* occurs via dedicated receptors and triggers phagocytosis of the pathogen followed by the subsequent exposure of ingested bacteria to a raft of bactericidal products including reactive oxygen species, antimicrobial peptides and proteases [11,12].

To combat the threat posed by neutrophils, *S. aureus* has evolved numerous mechanisms of evading opsoninic complement and antibody [12,13,14,15]. For example, *S. aureus* produces two immunoglobulin binding proteins, Spa and Sbi, that reduce antibody-mediated opsonisation, whilst the production of proteins such as SCIN, Efb and CHIPS reduces complement deposition, activation

63 and detection by immune cells [12-24]. As such, the bacterial cell surface is a critically important
64 determinant in immune detection of *S. aureus* and efforts by the pathogen to evade surveillance and
65 killing by host defences.

66 The staphylococcal cell envelope is a dynamic structure that responds to host-induced stresses [25-
67 28]. Consequently, *S. aureus* has a thicker cell wall *in vivo* than when growing *in vitro* [29], a phenotype
68 that is replicated when staphylococci are exposed to human serum or present within endothelial or
69 osteoblast cells [30,31,32]. In the case of serum, cell wall thickening is triggered when *S. aureus* detects
70 the presence of the host defence antimicrobial peptide LL-37 via the GraRS two component system
71 [30].

72 Host-induced changes to the cell wall are important for the ability of the pathogen to cause and sustain
73 infection. Cell wall thickening has been shown to reduce susceptibility to antibiotics, whilst mutant
74 strains lacking various cell wall synthetic enzymes are less virulent in infection models [29,30,33].
75 However, it is unknown whether host-induced changes to the bacterial cell wall affect the detection
76 and killing of *S. aureus* by the host immune system. To address this, we examined the impact of host-
77 induced changes to the staphylococcal cell envelope on subsequent interactions of *S. aureus* with
78 neutrophils. This revealed that cell wall thickening constitutes a previously unrecognised mechanism
79 of immune evasion that functions by concealing bound opsonins and thereby reducing
80 opsonophagocytic killing.

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82 ## Results

83 ### Host adaptation reduces killing of *S. aureus* by neutrophils

84 To understand the impact of host adaptation on staphylococcal susceptibility to host defences, we
85 either grew bacteria to exponential phase in tryptic soy broth (TSB) to represent standard laboratory
86 conditions or allowed bacteria to adapt to host conditions using a previously described *ex vivo* human
87 serum model that triggers cell wall thickening [30] (Fig. 1A). We then measured the survival of bacteria
88 prepared under each condition during incubation with purified *ex vivo* human neutrophils from male
89 and female healthy donors in the presence of serum to provide antibody- and complement-mediated
90 opsonisation [34,35]. We examined four distinct wild type *S. aureus* strains to represent both
91 methicillin resistant (USA300, Col) and methicillin susceptible (SH1000, Newman) organisms
92 [36,37,38,39].

93 For all four of the *S. aureus* strains tested, exponential phase bacteria were efficiently killed over time,
94 with <5% of bacteria remaining viable after 2 hours incubation with neutrophils (Fig. 1B,C,D,E).

95 However, host-adapted bacteria survived at levels 5-10 times greater than that seen for exponential
96 bacteria for all strains (Fig. 1B,C,D,E). In addition to demonstrating that host adaptation reduces
97 staphylococcal susceptibility to host immune defences, the high level of consistency observed across
98 all four strains indicated that this is a conserved phenotype.

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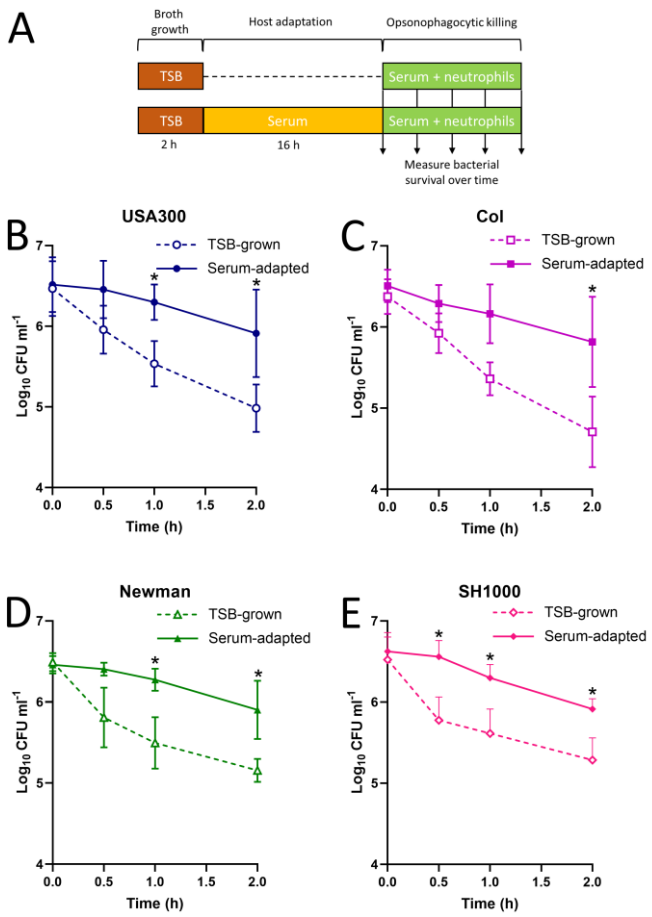

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102 **Figure 1. Incubation in human serum promotes tolerance to killing by neutrophils.** Bacteria were
103 grown in broth or allowed to adapt to human serum before incubation with human neutrophils in the
104 presence of serum and staphylococcal survival measured over time (**A**). Survival of TSB-grown and
105 serum-adapted cultures of *S. aureus* USA300 (**B**), Col (**C**), Newman (**D**) and SH1000 (**E**) during a 2 h
106 incubation with purified human neutrophils. Data represent the geometric mean ± geometric standard
107 deviation of at least three independent biological replicates. Data were analysed by a two-way ANOVA
108 with Sidak's *post-hoc* test (*, $P < 0.05$; Serum-adapted vs TSB-grown at indicated time-points).
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112 **Host adaptation reduces opsonin exposure and opsonophagocytosis**

113 Having found that host adaptation reduced staphylococcal susceptibility to host defences, we next
114 determined the mechanism(s) responsible. Given the consistency in survival data across all four strains
115 examined, we focussed on the USA300 lineage since it is both well characterised and clinically
116 important.

117 We started by assessing whether the increase in survival of host-adapted bacteria was due to impaired
118 phagocytosis, using two distinct assays. Bacteria were grown in broth or host-adapted by incubation
119 in serum, before washing in PBS and then incubation with neutrophils in the presence of fresh serum
120 to enable opsonisation (Fig. 2A). The first phagocytosis assay was a well-established flow cytometry-
121 based approach that determined how many fluorescently-labelled bacteria were associated (or not)
122 with neutrophils [35,40]. This revealed that the majority of both broth-grown and host-adapted *S.*
123 *aureus* were phagocytosed after 30 min incubation with neutrophils. However, whilst <3% of broth-
124 grown bacteria remained unphagocytosed, >20% of host-adapted bacteria were free from neutrophils
125 (Fig. 2B). This finding was replicated in a second phagocytosis assay that measured the viability of free
126 and neutrophil-associated bacteria [41], with >10% of host-adapted *S. aureus* unphagocytosed
127 compared with <1% of broth-grown *S. aureus* cells (Fig. 2C). Combined, these two assays
128 demonstrated that host-adapted *S. aureus* were significantly better at evading phagocytosis than
129 broth-grown bacteria.

