Bacterial adaptation strategies to host-derived fatty acids

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Abstract

Fatty acids (FAs) are potent antimicrobials, which hold great promise as viable alternatives or complements to conventional antibiotics. Intriguingly, bacteria are well equipped to use environmental FAs as energy sources and/or building blocks for their membrane lipids. Furthermore, these microbes display a wide array of mechanisms to prevent or mitigate FA toxicity. In this review, we discuss strategies that bacteria use to thrive despite extensive exposure to host-derived antimicrobial FAs. We also highlight the altered response of these FA-adapted bacteria to antibiotics. Given the ubiquitous nature of FAs in various host environments, deciphering bacterial adaptation strategies to FAs is of prime importance. This knowledge may pave the way for a rational design of FA-based combination therapies with antibiotics.

~~Antimicrobial~~Host-derived fatty acids: ~~potentially toxic~~ energy sources with antibacterial properties

The widespread expansion of multidrug-resistant bacteria and the paucity of new antibiotics have reignited scientific interest in non-conventional ~~antimicrobials~~antibiotics such as fatty acids (FAs). Indeed, a wide range of organisms (plants, nematodes, insects, mice, and humans) use antimicrobial FAs ~~(**Figure 1**)~~ to directly inhibit the proliferation of bacterial pathogens or activate host innate immune responses [1-5]. Importantly, bacteria, as well as their hosts, utilize FAs as energy sources or building blocks for their membranes. Hitherto, antibacterial activities have been reported for FAs with at least eight carbon atoms (medium-chain and long-chain FAs) [6]. Strikingly, for FAs with more than 16 carbons, antibacterial potency tends to increase with unsaturation (detailed in **Box1**). Polyunsaturated long-chain FAs, which most pathogenic bacteria are unable to synthesise, are potent host-derived antimicrobials [6-10]. It is not yet well understood how FAs inhibit bacteria. However, these mechanisms, which include membrane disruption and oxidative stress, appear to be concentration-dependent [6-9].

The appeal of FAs as ~~alternative~~ antimicrobials is boosted by their virulence- and biofilm-inhibitory capabilities, which have been reviewed elsewhere [11]. However, recent studies on critical human pathogens *Staphylococcus aureus* and *Streptococcus pneumoniae* have revealed that these Gram-positive bacteria express highly selective proteins to acquire host-derived FAs and incorporate these exogenous FAs into their membrane phospholipids [12, 13]. Moreover, major research on *Vibrio cholerae* has unveiled a cholera toxin-dependent increase in the concentration of long-chain FAs in the intestinal lumen of infected mice or rabbits, which in turn promotes bacterial growth [14]. The aforementioned examples show that bacteria are frequently exposed to

exogenous FAs. This review details our current understanding of bacterial responses to host-derived FAs (**Figure ~~2~~1**, **Key Figure**), and their possible implications for antibiotic susceptibility and discovery.

Bacterial metabolism of host-derived FAs

FA synthesis is widely conserved amongst eukaryotes and prokaryotes. It requires, however, a single, multifunctional enzyme in animals and fungi (type I fatty acid synthase) or multiple enzymes organized into a functional type II fatty acid synthase (FASII) in plants and most bacteria. FASII generates various acyl ~~groups bound individually to acyl~~ carrier protein (ACP~~) and ready to~~)-bound acyl groups, which can be used for phospholipid synthesis [15]. Considering the sophisticated machinery and the energy cost that are associated with FA synthesis, it is not surprising that all bacterial species studied to date have developed a specialized system to gain FAs directly from their environment [12, 13, 15, 16]. These exogenous FAs are activated prior to their degradation by β-oxidation or incorporation into membrane phospholipids.

Gram-negative bacteria require the outer membrane protein FadL for the translocation of exogenous long-chain FAs across the lipid bilayer of the outer membrane [17]. An undefined mode of transfer allows FAs to traverse the periplasm and enter the inner membrane bilayer, where the fate of exogenous FAs depends on the Gram-negative bacterial species. In Gammaproteobacteria, such as *Escherichia coli* and *Pseudomonas aeruginosa*, FadD fatty acyl-CoA synthetases convert free FAs into acyl coenzyme A (acyl-CoA). Other Gram-negative bacteria rely on acyl-ACP synthetases to activate exogenous FAs to acyl-ACP [15, 16, 18]. Despite its clear role for nutrient acquisition within the host [19-21], FA degradation in Gram-negative bacteria remains

poorly explored as a resistance strategy against toxic FAs. It was recently shown that β-oxidation was pivotal to *Acinetobacter baumannii* growth in the presence of polyunsaturated FAs [9]. By contrast, within-host evolution studies in patients with chronic obstructive pulmonary disease have revealed that *Haemophilus influezae* tended to inactivate FadL to resist host-derived antimicrobial FAs during lung adaptation [22].

Thus far, the mechanism by which Gram-positive organisms, such as *S. pneumoniae* and *S. aureus*, acquire and activate exogenous FAs appears to allow for highly selective acquisition of various types of FAs. Gram-positive bacteria utilise the fatty acid kinase system (FakAB) to generate acyl-phosphate from FAs. FakB is a membrane-tethered surface protein that recruits exogenous FAs for delivery to the membrane-embedded kinase FakA [23]. In *S. aureus*, FakB1 is responsible for the acquisition of saturated fatty acids, whereas FakB2 for unsaturated fatty acids [12]. *S. pneumoniae* employs three distinct FakB members, namely FakB1, FakB2 and FakB3 that allow for the selective acquisition of saturated, mono-unsaturated and poly-unsaturated FAs, respectively [13].

The Fak-mediated utilization of host-derived ~~FA~~FAs has been demonstrated for *S. aureus* in a thigh infection murine model, where deficiency in exogenous FA incorporation (Δ*fakA* or Δ*fakB1* Δ*fakB2* mutant) does not affect staphylococcal proliferation [24]. However, compared to isogenic wild-type strains, *S. aureus* mutants defective for unsaturated FA acquisition are outcompeted [25] or less abundant [26] in livers~~, but not kidneys,~~ of systemically infected mice. Moreover, the Δ*fakA* mutant provokes larger lesions than the wild type during murine skin infection [27]. These seemingly contradictory phenotypes stem, at least partly, from the pleiotropic effects caused by *fakA* inactivation in *S. aureus* [23, 28]. These effects include the increased

expression of the FA efflux pump FarE [29], and heightened resistance to antimicrobial FAs [29, 30]. Thus, ~~it is still unclear if FA~~whether the incorporation ~~*per se* protects~~of antimicrobial FAs by *S. aureus* ~~against toxic FAs.~~ is a resistance strategy is still unclear.

