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Review article

Staphylococcus aureus host interactions and adaptation

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Supplementary Material

Title: *Staphylococcus aureus* host interactions and adaptation.

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Supplementary Box 1: Interplay between other colonising species and *S. aureus* in niches such as anterior nares and gut

For reasons of tractability and practicality *S. aureus* is usually isolated and studied in pure culture in the absence of competing or co-existing microorganisms, and much has been learnt from this approach. Yet, this methodological convenience ignores the ensemble of bacteria and other microbes that share various niches of the human body with *S. aureus*. Bacterial community profiling using 16S rRNA amplicon sequencing, metagenomic and metabolomic microbiome analyses across various body sites are now revealing the complexity of interactions with *S. aureus* and other microbes. These interactions can influence *S. aureus* response to antibiotics, and swing from preventing colonisation to promoting *S. aureus* transition to invasive pathogen. The molecular mechanisms underlying these effects are only beginning to be understood but have been presaged in the literature for some years, including observations of the impact of microbial metabolites and small molecules on *S. aureus.* For instance, exposure to sub-clinical concentrations of fluoroquinolone antibiotics led to significant up-regulation of genes involved in the *S. aureus* SOS response [1]. In another example, three different *Pseudomonas aeruginosa* metabolites (an endopeptidase, rhamnolipids and 2-heptyl-4-hydroxyquinoline N-oxide) each influenced susceptibility to antibiotics (both antagonising and potentiating). These effects were modulated by the *S. aureus* genotype, other microbes present and the type of antibiotic [2].

Interactions in the nasal microbiome: Human colonisation cohort studies of *S. aureus* have shown that nasal carriage can be both dynamic and stable and that this phenotype is strongly influenced by 'host factors' [3,4]. The host factors that promote *S. aureus* nasal colonisation have been reviewed [5]. However, evidence is now accumulating for the complexity and importance of chemical signalling between *S. aureus* and other commensal bacteria. For instance, coproporphyrin III, a diffusible small molecule produced by *Propionibacterium spp.* (a nose and skin commensal) induces *S. aureus* clumping and surface-attached biofilms [6]. Most notable was the discovery of lugdunin, a novel class of cyclic peptide antibiotic produced by the nasal commensal and *Staphylococcus lugdunensis* that kills *S. aureus* and can prime anti-infection innate immune responses [7]. The role of coagulase negative staphylococci (CoNS) like *S. lugdunesis* on *S. aureus* skin colonisation has been reviewed recently [8]. Observations that *S. aureus* exhibits 'reduced virulence' phenotype when co-cultured with the nasal bacterium *Corynebacterium striatum*, including repression of *agr* quorum sensing, underscore the importance of chemical exchanges influencing key *S. aureus* behaviours [9]. Consistent with such findings, *S. aureus* also displayed diminished fitness during *in vivo* coinfection with

C. striatum when compared to monoinfection [9]. These data support a model in which *S. aureus* shifts from virulence toward a commensal state when exposed to commensal *Corynebacterium* species (Supplementary Figure 1). In a complementary approach to exploring *S. aureus* interactions with the nasal microbiome, 16S rRNA amplicon community profiling has shown that bacteria commonly found in the human nasal microbiome (including *Dolosigranulum pigrum*, *Corynebacterium accolens*, *Corynebacterium accolens_macginleyi_tuberculostearicum* and *Corynebacterium pseudodiphtheriticum*) were present when *S. aureus* was absent, suggesting these species might be promoting *S. aureus* colonization resistance [10,11]. Clinical correlates of increased post-surgical infection risk associated with nasal microbiome profiles have also been demonstrated [12]. Several groups have also noted the role of skin-associated *S. aureus* and specifically PSMα in promoting skin inflammation [13,14], and the inverse relationship between the presence of CoNS and *S. aureus* [15]. It was shown recently that alterations in the gut microbiome can have profound effects on the skin microflora leading to changes in CoNS colonisation [16] (Supplementary Figure 1).

Gut colonisation: Beyond the nasal microbiome, a human population-based study among a rural population in Thailand showed that *Bacillus subtilis* isolated from the gut of study participants produced lipopeptides called fengycins that quench *S. aureus agr* quorum sensing and prevent *S. aureus* gut colonisation [17]. This demonstration of the probiotic effect of *B subtilis* is also another example of how small molecule interactions influence *S. aureus* colonisation potential (Supplementary Figure 1) [6].

Contradictory roles of commensals in the augmentation of Staphylococcal infection: Contrary to the discussion above, interactions between *S. aureus* and resident microbes can also promote (augment) a shift to a virulence phenotype. For instance, peptidoglycan derived from skin-resident and other non-pathogen microbes can augment *S. aureus* virulence, and in a murine infection model at least, peptidoglycan from these bacteria increased *S. aureus* virulence by supressing ROS-mediated phagocytic killing [18,19]. The exopolysaccharides from Bacillus promote the activation of polarized macrophages (augmenting their INF-g expression) and lead to reduction of *S. aureus* infection burden [20].

