Environmental fatty acids enable emergence of infectious *Staphylococcus aureus* resistant to FASII-targeted antimicrobials

Claire Morvan1,†, David Halpern1, Gérald Kénanian1, Constantin Hays2,3,4,5, Jamila Anba-Mondoloni1, Sophie Brinster2,3,4,†, Sean Kennedy6,†, Patrick Trieu-Cuot7, Claire Poyart2,3,4,5, Gilles Lamberet1, Karine Gloux1 & Alexandra Gruss1

The bacterial pathway for fatty acid biosynthesis, FASII, is a target for development of new anti-staphylococcal drugs. This strategy is based on previous reports indicating that self-synthesized fatty acids appear to be indispensable for *Staphylococcus aureus* growth and virulence, although other bacteria can use exogenous fatty acids to compensate FASII inhibition. Here we report that staphylococci can become resistant to the FASII-targeted inhibitor triclosan via high frequency mutations in *fabD*, one of the FASII genes. The *fabD* mutants can be conditional for FASII and not require exogenous fatty acids for normal growth, and can use diverse fatty acid combinations (including host fatty acids) when FASII is blocked. These mutants show cross-resistance to inhibitors of other FASII enzymes and are infectious in mice. Clinical isolates bearing *fabD* polymorphisms also bypass FASII inhibition. We propose that fatty acid-rich environments within the host, in the presence of FASII inhibitors, might favour the emergence of staphylococcal strains displaying resistance to multiple FASII inhibitors.

1 Micalis Institute, INRA, AgroParisTech, Université Paris-Saclay, Jouy-en-Josas 78350, France. 2 ‘Barriers and Pathogens’ Team, INSERM U 1016, Institut Cochin, Paris F-75014, France. 3 CNRS UMR 8104, Paris F-75014, France. 4 Université Paris Descartes, Sorbonne Paris Cité, Paris F-75014, France. 5 Hôpitaux Universitaires Paris Centre Cochin-Hôtel Dieu-Broca, Assistance Publique Hôpitaux de Paris, Paris F-75014, France. 6 Metagenopolis-Micalis UMR 1319, Jouy en Josas 78352, France. 7 Biology of Gram-positive Pathogens Unit, Institut Pasteur 25-28 rue du docteur Roux, Paris 75015, France. 8 CNRS ERL3526, Paris 75015, France. † Present addresses: Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, Université Paris-Sud, Université Paris-Saclay, Orsay 91400, France (C.M.); Society Lubro, Sevrain 93270, France (S.B.); Pasteur Institute 25-28 rue du docteur Roux, Paris 75015, France (S.K.). Correspondence and requests for materials should be addressed to A.G. (email: alexandra.gruss@jouy.inra.fr).
**Staphylococcus aureus** is a remarkably versatile opportunistic pathogen that has confounded the medical community by its ability to escape antibiotic inhibition. Multidrug-resistant *S. aureus* are a main cause of hospital- and community-acquired infections, and treatment constitutes a major medical and economic challenge.

The fatty acid synthesis pathway (FASII) has been a leading candidate as a novel essential target for antimicrobial discovery. Numerous FASII inhibitors are in the pipeline or in clinical trials, most of which target FabI, the enoyl-acyl carrier protein (ACP) reductase (for example, AFN-1252 (now Debio-1452 and pro-drug Debio-1450), CG400549, CG400462 and MUT056399), or FabF, the β-ketoacyl-ACP synthase (for example, placulatin, placensimycin and fasamycins A and B).

Triclosan, a chemically synthesized biocide first introduced in 1965, is the best known FabI inhibitor developed thus far, and has had widespread use in hygiene, health care, and household products at concentrations of up to 1% (refs 12,13). In consequence, triclosan detection in human fluids is common, and has generated information on the limits of use of this anti-FASII, including safety issues.

Observed side effects of triclosan on the host may be caused by its general membrane-disrupting activity. Additionally, triclosan-resistant staphylococci, conferred by mutations that maintain FASII activity, or obtained by horizontal transfer of the *Staphylococcus haemolyticus fabI* gene (encoding a naturally occurring resistant FabI variant), are estimated to comprise 5–15% of *S. aureus* clinical isolates. Continued anti-FASII development is based on development of antimicrobials with greater FabI affinity and specificity that would curb its potentially dangerous side effects.

However, fatty acids are abundant in the host and can be used by several Gram-positive pathogens to overcome a FASII deficiency, which questions FASII targeting as a general strategy for antibacterial drug discovery. For example, exogenous fatty acids can fully compensate a FASII deficiency in *Streptococcus agalactiae*, and a FASII auxotroph was fully virulent in a murine septicemia infection model.

Nevertheless, the ability of *S. aureus* to override FASII inhibition in the presence of fatty acids has remained in question. *S. aureus* FASI_S expression is not blocked by exogenous fatty acids, whereas it is inhibited in streptococci. Rather than synthesizing unsaturated fatty acids to fluidify their membranes (as do the streptococci), *S. aureus* synthesizes branched chain fatty acids, of which the main one, anteiso C15:0 (aii15:0), is apparently required for phospholipid synthesis. Moreover, it has been proposed that FASI_S inhibition in *S. aureus* leads to accumulation of acyl-ACP intermediates, thereby limiting ACP availability for exogenous fatty acid utilization (see Supplementary Figs 1 and 2 for pathway and genetic contexts). In addition, while fatty acid auxotrophs (defective in *acc* genes encoding acetyl-CoA carboxylase) are viable, optimal growth requires *aii15:0*, and these *acc* mutants are avirulent in a mouse septicemia infection model.

The fundamental and medical importance of FASII-targeted drug development incited us to characterize *S. aureus* responses to anti-FASII drugs. Here we show that fatty acids, which are ubiquitous host constituents, can facilitate the emergence of *S. aureus* resistance to anti-FASII drugs.

### Results

**Fatty acids facilitate the emergence of triclosan resistance.**

Some Gram-positive bacteria can incorporate exogenous fatty acids, which enable resistance to anti-FASII drugs. In the case of *S. aureus*, we considered the possibility that arising mutations could facilitate fatty acid incorporation and lead to FASI bypass, as hypothesized in an earlier study. To reveal such fatty-acid-based triclosan resistant (FA-T*<sup>R</sup>*) mutants, we examined the response of the *S. aureus* Newman strain to the FabI inhibitor triclosan on fatty-acid-containing BHI solid medium. A fatty acid cocktail (referred hereafter as ‘FA’) was used comprising C14:0, C16:0, and C18:1, which are present in the human host.