Members of the order, Lactobacillales, which include lactobacilli and streptococci, utilize the transcriptional repressor FabT to potently restrict endogenous FA synthesis in response to activated exogenous FAs [15]. Interestingly, *fabT*-defective mutants often arise during streptococcal infections [31]. Growth in rich media has revealed that ~~*fabT*the~~ deletion of *fabT* rendered lactobacilli and *S. pneumoniae* resistant to antimicrobial FAs [32] and FA analogues [33], respectively. However, it was shown in *S. pneumoniae* that a Δ*fabT* mutant failed to inhibit ~~*fakB3*~~the transcription of *fakB3* in the presence of inhibitory concentrations of exogenous FAs [33], in clear contrast to the wild-type strain [8, 33]. Thus, it appears that these bacteria either ~~deactivate~~inactivate *fabT* or inhibit the activation of antimicrobial FAs. Both strategies enable the bacteria to derepress endogenous FA synthesis and restrict the accumulation of toxic amounts of unsaturated FAs within their membranes.

Bacterial barriers to antimicrobial FAs

The susceptibility of bacterial pathogens to host-derived, antimicrobial FAs greatly depends on the architecture of their cell surface. In many bacteria, the capsule is the outermost bacterial compartment, which interacts with the host environment. Owing to the presence of polysaccharides, the capsule is hydrophilic. Therefore, it is not surprising that capsules protect bacteria against hydrophobic, antimicrobial FAs, as shown in *S. aureus* [34] (**Figure ~~3~~2**~~). It is puzzling, however, that~~). Although *S. aureus*

strongly upregulate its capsule biosynthesis genes in response to long-chain FAs [22, 35], the impact of ~~antimicrobial~~these FAs on capsule production and function at the host–pathogen interface ~~has received so little attention, considering that bacteria strongly upregulate their capsule biosynthesis genes in response to long-chain FAs [22, 35].~~ is largely unexplored.

In Gram-positive bacteria, the cell wall has been characterized as a barrier against toxic FAs in addition to the capsule. Indeed, by virtue of their hydrophilicity, cell wall-associated glycopolymers, termed wall teichoic acids, ~~prevent FA binding to *S.*~~hamper the diffusion of FAs through the cell wall and their subsequent binding to the membrane of *S. aureus* [36] (**Figure ~~3~~2**). Accordingly, the genetic [7, 22, 36] or chemical [7] inhibition of wall teichoic acids renders *S. aureus* more susceptible to FAs. In addition to glycopolymers, Gram-positive bacteria utilize cell wall-anchored proteins to modulate their surface hydrophobicity. In *S. aureus*, the iron-regulated surface determinant A (IsdA) is the most abundant surface protein in iron-limiting conditions, which are reminiscent of the host environment. IsdA decreases bacterial hydrophobicity, which precludes FA binding [5] (**Figure ~~3~~2**). *S. aureus* SasF [35] and *S. saprophyticus* SssF [37] are additional cell wall proteins that mediate resistance to FAs by hitherto unknown, hydrophobicity-independent mechanisms (**Figure ~~3~~2**).

In Gram-negative bacteria, the outer membrane represents a permeability barrier to antimicrobial FAs. The outer membrane of most Gram-negative bacteria is an asymmetric bilayer ~~with~~composed of phospholipids in the inner leaflet and lipopolysaccharides (LPS) in the outer leaflet. An LPS macromolecule comprises lipid A, the core oligosaccharide, and the O-antigen. Anchored to the outer membrane by hydrophobic lipid A, LPS protrudes into the environment via its hydrophilic, oligosaccharide core that is connected to a component composed of repeating

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oligosaccharides (O-antigen). The extensive hydrophilic character provided to LPS by its sugar units renders the outer membrane particularly impermeable to hydrophobic FAs, which could explain why most Gram-negative bacteria are not susceptible to FAs. Accordingly, FAs display bactericidal activity against some Gram-negative bacteria (*Chlamydia trachomatis*, *Haemophilus influenzae*, *Neisseria gonorrhoeae*) whose LPS, referred to as lipooligosaccharides (LOS), lack O-antigens [6]. In *N. meningitidis*, mutants with a truncated or deleted LOS are more susceptible to FAs than otherwise isogenic wild-type strains [38]. Importantly, despite similarities in their LOS architecture, *N. gonorrhoeae* and *N. meningitidis* are not equally susceptible to FAs, with *N. meningitidis* being more resistant to FAs than *N. gonorrhoeae* [16, 38]. This discrepancy highlights the importance of structures other than LOS or LPS for bacteria to resist antimicrobial FAs (e.g.: efflux pumps).

The role of efflux systems in resistance to antimicrobial FAs

When antimicrobial compounds have accumulated at levels beyond the inhibitory threshold concentration, efflux into the extracellular milieu can provide an efficient means of detoxification. Antimicrobial FAs are no exception, as efflux systems from various organisms have been demonstrated to export these molecules across the bacterial membrane(s) thereby lowering their intracellular levels to promote cell survival. The primary efflux systems involved in this process are members of the resistance-nodulation-cell division (RND) superfamily. RND efflux systems are large protein complexes that consist of three subunits in Gram-negative bacteria: the inner membrane protein (three protomers per functional complex), the outer membrane β-barrel (three protomers per functional complex) and the periplasmic adaptor proteins

(highly variable number of protomers per complex) [39]. The inner membrane protein subunit dictates substrate specificity and mediates active substrate translocation from the cytoplasmic and/or periplasmic sides of the inner membrane. Hence, Gram-positive organisms often only express this component of the RND complex. The RND superfamily of transport systems consists of various subfamilies, such as the lipid transporters of the *Mycobacterium* membrane protein large (MmpL) subfamily [40]. Mycobacterial species utilise these systems to biosynthesise their complex pseudo-outer membrane and they generally encode various MmpL members per genome (up to 14). However, the MmpL-like efflux systems are widely distributed throughout other bacterial species and may assist in antimicrobial FA resistance, as observed for FarE in *S. aureus* [41] (**Figure ~~3~~2**). Additionally, various RND efflux systems with classical multidrug efflux capabilities (i.e., members of the hydrophobe/amphiphile efflux-1 [HAE-1] subfamily) enable the bacterial cell to resist antimicrobial FAs. This includes MtrCDE from *Neisseria gonorrhoea* [42] and AcrABC from *Bordetella* species [43]. *N. gonorrhoea* is susceptible to palmitic acid, an FA found in high abundance in the pathogen's primary environment, the urogenital tract. MtrCDE, in combination with an FA-specific efflux system, FarAB, from the Major Facilitatory Superfamily (MFS) [42, 44, 45], allows *N. gonorrhoea* to survive and mediate disease in this environment. Similarly, palmitic acid toxicity is alleviated by FarAB in *N. meningitidis* [38], an obligate human bacterial commensal that colonizes the nasopharynx of one in ten otherwise healthy individuals. Another natural inhabitant of the human nose, *S. aureus*, is also commonly exposed to antimicrobial FAs~~, major components of the host defence arsenal.~~. In addition to FarE, the bacterium utilises the MFS efflux pump Tet38 to limit the cellular accumulation of toxic FAs [41, 46] (**Figure ~~3~~2**).