We know that the transition of *S. aureus* from colonizing commensal to invasive pathogen is frequently accompanied by loss of its Agr quorum sensing (QS) function (Supplementary Table 2, Supplementary Figure 1). It is perhaps then no coincidence that some generic probiotic strains such as *Lactobacillus reuterii* interfere with the *S. aureus* Agr [21]. Our understanding of the breadth and mechanisms of Agr inhibition by nasal commensal bacteria is incomplete. However, evidence of spontaneous *S. aureus agrC* mutants resistant to secreted factors released

by *C. pseudodiphteriticum* is another indication that QS is likely targeted by microbial competitors [22]. The factors secreted by *C. pseudodiphteriticum* cause damage to *S. aureus* cell wall, reminiscent of the lytic activities of bacteriocins. Interestingly, it was observed that inhibition of *S. aureus* occurred in response to expression of PSM, which are controlled by Agr. Furthermore, the CoNS *Staphylococcus epidermidis,* normally insensitive to *C. pseudodiphteriticum* inhibition*,* becomes susceptible upon expression of PSMs. Further to this, the proteolytic activity of an *S. epidermidis* secreted protease (Esp) degrades many of the extracellular proteins used by *S. aureus* to adhere to the nasal epithelial cells [23]. By strongly stimulating antimicrobial peptides in murine nasal epithelial cells*, S. epidermidis* ST59 can exclude *S. aureus* from the nasal epithelium [24]. The role of different bacterial peptides, as either both direct agents of cell damage or signalling molecules is intriguing. These data suggests that *S. aureus* co-existence with specific commensal corynebacteria, CoNS and other species may result in the enrichment of *agr* non-functional *S. aureus* sub-populations within the polymicrobial, extra-cellular nasal niche. One could therefore envisage a model in which *S. aureus* shifts from a commensal state towards increased virulence potential when exposed to certain commensal bacteria such as *Corynebacterium* species, which in turn could have implications for potential transition of *S. aureus* towards a bloodstream pathogen (Box 1), (Supplementary Figure 1).

Supplementary Box 2: Genomic plasticity, drug resistance and persistence

Genetic changes linked to transition from coloniser to invasive pathogen: While several factors can be associated with a *S. aureus* invasive infection episode, including pathogen factors (eg. mutations in virulence and regulatory genes), host status (age, comorbidities), and triggers (eg. recent surgery or trauma) [25], genomic studies have highlighted bacterial adaptive changes linked to invasion (Supplementary Table 2). *S. aureus* clones that can survive the intracellular environment are under strong selective pressure and undergo adaptive evolution in genes linked to antibiotic resistance and pathogenesis with these adaptive mutations increasing during persistent infections [26,27]. Box 1 explores evolving understanding of the genomics of transition from colonisation to invasion and persistence, while Supplementary Figure 2 highlights the major genetic mechanisms of adaptation.

Evolving resistance to last line antimicrobials and impacts on host-pathogen interactions: *S. aureus* is naturally susceptible to almost all classes of antibiotics, however resistance has emerged rapidly after the introduction of new antibiotics into clinical practice contributing to distinct events linked to the emergence of new resistant clones [28] (Supplementary Figure 2). This phenomenon began with the rapid emergence of penicillin resistance in the mid 1940s [29], followed by the acquisition of *mecA* and global spread of MRSA in the 1960s [30] resulting in global "waves" of drug-resistant infections [28]. Methicillin-resistant clones have had a major impact on health globally, initially becoming a major concern in hospital settings followed by the more recent emergence in community settings [31]. Some clones of MRSA, such as ST239 that has been reported in healthcare settings around the world, are resistant to almost all classes of antibiotics making therapy for invasive infections challenging [32]. In many cases resistance in *S. aureus* has been acquired through horizontal gene transfer, however mutational resistance also plays a crucial role especially in the emergence of quinolone-resistant and rifampicin resistant strains [33,34]. The global spread of multi-drug resistant *S. aureus* has driven the clinical need to use "last-line" antibiotics for treatment. These include glycopeptides, especially vancomycin, the cyclic lipopeptide antibiotic daptomycin, and the oxazolidinone linezolid, as well as other newer agents [31]. Mutational resistance linked to antibiotics such as quinolones and rifampicin are well defined. Genomics has enabled comprehensive analysis of the adaptive genetic changes linked to reduced susceptibility/ drug-resistance in last line agents such as vancomycin, rifampicin and daptomycin [33,35-39], as well as providing new insights into adaptive mechanisms linked to low-level oxacillin-resistance [40].

Reduced vancomycin susceptibility in MRSA was first reported in the mid 1990's [41] with genome sequencing of paired clinical isolates able to identify and phenotypically characterise

with isogenic mutants the adaptive mutations leading to the phenotype [36]. Access to high throughput sequencing technologies permitted the characterisation of sequential isolates from patients with complex infections, where reduced vancomycin susceptibility emerged *in vivo* [36,38,42,43]. This has enabled the identification of numerous mutations as well as the involvement of chromosomal structural changes in reduced susceptibility but has also highlighted that the phenotype is polygenic and complex. A link between reduced susceptibility to vancomycin and attenuated virulence in both tissue culture (reduced adherence, invasion, and cytotoxicity) and a murine model of infection was identified. The adaption allowed these isolates to circumvent the host immune response in favour of a persistent infection over acute virulence [44].