Overnight *S. aureus* BHI cultures were plated on medium containing FA and 0.25 μg ml<sup>−1</sup> triclosan; as controls, they were also plated on medium without FA and/or triclosan. Triclosan-resistant colonies arose at about 100-fold higher frequencies (~10<sup>−6</sup>) in the presence of FA. The FA-*<sup>R</sup>* colonies were screened for growth on BHI, BHI-triclosan (BHI-T), BHI-FA and BHI-FA-triclosan (BHI-FA-T) plates (Supplementary Fig. 3). A minority of clones grew on all four media (FA prototrophic triclosan resistant mutants, T*<sup>R</sup>* mutants). The remaining clones grew either on BHI, BHI-FA and BHI-FA-T (FA prototrophs), or on FA-containing media but very poorly or not at all on BHI. The latter were thus considered as fatty-acid-dependent for normal growth and were designated DepT*<sup>R</sup>* mutants. Fatty acids thus appear to facilitate the emergence of triclosan resistance in *S. aureus*.

**Three classes of *S. aureus* FA-T*<sup>R</sup>* mutants.** Fatty acid profiles of randomly selected FA-*<sup>R</sup>* mutants (16 prototrophs and 20 DepT*<sup>R</sup>* mutants) were determined from whole cell extracts of cultures prepared in BHI-FA-T medium. Representative profiles of each class of mutants are shown (Fig. 1). Among prototrophs, a minor class (3/16) comprised mutants with endogenous (EndoT*<sup>R</sup>*) fatty acid profiles, indicating that the FASII pathway was active. The dominant class of prototrophs (13/16) comprised mutants having media-dependent fatty acid profiles, that is, with exogenous fatty acids in BHI-FA-T, and endogenous fatty acids in BHI (conditional triclosan-resistant mutants, referred to as CondT*<sup>R</sup>*). Mutants of this class have not previously been reported. All the DepT*<sup>R</sup>* clones showed poor or no growth on BHI plates, but displayed exogenous profiles in BHI-FA-T medium. These results show that the majority of isolated FA-*<sup>R</sup>* mutants were able to bypass a FASII block by incorporating exogenous fatty acids.

**Gain of function in an *S. aureus* conditional mutant.** Growth and fatty acid profiles of CondT*<sup>R</sup>*-17 and the Newman strain were compared in the absence or presence of fatty acids and triclosan (Fig. 2a). Growth of both strains was comparable in BHI medium. The addition of fatty acids expectedly slowed strain growth, which was also comparable for both strains (Fig. 2a, centre).

When triclosan was added, CondT*<sup>R</sup>*-17 maintained growth, but not the Newman strain. Fatty acid profiles confirmed CondT*<sup>R</sup>* strain capacity to bypass FASII inhibition and shift to the exogenous FA source (Fig. 2b). Thus, this conditional mutant can use exogenous fatty acids upon FASII block, while displaying normal growth otherwise.

**Numerous FASII-bypass mutations map to fabD.** Known *S. aureus* triclosan-resistant mutants carry mutations in the gene encoding FabI, the triclosan target: such mutant enzymes fail to bind triclosan but remain enzymatically active. Our FA-*<sup>R</sup>* clones with EndoT*<sup>R</sup>* fatty acid profiles resembled previously described strains carrying mutations in *fabI*. The role of fatty acids in obtaining such mutants is unknown. In contrast, all tested mutants with exogenous fatty acid profiles (DepT*<sup>R</sup>* and CondT*<sup>R</sup>* strains) carried wild type *fabI* genes (Table 1).
Previous isolated *S. aureus* fatty acid auxotrophs mapped to FASII initiation genes *accC* and *accD*, encoding acetyl-CoA carboxylase subunits (biotin carboxylase and β-carboxytransferase respectively) for malonyl-CoA synthesis\(^{27}\) (Supplementary Fig. 1). Among five tested *DepTR* mutants, one (*DepTR*-5) carried a single nucleotide substitution generating FabD\(^{G196R}\); *DepTR*-5 cannot grow in BHI media (Fig. 3c) or in medium supplemented with liver and kidney extracts (Supplementary Fig. 3). The results suggested that other fatty acids can replace *ai15:0* when FASII was blocked (Fig. 3). The *DepTR*-5 mutant was grown in medium containing triclosan and fatty acid cocktails with 33, 3 or 0% *C14:0*. For each cocktail, the amounts of *C14:0* in the medium, indicating that *C14:0* is not preferentially incorporated for FASII bypass.

**Diverse fatty acid combinations allow FASII bypass.** Previous studies suggested that *ai15:0* was strictly required at the second acyl position of the *S. aureus* phospholipid\(^{24,27,32,43}\). However, our results indicated that other fatty acids can replace *ai15:0*. We explored the possibility that a fatty acid of similar length, like *C14:0* (33% of our FA cocktail), might substitute for *ai15:0* when FASII was blocked (Fig. 3). The *DepTR*-5 mutant was grown in medium containing triclosan and fatty acid cocktails with 33, 3 or 0% *C14:0*. For each cocktail, the amounts of *C14:0* in *S. aureus* reflected its proportions among fatty acids in the medium, indicating that *C14:0* is not preferentially incorporated for FASII bypass.

A mix of the four principal fatty acids in human serum in physiological proportions (*C16:0, C18:0, C18:1 and C18:2*) was also tested. When added in purified form, the free fatty acid cocktail was toxic to staphylococci, likely due to *C18:2* (linoleic acid), which makes up ~35% of serum fatty acids\(^{35}\). However, growth was restored when 10% delipidated serum was added, and *DepTR*-5 displayed an exogenous fatty acid profile (Fig. 3e).

To mimic the within-host environment during infection, we grew *S. aureus* wild type and *DepTR*-5 strains in medium containing sterile-filtered pig liver and kidney extracts. The incorperated fatty acids reflected those present in the medium (shown for liver; Fig. 3f, see Supplementary Fig. 4 for organ extract profile). Unexpectedly, long-chain unsaturated fatty acids *C18:2* and *C20:4* were efficiently incorporated. *C16:0* was the shortest abundant fatty acid incorporated, indicating that *DepTR*-5 *fabD* mutant growth was not dependent on *ai15:0* or other short-chain fatty acids.