Noteworthily, AdeIJK from *A. baumannii* was found to provide resistance to antimicrobial long-chain polyunsaturated FAs, but subsequent FA accumulation assays revealed that ~~antimicrobial FA resistance~~this was not a result of their direct efflux. Instead, AdeIJK was shown to contribute to the efflux of endogenous FAs and membrane lipid homeostasis [9]. The role of AdeIJK in FA resistance is therefore indirect. In addition to altering the membrane phospholipid composition, AdeIJK modulates other physiological processes, such as biofilm formation and surface motility, in which the expelled FAs may participate [9]. The role of AdeIJK in the efflux of endogenous FAs is reminiscent of other efflux pumps, as previously reported for AcrAB-TolC from *E. coli* or *Salmonella enterica*, and EmrC from *Pseudomonas fluorescens* [47-51]. These systems contribute to bacterial lipid homeostasis by exporting endogenously generated free FAs, but whether these systems play a role in antimicrobial FA resistance has yet to be studied. Overall, considering their broad substrate range and localisation in the bacterial membrane, efflux systems are key facilitators of antimicrobial FA resistance. However, there is a critical need to consider their endogenous functions, including lipid homeostasis, when interpreting their roles in antimicrobial resistance, as illustrated in the case of AdeIJK and the well-studied multidrug efflux system QacA from *S. aureus* [52].

Bacterial detoxification of antimicrobial FAs

To prevent the detrimental accumulation of unsaturated, antimicrobial FAs, bacteria are equipped with enzymes that detoxify FAs. Of these enzymes, only fatty acid-modifying enzyme (FAME) and oleate hydratase have been characterized to date. ~~Additionally, LoxA, a lipoxygenase secreted by *P. aeruginosa*, oxidises~~

~~polyunsaturated FAs to interfere with lipid-mediated host signalling. By decreasing FA unsaturation, LoxA potentially reduces FA toxicity.~~

Fatty acid-modifying enzyme (FAME)

~~*S. aureus* strains that are able to replicate in kidneys or survive within intraperitoneal abscesses in murine infection models also exhibit high FAME activities *in vitro* [53].~~ FAME esterifies unsaturated FAs to certain alcohols, with a preference for cholesterol. Esterified FAs display a markedly decreased bactericidal effect against *S. aureus* (**Figure ~~3~~2**). FAME activity was initially observed in *S. aureus* culture filtrates [53]. While filtrates of several coagulase-negative staphylococci (CoNS; e.g.: *S. epidermidis*, *S. hominis* and *S. capitis*) were subsequently also shown to esterify FAs [54], the protein(s) responsible for this esterification (FAME activity) remained elusive for more than two decades. Recently, *S. aureus* secreted lipases Sal1 and Sal2 were uncovered to moonlight as potent esterases that are strongly reminiscent of FAME [55]. Although this finding is in accordance with the previous observation that lipases and FAME often co-occur in *S. aureus* [54], it is yet to be demonstrated whether FAME activity is compromised in the absence of Sal1 and/or Sal2. Furthermore, several CoNS show FAME activity in the absence of any detectable lipase [54], suggesting that ~~FAME is not yet fully understood.~~the protein(s) with this enzymatic activity may vary among staphylococcal species. Still, there is mounting evidence that lipases play a central role in host-bacteria interactions. For instance, following intracellular proliferation, *Vibrio parahaemolyticus* utilizes its secreted lipase VPA0226 to esterify host-derived polyunsaturated FAs to membrane cholesterol, thereby weakening the membrane of the invaded cell, which promotes the subsequent egress of the bacterium [56].

Oleate dehydratases (OhyAs)

OhyAs belong to a family of enzymes that are widely distributed amongst bacteria, which catalyse the hydration of double bonds in unsaturated FAs. Most OhyAs exclusively detoxify FAs containing *cis*-9 double bonds. However, some OhyAs, such as that of *Streptococcus pyogenes* ~~OhyA~~ (formerly referred to as myosin cross-reactive antigen), catalyse the hydration of *cis*-9 and *cis*-12 double bonds [57]. In the case of *S. pyogenes* ~~and~~or *S. aureus*~~, two major human pathogens~~, the deletion of *ohyA* increases bacterial susceptibility to FAs with *cis*-9 double bonds [57, 58]. Since *S. aureus* is unable to synthesise unsaturated FAs, the substrates of its OhyA are environmental, unsaturated FAs that are metabolized into less toxic, hydroxy-FAs, which are not used by the bacterium for membrane phospholipids~~, suggesting that~~. OhyA activity ~~is~~appears to be primarily against exogenous, antimicrobial FAs [58]. Intriguingly, as a plausible countermeasure, humans are the only mammals able to produce an antimicrobial, *cis*-6 FA (sapienic acid), against which OhyAs are inefficient. *S. aureus* ~~upregulates~~induces the expression of *ohyA* ~~transcription~~ in response to *cis*-6 [22] or *cis*-9 [58] FA exposure, suggesting that bacteria sense toxic, *cis*-6 or *cis*-9 FAs similarly to activate FA resistance strategies. These strategies also include the release of membrane vesicles (MVs), which act as decoys to protect bacteria against antimicrobial FAs, including sapienic acid. Interestingly, OhyA is abundant in MVs released in the presence of *cis*-9 FAs [59]. It is yet unclear whether MV-associated OhyA can detoxify exogenous FAs. Regardless, microbiota-derived hydroxy-FAs possess anti-inflammatory properties [60-62], suggesting that bacteria hijack antimicrobial FAs to manipulate their host.

Lipoxygenase LoxA

Lipoxygenases~~,~~ are lipid-oxidising enzymes, ~~are rare~~which rarely occur in bacteria. For instance, *P. aeruginosa* is currently the only opportunistic human pathogen known to possess a lipoxygenase (LoxA). LoxA converts various host-derived, unsaturated FAs into hydroperoxy derivatives, which can be further metabolized to bioactive lipid mediators with potent immunoregulatory properties such as lipoxins, and resolvins [63, 64]. Therefore, bacteria are thought to secrete LoxA to manipulate the host environment and cause inflammation. Strikingly, LoxA expression, which is rather low in *P. aeruginosa* grown in rich media devoid of polyunsaturated FAs, is enhanced within biofilms [65] or when planktonic growth temperature is decreased from 37 °C to 28°C [63]. This suggests that LoxA production is tightly controlled by certain environmental cues. However, it remains to be investigated if *P. aeruginosa* tailors LoxA expression in response to host-derived FAs to manipulate its niche, where recombinant LoxA was recently shown to promote bacterial persistence in a pneumonia mouse model [63]. Besides, by targeting FA *cis*-double bonds, LoxA potentially decreases FA toxicity towards *P. aeruginosa*.