130 An additional finding was that there were equal numbers of viable host-adapted and broth-grown *S.*
131 *aureus* cells associated with neutrophils. This indicated that host-adapted *S. aureus* were as
132 susceptible to the microbicides produced by the neutrophils as broth-grown cells (Fig. 2C). Therefore,
133 we concluded that the enhanced survival of host-adapted bacteria compared with broth-grown
134 bacteria (Fig. 1) was due to enhanced evasion of phagocytosis, rather than resistance to the
135 antibacterial products of neutrophils.

136 To understand why host-adapted *S. aureus* were phagocytosed much less efficiently than broth-grown
137 cells, we examined the degree of opsonisation of bacteria by antibody and complement using western
138 blotting. In keeping with previous work [42,43], for this experiment we used a mutant strain of USA300
139 lacking Spa and Sbi to avoid interference caused by these immunoglobulin binding proteins. Broth-
140 grown or host-adapted bacteria were washed in PBS and then incubated, or not, in fresh serum to
141 enable opsonin binding as used in the opsonophagocytosis assays described above (Fig. 1A) before
142 detection of bound antibody and complement component C3 (Fig. 2D).

143 Despite their reduced phagocytosis by neutrophils, there was more antibody and complement bound
144 to host-adapted cells than to broth-grown, suggesting that a lack of bound opsonins did not explain
145 the immune evasion phenotype of host-adapted bacteria (Supplementary Fig. S1).

146 To understand why host-adapted cells had high levels of bound antibody and complement but low
147 levels of phagocytosis, bacteria were prepared as described above for opsonophagocytosis assays and
148 then the levels of surface-exposed antibody and the complement component C3 quantified using flow
149 cytometry (Fig. 2D). Broth-grown bacteria that had been exposed to PBS instead of serum acted as a
150 negative control and confirmed that antibodies used in the assay did not bind non-specifically to *S.*
151 *aureus* cells (Fig. 2D,E,F). We then showed that, as expected, broth-grown bacteria that were
152 incubated in human serum for 30 min were very strongly bound by both IgG and the complement
153 component C3 (Fig. 2E,F).

154 Next, we examined host adapted bacteria and found that they had a very low level of exposed
155 opsonins, regardless of whether they had been incubated in fresh serum for 30 min or not (Fig. 2E,F).
156 Therefore, despite prolonged incubation in serum and high levels of bound antibody and complement
157 (supplementary Fig. S1), host-adapted cells had significantly reduced exposure of opsonins on their
158 cell surface relative to broth-grown bacteria that had been opsonised.

159 A further conclusion from these findings was that host adapted bacteria did not have any immuno-
160 reactive epitopes on their cell surface, since the second incubation in fresh serum did not enhance
161 opsonin exposure above that seen for the PBS control (Fig. 2E,F).

162 Taken together, these experiments revealed that host-adapted bacteria are better able to survive
163 exposure to neutrophils than broth-grown *S. aureus* because they are less likely to be phagocytosed,
164 apparently due to weak surface exposure of bound IgG and complement.

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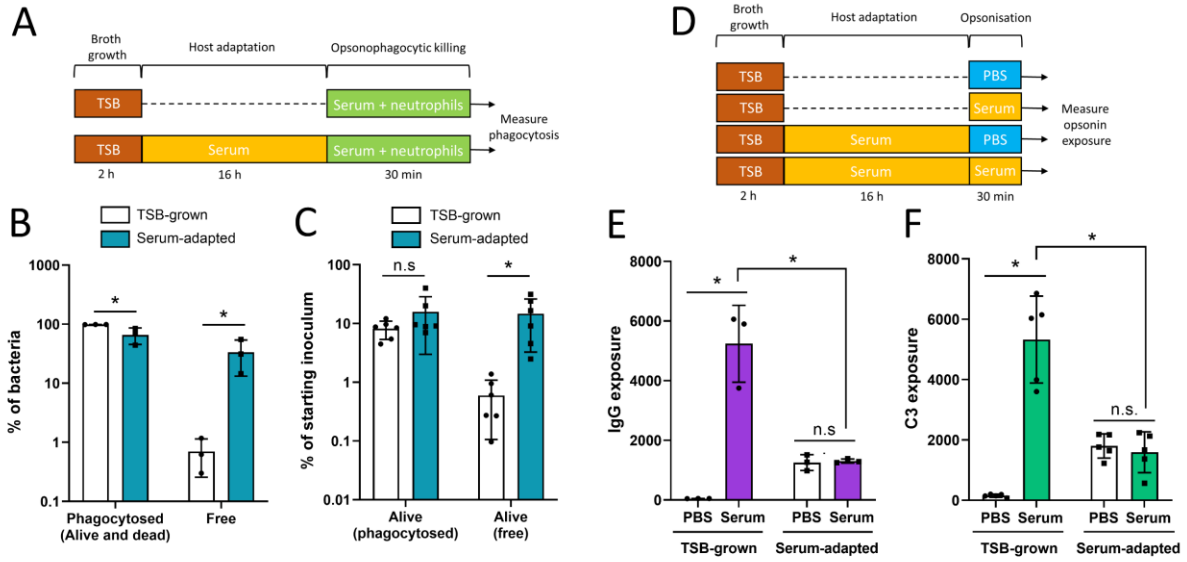

Figure 2. Incubation in human serum reduces opsonophagocytosis and surface-exposed IgG and complement. TSB-grown and serum-adapted cultures of *S. aureus* USA300 were incubated with purified human neutrophils for 30 min (**A**) before the percentage of neutrophil-associated (phagocytosed) and non-neutrophil associated (free) bacteria were determined by (**B**) flow cytometry and (**C**) CFU counts. TSB-grown and serum-adapted *S. aureus* cells were incubated for 30 min with PBS or 10 % serum (**D**) before the levels of surface-exposed bound (**E**) IgG and (**F**) C3 were determined by flow cytometry. Data in **B** and **C** show values for each independent experiment, with bars representing the mean ± standard deviation. In **E** and **F**, each biological repeat is represented by the median fluorescence value of 10,000 bacterial cells. Data in **B, C, E, F** were analysed by two-way ANOVA with Sidak's *post-hoc* test (*, $P < 0.05$; n.s, $P \geq 0.05$ for the indicated comparisons).

Cell wall accumulation impairs opsonophagocytosis by concealing bound opsonins

Since *S. aureus* accumulates peptidoglycan during incubation in serum, we tested whether this concealed bound antibody and complement. To do this, host-adapted bacteria were incubated with a range of concentrations of the enzyme lysostaphin, which cleaves peptidoglycan. This resulted in a dose-dependent increase in exposure of bound IgG and complement (Fig. 3A,B), and a corresponding increase in phagocytosis of host-adapted *S. aureus* by neutrophils (Fig. 3C,D). Furthermore, lysostaphin treatment of host-adapted *S. aureus* rendered them as susceptible to neutrophil-mediated killing as broth-grown bacteria (Fig. 3E).

Combined these findings indicated that peptidoglycan accumulation was responsible for the concealment of bound opsonins (Fig. 3F). However, it did not determine whether peptidoglycan-bound proteins or WTA were also required. We ruled out a role for peptidoglycan-bound proteins by

198 showing that host-adapted mutants defective for SrtA or SrtB, which cannot anchor proteins to
199 peptidoglycan, survived incubation with neutrophils as well as host-adapted wild type bacteria
200 (Supplementary Fig. S2A,B). We also showed that serum-induced accumulation of WTA did not
201 contribute to immune evasion using tunicamycin to block the synthesis of this polymer [30]
202 (Supplementary Fig. S2C).