Rifampicin is a potent inhibitor of the bacterial RNA polymerase and used clinically in combination with other agents to treat invasive *S. aureus* infections [39]. Mutations in the *rpoB* gene, encoding the beta-subunit of RNA polymerase, and causing rifampicin resistance are well characterised across bacterial species, including in *S. aureus*. However, through studies exploring the impact of mutations identified during persistent and drug-resistant staphylococcal infections it is emerging that rifampicin resistance mutations in *rpoB* have pleiotropic effects beyond rifampicin resistance. Exploiting targeted mutagenesis and examining the prevalent $RpoB^{H481Y}$ mutation demonstrated, reduced susceptibility to vancomycin, daptomycin and host antimicrobial peptides, while exhibiting impaired virulence leading to increased persistence [43,45]. Using global *S. aureus* genome data (>7000 sequences) the relevance of mutations with the strongest signature of convergent evolution has been explored using targeted mutagenesis [35]. This study identified that RpoB mutations affecting residue 481 (H481N/Y) were the most common globally and associated with worldwide expansion of rifampicin-resistant clones spanning decades. The eight most common RpoB mutations were recreated and demonstrated frequent cross-resistance to other last line antimicrobials (vancomycin and daptomycin) [35].

Daptomycin is a lipopeptide antibiotic that interacts with the cell membrane, important for treating multi-drug resistant MRSA infections. Like reduced vancomycin susceptibility described above, resistance often emerges *in vivo* during persistent infections [46-48]. Gain-of-function mutations in the gene encoding the multiple peptide resistance factor (*mprF*) have been frequently reported in resistant isolates [46], as well as mutations in *cls2* resulting in enhanced cardiolipin biosynthesis and membrane composition, blocking the cell membrane mediated activity of daptomycin [37]. Laboratory correlates linking drug-resistance with persistence in this study included reduced neutrophil recruitment and enhanced bacterial persistence in *cls2* mutants in a zebrafish model [37]. A transposon sequencing approach

indicated additional genetic heterogeneity in the mechanism of daptomycin resistance across *S. aureus* clones [49].

Genetic heterogeneity linked to chromosomal changes and insertion sequence mobility: Phenotypic heterogeneity, which is commonly seen clinically in persistent *S. aureus* SCVs [50], and antibiotic heteroresistance such as that seen with low-level vancomycin resistant *S. aureus* [39], can occur when a bacterial population contains a subpopulation with a niche-specific survival or antibiotic resistance phenotype [51]. Single nucleotide polymorphisms (SNPs) or small insertions and deletions (InDels) have been well described in *S. aureus* to result in phenotypic heterogeneity and antibiotic resistance. However, advances in bioinformatic approaches and increasing use of long-read sequencing is now providing additional insights into other mechanisms of *S. aureus* mutation and genome plasticity that need to be considered in experimental models and in the clinic (Box 1, Supplementary Figure 2). These new approaches are revealing for instance that large chromosomal duplications [43], and insertion sequence mobility [27] arise during persistent clinical infections and reduce antibiotic susceptibility. For instance, IS*256* insertions in the WalKR promoter alter transcription result in reduced vancomycin susceptibility [26,52]. It is important to gain an understanding of genetic heterogeneity in *S. aureus*, especially as it relates to clinically important phenotypes such as SCVs and heteroresistance. A recent study of *S. aureus* with an unstable (reversible) SCV phenotype highlighted the utility of long-read DNA sequencing to identify novel chromosome structural variants. No genetic differences were observed between the wild type and SCV using short-read sequence analysis. However, long read sequencing revealed a large chromosomal inversion that explained the SCV phenotype [53]. Importantly, the chromosomal inversion was the result of recombination between near identical *hsdM* genes (part of the Type I restriction modification system) separated by half the chromosome. Analysis of publicly available *S. aureus* genome data indicated that chromosomal inversions had occurred repeatedly during the evolution of *S. aureus* clones. An intriguing link between the SCV phenotype and immune evasion and persistence was uncovered, as the chromosomal inversion was associated with activation of the immune evasion prophage (ϕ3) containing the immune evasion gene cluster [53].