Global bacterial responses to antimicrobial FAs

Aided by numerous strategies employed to prevent host-derived FA binding or expel/detoxify bound FAs, bacteria can replicate in the presence of ~~a~~ subinhibitory ~~concentration~~concentrations of FAs. However, it is becoming apparent that growth in FA-~~containing~~supplemented media or FA-rich host environments elicits a strong transcriptional reprogramming in bacteria (e.g.: one in two transcripts is differentially expressed in *S. aureus* [22]), which greatly alters their pathogenicity. Since FA-mediated inhibition of bacterial virulence was recently reviewed elsewhere [11], this

296 section only includes instances where bacterial adaptive responses to FAs promote
297 virulence.

298 ***Virulence induction in response to antimicrobial FAs***

299 By virtue of their antimicrobial and immunomodulatory properties, free FAs
300 ~~are~~~~constitue~~constitute key components of the innate immune system. Notably, some
301 pathogens utilize host-derived FAs as cues to fine-tune their virulence factors. For
302 instance, in response to certain long-chain FAs, the swarming migration of *Proteus*
303 *mirabilis* was enhanced [66], which is known to improve its colonization capacity.
304 Similarly, *P. aeruginosa* preferentially migrates towards long-chain FAs or FA-rich
305 human bronchoalveolar lavage fluid (BALF) [67]. Upon incubation with mouse BALF,
306 *P. aeruginosa*~~,~~ exhibits an upregulation of genes involved in the digestion of FAs [68].
307 The expression of these genes is pivotal for bacterial virulence as assessed in a
308 murine acute pneumonia model [68]. Furthermore, *P. aeruginosa* upregulates
309 ~~T3SS~~the genes of the type 3 secretion system and other virulence determinants upon
310 serial passages in *Galleria mellonella* larvae or rich media supplemented with
311 polyunsaturated FAs to mimic the haemolymph of these larvae [69]. These passages
312 induce a robust virulence phenotype without genetic variations, but an altered
313 transcriptional landscape, suggesting a programmed response to FAs. Interestingly,
314 FA-enhanced bacterial virulence persists for many generations, even in the absence
315 of polyunsaturated FAs [69].

316 The type VII secretion system (T7SS) is critical for *S. aureus* virulence in murine
317 models of pneumonia [70] and systemic infection [26]. Importantly, *S. aureus* activates
318 its T7SS, when grown in rich media supplemented with antimicrobial FAs [10, 26, 35,
319 70] or FA-rich bovine pulmonary surfactant [70]. This T7SS activation is attributed to

the FA-mediated inhibition of the SaeRS two-component system~~. In contrast to~~, which represses T7SS~~,~~ while activating many virulence factors, including α-hemolysin toxin and the immune evasion proteins Ehp and Sbi~~, are activated by SaeRS signalling~~ [28]. The mechanistic basis of SaeRS inhibition by free FAs remains elusive. However, ~~it~~this inhibition shows how bacterial virulence within FA-rich, host environments may exhibit many layers of complexity. Another example of the complex role of FAs in virulence is provided by *V. cholerae*, where long-chain FAs inhibit the master virulence activator ToxT [11]. However, ToxT-mediated cholera toxin production by *V. cholerae* enables the bacterium to release long-chain FAs within the rabbit or mouse small intestine (**Figure 43**). There, *V. cholerae* mutants that are defective for the long-chain FA transporter FadL have a fitness disadvantage compared to wild-type bacteria [14]. Similarly, although the pathogenicity island SPI1 of *Salmonella enterica* is inhibited by exogenous FAs *in vitro* [51], the bacterium requires FA import and metabolism genes for successful mouse colonisation [20]. However, studies on a murine model for acute infection have shown that *S. enterica* mutants that are defective in import of FAs outcompete the wild type [51]. Thus, it is conceivable that the nutritional landscape of their host dictates how bacteria fine-tune their virulence in response to FAs, with exogenous FAs being either inhibitory or permissive to pathogen proliferation depending on FA species and their concentrations. Host-mimicking media and growth conditions appear to be instrumental in deciphering the extent to which host-derived FAs modulate pathogen virulence (see **Outstanding questions**).

Membrane and metabolic adaptations to ~~resist~~ antimicrobial FAs

Emerging data suggest that bacterial cells trigger adaptive cellular responses upon alterations in their membrane viscosity resulting from certain environmental cues such as being exposed to exogenous, unsaturated FAs [26]. Since these FAs tend to

increase membrane fluidity, strategies that allow for the maintenance of membrane homeostasis enable bacteria to thrive in FA-rich environments. In such environments, bacteria alter their transcriptional landscape and protein content, as well as their lipid composition [10]. Indeed, grown in the presence of exogenous FAs, bacteria incorporate these FAs into novel phospholipid species, while inhibiting endogenous FA and phospholipid synthesis [8, 10, 16]. Yet, it is unclear if lipid remodelling helps bacteria resist antimicrobial FAs. Further investigations are warranted to fully understand how bacteria monitor changes in the physical properties of their membranes, which result from the incorporation of antimicrobial FAs.

Besides lipid remodelling, *S. aureus* utilizes the golden carotenoid pigment, staphyloxanthin, which aids in membrane stability, and thereby promotes resistance to long-chain unsaturated FAs [35]. Similarly, *S. aureus* T7SS, which is thought to contribute to the membrane architecture, helps the bacterium mitigate FA toxicity [10] (**Figure ~~3~~2**). Strikingly, in response to free FAs, *S. aureus* swiftly activates its T7SS. The more toxic FAs are, the stronger they can induce *S. aureus* T7SS activation [26]. T7SS activation by membrane-disrupting FAs is concomitant with metabolic adaptation, which likely sustains ATP synthesis and energy generation. Of the metabolic pathways that are upregulated in the presence of FAs, the arginine deiminase pathway, which results in ammonia production upon the anaerobic catabolism of arginine, was shown to contribute to *S. aureus* FA resistance [22, 35]. Furthermore, mannitol metabolism is required for the survival of the bacterium upon exposure to FAs [71]. However, the detailed molecular underpinnings of how these metabolic pathways enable bacteria to withstand antimicrobial FAs are lacking. Likewise, it is unclear if other metabolic pathways (e.g.: glycolysis [22] or glycerol

metabolism [69]) that ~~bacteria upregulate~~are upregulated in response to antimicrobial FAs represent resistance strategies.