203 Taken together, the experiments described here demonstrate that host-adapted *S. aureus* cells are
204 bound by high levels of antibody and complement but the accumulation of cell wall peptidoglycan
205 conceals these bound opsonins, enabling bacterial evasion of phagocytosis and killing by neutrophils.

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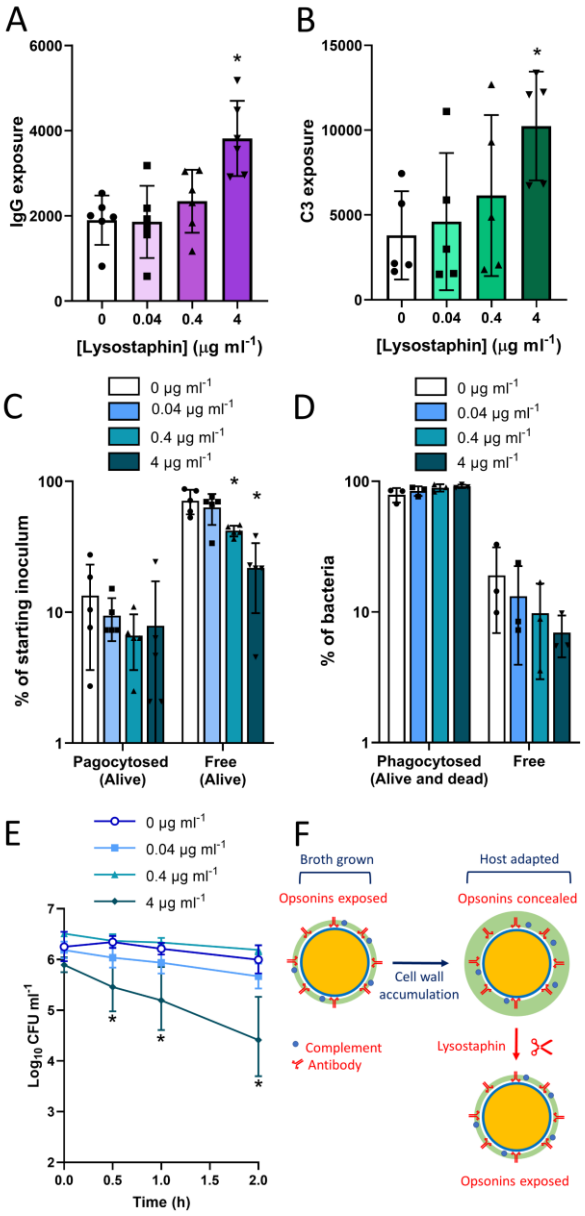

Figure 3. Cell wall accumulation impairs opsonophagocytosis by concealing bound opsonins. Host-adapted cultures of *S. aureus* USA300 were treated with indicated concentrations of lysostaphin for 20 min to partially digest the cell wall before levels of surface-exposed (**A**) IgG and (**B**) complement component C3 were determined by flow cytometry. Additionally, these cells were incubated with purified human neutrophils for 30 min before the percentage of neutrophil-associated (phagocytosed) and non-neutrophil associated (free) bacteria were determined by (**C**) CFU counts and (**D**) flow cytometry. (**E**) Survival of lysostaphin-treated cells during a 3 h incubation with purified human neutrophils. Summary diagram of the effect of lysostaphin on host-adapted bacteria, with lysostaphin-mediated degradation of the cell wall exposing bound opsonins that had been obscured by accumulated peptidoglycan (**F**).

Data in **A** – **D** represent the mean ± standard deviation of the indicated number of independent biological replicates. Data in **E** represent the geometric mean ± geometric standard deviation of 4 independent experiments. In **A** and **B**, each biological repeat is represented by the median fluorescence value of 10,000 bacterial events. Data in **A** and **B** were analysed by one-way ANOVA with Dunnett's *post-hoc* test. Data in **C** – **E** were analysed by two-way ANOVA with Dunnett's *post-hoc* test (*, $P \leq 0.05$; Lysostaphin-treated vs non-lysostaphin treated).

242 **Antibiotic-mediated inhibition of cell wall accumulation maintains opsonin exposure and efficient**
243 **opsonophagocytosis**

244 To further test whether host adaptation reduced phagocytosis via peptidoglycan-mediated
245 concealment of bound opsonins, and to explore potential therapeutic approaches to enhance
246 neutrophil-mediated killing, we carried out host adaptation in the presence of a sub-lethal
247 concentration of the antibiotic fosfomycin. This antibiotic inhibits peptidoglycan synthesis and
248 prevents cell wall thickening from occurring and has been used clinically in anti-staphylococcal
249 combination therapies [30,44,45].

250 As above, the adaptation of bacteria to serum resulted in a significant reduction in opsonisation, as
251 determined by exposure of IgG and complement, relative to broth-grown bacteria (Fig. 4A,B).
252 However, the presence of fosfomycin in serum significantly reduced opsonin concealment,
253 maintaining IgG and complement exposure at similar levels to that seen for broth-grown bacteria (Fig.
254 4A,B). In keeping with this, bacteria adapted to serum in the presence of fosfomycin were
255 phagocytosed as efficiently as broth-grown bacteria, as determined by both phagocytosis assays (Fig.
256 4C,D). Furthermore, bacteria that were adapted in serum with fosfomycin were killed by neutrophils
257 as efficiently as broth-grown bacteria, whereas host-adapted bacteria not exposed to fosfomycin
258 survived at significantly higher levels (Fig. 4E).

259 Taken together, these findings provided additional evidence that cell wall accumulation conceals
260 bound opsonins, which in turn compromises phagocytosis and killing by neutrophils. They also indicate
261 that the antibiotic fosfomycin, in addition to its own antibacterial activity, may aid the clearance of
262 infection by preventing concealment of opsonins.

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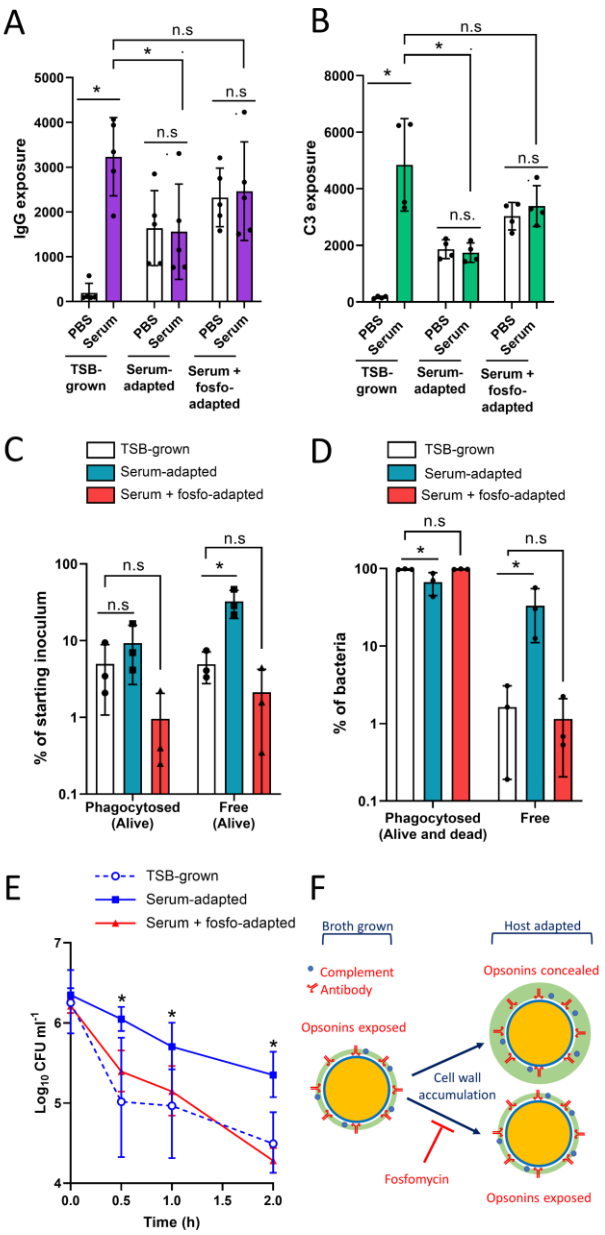