Supplementary Table 1. Major characteristics of *S. aureus* immune evasion factors relevant to human infections[a]

Name	Gene	Target	Action	Key characteristics
Adenosine synthase A	*adsA*	ATP dAMP	Increase adenosine, anti-inflammatory, suppress ROS in phagocytes Generate dAdo from NETs, blocks caspase 3, apoptosis of macrophages Inhibits Th1/Th17 immunity by attenuating NLRP3-mediated release of IL-1β [54]	Cell wall anchored enzyme; core genome encoded
Aureolysin	*aur*	C3	Zinc-dependent metalloprotease Inhibits phagocytosis and complement	Core genome
Capsule	Cap operon (*capA-P*)	Unknown	Inhibition of phagocytosis	Serotype 5 and 8 dominant in clinical isolates
Chemotaxis inhibitory protein (CHIPS)	*chp*	FPR1, C5aR	Inhibition of chemotaxis	Core genome encoded
Clumping factor A (ClfA)	*clfA*	γ-fibrinogen and factor I	Attachment; inhibition of phagocytosis	Core genome encoded
Collagen adhesin	*cna*	C1q	Adhesion (collagen), C1q binding	Variable
Coagulase	*coa*	Prothrombin, fibrinogen	Inhibition of phagocytosis	Core genome encoded
Extracellular adherence proteins (Eap, EAPH1, EapH2)	*Eap, eapH1, eapH2*	ICAM1, C4b (*eap*) elastase, cathepsin G and proteinase 3 (*eap, eapH1,H2*)	Inhibition of phagocytic killing; preserve PSM function [55] (*eap, eapH1, eapH2*) Complement inhibition (*eap*)	Core genome encoded
Enterotoxins B, C, -like X	*seb, sec, selX*	Vβ TCR (*seb, sec*) PSGL1 (*selX*)	T cell superantigen	Pathogenicity island (*seb, sec*) Core (*selX*)
Ecb, Efb	*ecb, efb*	C3d (*ecb, efb)* αMβ2 integrin (*efb)*	Inhibition of complement	IEC2 (immune evasion cluster/island)
FLIPr, FLIPrL	*flipr, fliprl*	FPR2 (*flipr, fliprl)* FPR1 (*fliprl*)	Inhibition of chemotaxis	IEC2 (immune evasion cluster/island)

Fibronectin-binding protein A (FnBPA)	*fnbpA*	γ-fibrinogen and fibronectin	Inhibition of phagocytosis, invasion	Core genome encoded
Fibronectin-binding protein B (FnBPB)	*fnbpB*	Histones, plasminogen	Protects against antimicrobial activity of histones[56]	Core genome encoded
γ-haemolysin AB (HlgAB) and CB (HlgCB)	*hlgAB, hlgCB*	CXCR1, CXCR2, CCR2, DARC (HlgAB) C5aR1/2 (HlgCB)	Phagosome escape	Secreted bicompenent leukocidins. Both high activity against human cells. HlgCB low activity in mice.
Leukocidin AB (LukAB) [LukGH]	*lukAB*	CD11b HVCN1 [57]	Neutrophil lysis Contribution to dendritic cell inhibition [58]	High activity against human cells, low activity in mice Clonal complex specific sequence variability linked to alternate targets [57]
Leukocidin ED	*lukED*	CXCR1, CXCR2, CCR5, DARC	Neutrophil lysis	Genomic island β (Giβ)
Leukocidin SF-PV (PVL)	*lukFS*	hC5aR1/2 (S-component) CD45 (F-component) [59]	Neutrophil lysis	Encoded on PVL prophage ΦSa2. Highly active against human cells, no activity against mouse cells hC5aR1 dissociation from toxin may amplify inflammatory response [60]
Phenol soluble modulins (PSMs) PSMα1-4 PSMβ1-2	 *psmα1-4* *psmβ1-2*	FPR2	Neutrophil lysis	Core genome encoded
Delta-toxin (δ)	*hld*	Not known	Mast cell degranulation	PSM family. Core genome encoded, allelic variants have impact on pathogenesis
Protein A (SpaA)	*spaA*	Ig Fc, Ig Fab	Inhibition of phagocytosis, B cell superantigen Inhibit IgG hexamerization and complement [61]	Core genome encoded, Ig binding domain highly conserved, variable cell wall spanning domain
Staphylococcal binder of immunoglobulin (Sbi)	*sbi*	IgG Fcγ, C3, factor H	Inhibition of phagocytosis	
Serine-aspartate repeat protein D (SdrD)	*sdrD*	Dsg 1, uncertain	Adherence and abscess formation	Variably present