Bacterial responses to FA-~~provoked~~induced oxidative stress

Reactive oxygen species (ROS) swiftly kill invading bacteria within host phagocytes, where polyunsaturated FAs are abundant. Intriguingly, it has been speculated for more than three decades that FA toxicity in *S. aureus*, is partly mediated by FA peroxidation [72]. However, it has been shown only recently that the susceptibility of *S. aureus* to otherwise inhibitory FA concentrations was abrogated when the bacterium was co-treated with 5'-*O*-pentyl-pyridoxamine, a scavenger of peroxidated FAs [7]. Consistently, antioxidants (such as manganese and α-tocopherol) have been shown to inhibit FA toxicity, whereas ROS-generating agents synergise with FAs to ~~strongly impede~~inhibit *S. aureus* growth [7, 72]. Catalase, another antioxidant, substantially enhances *S. aureus* survival in the presence of bactericidal FAs [72]. However, the deletion of the catalase-encoding gene *katA* in *S. aureus* appears not to ~~change~~increase the ~~bacterial~~bacterium's susceptibility to long-chain FAs [35]. Notwithstanding this puzzling observation, it seems that the amounts of cellular/endogenous ROS prior to exposure to antimicrobial FAs dictate *S. aureus* survival. For instance, strains lacking the LytR-CpsA-Psr protein A (LCPA) produce less ROS and exhibit increased resistance to antimicrobial FAs [7]. It is unknown how LCPA, which covalently attaches wall teichoic acid precursors to nascent peptidoglycans, would augment bacterial ROS production. ~~The~~Further, the exposure of *Clostridioides difficile* to antimicrobial FAs has been shown to increase intracellular ROS levels [73]. ~~How~~However, how *C. difficile* withstands this ~~oxidative challenge~~stress is unclear.

Importantly, *E. coli*, which is by far more resistant to antimicrobial FAs than *S. aureus* [72], must also cope with the long-chain FA-mediated increase in cellular ROS levels [74]. To mitigate this oxidative stress *E. coli* appears to rely primarily on ubiquinone as an antioxidant, since the bacterium increases its ubiquinone production upon FA metabolism, and mutants that are defective in ubiquinone biosynthesis show strong growth defects in media that are supplemented with various FAs as sole carbon sources [74]. It would be of interest to determine whether Gram-negative pathogens, such as *P. aeruginosa, S. enterica*, and *V. cholerae* generate ROS by metabolizing host-derived FAs~~, and~~ as well as the counterstrategies that are employed by these bacteria in response to oxidative stress.

Antibiotic resistance in ~~FA-adapted~~ bacteria exposed to antimicrobial FAs

By virtue of their potent antimicrobial properties FAs represent very attractive alternatives to conventional antibiotics, especially to combat recalcitrant, multidrug-resistant pathogens. These pathogens can even be rendered vulnerable to antibiotics if co-treated with FAs, as demonstrated in the case of vancomycin-resistant *Enterococcus faecalis* [75]. The growing body of knowledge in this emergent and exciting field has been reviewed recently [76]. However, most studies disregard that bacteria are exposed to free FAs in their host environment prior to antibiotic exposure. In light of the pleiotropic effects of FAs on bacterial cells within different niches, it is apparent that *in vitro* antibiotic susceptibility testing upon bacterial growth in the presence of host-specific FAs recapitulates some aspects of the *in vivo* scenario. For instance, *S. aureus* displays an increased vulnerability to daptomycin, when the bacterium incorporates unsaturated FAs into its membrane [77]. By contrast, the

incorporation of host-derived, unsaturated FAs ~~promotes~~by *S. aureus* ~~survival~~enables the bacterium to survive FASII-targeting antibiotics, as seen in a mouse septicaemia model [25].

For the Gram-positive bacterium, *E. faecalis*, growth in the presence of unique unsaturated FAs or FA-rich human serum leads to the membrane incorporation of exogenous FAs and improved survival ~~to subsequent~~upon exposure to membrane damaging agents, including daptomycin [78]. This tolerance phenotype is neither due to the selection of genetic mutants nor to a change in the charge associated with the cell envelope [79]. Thus, the impact of FA metabolism on antibiotic susceptibility is still poorly understood for *E. faecalis* as it is for other clinically relevant bacteria.

Concluding remarks

~~Antimicrobial~~The antimicrobial properties of FAs have been known for more than a century. The rise of multidrug-resistant bacterial pathogens and the paucity of novel antibiotic classes have revived interest in FAs as alternative antimicrobials with anti-biofilm and anti-virulence capacities. However, the extensive exposure of bacteria to host-derived FAs has enabled these organisms to evolve a wide range of strategies to fine-tune their interactions with FAs. Irrespective of how susceptible bacteria are to exogenous FAs, they have to find ways to mitigate FA-provoked cellular stress. There are similarities between the adaptation strategies to antimicrobial FAs and resistance mechanisms to conventional antibiotics (e.g.: efflux pumps). This suggests that FA-adapted and FA-naïve bacteria would respond differently to antibiotics. Despite obvious species-specific variations, a comprehensive understanding of these

responses is needed to better grasp bacterial persistence within the FA-rich host and its contribution to antibiotic resistance.

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References

1 Anderson, S.M., *et al.* (2019) The fatty acid oleate is required for innate immune activation and pathogen defense in *Caenorhabditis elegans*. *PLoS Pathog* 15, e1007893

2 Georgel, P., *et al.* (2005) A toll-like receptor 2-responsive lipid effector pathway protects mammals against skin infections with gram-positive bacteria. *Infect Immun* 73, 4512-4521

3 Hasan, M.A., *et al.* (2019) Biosynthetic pathway of arachidonic acid in *Spodoptera exigua* in response to bacterial challenge. *Insect Biochem Mol Biol* 111, 103179

4 Yu, K., *et al.* (2013) A feedback regulatory loop between G3P and lipid transfer proteins DIR1 and AZI1 mediates azelaic-acid-induced systemic immunity. *Cell Rep* 3, 1266-1278

5 Clarke, S.R., *et al.* (2007) The *Staphylococcus aureus* surface protein IsdA mediates resistance to innate defenses of human skin. *Cell Host Microbe* 1, 199-212

6 Yoon, B.K., *et al.* (2018) Antibacterial free fatty acids and monoglycerides: biological activities, experimental testing, and therapeutic applications. *Int J Mol Sci* 19

7 Beavers, W.N., *et al.* (2019) Arachidonic acid kills *Staphylococcus aureus* through a lipid peroxidation mechanism. *mBio* 10