Figure 4. Antibiotic-mediated inhibition of cell wall accumulation maintains opsonin exposure and efficient opsonophagocytosis. TSB-grown, serum-adapted and serum + fosfomycin-adapted cultures of *S. aureus* USA300 were incubated for 30 min with PBS or 10 % serum before the levels of surface-exposed (**A**) IgG and (**B**) complement component C3 were determined by flow cytometry. Additionally, these cells were incubated with purified human neutrophils for 30 min before the percentage of neutrophil-associated (phagocytosed) and non-neutrophil associated (free) bacteria were determined by (**C**) CFU counts and (**D**) flow cytometry. (**E**) Survival of TSB-grown, serum-adapted and serum + fosfomycin-adapted cultures during a 2 h incubation with purified human neutrophils. Summary diagram of the effect of fosfomycin on opsonin exposure during host-adaptation, with antibiotic-mediated inhibition of peptidoglycan accumulation preserving the exposure of bound antibody and complement (**F**). Data in **A** – **D** represent the geometric mean ± geometric standard deviation of the

284 indicated number of independent biological replicates. Data in **E** are presented as the geometric mean
285 ± geometric standard deviation of three independent experiments. In **A** and **B**, each biological repeat
286 is represented by the median fluorescence value of 10,000 bacterial events. Data in **A** and **B** were
287 analysed by two-way ANOVA with Tukey's *post-hoc* test. Data in **C** – **E** were analysed by two-way
288 ANOVA with Dunnett's *post-hoc* test (*, $P < 0.05$; n.s, $P \geq 0.05$; comparisons are indicated in **A** – **D** and
289 serum/serum + fosfomycin-adapted vs TSB-grown at each time-point in **E**).

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291

292 **Discussion**

293 The binding of antibody and complement to the bacterial cell surface enables the detection and
294 destruction of pathogens by phagocytic immune cells [10,11,13]. The data presented here
295 demonstrate that *S. aureus* can conceal bound opsonins via cell wall accumulation, significantly
296 reducing phagocytosis and killing by neutrophils. Crucially, once bound opsonins were concealed, it
297 was not possible to restore full opsonisation with fresh serum, demonstrating that host-adapted cells
298 with thickened cell walls have reduced exposure of immune-reactive epitopes.

299 Previous work has indicated that the thickened cell wall associated with vancomycin resistance
300 reduces staphylococcal susceptibility to intracellular killing by neutrophils [46]. However, our data did
301 not show a difference in staphylococcal survival within neutrophils, with similar numbers of
302 intracellular viable broth-grown and host-adapted bacteria. Instead, the survival advantage of host
303 adaptation appeared to be entirely due to enhanced evasion of phagocytosis.

304 We do not yet know if these findings are applicable to other Gram-positive pathogens. However, since
305 previous work has shown that serum triggers cell wall thickening in *Enterococcus faecalis* and viridans
306 group streptococci, it is possible that our findings with *S. aureus* represent a broadly conserved
307 mechanism of immune evasion [47].

308 Cell wall thickening in *S. aureus* is triggered by bacterial sensing of the host defence antimicrobial
309 peptide LL-37 via the GraRS system [30]. Since LL-37 is present in most tissues and amongst the earliest
310 host responses to infection or trauma [49,50], we hypothesise that *S. aureus* has evolved to sense this
311 AMP as an early indicator that it is subject to immune attack and provides an opportunity to employ
312 defensive measures against the impending arrival of neutrophils (Supplementary figure S3). In support
313 of this hypothesis, GraRS, the two-component system that detects LL-37, is activated in the early
314 stages of staphylococcal skin colonisation, whilst *S. aureus* mutants lacking GraRS are significantly less
315 virulent than wild type strains in invasive infection models [51,52,53].

316 In addition to providing protection against opsonophagocytosis, LL-37 exposure triggers reduced
317 susceptibility to the antibiotics daptomycin and vancomycin [30,54], suggesting *S. aureus* employs
318 strategies that are broadly protective against the twin threats of host immunity and antibiotic therapy.
319 This is similar to our previous work showing that induction of the *S. aureus* general stress response
320 regulated by the alternative sigma factor SigB can promote survival of bacteria exposed to host
321 defences and various classes of antibiotics [55]. Further support for the link between *S. aureus*-
322 immune interactions and antibiotic tolerance comes from studies showing that oxidative stress
323 conferred by phagocytic cells reduce staphylococcal susceptibility to antibiotics [56,57].

324 Whilst the immune response may compromise the efficacy of antibiotic therapy under certain
325 circumstances, our study also highlights how antibiotics and the immune response can work
326 synergistically by showing that fosfomycin blocked LL-37 induced cell wall thickening and thereby
327 maintained exposure of bound opsonins, leading to efficient opsonophagocytic killing. Additionally,
328 previous work has suggested that fosfomycin also promotes killing of *S. aureus* via enhanced
329 production of the neutrophil oxidative burst [58]. However, whilst we exposed *S. aureus* to fosfomycin
330 in serum, this was removed by washing prior to incubation with neutrophils and thus does not explain
331 the enhanced killing effect observed in our assays. This strongly suggests that there are at least two
332 mechanisms by which fosfomycin and neutrophils synergise against *S. aureus* and a greater
333 understanding of this may contribute to more effective therapeutic approaches that reduce the high
334 incidence of relapsing or chronic staphylococcal infections [59].

335 In summary, we show that *S. aureus* cells are heavily opsonised upon initial exposure to serum.
336 However, *S. aureus* responds to serum by accumulating peptidoglycan, which conceals bound
337 opsonins, reducing phagocytosis and killing by neutrophils.

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341 Materials and methods

342 Bacterial strains and growth conditions

343 Bacterial strains used in this study are shown in Table 1. Strains were grown at 37 °C on tryptic soy
344 agar (TSA) or in tryptic soy broth (TSB) with shaking (180 r.p.m.) supplemented with erythromycin (10
345 µg ml^{-1}) or kanamycin (90 µg ml^{-1}) when required.

Strain	Relevant characteristics	Reference/source
USA300 JE2	LAC strain of the USA300 lineage of community acquired MRSA cured of plasmids	36
JE2 *sbi*::Tn *spa*::*kan*	JE2 strain defective for immunoglobulin-binding proteins Spa and Sbi	This study
JE2 *srtA*::Tn	JE2 strain defective for sortase A, which anchors LPxTG motif-containing proteins to peptidoglycan	60
JE2 *srtB*::Tn	JE2 strain defective for sortase B, which anchors NPQTN motif-containing IsdC protein to peptidoglycan	60
SH1000	*rsbU*+ derivative of laboratory strain 8325-4	37
Col	Early hospital-associated MRSA isolate, commonly used in laboratory experiments	38
Newman	Clinical isolate, methicillin susceptible, used widely in animal and laboratory experiments	39

346

347 **Table 1.** Strains used in this study.