			Promotes survival in human blood [62]	Well described to promote binding to nasal epithelium and promote abscess formation. Innate immune evasion function [62]
Serine-aspartate repeat protein E (SdrE)	*sdrE*	Complement factor H	Inhibition of complement activity [63]	
SEIW	*selw*	T-cell	T-cell mitogen	Present in majority of *S. aureus*. Truncated in some clones [64]
staphylococcal enterotoxin-like toxin X (SEIX) [65]	*selX*	PSGL 1 [66]	Superantigen activity Survival in human blood	Core genome encoded An "SSL-like" sAg [66]
Staphylococcal superantigen-like protein 1 (SSL1)	*ssl1*	MMPs	Inhibition of matrix metalloproteinases and neutrophil activation [67]	Genomic island α (Giα)
Staphylococcal superantigen-like protein 3 (SSL3)	*ssl3*	TLR2	Toll-like receptor inhibition	Genomic island α (Giα)
Staphylococcal superantigen-like protein 5 (SSL5)	*ssl5*	PSGL1, GPCRs, GPIbα, GPVI, MMPs	Chemotaxis and platelet inhibition Inhibition **of** matrix metalloproteinases and neutrophil activation [67]	Genomic island α (Giα)
Staphylococcal superantigen-like protein 6 (SSL6)	*ssl6*	PSGL1	Inhibition of chemotaxis	Genomic island α (Giα)
Staphylococcal superantigen-like protein 7 (SSL7)	*ssl7*	C5, IgA	Inhibition of phagocytosis	Genomic island α (Giα)
Staphylococcal superantigen-like protein 10 (SSL10)	*ssl0*	IgG, fibrinogen, fibronectin, thrombin, factor Xa	Inhibition of phagocytosis	Genomic island α (Giα)
Staphylococcal superantigen-like protein 11 (SSL11)	*ssl1*	PSGL1	Inhibition of chemotaxis	Genomic island α (Giα)
Staphylococcal superantigen-like protein 13 (SSL13)	*Ssl13*	FPR2	Promotes neutrophil recruitment [68]	Genomic island α (Giα)
Staphylococcal complement inhibitor (SCIN) SCIN, SCIN-B, SCIN-C	*scn, scnB, scnC*	C3bBb	Inhibition of complement	IEC2 (immune evasion island)
Staphylococcocal peroxidase inhibitor (SPIN) [69]	*spn*; NWMN_0402	Myeloperoxidase (MPO)	Upregulated following phagocytosis Evasion of MPO-dependent killing	Located in conserved region of genomic island νSaα

Staphylokinase	*sak*	Plasminogen, fibronectin, C3, IgG Antimicrobial peptides	Inhibition of phagocytosis Inhibition of action [70]	IEC1 (immune evasion island)
Staphopain cysteine protease	*scpA*	CXCR2	Inhibition of chemotaxis	Core genome encoded
Toxic shock syndrome toxin-1 (TSST1)	*tst*	Vβ2 TCR, MHC class II α-chain	T cell superantigen	Pathogenicity island, SaPI1
Von Willebrand factor-binding protein (vWbp)	*vwb*	Prothrombin, fibrinogen, factor XIII, fibronectin	Inhibition of phagocytosis	Core genome encoded

[a]This table incorporates data from a previous comprehensive review [71], and highlights in red recent advances (with references) in identification of new immune evasion factors or advances in understanding new mechanisms of action of previously described factors. The table includes all know extracellular proteins and polysaccharide factors for completeness. Genomic studies of staphylococcal immune evasion factors highlight sequence variability within many factors [72], which may impact activity. Note: C5aR, C5a receptor; CCR, CC-chemokine receptor; CXCR, C-X-C chemokine receptor; DARC, Duffy antigen receptor for chemokines; dAdo, deoxyadenosine; Ecb, extracellular complement-binding protein; Efb, extracellular fibrinogen-binding protein; FLIPr, formyl peptide receptor-like 1 inhibitor; FLIPrL, FLIPr-like; FPR, formyl-peptide receptor; MHC, major histocompatibility complex; NETs, neutrophil extracellular traps; PSGL1, P-selectin glycoprotein ligand 1; TCR, T cell receptor

Supplementary Table 2. Summary of genetic changes used by *S. aureus* to switch from colonizer to pathogen to persistence

Functional category	Gene/ operon	Description	Dominant genetic change	Localisation of adaptive mutations[1]	Ecological niche[2]			Statistical evidence[3]	Functional evidence[4]
					Colonisation	Colonisation to invasion switch	Early Invasion to persistence		
Global regulators	Agr	Accessory gene regulator	Protein truncating, IS insertions	All over the coding sequence (*agrA*, *agrC*)	+++	+++	+++	[73-75]	
	rsp	Repressor of surface proteins	Protein truncating	All over the coding sequence		+			[76,77]
	purR	Purine biosynthesis regulator	Protein truncating, substitutions	All over the coding sequence			++	[74]	
	yjbH	Adaptor protein, negative regulator of Spx	Protein truncating	All over the coding sequence			++	[74]	
	gdpP	cyclic-di-AMP phosphodiesterase	Protein truncating	All over the coding sequence			+		[40]
	rsbU	sigma-B regulation protein	Substitutions	All over the coding sequence	+++			[75]	
Resistance determinants	*walRKHI*	Two-component system, cell-wall regulator (resistance to vancomycin)	Substitutions, IS insertions	All over the coding sequence (walR, walk, promoter)	++		+++	[74,75]	[36]
	mprF	Phosphatidylglycerol lysyltransferase (resistance to	Gain-of-function substitutions,	Concentrated on specific residues			+++	[74]	[47]