8 Eijkelkamp, B.A., *et al.* (2018) Arachidonic acid stress impacts pneumococcal fatty acid homeostasis. *Front Microbiol* 9, 813
9 Jiang, J.H., *et al.* (2019) Identification of novel *Acinetobacter baumannii* host fatty acid stress adaptation strategies. *mBio* 10
10 Kengmo Tchoupa, A., *et al.* (2020) The type VII secretion system protects *Staphylococcus aureus* against antimicrobial host fatty acids. *Sci Rep* 10, 14838
11 Kumar, P., *et al.* (2020) Fatty acids as antibiofilm and antivirulence agents. *Trends Microbiol* 28, 753-768
12 Cuypers, M.G., *et al.* (2019) Acyl-chain selectivity and physiological roles of *Staphylococcus aureus* fatty acid-binding proteins. *J Biol Chem* 294, 38-49
13 Gullett, J.M., *et al.* (2019) A fatty acid-binding protein of *Streptococcus pneumoniae* facilitates the acquisition of host polyunsaturated fatty acids. *J Biol Chem* 294, 16416-16428
14 Rivera-Chavez, F. and Mekalanos, J.J. (2019) Cholera toxin promotes pathogen acquisition of host-derived nutrients. *Nature* 572, 244-248
15 Yao, J. and Rock, C.O. (2017) Exogenous fatty acid metabolism in bacteria. *Biochimie* 141, 30-39
16 Yao, J., *et al.* (2016) Activation of exogenous fatty acids to acyl-acyl carrier protein cannot bypass fabi inhibition in *Neisseria*. *J Biol Chem* 291, 171-181
17 Hearn, E.M., *et al.* (2009) Transmembrane passage of hydrophobic compounds through a protein channel wall. *Nature* 458, 367-370
18 Radka, C.D., *et al.* (2020) Fatty acid activation and utilization by *Alistipes finegoldii*, a representative Bacteroidetes resident of the human gut microbiome. *Mol Microbiol* 113, 807-825
19 Zarzycki-Siek, J., *et al.* (2013) Elucidating the *Pseudomonas aeruginosa* fatty acid degradation pathway: identification of additional fatty acyl-CoA synthetase homologues. *PLoS One* 8, e64554
20 Reens, A.L., *et al.* (2019) *Salmonella enterica* requires lipid metabolism genes to replicate in proinflammatory macrophages and mice. *Infect Immun* 88
21 Gao, L., *et al.* (2020) Gonococcal adaptation to palmitic acid through farAB expression and FadD activity mutations increases in vivo fitness in a murine genital tract infection model. *J Infect Dis*
22 Moran, J.C., *et al.* (2017) Comparative transcriptomics reveals discrete survival responses of *S. aureus* and *S. epidermidis* to sapienic acid. *Front Microbiol* 8, 33
23 Parsons, J.B., *et al.* (2014) Identification of a two-component fatty acid kinase responsible for host fatty acid incorporation by *Staphylococcus aureus*. *Proc Natl Acad Sci U S A* 111, 10532-10537
24 Frank, M.W., *et al.* (2020) Host fatty acid utilization by *Staphylococcus aureus* at the infection site. *mBio* 11
25 Kenanian, G., *et al.* (2019) Permissive fatty acid incorporation promotes staphylococcal adaptation to FASII antibiotics in host environments. *Cell Rep* 29, 3974-3982 e3974
26 Lopez, M.S., *et al.* (2017) Host-derived fatty acids activate type VII secretion in *Staphylococcus aureus*. *Proc Natl Acad Sci U S A* 114, 11223-11228
27 Ridder, M.J., *et al.* (2020) *Staphylococcus aureus* fatty acid kinase FakA modulates pathogenesis during skin infection via proteases. *Infect Immun* 88
28 Ericson, M.E., *et al.* (2017) Role of fatty acid kinase in cellular lipid homeostasis and SaeRS-Dependent virulence factor expression in *Staphylococcus aureus*. *mBio* 8

29 Alnaseri, H.*, et al.* (2019) DNA binding and sensor specificity of FarR, a novel TetR family regulator required for induction of the fatty acid efflux pump FarE in *Staphylococcus aureus*. *J Bacteriol* 201
30 Krute, C.N.*, et al.* (2019) Inactivation of the exogenous fatty acid utilization pathway leads to increased resistance to unsaturated fatty acids in *Staphylococcus aureus*. *Microbiology (Reading)* 165, 197-207
31 Eraso, J.M.*, et al.* (2016) Genomic landscape of intrahost variation in Group A *Streptococcus*: repeated and abundant mutational inactivation of the *fabt* gene encoding a regulator of fatty acid synthesis. *Infect Immun* 84, 3268-3281
32 Di Rienzi, S.C.*, et al.* (2018) Resilience of small intestinal beneficial bacteria to the toxicity of soybean oil fatty acids. *Elife* 7
33 Reithuber, E.*, et al.* (2020) The bactericidal fatty acid mimetic 2CCA-1 selectively targets pneumococcal extracellular polyunsaturated fatty acid metabolism. *mBio* 11
34 Mortensen, J.E. and Kapral, F.A. (1992) Effect of capsulation on the resistance of *Staphylococcus aureus* to the bactericidal lipids produced in abscesses. *J Med Microbiol* 36, 337-340
35 Kenny, J.G.*, et al.* (2009) The *Staphylococcus aureus* response to unsaturated long chain free fatty acids: survival mechanisms and virulence implications. *PLoS One* 4, e4344
36 Kohler, T.*, et al.* (2009) Wall teichoic acid protects *Staphylococcus aureus* against antimicrobial fatty acids from human skin. *J Bacteriol* 191, 4482-4484
37 King, N.P.*, et al.* (2012) Characterisation of a cell wall-anchored protein of *Staphylococcus saprophyticus* associated with linoleic acid resistance. *BMC Microbiol* 12, 8
38 Schielke, S.*, et al.* (2010) The transcriptional repressor FarR is not involved in meningococcal fatty acid resistance mediated by the FarAB efflux pump and dependent on lipopolysaccharide structure. *Appl Environ Microbiol* 76, 3160-3169
39 Piddock, L.J. (2006) Clinically relevant chromosomally encoded multidrug resistance efflux pumps in bacteria. *Clin Microbiol Rev* 19, 382-402
40 Nikaido, H. (2018) RND transporters in the living world. *Res Microbiol* 169, 363-371
41 Alnaseri, H.*, et al.* (2015) Inducible expression of a resistance-nodulation-division-type efflux pump in *Staphylococcus aureus* provides resistance to linoleic and arachidonic acids. *J Bacteriol* 197, 1893-1905
42 Lee, E.H.*, et al.* (2003) FarR regulates the farAB-encoded efflux pump of *Neisseria gonorrhoeae* via an MtrR regulatory mechanism. *J Bacteriol* 185, 7145-7152
43 MacArthur, I.*, et al.* (2019) The evolution of *Bordetella pertussis* has selected for mutations of acr that lead to sensitivity to hydrophobic molecules and fatty acids. *Emerg Microbes Infect* 8, 603-612
44 Lee, E.H.*, et al.* (2006) Integration Host Factor is required for FarR repression of the farAB-encoded efflux pump of *Neisseria gonorrhoeae*. *Mol Microbiol* 60, 1381-1400
45 Lee, E.H. and Shafer, W.M. (1999) The farAB-encoded efflux pump mediates resistance of gonococci to long-chained antibacterial fatty acids. *Mol Microbiol* 33, 839-845
46 Truong-Bolduc, Q.C.*, et al.* (2014) Native efflux pumps contribute resistance to antimicrobials of skin and the ability of *Staphylococcus aureus* to colonize skin. *J Infect Dis* 209, 1485-1493
47 Lennen, R.M.*, et al.* (2013) Identification of transport proteins involved in free fatty acid efflux in *Escherichia coli*. *J Bacteriol* 195, 135-144