348

349 **Construction of strains**

350 The JE2 *sbi*::Tn/*spa*::*kan* double mutant was constructed via transduction of the kanamycin resistance
351 marker from Newman *spa*::*kan* [61] into the *sbi*::Tn mutant present in the NARSA transposon mutant
352 library [60] using ϕ11.

353 **Generation of broth-grown and host-adapted bacterial cultures**

354 To generate broth-grown bacteria, cultures were grown for 16 h in TSB to stationary phase. These
355 were then diluted to 10^7 CFU ml^{-1} in fresh TSB and incubated for 2 h at 37 °C until 10^8 CFU ml^{-1} was
356 reached.

357 To generate host-adapted bacteria, broth-grown cultures were centrifuged (3,200 x *g* for 10 min),
358 resuspended in an equal volume of human serum from human male AB plasma (Sigma) and incubated
359 for 16 h at 37 °C. As *S. aureus* is unable to replicate in human serum, these cultures were also at 10^8
360 CFU ml^{-1}. Where appropriate, serum was supplemented with a sub-lethal concentration of fosfomycin
361 (8 µg ml^{-1}) or tunicamycin (128 µg ml^{-1}).

362 Where appropriate, the cell walls of serum-adapted cultures were degraded by lysostaphin. To do this,
363 1 ml aliquots of serum-adapted bacteria were washed in PBS and resuspended in 1 ml PBS
364 supplemented with indicated concentrations of lysostaphin (between 0.04 and 4 µg ml^{-1}). Bacteria
365 were incubated statically for 20 min at 37 °C before being washed by 3 rounds of centrifugation in
366 PBS.

367 **Purification of neutrophils**

368 Ethical approval for drawing and using human blood was obtained from the Regional Ethics Committee
369 and the Imperial NHS Trust Tissue Bank (REC Wales approval no. 12/WA/0196 and ICHTB HTA license
370 no. 12275). Neutrophils were extracted from 45 ml human blood, collected in heparin tubes to prevent
371 coagulation. Blood (15 ml) was carefully layered over 30 °C PolymorphPrep (20 ml) and centrifuged
372 for 1 h at 500 x *g* to separate the different cell types. Neutrophils were collected, washed with Hanks
373 balanced salt solution (HBSS) and adjusted to 5 x 10^6 cells ml^{-1} in HBSS.

374 **Determination of bacterial killing by neutrophils and phagocytosis by CFU counts**

375 Neutrophils were adjusted to 5 x 10^6 cells ml^{-1} in HBSS supplemented with 10 % human serum, 0.1
376 mM $CaCl_2$ and 0.1 mM $MgCl_2$. In the case of lysostaphin-treated bacteria, 10 % serum was omitted
377 from the HBSS.

378 TSB-grown/host-adapted bacteria were generated as described above, washed three times in PBS and
379 then added to neutrophils at 5 x 10^6 CFU ml^{-1}. Tubes were incubated with end-over-end mixing at 37°
380 C for 3 h and at each time-point (0, 0.5, 1 and 2 h) aliquots were removed, serially diluted 10-fold in
381 PBS and plated to enumerate CFU ml^{-1}.

382 In addition, the number of phagocytosed/unphagocytosed bacteria was also enumerated at the 0.5 h
383 time-point. A 500 µl aliquot of the neutrophil/bacteria mixture was taken and centrifuged at 500 x *g*
384 for 1 min to pellet the neutrophils, along with any neutrophil-associated bacteria. The supernatant
385 (containing unphagocytosed bacteria) was serially diluted 10-fold in PBS and plated for CFU counts
386 and the pellet was resuspended in 500 µl PBS, serially diluted 10-fold in PBS and plated for CFU counts.
387 The CFU ml^{-1} values in the pellet and the supernatant were divided by the CFU ml^{-1} of the starting
388 inoculum to generate the percentage of CFU ml^{-1} phagocytosed and unphagocytosed, respectively.

389 **Measurement of phagocytosis by flow cytometry**

390 To measure phagocytosis by flow cytometry neutrophils and bacteria were prepared as described
391 above except that immediately before bacteria were added to the neutrophils the bacteria were

392 incubated with 10 µg ml^{-1} fluorescein isothiocyanate for 30 min at room temperature and then washed
393 three times in PBS.

394 As above, 5 x 10^6 CFU ml^{-1} bacteria were added to 5 x 10^6 cells ml^{-1} in HBSS supplemented with 10 %
395 human serum, 0.1 mM $CaCl_2$ and 0.1 mM $MgCl_2$. In the case of lysostaphin-treated bacteria, 10 %
396 serum was omitted from the HBSS. After a 30 min incubation at 37˚C with end-over-end mixing in the
397 dark, cultures were fixed by the addition of an equal volume of 4 % paraformaldehyde (PFA). Samples
398 were then analysed by flow cytometry using an Amnis CellStream. Bacteria were detected using the
399 488 nm laser and at least 10,000 bacterial events were recorded. Events with FITC ≥ 2 x 10^3 were
400 counted as bacteria. Events with an FCS of ≥ 3000 were counted as phagocytosed and ≤ 3000 were
401 counted as unphagocytosed/free.

402 **Measurement of IgG and complement surface exposure by flow cytometry**

403 TSB-grown and serum-adapted cultures were prepared as described above. A *spa/sbi* double mutant
404 was used to prevent non-specific antibody binding. Aliquots (500 µl) were incubated for 30 min at
405 room temperature in either PBS or 10 % human serum. Samples were washed by three rounds of
406 centrifugation in PBS (13,000 x *g* for 1 min) and blocked for 1 h in 4 % BSA in PBS. Samples were washed
407 once in PBS before IgG was detected with a 1:1000 dilution of goat anti-human IgG antibody labelled
408 with the BV421 fluorophore (Jackson ImmunoResearch) or C3 was detected with a 1:1000 dilution of
409 goat anti-human C3 $F(ab')_2$ labelled with FITC (Protos Immunoresearch). Antibody incubations were
410 carried out statically for 1 h at room temperature in the dark. Samples were washed with PBS by three
411 rounds of centrifugation (13,000 x *g* for 1 min) and fixed in 4 % PFA. Samples were analysed by flow
412 cytometry using an Amnis CellStream. IgG was detected using the 405 nm laser and C3 using the 488
413 nm laser. At least 10,000 bacterial events were recorded and the median value recorded.

414 **Measurement of IgG and complement by western blotting**

415 Cultures of TSB-grown and serum-adapted bacteria (1 ml at 10^8 CFU ml^{-1}) were prepared as described
416 above, washed by three rounds of centrifugation in PBS (13,000 x *g* for 1 min) and resuspended in 100
417 µl PBS. A *spa/sbi* double mutant was used to prevent non-specific antibody binding. Lysostaphin (10
418 µg ml^{-1}) was added and bacteria incubated statically for 1 h at 37˚C. Sample buffer (187.5 mM Tris-
419 HCl (pH 6.8), 6 % SDS, 30 % glycerol, 0.03 % bromophenol blue and 15 % beta-mercaptoethanol; 50
420 µl) was added and samples were incubated at 95˚C for 10 min before 15 µl was loaded onto 10 %
421 polyacrylamide gels. Gels were run in Tris-Glycine running buffer (25 mM Tris, 192 mM glycine, 0.1 %
422 SDS, pH 8.4) at 100 V for 10 min followed by 200 V for 50 min before being transferred onto PVDF
423 membranes (10 V for 60 min). Membranes were blocked for 1 h at room temperature in 5 % milk and

424 1 % BSA in TBST. IgG was detected using 1:10000 dilution of donkey anti-human IgG conjugated to
425 HRP (Abcam) and C3 was detected by 1:5000 dilution of rabbit anti-C3 (Abcam) followed by 1:10000
426 dilution of goat anti-rabbit IgG conjugated to HRP (Abcam). Blots were developed using SuperSignal
427 West Pico PLUS chemiluminescent substrate (Thermo scientific) and imaged using the Bio-rad
428 ChemiDoc MP imaging system.