Thus, the *S. aureus* *fabD* mutant can use diverse fatty acids, including those available in the host, and can dispense with branched chain fatty acids. These results indicate that the composition of the *S. aureus* membranes can be more flexible than generally supposed\(^{24,27,32,43}\).

**Role of the *S. aureus* fabD allele in FASII bypass.** To confirm that the *fabD* mutation is responsible for fatty acid dependency of *DepTR*-5, the wild type *fabD* gene was cloned in expression vector pJ (resulting in pJ-fabD) and established in the mutant strain background. To simulate in vivo conditions and obtain insight into *S. aureus* *fabD* mutant behaviour in the animal host, tests were performed in medium supplemented with liver and kidney extracts as fatty acid sources. In non-supplemented medium, the pJ-fabD plasmid restored *ai15:0* synthesis and growth to the *DepTR*-5 strain, confirming that the *fabD* mutation was responsible for the FASII defect (Fig. 4a). Importantly, growth of *DepTR*-5 (pJ) in host organ extracts was comparable to that of the Newman (pJ) strain, indicating that *DepTR*-5 can grow robustly while using exclusively host fatty acids. In this condition, complementation with pJ-fabD also resulted in *ai15:0* synthesis.
In triclosan plus host organ extracts, growth of DepTR-5 (pJ-fabD) lagged slightly behind that of DepTR-5 (pJ) (Fig. 4c, Supplementary Fig. 5a). The intermediate effect could indicate that triclosan exerts a counter-selective effect on the plasmid-carried fabD copy.

CondTR-17 grew both without and with fatty acid supplementation, and as expected, strong phenotypic differences were not observed in CondTR-17 complementation tests. The CondTR-17 (pJ-fabD) strains grew like Newman (pJ) in host organ extracts (data not shown). However, as above, CondTR-17 (pJ-fabD) displayed a slight growth retardation compared with CondTR-17 (pJ) in fatty-acid-triclosan-containing media as shown in the presence of kidney extracts (Supplementary Fig. 5b). In view of the absence of other mutations in the WT a BHI-FA-TriclosanBHI n.g..
Platensimycin was poorly effective in both media at the tested concentration. We also noted a pronounced decrease in the inhibitory zone diameter for triclosan on BHI-FA compared with BHI plates (compare Fig. 5a,b). In contrast, both DepTR-5 and CondTR-17 strains grew, albeit more slowly, within inhibitory zones of the 3 FASII inhibitors on BHI-FA plates (Fig. 5c,d).

Cross-resistance was then checked for colonies isolated from WT BHI-FA plates as triclosan, AFN-1252 or platensimycin resistant (Fig. 5b). Colonies were patched on BHI-FA medium containing triclosan (0.25 µg ml⁻¹) or AFN-1252 (0.08 µg ml⁻¹) (Fig. 5e). Fatty acid profiles of colonies as determined on primary selections and cross-selections were all exogenous (not shown). In addition, liquid cultures of DepTR-5 and CondTR-17 confirmed the capacity of DepTR-5 and CondTR-17 to grow on platensimycin and incorporate exogenous fatty acids (Supplementary Fig. 6).

These results further support a role of fatty acids in facilitating S. aureus escape from anti-FASII inhibition, and show that mutants selected by the use of a FASII inhibitor can be resistant to other FASII inhibitors.

Infection by S. aureus fabD mutants. Infections by fabD DepTR-5, fabD CondTR-17 or the WT strain were compared in a mouse sepsis model. A low inoculum (10⁶ CFU) was used to avoid the possibility of false-positive virulence assignments. The conditional FA-TR mutant, CondTR-17, was as infective as the parental Newman strain and gave equivalent CFUs in both liver and kidneys (Fig. 6). These results indicate that this strain, and possibly similar variants, can retain full virulence.

The DepTR-5 mutant showed delayed kinetics of infection. At two days post-infection, significantly lower CFUs were present in both liver and kidneys compared with the WT strain (P = 0.001 in both organs using the Mann–Whitney U-test). However, these differences narrowed at 6 days post-infection, particularly in kidneys, where DepTR-5 CFUs increased and were not statistically different from WT CFUs (P = 0.77 in kidneys) (Fig. 6). Colonies isolated from the 6-day infection were confirmed to maintain fatty acid growth dependency, and two colonies selected at random from each organ were confirmed to carry the original fabD mutation. Our results indicate that both CondTR and DepTR mutants would resist challenge by a FASII inhibitor in vivo. As DepTR-5 and CondTR-17 also proliferate and incorporate fatty acids in the presence of AFN-1252 and platensimycin (Fig. 5c,d), we speculate that both types of mutants might override FASII inhibition regardless of the FASII inhibitor.

FASII bypass in natural isolates carrying FabD polymorphisms. Our results show that the CondTR-17 G196R substitution correlates with FASII bypass via incorporation of exogenous fatty acids. We searched public databases for natural S. aureus isolates carrying FabD modifications at the G196 position, and found three such isolates. Two independent sputum and nare isolates carry FabD196R; M0251 (a methicillin-resistant S. aureus (MRSA) clonal complex CCS isolate, BioSample accession code SAMN02352289) and M1532 (BioSample accession code SAMN02364048). The third strain, SA40, is a community- and epidemic-associated methicillin-resistant sequence type ST59 that does not carry the FabD196 polymorphism45. These results lead us to conclude that the fabD allele is responsible for the FASII defect in DepTR-5 and CondTR-17 strains. Both mutants grew comparably to the WT strain in host-simulated conditions, suggesting that these mutants would behave like the wild type strain in host compartments.
Figure 4 | Complementation of DepTR-5 by the wild type fabD allele. Overnight LB-FA cultures of WT and DepTR-5 strains carrying pJ (control plasmid), or pJ-fabD (expressing the fabD gene) were washed twice in LB, and cultures were inoculated at a starting OD$_{600}$ = 0.05 in (a) LB (Cm 3 µg ml$^{-1}$ and Atc 50 µg ml$^{-1}$), (b) the same medium containing sterile-filtered pig kidney extract (3% final volume) without or (c) with triclosan (0.5 µg ml$^{-1}$). OD$_{600}$ was followed over 8 h. Fatty acid profiles were determined from harvested cells, as shown below the corresponding growth condition. N.g., no growth. Reference fatty acid profile and peak identification of kidney extract is in Supplementary Fig. 4. Growth results show the average with standard deviation of three experiments that gave comparable results (Supplementary Fig. 5). However, despite precautions, growth was variable between independent experiments, which may be due to the counter-selective effect of triclosan on the pJ-fabD plasmid.