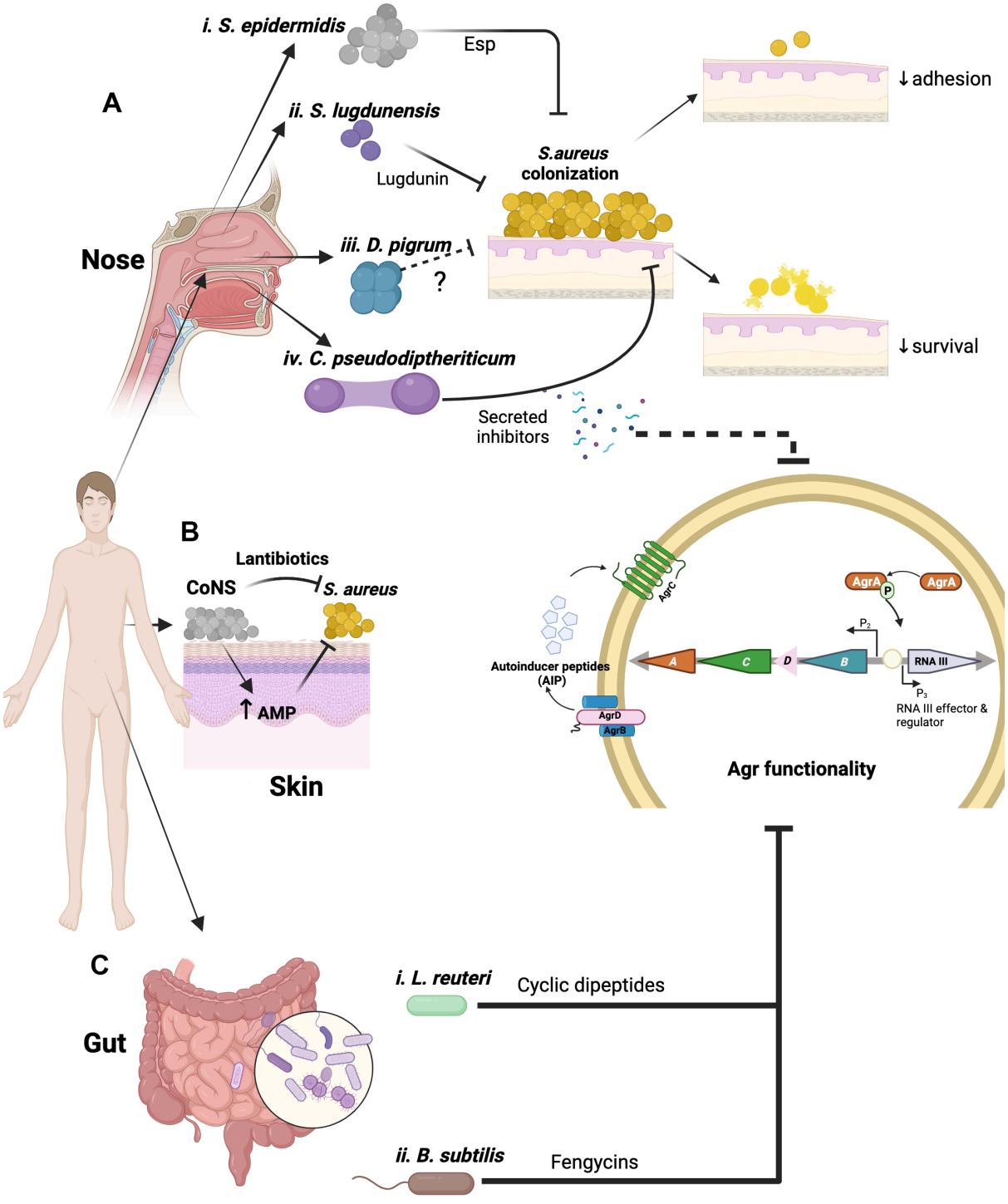
		vancomycin, daptomycin)	copy number variations						
	rpoB	RNA polymerase subunit b (resistance to rifampicin, vancomycin)	Substitutions (gain-of-function?)	Concentrated on specific residues			+++	[74,75]	[45]
	fmtB	Methicillin resistance determinant	Substitutions	All over the coding sequence	+++			[75]	
	rpsJ	Ribosomal S10 protein (resistance to tigecycline)	Substitutions (gain-of-function?)	Concentrated on specific residues	+++			[75]	
	dfrB	Dihydrfolate reductase (resistance to trimethoprim)	Substitution	Concentrated on specific residues	++			[78]	
Metabolic genes	*sucA-sucB*	Two components of the α-ketoglutarate dehydrogenase (tricarboxylic acid cycle [TCA])	Substitutions	All over the coding sequence (sucA, sucB)	+	+	++	[74,79]	
	citZ	Citrate synthase	Substitutions	All over the coding sequence			+++	[79]	[79]
	thyA	Thymidylate synthase	Substitutions	All over the coding sequence	+++			[75]	

[1] Gain-of-function are expected to be concentrated on specific residues, while loss-of-function mutations are spread across the coding sequence. Mutations in promoters are expected to impact the operon transcription.