48 Bellefleur, M.P.A., *et al.* (2019) Characterizing active transportation mechanisms for free fatty acids and antibiotics in *Synechocystis* sp. PCC 6803. *BMC Biotechnol* 19, 5
49 Kato, A., *et al.* (2015) Identification of a cyanobacterial RND-type efflux system involved in export of free fatty acids. *Plant Cell Physiol* 56, 2467-2477
50 Adebusuyi, A.A. and Foght, J.M. (2011) An alternative physiological role for the EmhABC efflux pump in *Pseudomonas fluorescens* cLP6a. *BMC Microbiol* 11, 252
51 Golubeva, Y.A., *et al.* (2016) Intestinal long-chain fatty acids act as a direct signal to modulate expression of the *Salmonella* pathogenicity island 1 type III secretion system. *mBio* 7, e02170-02115
52 Bayer, A.S., *et al.* (2006) Low-level resistance of *Staphylococcus aureus* to thrombin-induced platelet microbicidal protein 1 in vitro associated with *qacA* gene carriage is independent of multidrug efflux pump activity. *Antimicrob Agents Chemother* 50, 2448-2454
53 Mortensen, J.E., *et al.* (1992) Modification of bactericidal fatty acids by an enzyme of *Staphylococcus aureus*. *J Med Microbiol* 36, 293-298
54 Long, J.P., *et al.* (1992) The production of fatty acid modifying enzyme (FAME) and lipase by various staphylococcal species. *J Med Microbiol* 37, 232-234
55 Saylor, B.D. and Love, J.J. (2016) A secreted *Staphylococcus aureus* lipase engineered for enhanced alcohol affinity for fatty acid esterification. *Journal of Molecular Catalysis B: Enzymatic* 133, S44-S52
56 Chimalapati, S., *et al.* (2020) Vibrio deploys type 2 secreted lipase to esterify cholesterol with host fatty acids and mediate cell egress. *Elife* 9
57 Volkov, A., *et al.* (2010) Myosin cross-reactive antigen of *Streptococcus pyogenes* M49 encodes a fatty acid double bond hydratase that plays a role in oleic acid detoxification and bacterial virulence. *J Biol Chem* 285, 10353-10361
58 Subramanian, C., *et al.* (2019) Oleate hydratase from *Staphylococcus aureus* protects against palmitoleic acid, the major antimicrobial fatty acid produced by mammalian skin. *J Biol Chem* 294, 9285-9294
59 Kengmo Tchoupa, A. and Peschel, A. (2020) *Staphylococcus aureus* releases proinflammatory membrane vesicles to resist antimicrobial fatty acids. *mSphere* 5
60 Bergamo, P., *et al.* (2014) Immunomodulatory activity of a gut microbial metabolite of dietary linoleic acid, 10-hydroxy-cis-12-octadecenoic acid, associated with improved antioxidant/detoxifying defences. *Journal of Functional Foods* 11, 192-202
61 Miyamoto, J., *et al.* (2015) A gut microbial metabolite of linoleic acid, 10-hydroxy-cis-12-octadecenoic acid, ameliorates intestinal epithelial barrier impairment partially via GPR40-MEK-ERK pathway. *J Biol Chem* 290, 2902-2918
62 Miyamoto, J., *et al.* (2019) Gut microbiota confers host resistance to obesity by metabolizing dietary polyunsaturated fatty acids. *Nat Commun* 10, 4007
63 Morello, E., *et al.* (2019) *Pseudomonas aeruginosa* lipoxygenase LoxA contributes to lung infection by altering the host immune lipid signaling. *Front Microbiol* 10, 1826
64 Banthiya, S., *et al.* (2016) Structural and functional basis of phospholipid oxygenase activity of bacterial lipoxygenase from *Pseudomonas aeruginosa*. *Biochim Biophys Acta* 1861, 1681-1692
65 Deschamps, J.D., *et al.* (2016) Biochemical and cellular characterization and inhibitor discovery of *Pseudomonas aeruginosa* 15-Lipoxygenase. *Biochemistry* 55, 3329-3340
66 Liaw, S.J., *et al.* (2004) Modulation of swarming and virulence by fatty acids through the RsbA protein in *Proteus mirabilis*. *Infect Immun* 72, 6836-6845