429 **Statistical analyses**

430 CFU counts were log10 transformed and displayed as the geometric mean ± geometric standard
431 deviations [62]. Other data are displayed as the mean ± standard deviation. For all experiments, three
432 or more independent replicates were performed as indicated by individual data points. Data were
433 analysed by one-way ANOVA or two-way ANOVA with appropriate post-hoc multiple comparison test
434 as detailed in figure legends using GraphPad Prism (V8.0).

435

436 Acknowledgements

437 We thank the blood donors, without whom this study would not have been possible. Joan Geoghegan
438 (University of Birmingham) and Angelika Grundling (Imperial College London) are thanked for
439 providing strains. EVKL was supported by a Wellcome Trust PhD Studentship (203812/Z/16/Z). We
440 acknowledge the technical support and the use of equipment from the SAFB Flow Cytometry Facility
441 (Imperial College London). AME acknowledges funding from the Rosetrees Trust and from the Imperial
442 NIHR Biomedical Research Centre, Imperial College London. All authors acknowledge the provision of
443 strains by the Network on Antimicrobial Resistance in *Staphylococcus aureus* (NARSA) Program: under
444 NIAID/ NIH Contract No. HHSN272200700055C.

445

446 Contributions and study design

447 EVKL and AME designed the experiments, conducted the experiments, analyzed the data, and wrote
448 the manuscript. The funders had no role in the study design, interpretation of the findings or the
449 writing of the manuscript.

450

451 Conflict of interest

452 The authors declare no conflict of interest.

References

1. **Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler VG Jr.** *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. *Clin Microbiol Rev*. 28(3):603-61. 2015.
2. **Yang Z, Wang J, Wang W, Zhang Y, Han L, Zhang Y, Nie X, Zhan S.** Proportions of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* in patients with surgical site infections in mainland China: a systematic review and meta-analysis. *PLoS One*. 10(1):e0116079. 2015.
3. **Kuehl R, Morata L, Boeing C, Subirana I, Seifert H, Rieg S, Kern WV, Kim HB, Kim ES, Liao CH, Tilley R, Lopez-Cortés LE, Llewelyn MJ, Fowler VG, Thwaites G, Cisneros JM, Scarborough M, Nsutebu E, Gurgui Ferrer M, Pérez JL, Barlow G, Hopkins S, Ternavasio-de la Vega HG, Török ME, Wilson P, Kaasch AJ, Soriano A; International *Staphylococcus aureus* collaboration study group and the ESCMID Study Group for Bloodstream Infections, Endocarditis and Sepsis.** Defining persistent *Staphylococcus aureus* bacteraemia: secondary analysis of a prospective cohort study. *Lancet Infect Dis*. 20(12):1409-1417. 2020.
4. **Bergin SP, Holland TL, Fowler VG Jr, Tong SYC.** Bacteremia, Sepsis, and Infective Endocarditis Associated with *Staphylococcus aureus*. *Curr Top Microbiol Immunol*.409:263-296. 2017.
5. **Fowler VG Jr, Olsen MK, Corey GR, Woods CW, Cabell CH, Reller LB, Cheng AC, Dudley T, Oddone EZ.** Clinical identifiers of complicated *Staphylococcus aureus* bacteremia. *Arch Intern Med*. 22;163(17):2066-72. 2003.
6. **Nguyen TH, Cheung GYC, Rigby KM, Kamenyeva O, Kabat J, Sturdevant DE, Villaruz AE, Liu R, Piewngam P, Porter AR, Firdous S, Chiou J, Park MD, Hunt RL, Almufarriji FMF, Tan VY, Asiamah TK, McCausland JW, Fisher EL, Yeh AJ, Bae JS, Kobayashi SD, Wang JM, Barber DL, DeLeo FR, Otto M.** Rapid pathogen-specific recruitment of immune effector cells in the skin by secreted toxins. *Nat Microbiol*. 7(1):62-72. 2022.
7. **DeLeo FR, Diep BA, Otto M.** Host defense and pathogenesis in *Staphylococcus aureus* infections. *Infect Dis Clin North Am*. 23(1):17-34. 2009.
8. **Ellson CD, Davidson K, Ferguson GJ, O'Connor R, Stephens LR, Hawkins PT.** Neutrophils from p40phox-/- mice exhibit severe defects in NADPH oxidase regulation and oxidant-dependent bacterial killing. *J Exp Med*. 203(8):1927-37. 2006.
9. **Ha KP, Clarke RS, Kim GL, Brittan JL, Rowley JE, Mavridou DAI, Parker D, Clarke TB, Nobbs AH, Edwards AM.** Staphylococcal DNA Repair Is Required for Infection. *mBio*. 11(6):e02288-20. 2020.
10. **Scribner DJ, Fahrney D.** Neutrophil receptors for IgG and complement: their roles in the attachment and ingestion phases of phagocytosis. *J Immunol*. 116(4):892-7. 1976.
11. **Wright AE, Douglas SR.** An experimental investigation of the rôle of the blood fluids in connection with phagocytosis. *Proc R Soc Lond*. 72357–370. 1904.
12. **Leuzzi R, Bodini M, Thomsen IP, Soldaini E, Bartolini E, Muzzi A, Clemente B, Galletti B, Manetti AGO, Giovani C, Censini S, Budroni S, Spensieri F, Borgogni E, Rossi Paccani S, Margarit I, Bagnoli F, Giudice GD, Creech CB.** Dissecting the Human Response to *Staphylococcus aureus* Systemic Infections. *Front Immunol*. 12:749432. 2021.
13. **Guerra FE, Borgogna TR, Patel DM, Sward EW, Voyich JM.** Epic Immune Battles of History: Neutrophils vs. *Staphylococcus aureus*. *Front Cell Infect Microbiol*. 7:286. 2017.
14. **de Jong NWM, van Kessel KPM, van Strijp JAG.** Immune Evasion by *Staphylococcus aureus*. *Microbiol Spectr*. 7(2). 2019.
15. **Thammavongsa V, Kim HK, Missiakas D, Schneewind O.** Staphylococcal manipulation of host immune responses. *Nat Rev Microbiol*. 13(9):529-43. 2015.
16. **Cruz AR, Boer MAD, Strasser J, Zwarthoff SA, Beurskens FJ, de Haas CJC, Aerts PC, Wang G, de Jong RN, Bagnoli F, van Strijp JAG, van Kessel KPM, Schuurman J, Preiner J, Heck AJR, Rooijakkers SHM.** Staphylococcal protein A inhibits complement activation by interfering with IgG hexamer formation. *Proc Natl Acad Sci U S A*. 118(7):e2016772118. 2021.