Figure 5 | S. aureus cross-resistance to anti-FASII drugs in the presence of exogenous fatty acids. Overnight BHI-FA-grown cultures of Newman WT, DepTR-5, and CondTR-17 were washed twice in BHI and plated on BHI solid medium without (a) (for Newman strain) or with fatty acids (b–d). Plates were spotted with triclosan (‘T’, 1.5 µg), AFN-1252 (‘A’, 1 µg), and platensimycin (‘P’, 8 µg), respectively 100, 100 and 16 times the MIC. Photographs were taken after 48 h at 37°C. (c) DepTR-5 and (d) CondTR-17 strains accrue resistance to all three drugs on FA plates as seen by growth within the halo region, as compared with (b) the WT strain. The small inhibitory zone around triclosan is attributed to non-specific membrane effects12. (e) Colonies that appeared within the inhibitory zones of WT strains on BHI-FA plates were cross-patched on BHI, BHI-FA, BHI-FA-AFN-1252 (80 ng ml$^{-1}$) and FA-triclosan (250 ng ml$^{-1}$). All colonies picked in fatty acids as triclosan-resistant were AFN-1252-resistant, and conversely. Fatty acid profiles (not shown) determined for colonies from each anti-FASII selective plate were fully exogenous.
support the correlation between \( fabD^{196} \) polymorphism and anti-FASII resistance, and show the capacity of \( S. aureus \) human isolates to use exogenous fatty acids to overcome FASII inhibitors. They also indicate that anti-FASII-resistance is present in some commensal and clinical isolates.

Redistribution of acyl-ACP pools in \( fabD \) variants. FabD catalyses the transfer of malonyl from malonyl-CoA to acyl-carrier proteins (ACPs), yielding malonyl-ACP (Supplementary Fig. 1). We considered that FabD polymorphisms near the active site (that is, position 196) might lower the efficiency of this reaction, thus leaving free ACP available for exogenous fatty acid incorporation. Two strain pairs were used to test this hypothesis: Newman strain (\( fabDG^{196} \))–CondTR-17 (\( fabDR^{196} \)) and ST59 clinical strains SA957 (\( fabDG^{196} \))–SA40 (\( fabDR^{196} \)). Cell extracts and culture supernatants were prepared from 4h cultures in BHI-FA-T. Short- and long-chain acyl-ACP were assessed on Western blots of conformation-sensitive gels using anti-ACP antibodies\(^{27,46}\); fatty acid profiles of cell extracts were done in parallel (Fig. 7). A dithiothreitol (DTT)-treated extract of BHI-grown Newman strain was used to identify the migration position of free ACP\(^{27,46,47}\).

Acyl-ACP species were redistributed in both \( fabD \) variants (CondTR-17 and SA40) compared with respective reference strains. Short-chain acyl-ACP pools were decreased in both variant strains, which is consistent with a reduced FabD activity as hypothesized. In addition, the \( fabD \) mutants contain long-chain acyl-ACPs that are not detected in reference strains, and their fatty acid profiles (but not those of the reference strains) are fully exogenous. Certain fatty acids are known to induce ACP release from \( S. aureus \) cells\(^{48}\); however, we found that at 4h, supernatants of all strains produced weak signals that did not differ significantly between strain pairs (Supplementary Fig. 7). Thus, we hypothesize that the FabDR\(^{196} \) and FabD\(^{196} \) mutant enzymes may be less active than FabDG\(^{196} \), allowing free ACPs to be incorporated into membrane phospholipids.

Discussion

The present work shows that a fatty-acid-rich environment can favour the emergence of \( S. aureus \) strains displaying resistance to multiple FASII inhibitors. Point mutations in \( fabD \) appear to facilitate FASII bypass by enhancing incorporation of exogenous fatty acids. Conditional (CondTR) \( fabD \) mutants can grow normally, and can also bypass FASII in the presence of exogenous fatty acids, which suggests that they might be able to spread successfully in environments such as host organs. The \( S. aureus \) \( fabD \) mutants can use various fatty acid combinations (Fig. 3). This flexibility indicates that, contrary to previous assumptions\(^{24,27,43}\), the branched-chain fatty acid requirement in \( S. aureus \) is not strict, as shown here for \( fabD^{196} \) variants in laboratory and clinical strains from major lineages (CC8, CC5 and ST59). It may also help survival of anti-FASII-resistant bacteria in different host compartments that vary in fatty acid composition. Therefore, we propose that all these factors need to be considered when developing anti-FASII drugs for treating \( S. aureus \) infection\(^{4,5,8,10,11,48,49}\).

In FASII-bypass conditions, \( fabD^{196} \) variant strains show decreased levels of short-chain acyl-ACPs and increased levels of long-chain acyl-ACPs, in comparison with reference strains (Fig. 7). The decrease in short-chain acyl-ACP is consistent with our proposal that FabD\(^{196} \) variants may have reduced enzyme activity. We speculate that the detected long-chain acyl-ACP species correspond to exogenous fatty-acyl-ACP intermediates in phospholipid synthesis, which is consistent with the exogenous fatty acid profiles of \( fabD^{196} \) variants (Fig. 7). FASII bypass might depend on the outcome of competition for ACP between two

![Image](68x414 to 163x493)