67 Miller, R.M.*, et al.* (2008) *Pseudomonas aeruginosa* twitching motility-mediated chemotaxis towards phospholipids and fatty acids: specificity and metabolic requirements. *J Bacteriol* 190, 4038-4049
68 Pan, X.*, et al.* (2020) PvrA is a novel regulator that contributes to *Pseudomonas aeruginosa* pathogenesis by controlling bacterial utilization of long chain fatty acids. *Nucleic Acids Res* 48, 5967-5985
69 Kordes, A.*, et al.* (2019) Establishment of an induced memory response in *Pseudomonas aeruginosa* during infection of a eukaryotic host. *ISME J* 13, 2018-2030
70 Ishii, K.*, et al.* (2014) Induction of virulence gene expression in *Staphylococcus aureus* by pulmonary surfactant. *Infect Immun* 82, 1500-1510
71 Kenny, J.G.*, et al.* (2013) Mannitol utilisation is required for protection of *Staphylococcus aureus* from human skin antimicrobial fatty acids. *PLoS One* 8, e67698
72 Knapp, H.R. and Melly, M.A. (1986) Bactericidal effects of polyunsaturated fatty acids. *J Infect Dis* 154, 84-94
73 Yang, H.T.*, et al.* (2017) Lauric acid is an inhibitor of *Clostridium difficile* growth in vitro and reduces inflammation in a mouse infection model. *Front Microbiol* 8, 2635
74 Agrawal, S.*, et al.* (2017) A genome-wide screen in *Escherichia coli* reveals that ubiquinone is a key antioxidant for metabolism of long-chain fatty acids. *J Biol Chem* 292, 20086-20099
75 Cheung Lam, A.H.*, et al.* (2016) Assessment of free fatty acids and cholesteryl esters delivered in liposomes as novel class of antibiotic. *BMC Res Notes* 9, 337
76 Casillas-Vargas, G.*, et al.* (2021) Antibacterial fatty acids: an update of possible mechanisms of action and implications in the development of the next-generation of antibacterial agents. *Prog Lipid Res* 82, 101093
77 Boudjemaa, R.*, et al.* (2018) Impact of bacterial membrane fatty acid composition on the failure of daptomycin to kill *Staphylococcus aureus*. *Antimicrob Agents Chemother* 62
78 Saito, H.E.*, et al.* (2014) Incorporation of exogenous fatty acids protects *Enterococcus faecalis* from membrane-damaging agents. *Appl Environ Microbiol* 80, 6527-6538
79 Brewer, W.*, et al.* (2020) Induction of daptomycin tolerance in *Enterococcus faecalis* by fatty acid combinations. *Appl Environ Microbiol* 86
80 Bomar, L.*, et al.* (2016) *Corynebacterium accolens* releases antipneumococcal free fatty acids from human nostril and skin surface triacylglycerols. *mBio* 7, e01725-01715

Box 1 Fatty acids and where to find them

FAs comprise a carboxyl group (–COOH) hydrophilic head and a hydrophobic hydrocarbon tail (**Figure ~~1~~1**). Based on the length of their aliphatic tail, FAs are generally grouped into short-chain FAs (less than 6 carbons), medium-chain FAs (between 6 and 11 carbons), and long-chain FAs (more than 11 carbons) [6]. Besides length, the degree of saturation of the hydrocarbon chain dictates FA physical properties. Unsaturated FAs display at least one carbon-carbon double bond that is absent in saturated FAs. The orientation of this double bond (*cis* or *trans*) determines whether unsaturated FAs appear straight (*trans*) or bent (*cis*). Long-chain polyunsaturated FAs predominantly entail *cis* double bonds and are therefore bulky. These FAs strongly increase the fluidity and permeability of bacterial membranes, which, at least partly, explains their antimicrobial properties [6-10].

FAs are synthesised by both bacteria and their eukaryotic hosts. Historically, FAs were trivially, but uniquely, named according to organisms (or organs) from which they were first isolated or to formerly characterised FAs with similar features (e.g.: sapienic acid – humans; palmitic acid – palm oil; palmitoleic acid – palmitic acid). Nowadays, this trivial nomenclature coexists with more systematic ones, which specify the FA length as well as unsaturation levels and double bond positions. For instance, the Omega nomenclature (ω-x), popular in nutritional literature, numbers carbon atoms counting from the terminal methyl group (–CH3). Meanwhile, the carboxyl group is used as starting point for the numbering of FA carbon atoms in the Delta nomenclature, which is preferred by chemists. Thus, palmitoleic acid can also be referred to as 9-*cis*-hexadecenoic acid, 16:1 Δ^9, or 16:1 ω-7. (Figure 1 Box1).

Fatty acids are building blocks for various lipids from which they can be released by endogenous lipases or from the resident microbiota. A human skin and nose commensal bacterium, *Corynebacterium accolens*, which is defective in fatty acid synthase, seems to employ a lipase (LipS1) to free host-derived FAs required for the bacterium to grow while incidentally inhibiting FA-susceptible bacterial competitors within the same niche [80]. In addition to the nose and the skin, the serum [25], the pulmonary surfactant [70], the gastrointestinal tract [32], the genital tract [21], and arguably all mucosal surfaces are rich in long-chain FAs with antimicrobial properties.

Figure legends

Figure ~~1.~~I (Box1). Examples of fatty acids encountered by bacteria in their human host. (a-d) Depicted are the chemical structures, and trivial, systematic and omega names of exemplary fatty acids with antibacterial activity. (a) Palmitic acid is found in the pulmonary surfactant, the genital tract, and various mucosal surfaces. (b) Palmitoleic acid is abundant in the nasal fluid and lungs. (c) Sapienic acid is solely produced by human skin. (d) Linoleic acid is one of the most common free fatty acids throughout the human body.

Figure ~~2~~1 (Key Figure). Bacterial responses to exogenous fatty acids. An overview of the strategies used by bacteria to mitigate the toxicity of host-derived fatty acids (FAs). These antimicrobial FAs are depicted in red with two kinks per molecule. (a) Bacteria release enzymes and membrane vesicles that inactivate toxic exogenous FAs. Detoxified FAs, depicted in green with one kink per molecule, can also be generated intracellularly prior to their release to the bacterial environment. (b) The cell envelope of bacteria is a formidable, permeability barrier against antimicrobial FAs. (c)

Gram-negative bacteria require a specific transporter (FadL) to take up long-chain FAs, which can readily diffuse through the membrane in Gram-positive bacteria. (d) The intracellular accumulation of toxic levels of FAs is prevented by efflux pumps. (e) Intracellular FAs can also be degraded by β-oxidation or (f) incorporated into membrane lipids.

Figure ~~3~~2. Resistance to antimicrobial fatty acids by *Staphylococcus aureus*. ~~For clarity, mannitol catabolism~~(a) The capsule, wall teichoic acids and ~~oxidative stress mitigation were not included~~the iron-regulated surface determinant A (IsdA) constitute a hydrophilic barrier that impede the diffusion of hydrophobic, antimicrobial FAs. (b) The efflux pumps FarE and Tet38 restrict the intracellular accumulation of FAs to toxic levels. (c) *S. aureus* utilises enzymes (FAME and oleate hydratase) and membrane vesicles to detoxify antimicrobial FAs. The unknown identity of the protein responsible for FAME (fatty acid modifying enzyme) activity observed in *S. aureus* culture supernatants is indicated by a question mark. (d) The ~~depiction of the~~ type VII secretion system (T7SS) and ~~other cellular components is purely for illustrative purposes.~~ staphyloxanthin limit membrane disruption by FAs. (e) The cell wall-anchored protein SasF, the arginine degradation pathway, and mannitol catabolism protect *S. aureus* against FA toxicity via unknown mechanisms.

Figure ~~4~~3. *Vibrio cholerae* utilizes host-derived fatty acids for proliferation in the gut. In the host intestine, *V. cholerae* releases cholera toxin, which activates a signalling cascade leading to host lipase activation and long-chain FA release. The bacterium utilises FadL to import long-chain FAs that promote bacterial proliferation while attenuating virulence.