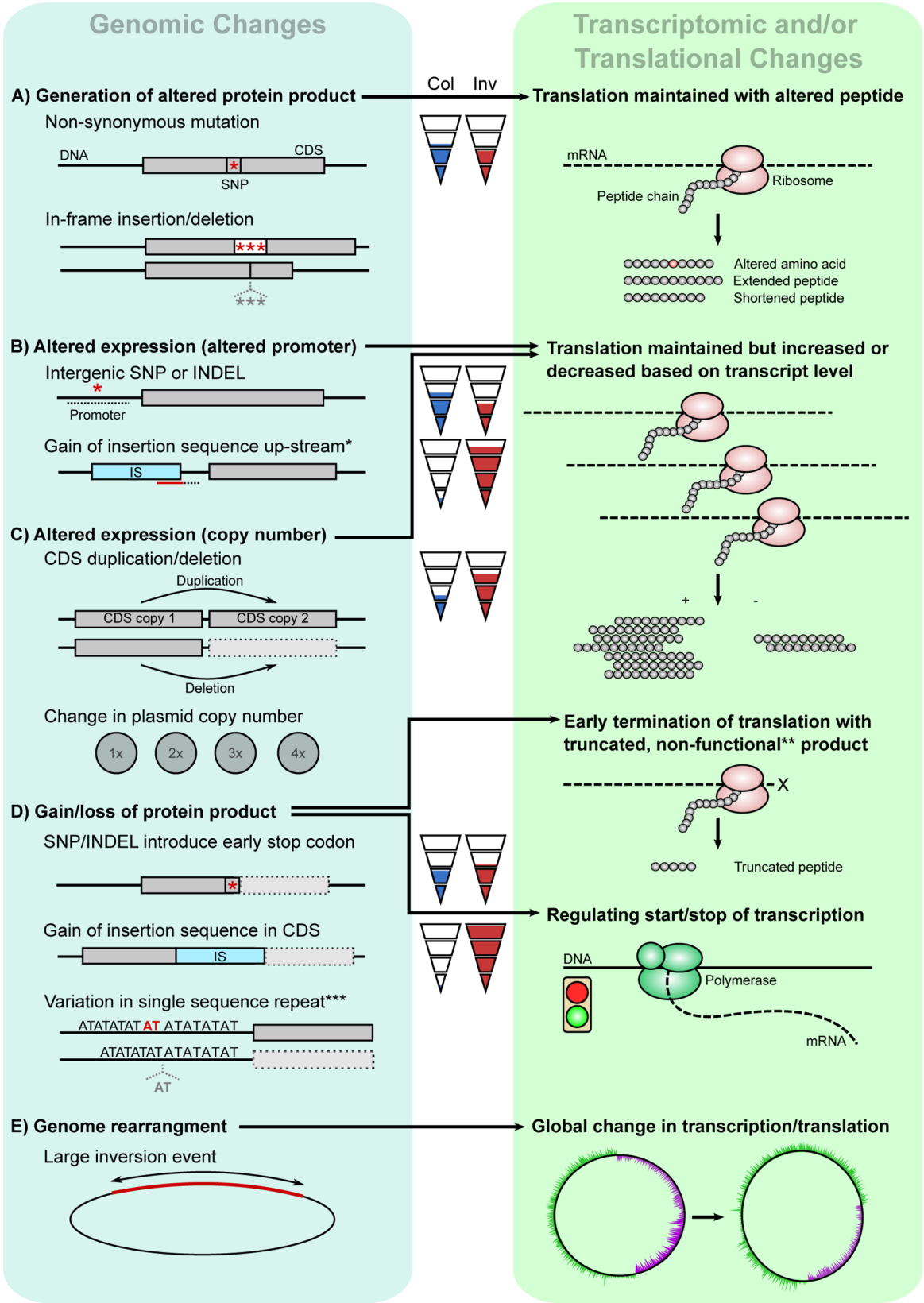
[2] When considering the evolutionary model of *S. aureus* infections (see Box 1), whether enrichment of variants is found within colonising strains, between colonising and invasive strains or within invasive strains (+ suggestive enrichment, no statistical support; ++ statistical support, didn't reach genome-wide significance; +++ strong statistical support)

[3] Statistical significant enrichment of variants (within-host evolution studies) or GWAS results.

[4] Functional confirmation of variants identified in clinical isolates.