**Figure 6 | Infection and persistence of \( fabD \) isolates in a mouse model.**

Newman WT (black), CondTR-17 (\( fabD \) conditional; blue), and DepTR-5 (\( fabD \) fatty-acid-dependent; red) strains were prepared. 10\(^{6} \) CFU were injected in the tail veins of 6-week female BALB/c mice (in groups of 8–11). In these experiments, one WT-infected and one CondTR-17-infected mouse died 4 days post-infection. At days 2 (D2) and 6 (D6), animals from each group were sacrificed and dissected. Liver and kidney were homogenized by ultra-turrax and CFU per organ were determined by serial dilutions on BHI-FA plates containing 10% serum. Results are pooled from two independent experiments. Statistical tests were done using Mann–Whitney U to determine differences in CFU between groups on D2 and/or D6. No significant differences were observed between WT and CondTR-17 in any test condition. Notably, no significant difference was observed between WT and DepTR-5 CFUs in kidneys at D6 (\( P = 0.77 \)). NS, non-significant differences. The detection limit was 10\(^{2} \) CFU per organ; samples with no CFUs were plotted as ‘0’. In DepTR-5 samples, one mouse showed no detectable counts in liver or kidney.

| Strain          | Origin      | FabD\(^{196} \) locus | Growth on     | FA profile on FA-T |
|-----------------|-------------|-----------------------|---------------|--------------------|
| Newman          | Clinical-lab| \( fabDG^{196} \)     | + + + +        | -                  |
| SA957           | Blood       | FabD\(^{196} \)       | + + +          | -                  |
| SA40            | Nare        | FabD\(^{196} \)       | + + + +        | -                  |
| MO251           | Sputum      | FabD\(^{196} \)       | + + + +        | + + +              |
| MO1532          | Nare        | FabD\(^{196} \)       | + + + +        | -                  |
| CondTR-4        | FAT\(^{R} \)| FabD\(^{196} \)       | + + + +        | + + +              |
| CondTR-17       | FAT\(^{R} \)| FabD\(^{196} \)       | + + +          | Exo                |

The NCBI database was searched for natural \( S. aureus \) isolates bearing \( fabD \) proteins with residues other than G196. Three such strains were received: CC5 isolate M0251, M1532 (Brigham and Women’s Hospital, Boston; Mary Delaney and Michael Calderwood), and ST59 strain SA40 a Taiwanese commensal strain (Götz and Stemmler\(^{45} \)). All strains listed above were grown overnight in BHI, BHI-FA (not presented) and BHI-FA-T. Overnight OD\(_{600}\) = 0.5–1; no growth. Growth results were within this range in three biological replicates. Fatty acid profiles for all strains but Newman and SA957 were determined on BHI-FA-T. FA, fatty acid; FA-T, BHI-FA-T medium. Exo, exogenous fatty acid profile.
enzymes at opposing ends of the pathway: the FASII initiation enzyme FabD (which uses ACP to synthesize malonyl-ACP) and the post-FASII termination enzyme PIsX (which catalyses the reversible formation of acyl-phosphate from acyl-ACP)\textsuperscript{43,50}. Lower FabD activity would leave more ACP available for PIsX, to drive the reaction towards the synthesis of long-chain acyl-ACP (the phospholipid synthesis precursor) from exogenous fatty acids, and thus lead to FASII bypass (Fig. 8). At present, this or other roles for long-chain acyl-ACP, for example, in modulating FASII enzyme activities, need to be demonstrated.

Previous results showing that fatty acid auxotrophs carrying mutations in \textit{acc} genes are avirulent led to the hypothesis that FASII-bypass mutants would not survive in the host\textsuperscript{27,32}. In contrast, we have shown here that the \textit{fabD} mutants \textit{Dept}\textsuperscript{K-5} and \textit{CondT}\textsuperscript{K-17} are infectious and persist in the host, and that clinical isolates chosen as bearing \textit{fabD}\textsubscript{196} polymorphisms can bypass FASII inhibition (Fig. 6 and Table 2). We speculate that this apparent discrepancy may be due to the different effects of the \textit{acc} and \textit{fabD} mutations on malonyl-ACP pools. FapR is a transcriptional repressor of FASII genes and of phospholipid synthesis genes \textit{pIsX} and \textit{pIsC}\textsuperscript{51} (Supplementary Fig. 1). FapR activity is alleviated by malonyl-CoA\textsubscript{51-53}. Mutations in \textit{acc} leading to reduced synthesis of malonyl-CoA would result in FapR-mediated repression of phospholipid synthesis. On the contrary, malonyl-CoA would not be affected in the \textit{fabD} mutants; it is even possible that the \textit{fabD} mutants may accumulate malonyl-CoA, thus alleviating FapR-mediated repression of phospholipid synthesis genes. This situation would favour FASII bypass in environments rich in fatty acids.

Our study revealed that FASII bypass mutants need not be fatty-acid auxotrophs. The \textit{CondT}\textsuperscript{K} mutant is resistant to several FASII inhibitors, yet grows normally and is infectious, indicating a gain of function. \textit{CondT}\textsuperscript{K}-like variants may thus present a risk of dissemination in the environment. The three tested clinical and commensal isolates with \textit{fabD}\textsubscript{196} mutations were not identified previously because of differences in experimental conditions, for example, in the choice of fatty acids and/or FASII inhibitors used in selections. Alternatively, strains might differ in their capacity to mediate FASII-bypass \textit{via} mutations. Nevertheless, identification of \textit{fabD} variant strains belonging to CC5, CC8, and ST59 groups and including MRSA isolates suggests that FASII-bypass variants may emerge in these dominant lineages.

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**Figure 7 | Shift in ACP species in strains with \textit{fabD}\textsuperscript{196} polymorphisms.**

The impact of an altered amino acid at FabD position 196 on acyl-ACP pools was tested by comparing strain pairs: Newman and \textit{CondT}\textsuperscript{K-17} (G196 and R196 respectively), and the ST59 pair SA957 (virulent) and SA40 (nasal isolate)\textsuperscript{45} (G196 and S196 respectively). Extracts prepared from 4 h cultures in BHI-FA-T medium were run on conformation-sensitive gels. Anti-ACP antibodies were used to identify ACP moieties after gel transfer. In these gels, migration of acyl-ACP species increases with longer acyl chain length\textsuperscript{46,66}. DTT treatment reduces acyl-ACP thioesters and releases free ACP\textsuperscript{47}; the DTT-treated Newman protein extract from BHI-grown cells was used as migration control. Short-chain acyl-ACP is predominant in reference strains, and long-chain acyl-ACP moieties (arrows) are detected only in extracts of \textit{fabD} variants. Results of fatty acid profiles of reference strains and \textit{fabD} variants are indicated below respective lanes. Nwmn, Newman strain; *, fatty acid profiles derived from non-growing cultures. The Western blot shown above corresponds to the entire gel.