503 17. **Falugi F, Kim HK, Missiakas DM, Schneewind O.** Role of protein A in the evasion of host adaptive
504 immune responses by *Staphylococcus aureus*. *mBio*. 4(5):e00575-13. 2013.
505 18. **Zhang L, Jacobsson K, Vasi J, Lindberg M, Frykberg L.** A second IgG-binding protein in
506 *Staphylococcus aureus*. *Microbiology*. 144(4):985-991. 1998.
507 19. **Smith EJ, Visai L, Kerrigan SW, Speziale P, Foster TJ.** The Sbi protein is a multifunctional immune
508 evasion factor of *Staphylococcus aureus*. *Infect Immun*. 79(9):3801-9. 2011.
509 20. **de Haas CJ, Veldkamp KE, Peschel A, Weerkamp F, Van Wamel WJ, Heezius EC, Poppelier MJ,**
510 **Van Kessel KP, van Strijp JA.** Chemotaxis inhibitory protein of Staphylococcus aureus, a bacterial
511 antiinflammatory agent. *J Exp Med*. 199(5):687-95. 2004.
512 21. **Ko YP, Kuipers A, Freitag CM, Jongerius I, Medina E, van Rooijen WJ, Spaan AN, van Kessel KP,**
513 **Höök M, Rooijakkers SH.** Phagocytosis escape by a *Staphylococcus aureus* protein that connects
514 complement and coagulation proteins at the bacterial surface. *PLoS Pathog*. 9(12):e1003816.
515 2013.
516 22. **Rooijakkers SH, Ruyken M, Roos A, Daha MR, Presanis JS, Sim RB, van Wamel WJ, van Kessel KP,**
517 **van Strijp JA.** Immune evasion by a staphylococcal complement inhibitor that acts on C3
518 convertases. *Nat Immunol*. 6(9):920-7. 2005.
519 23. **Rooijakkers SH, Ruyken M, van Roon J, van Kessel KP, van Strijp JA, van Wamel WJ.** Early
520 expression of SCIN and CHIPS drives instant immune evasion by *Staphylococcus aureus. Cell*
521 *Microbiol*. 8(8):1282-93. 2006.
522 24. **Howden BP, Giulieri SG, Wong Fok Lung T, Baines SL, Sharkey LK, Lee JYH, Hachani A, Monk IR,**
523 **Stinear TP.** *Staphylococcus aureus* host interactions and adaptation. *Nat Rev Microbiol*. 1-16.
524 2023.
525 25. **Pidgeon SE, Pires MM.** Cell Wall Remodeling of *Staphylococcus aureus* in Live *Caenorhabditis*
526 *elegans*. *Bioconjug Chem*. 28(9):2310-2315. 2017.
527 26. **Sobral R, Tomasz A.** The Staphylococcal Cell Wall. *Microbiol Spectr*. 7(4). 2019.
528 27. **Salamaga B, Kong L, Pasquina-Lemonche L, Lafage L, von Und Zur Muhlen M, Gibson JF,**
529 **Grybchuk D, Tooke AK, Panchal V, Culp EJ, Tatham E, O'Kane ME, Catley TE, Renshaw SA, Wright**
530 **GD, Plevka P, Bullough PA, Han A, Hobbs JK, Foster SJ.** Demonstration of the role of cell wall
531 homeostasis in *Staphylococcus aureus* growth and the action of bactericidal antibiotics. *Proc Natl*
532 *Acad Sci U S A*. 118(44):e2106022118. 2021.
533 28. **Monteiro JM, Fernandes PB, Vaz F, Pereira AR, Tavares AC, Ferreira MT, Pereira PM, Veiga H,**
534 **Kuru E, VanNieuwenhze MS, Brun YV, Filipe SR, Pinho MG.** Cell shape dynamics during the
535 staphylococcal cell cycle. *Nat Commun*. 6:8055. 2015.
536 29. **Sutton JAF, Carnell OT, Lafage L, Gray J, Biboy J, Gibson JF, Pollitt EJG, Tazoll SC, Turnbull W,**
537 **Hajdamowicz NH, Salamaga B, Pidwill GR, Condliffe AM, Renshaw SA, Vollmer W, Foster SJ.**
538 *Staphylococcus aureus* cell wall structure and dynamics during host-pathogen interaction. *PLoS*
539 *Pathog*. 17(3):e1009468. 2021.
540 30. **Ledger EVK, Mesnage S, Edwards AM.** Human serum triggers antibiotic tolerance in
541 *Staphylococcus aureus. Nat Commun*. 13(1):2041. 2022.
542 31. **Ellington JK, Harris M, Hudson MC, Vishin S, Webb LX, Sherertz R.** Intracellular Staphylococcus
543 aureus and antibiotic resistance: implications for treatment of staphylococcal osteomyelitis. *J*
544 *Orthop Res*. 24(1):87-93. 2006.
545 32. **Raineri EJM, Yedavally H, Salvati A, van Dijl JM.** Time-resolved analysis of *Staphylococcus aureus*
546 invading the endothelial barrier. Virulence. 11(1):1623-1639. 2020.
547 33. **Reed P, Atilano ML, Alves R, Hoiczyk E, Sher X, Reichmann NT, Pereira PM, Roemer T, Filipe SR,**
548 **Pereira-Leal JB, Ligoxygakis P, Pinho MG.** *Staphylococcus aureus* Survives with a Minimal
549 Peptidoglycan Synthesis Machine but Sacrifices Virulence and Antibiotic Resistance. *PLoS Pathog*.
550 11(5):e1004891. 2015.
551 34. **Painter KL, Hall A, Ha KP, Edwards AM.** The Electron Transport Chain Sensitizes *Staphylococcus*
552 *aureus* and *Enterococcus faecalis* to the Oxidative Burst. *Infect Immun*. 85(12):e00659-17. 2017.

35. **Ha KP, Clarke RS, Kim GL, Brittan JL, Rowley JE, Mavridou DAI, Parker D, Clarke TB, Nobbs AH, Edwards AM.** Staphylococcal DNA Repair Is Required for Infection. *mBio*. 11(6):e02288-20. 2020.
36. **Fey PD, Endres JL, Yajjala VK, Widhelm TJ, Boissy RJ, Bose JL, Bayles KW.** A genetic resource for rapid and comprehensive phenotype screening of nonessential *Staphylococcus aureus* genes. *mBio*. 4(1):e00537-12. 2013.
37. **Horsburgh MJ, Aish JL, White IJ, Shaw L, Lithgow JK, Foster SJ.** sigmaB modulates virulence determinant expression and stress resistance: characterization of a functional *rsbU* strain derived from *Staphylococcus aureus* 8325-4. *J Bacteriol*. 184(19):5457-67. 2002.
38. **Gill SR, Fouts DE, Archer GL, Mongodin EF, Deboy RT, Ravel J, Paulsen IT, Kolonay JF, Brinkac L, Beanan M, Dodson RJ, Daugherty SC, Madupu R, Angiuoli SV, Durkin AS, Haft DH, Vamathevan J, Khouri H, Utterback T, Lee C, Dimitrov G, Jiang L, Qin H, Weidman J, Tran K, Kang K, Hance IR, Nelson KE, Fraser CM.** Insights on evolution of virulence and resistance from the complete genome analysis of an early methicillin-resistant *Staphylococcus aureus* strain and a biofilm-producing methicillin-resistant *Staphylococcus epidermidis* strain. *J Bacteriol*. 2005 187(7):2426-38. 2005.
39. **Baba T, Bae T, Schneewind O, Takeuchi F, Hiramatsu K.** Genome sequence of *Staphylococcus aureus* strain Newman and comparative analysis of staphylococcal genomes: polymorphism and evolution of two major pathogenicity islands. *J Bacteriol*. 190(1):300-10. 2008.
40. **Surewaard BG, de Haas CJ, Vervoort F, Rigby KM, DeLeo FR, Otto M, van Strijp JA, Nijland R.** Staphylococcal alpha-phenol soluble modulins contribute to neutrophil lysis after phagocytosis. *Cell Microbiol*. 15(8):1427-37. 2013.
41. **Nagl M, Kacani L, Müllauer B, Lemberger EM, Stoiber H, Sprinzl GM, Schennach H, Dierich MP.** Phagocytosis and killing of bacteria by professional phagocytes and dendritic cells. *Clin Diagn Lab Immunol*. 9(6):1165-8. 2002.
42. **Wonfor T, Li S, Dunphy RW, Macpherson A, van den Elsen J, Laabei M.** Novel method for detecting complement C3 deposition on *Staphylococcus aureus*. *Sci Rep*. 12(1):15766. 2022.
43. **Boero E, Brinkman I, Juliet T, van Yperen E, van Strijp JAG, Rooijakkers SHM, van Kessel KPM.** Use of Flow Cytometry to Evaluate Phagocytosis of *Staphylococcus aureus* by Human Neutrophils. *Front Immunol*. 12:635825. 2021.
44. **Silver LL.** Fosfomycin: Mechanism and Resistance. *Cold Spring Harb Perspect Med*. 7:a025262. 2017.
45. **Pujol M, Miró JM, Shaw E, Aguado JM, San-Juan R, Puig-Asensio M, Pigrau C, Calbo E, Montejo M, Rodriguez-Álvarez R, Garcia-Pais MJ, Pintado V, Escudero-Sánchez R, Lopez-Contreras J, Morata L, Montero M, Andrés M, Pasquau J, Arenas MD, Padilla B, Murillas J, Jover-Sáenz A, López-Cortes LE, García-Pardo G, Gasch O, Videla S, Hereu P, Tebé C, Pallarès N, Sanllorente M, Domínguez MÁ, Càmara J, Ferrer A, Padullés A, Cuervo G, Carratalà J; MRSA Bacteremia (BACSARM) Trial Investigators.** Daptomycin Plus Fosfomycin Versus Daptomycin Alone for Methicillin-resistant *Staphylococcus aureus* Bacteremia and Endocarditis: A Randomized Clinical Trial. *Clin Infect Dis*. 72(9):1517-1525. 2021.
46. **Falcón R, Martínez A, Albert E, Madrid S, Oltra R, Giménez E, Soriano M, Vinuesa V, Gozalbo D, Gil ML, Navarro D.** High vancomycin MICs within the susceptible range in *Staphylococcus aureus* bacteraemia isolates are associated with increased cell wall thickness and reduced intracellular killing by human phagocytes. *Int J Antimicrob Agents*. 47(5):343-50. 2016.
47. **Tickle ARH, Ledger EVK, Edwards AM.** Human serum induces daptomycin tolerance in *Enterococcus faecalis* and viridans group streptococci. *Microbiology*. 168(12). 2022.
48. **Ong PY, Ohtake T, Brandt C, Strickland I, Boguniewicz M, Ganz T, Gallo RL, Leung DY.** Endogenous antimicrobial peptides and skin infections in atopic dermatitis. *N Engl J Med*. 347(15):1151-60. 2002.
49. **Nizet V, Ohtake T, Lauth X, Trowbridge J, Rudisill J, Dorschner RA, Pestonjamasp V, Piraino J, Huttner K, Gallo RL**. Innate antimicrobial peptide protects the skin from invasive bacterial infection. *Nature*. 414(6862):454-7. 2001.

604 50. **Dorschner RA, Pestonjamasp VK, Tamakuwala S, Ohtake T, Rudisill J, Nizet V, Agerberth B,**
605 **Gudmundsson GH, Gallo RL.** Cutaneous injury induces the release of cathelicidin antimicrobial
606 peptides active against group A *Streptococcus*. *J. Invest. Dermatol.* **117**, 91–97 (2001).
607 51. **Burian M, Plange J, Schmitt L, Kaschke A, Marquardt Y, Huth L, Baron JM, Hornef MW, Wolz C,**
608 **Yazdi AS.** Adaptation of *Staphylococcus aureus* to the Human Skin Environment Identified Using
609 an *ex vivo* Tissue Model. *Front Microbiol*. 12:728989. 2021.
610 52. **Kraus D, Herbert S, Kristian SA, Khosravi A, Nizet V, Götz F, Peschel A.** The GraRS regulatory
611 system controls *Staphylococcus aureus* susceptibility to antimicrobial host defenses. *BMC*
612 *Microbiol*. 8:85. 2008.
613 53. **Cheung AL, Cho J, Bayer AS, Yeaman MR, Xiong YQ, Donegan NP, Mikheyeva IV, Lee GY, Yang SJ.**
614 Role of the *Staphylococcus aureus* Extracellular Loop of GraS in Resistance to Distinct Human
615 Defense Peptides in PMN and Invasive Cardiovascular infections. *Infect Immun*. 89(10):e0034721.
616 2021.
617 54. **Friberg C, Haaber JK, Vestergaard M, Fait A, Perrot V, Levin BR, Ingmer H.** Human antimicrobial
618 peptide, LL-37, induces non-inheritable reduced susceptibility to vancomycin in *Staphylococcus*
619 *aureus*. *Sci Rep*. 10(1):13121. 2020.
620 55. **Ranganathan N, Johnson R, Edwards AM.** The general stress response of *Staphylococcus aureus*
621 promotes tolerance of antibiotics and survival in whole human blood. *Microbiology*. 166(11):1088-
622 1094. 2020.
623 56. **Rowe SE, Wagner NJ, Li L, Beam JE, Wilkinson AD, Radlinski LC, Zhang Q, Miao EA, Conlon BP.**
624 Reactive oxygen species induce antibiotic tolerance during systemic *Staphylococcus aureus*
625 infection. *Nat Microbiol*. 5(2):282-290. 2020.
626 57. **Beam JE, Rowe SE, Conlon BP.** Shooting yourself in the foot: How immune cells induce antibiotic
627 tolerance in microbial pathogens. *PLoS Pathog*. 17(7):e1009660. 2021.
628 58. **Shen F, Tang X, Cheng W, Wang Y, Wang C, Shi X, An Y, Zhang Q, Liu M, Liu B, Yu L.** Fosfomycin
629 enhances phagocyte-mediated killing of *Staphylococcus aureus* by extracellular traps and reactive
630 oxygen species. *Sci Rep*. 6:19262. 2016.
631 59. **Berti A, Rose W, Nizet V, Sakoulas G.** Antibiotics and Innate Immunity: A Cooperative Effort
632 Toward the Successful Treatment of Infections. *Open Forum Infect Dis*. 7(8):ofaa302. 2020.
633 60. **Fey PD, Endres JL, Yajjala VK, Widhelm TJ, Boissy RJ, Bose JL, Bayles KW.** A genetic resource for
634 rapid and comprehensive phenotype screening of nonessential *Staphylococcus aureus* genes.
635 *mBio*. 4(1):e00537-12. 2013.
636 61. **Higgins J., Loughman A., van Kessel K. P., van Strijp J. A., Foster T. J.** Clumping factor A of
637 *Staphylococcus aureus* inhibits phagocytosis by human polymorphonuclear leucocytes. *FEMS*
638 *Microbiol. Lett*. 258:290–296. 2006.
639 62. **Olsen CH**. Review of the use of statistics in infection and immunity. *Infect Immun*. 1(12):6689-92.
640 2003.
641 63. **Cunnion KM, Hair PS, Buescher ES.** Cleavage of complement C3b to iC3b on the surface of
642 *Staphylococcus aureus* is mediated by serum complement factor I. *Infect Immun*. 72(5):2858-63.
643 2004.