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**Figure 8 | Model of FASII bypass by \textit{S. aureus} \textit{fabD}\textsuperscript{196} variants based on competition for ACP.** A role for FabD in ACP balance and as a determining factor of FASII bypass is proposed. Indicated pathways and regulatory functions are as previously identified\textsuperscript{43,67}. In grey, the inhibited FASII elongation pathway. FASII initiation enzyme FabD uses ACP and malonyl-CoA to synthesize malonyl-ACP\textsuperscript{50}. In FASII inhibitory conditions, PIsX would compete for ACP pools to convert exogenous fatty acids (turquoise) into phospholipids. Less efficient activity of FabD\textsubscript{196} variants (Fig. 7) would leave ACP available for the PIsX reaction in the indicated direction (PIsX is a potentially reversible enzyme\textsuperscript{49}), as indicated by orange dashed arrow. Fak-PlsY and Fak-PlsX-PlsC as proposed here, mediate acyl group joining respectively to positions 1 and 2 of the glycerol-3-phosphate (G3P) backbone\textsuperscript{51,43}. FA, fatty acid; FA-ACP, acyl-ACP; FA-PO\textsubscript{4}, acyl-phosphate.
DepTR-like mutants are also likely to be relevant to infection. Results presented here indicate that DepTR-5 persists in the kidney over a six-day infection period (Fig. 6), indicating that these bacteria survive in the infected host. A recent study identified a FASII defect among clinical S. aureus small colony variants (SCV). SCV growth was restored to normal by fatty acid supplementation55, strongly pointing to a DepTR-like phenotype and implicating FASII bypass in chronic infection. Similar findings were reported for the opportunist pathogen Enterococcus faecalis66. Abscess formation during S. aureus infection is common, and it is tempting to speculate that this stage of infection may give privileged access to host fatty acids that support growth of both CondTR and DepTR mutants.

Fatty-acid-rich skin56,57, possibly enriched by fatty-acid-containing skin products58, may be an ideal medium on which FASII inhibitors would select for FASII-bypass-competent strains. The selection strategy used in our study mimicked this situation. Emergence of versatile S. aureus FASII bypass variants upon anti-FASII treatment may generate a long-term risk for persistence and infection59, requiring new rounds of antimicrobial therapy.

Methods

Bacterial strains and growth conditions. Experiments were performed using the S. aureus Newman strain60, SA40 (an Asian-Pacific strain from MRA isolate from a healthy child) and SA957 (a virulent Thai strain) were kindly provided by Friedrich Götz and Regine Stechmiller (U. Tübingen, Germany)65. The M0251 (MRSA, clonal complex CC5) and M1532 strains are respectively nasal and sputum isolates that were generously provided by Mary Delaney and Michael Calderwood, and are part of the Brigham and Women’s Hospital collection (Boston, USA). Plasmid cloning and extraction were performed in E. coli strains Top10 (Invitrogen, France) and MG1655 (ref. 61). BHI and LB media were used for S. aureus, and LB for E. coli growth. The fatty-acid mixture (referred to as ‘FA’) comprises C14:0 (myristic acid), C16:0 (palmitic acid), and C18:1 (oleic acid); 100 mM fructose and 0.5 g sodium sulphate (DNS) was added to each fatty acid with a 1:1:1 ratio, and then diluted 1:2000 for use in liquid or solid medium. Other fatty acid mixtures were prepared similarly, such that the final molar concentration of fatty acids was 0.5 mM (that is, 0.17 mM each fatty acid). All fatty acids were from Larodan Fine Chemicals (Sweden). Newborn calf serum (Sigma-Aldrich, France) and delipidated calf serum (Eurobio, France) were added to growth medium (10% final concentration) as indicated. Triclosan (Irgasan; Sigma-Aldrich) was routinely used at 0.25 µg ml−1 in liquid cultures; this is 15 times the MIC for S. aureus Newman strain in BHI medium. Platensimycin (Tebu-Bio, France) and AFN-1252 (MedChem Express) were used respectively at 2 gm l−1 and 0.5 gm l−1 for S. aureus Newman strain in BHI medium. Phenytoin (DepTR-5). Cells were grown in the same medium to OD600 = 0.05, in the following media: (i) LB containing 3 µg ml−1 Cm and 20 gm l−1 triclosan. Growth was monitored in three independent experiments (ii) compared with DepTR-5 (p) and, of CondTR-17 (p) compared with CondTR-17 (p) were determined as a ratio in individual experiments, and plotted as the ratio of the OD600 values at different time points (Supplementary Fig. 5).

WT and fabD mutant resistance to FASII inhibitors. WT, DepTR-5 and CondTR-17 strains were grown overnight in BHI-FA, washed twice in BHI, and 100 µl of OD600 = 0.1 bacterial suspension were plated on solid BHI or BHI-FA medium to obtain a lawn. Triclosan (1.5 µg in 3 µl), AFN-1252 (1 µg in 3 µl), and platensimycin (0.5 µg in 4 µl) were spotted immediately after plating. Plates were incubated at 37 °C and photographed after 48 h. Colonies growing within the inhibition zone of the anti-FASII drugs were patched to BHI, or to BHI-FA medium with or without inserts were transformed by electroporation into S. aureus strain RN4220 (ref. 60), re-extracted and transformed into Newman WT and fabD mutants. Standard electroporation protocols were followed62, except that DepTR-5 competent cells were prepared in BHI medium containing 10% heat-inactivated newborn calf serum and FA.

DNA sequencing. The fabI, abcABC and fabD genes of selected S. aureus Newman strain FA-10 clones were sequenced. Primer pairs used for DNA amplification were: for fabI: FabId 5′-GATACAGAAGGCTACATTTACAA-3′ and FabId 5′-TTTCTTACGTCTCAGGATATTATA-3′ (ref. 20); for accDA: AccDAid 5′-AACAATGTTATGATGTTGAAG-3′ and AccDArev 5′-AAATCCACCGCTCAACAGG-3′; for accBC: two sets were used AccBCid 5′-ACCGGTATAGGAAACAAACAC-3′ and AccBCrev 5′-TCTTTCTTACCATCAGGCAA-3′, and AccBC2rev 5′-CAGCATGATCCTGCAGTTCC-3′; for fabD: FabDId 5′-GAAGAGTCAGTAGTTAAGAACCACG-3′ and FabDrev 5′-GCTTGTATCTTCTGACATCGTTC-3′. Whole genome sequencing was done on DepTR-5 isolates DepTR-5, DepTR-5, and CondTR-17, and our parental Newman strain. Libraries were prepared with genomic DNA using the Illumina MiSeq next generation sequencing system (IMGM, Germany). The 2 × 250 base pair reads were aligned and sequence variations were analysed using CLC Genomics Workbench 5.5.2 software (CLC Bio). SNPs were found when comparing our Newman strain to that in the Newman reference sequence (GenBank Nucleotide accession code NC_009641). Only differences between our Newman strain and FA-T mutants were considered in genome comparisons.

Cloning of fabD and acp. The fabD gene (locus NWNN-1140) and the fabD50 variant gene were PCR-amplified from chromosomal DNA prepared from the Newman strain using the forward primer fabD-5′-GGGGGATTCCACTAATGAGTAAAACAGCAATTATT-3′ and reverse primer fabD-3′ (5′-GGGCGGATATTACCCCTCAGTAAAGGTTATAGTTTTTCTACTCATTCCTTACAC-3′). Amplified DNA was cloned by Gibson assembly63. The vector, pBluescribe-pET-βLac (Invitrogen, France), was transformed into E. coli Top10, and the resulting plasmid was sequenced. The fabD gene was amplified from chromosomal DNA of S. aureus strains using the primers fabD-l and fabD-r (5′-GCAAGTATGTTACGTTTGATTTTGCTCTGGG-3′ and 5′-GGGAGTACCCGCCGCTAT-3′, respectively). The amplicons were cloned in pET28a(+) (Novagen, Madison, WI, USA) and sequenced.

Selection for triclosan resistance on FA plates. Dilutions of overnight S. aureus Newman cultures were plated on four types of medium: non-selective (BHI); BHI plus FA (BHI-FA; FA are as described above); BHI with triclosan (BHI-T (0.25 µg ml−1)), BHI containing FA and triclosan (BHI-FA-T (0.25 µg ml−1)). Plates were incubated at 37 °C and scored after 24 h. Colonies were selected for phenotypic confirmation and characterization.

 Colony forming units (CFU) on plates were compared with determine FA-TR frequencies. Colonies that appeared on BHI-FA-T plates were then patched on the plates with different fatty acid mixtures. Proportions of DepTR and CondTR mutants varied in independent experiments, possibly suggesting that slight variations in conditions may impact mutant phenotypes. Changes in fatty acid profiles of some FA-TR isolates were observed upon FA-T selection, particularly after storage, suggesting that adaptation might occur during selection and/or storage. Care was therefore taken in analysing profiles directly from selective plates and before storage.

Determination of fatty acid profiles. Whole cell esterified fatty acid determinations were done essentially as described62. Briefly, 1–2 ml S. aureus cultures (OD600 = ≥ 1) were centrifuged, washed once in 0.9% NaCl containing 0.02% Triton X-100, then washed twice in 0.9% NaCl. Cell pellets were treated with 0.5 ml of 1 N sodium methoxide. Heptane (200 µl) was then added, together with methyl-10-undecenoate (Sigma-Aldrich) as internal standard, vortexed for 1 min, and centrifuged. Fatty acid methyl esters were recovered in the hexane phase. GC analyses were performed in a splitless-injection mode on an AutoSystem XL Gas Chromatograph (Perkin-Elmer) equipped with a ZB-Wax capillary column (30 m × 0.25 mm × 0.25 µm; Phenomenex, France). Data were recorded and analysed by TotalChrom Workstation (Perkin-Elmer). S. aureus fatty acid peaks were detected between 12 and 30 min of elution.
resuspended in 0.9% NaCl to obtain 2 × 10^6 cells per mL, as confirmed by plating on BHI-FA plus 1% serum solid medium. Six-week female BALB/c mice (Janvier Labs, France) were injected in the tail vein with 10^6 cells in 500 µL 0.9% NaCl (8–11 animals per group). Animals were sacrificed at day 2 and day 6 (4–6 animals per time point) and dissected. Liver and kidneys were suspended in phosphate-buffered saline and homogenized with an ultra-turrax. CFUs were determined per organ type on BHI-FA plates containing 10% serum. DepT5–8 and CondT17 strains, each mutant per organ, isolated from day 6 platings, were randomly selected to verify fatty-acid-dependent and conditional phenotypes; conservation of the mutated alleles was further confirmed in two clones from each organ by PCR-sequencing. The presented data are pooled from animal studies performed as two independent experiments.

**Statistical analyses.** The Mann–Whitney U-test was used to evaluate whether differences in CFUs in mice infected by Newman or mutant derivatives were statistically significant. Differences were compared for day 2 or day 6 between Newman and mutant derivatives. Analyses were performed with GraphPad Prism Software Version 6.00 (GraphPad Software, USA). Statistical significance was given as P values.

**Growth capacity of fabD clinical variants and mutants on FA-triclosan.** Precultures of Newman, M0251, M1532, SA957, SA40, CondT, and CondT17 strains were prepared in BHI medium, and used to inoculate BHI or BHI-FA-T medium at an initial OD_{590} = 0.01. Cell density was determined as OD_{590} measurements after 16 h growth. FA profiles were determined for cultures that reached OD_{590} values above 0.5, which was defined as the growth threshold. Experiments were repeated three times.

**ACP assessment by conformation-sensitive gels.** Anti-S. aureus-ACP antibodies were prepared using ACP purified from E. coli TG1 carrying pMalCACP-ACP. For this purpose, cultures were grown in LB ampicillin (100 µg mL^-1) and ACP-MBP expression was induced by IPTG 0.5 mM for 2 h during mid-exponential growth. The ACP-MBP fusion was recovered on a maltose resin and eluted with 20 mM maltose. The protein fusion was digested with Xa protease as per manufacturer’s instructions (New England Biolabs). Purified ACP was recovered from polyacrylamide gel electrophoresis–SDS (PAGE–SDS) gels and used for rabbit antibody preparation (Covalabs). The antibody preparation was validated on purified ACP protein, and used at 1:15,000 dilution. Conformation-sensitive (non-denaturing) gels were done as described. Precultures of test strains were grown to OD_{590} = 0.5 in BHI medium at 37°C. They were then split in equal aliquots, and further grown for 4 h in BHI or BHI-FA-T. Cultures were harvested for preparation of protein extracts as described from the equivalent of OD_{590} = 5. DTT-treated extracts from BHI-grown S. aureus Newman were used as reference for the position of holo-ACP. Precultures (equivalent to OD_{590} = 0.25 in 10 µl) were loaded on 13% polyacrylamide gels containing 1 M urea, which were run at 100 volts for 3 h, and then transferred to PVDF membranes (0.2 µm: BioRad) for Western blotting and exposure using an ECL kit (Perkin-Elmer) as per supplier’s instructions.

**Ethics statement.** Animal experiments were performed at the animal facilities of HUPC in strict accordance with European Union guidelines for handling of laboratory animals, approved by the Ethics Committee for Animal Experimentation at the University of Paris-Des cartes (CEEA 34), registration number 14-077.

**Data availability.** Genome sequence data for DepT5, DepT8, DepT17, and our parental Newman strain have been deposited in the European Nucleotide Archive and can be accessed at http://www.ebi.ac.uk/ena/data/view/PRJEB11476. The authors declare that all other data supporting the findings of this study are available within the article and its Supplementary Information files, or from the corresponding author upon request.

**References**

1. Lowy, F. D. Methicillin-resistant Staphylococcus aureus: where is it coming from and where is it going? JAMA Internal Med. 173, 1978–1979 (2013).
2. Uhlemann, A. C., Otto, M., Lowy, F. D. & DeLeo, F. R. Evolution of community- and healthcare-associated methicillin-resistant Staphylococcus aureus. Nat. Rev. Microbiol. 21, 563–574 (2017).
3. Banevicius, M. A., Kaplan, N., Hafkin, B. & Nicolau, D. P. Pharmacokinetics, pharmacodynamics and efficacy of novel FabI inhibitor AFN-1252 against MSSA and MRSA in the murine thug infection model. J. Chemother. 25, 26–31 (2013).
4. Escaich, S. et al. The MUT05639 inhibitor of FabI is a new antistaphylococcal compound. Antimicrob. Agents Chemother. 55, 4692–4697 (2011).
5. Feng, Z., Chakraborty, D., Dewell, S. B., Reddy, B. V. & Brady, S. F. Environmental DNA-encoded antibiotics fasamycins A and B inhibit FabI in type II fatty acid biosynthesis. J. Am. Chem. Soc. 134, 2981–2987 (2012).
6. Moir, D. T., Opperman, T. J., Butler, M. M. & Bowlin, T. L. New classes of fatty-acid-dependent and conditional phenotypes; conservation of the mutated alleles was further confirmed in two clones from each organ by PCR-sequencing. The presented data are pooled from animal studies performed as two independent experiments.

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36. Ni Raghallaigh, S., Bender, K., Lacey, N., Brennan, L. & Powell, F. C. The fatty acid profile of the skin surface lipid layer in papulopustular rosacea. Br. J. Dermatol. 166, 279–287 (2012).
37. Greenway, D. L. & Dyke, K. G. Mechanism of the inhibitory action of linoleic acid on the growth of Staphylococcus aureus. J. Gen. Microbiol. 115, 233–245 (1979).
38. Parsons, J. B., Yao, J., Frank, M. W., Jackson, P. & Rock, C. O. Membrane disruption by antimicrobial fatty acids releases low-molecular-weight proteins from Staphylococcus aureus. J. Bacteriol. 194, 5294–5304 (2012).
39. Schluepen, C. et al. Mining the bacterial unknown proteome: identification and characterization of a novel family of highly conserved proteomic antigens in Staphylococcus aureus. Biochem. J. 455, 273–284 (2013).
40. Krishnamurthy, M., Tadesse, S., Rothenhaus, K. & Graumann, P. L. A novel SMC-like protein, SbcE (YhaN), is involved in DNA double-strand break repair and competence in Bacillus subtilis. Nucleic Acids Res. 38, 455–466 (2010).
41. Hong, S. K. et al. New design platform for malonyl-CoA acyl carrier protein transacylase. FERS Lett. 584, 1240–1244 (2010).
42. Serre, L., Verbree, E. C., Dauter, Z., Stutjte, A. R. & Derewenda, Z. S. The Escherichia coli malonyl-CoA acyl carrier protein transacylase at 1.5-A resolution. Crystal structure of a fatty acid synthase component. J. Biol. Chem. 270, 12961–12964 (1995).
43. Parsons, J. B., Frank, M. W., Jackson, P., Subramanian, C. & Rock, C. O. Incorporation of extracellular fatty acids by a fatty acid kinase-dependent pathway in Staphylococcus aureus. Mol. Microbiol. 92, 234–245 (2014).
44. Haifkin, B., Kaplan, N. & Murphy, B. Efficacy and safety of AFN-1252, the first FabI inhibitor, in the presence of serum and in combination with other antimicrobials. Methods Enzymol. 459, 273–284 (2013).
45. Chen, C. J. et al. Characterization and comparison of 2 distinct epidemic community-associated methicillin-resistant Staphylococcus aureus clones of ST359 lineage. PLoS One 8, e63210 (2013).
46. Gronan, J. E. & Thomas, J. Bacterial fatty acid synthesis and its relationships to membrane lipid homeostasis in Streptococcus agalactiae. J. Biol. Chem. 282, 1858–1865 (1991).
47. Zhang, Y. M. & Rock, C. O. Membrane lipid homeostasis in bacteria. Nat. Rev. Microbiol. 6, 222–233 (2008).

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Author contributions
C.M., D.H., G.K., J.A., C.P. and P.T.-C. designed experiments. C.M., D.H., G.K., J.A., C.H., S.B., S.K., G.L., K.G. and A.G. performed experiments. C.M., D.H., G.K., J.A., P.T.C., C.P., G.L., K.G. and A.G. supervised the project. A.G. supervised the project. C.M., D.H., G.K., J.A., P.T.C., C.P., G.L., K.G. and A.G. designed experiments. C.M., D.H., G.K., J.A., C.H., S.B., S.K., G.L., K.G. and A.G. performed experiments. C.M., D.H., G.K., J.A., P.T.C., C.P., G.L., K.G. and A.G. supervised the project.

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