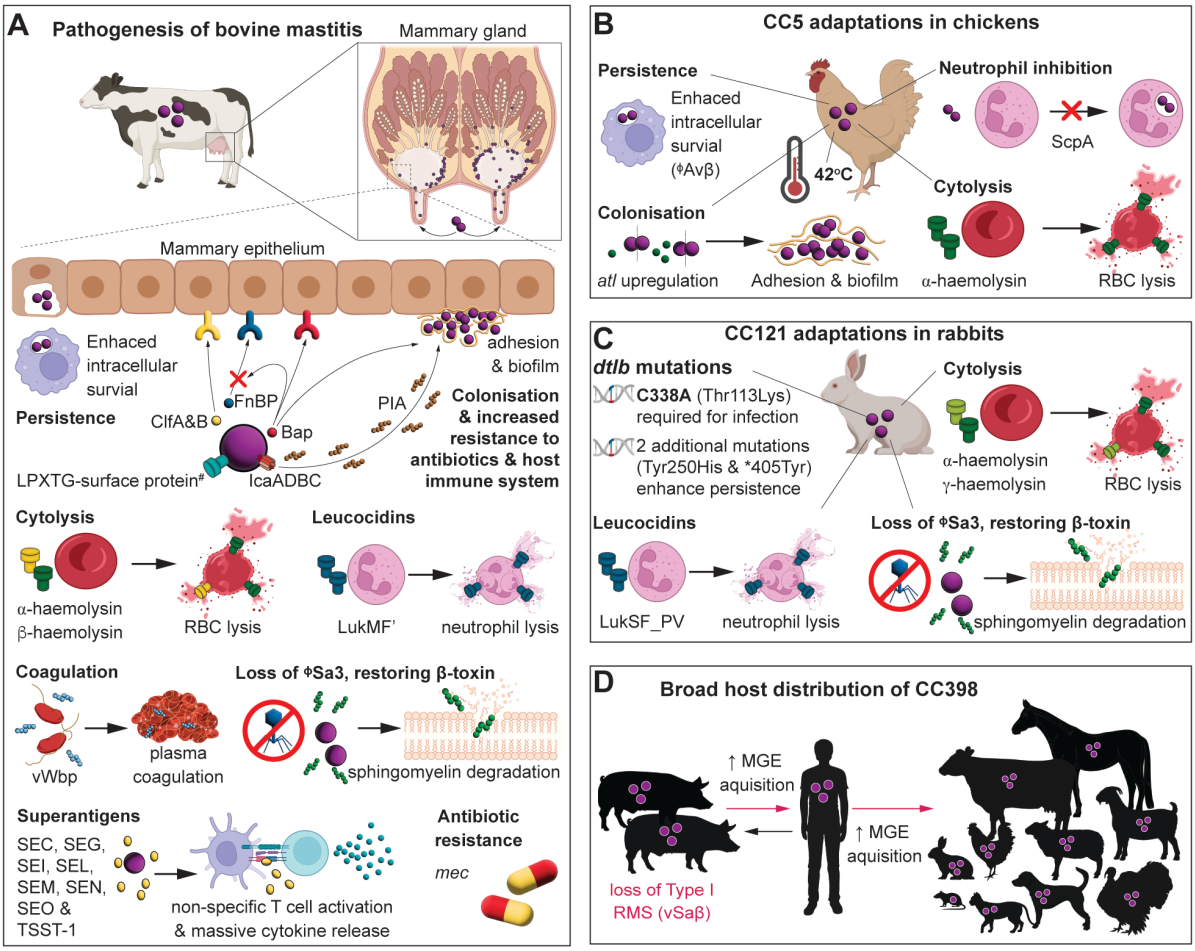


***Supplementary Figure 1. Staphylococcus aureus* colonization in human tissue niches.** Interactions of colonising *S. aureus* with commensal bacteria in various tissue sites in the human body, including (A) the human nasal epithelium (in the anterior nares), (B) the skin, and (C) the gut during healthy and disease states. Shown are examples of the interference with *S. aureus* colonization and viability, and the central role of the *agr* quorum sensing system.



Supplementary Figure 2. **Genome plasticity in *Staphylococcus aureus.*** The genetic changes associated with genome plasticity in *S. aureus*. The panel in blue shows a range of genomic changes that can occur and grouped by common impact on transcription/translation. The green panel shows the transcription/translational impacts. In the centre the pyramids show the

relative proportion of each change between invasive and colonising isolates based on data from [26] (left black if a suitable "mutation" category not available). For each pair of pyramids the colonizing and invasive category together result in 100% of that mutation type seen in the study dataset. Notably, intergenic IS insertions need to overlap/replace all or part of the promoter sequence to have the illustrated impact *; truncated products may have some function, dependent on the location of the truncation and amount of the peptide maintained **. Single sequence repeats (SSRs) can turn on/off transcript through alteration of the promoter and SSRs within CDS can also impact on the protein product in different ways through early or delayed stop codons, and extra/reduced amino acid in the peptide chain***.



***Supplementary Figure 3. S. aureus* host species adaptation.** (A) Pathogenesis of bovine mastitis. Multiple human *S. aureus* lineages have adapted to bovine hosts through the acquisition of host specific mobile genetic elements (MGEs). *S. aureus* surface proteins interact with bovine epithelium to promote bacterial adhesion and invasion. Haemolysins promote mammary gland necrosis and liberate iron from erythrocytes (Adapted from [80,81]). # Putative adhesin. (B) Chickens. The CC5 lineage is thought to have transmitted from human to chickens approximately 40 years ago, with evidence of subsequent genetic recombination resulting in host adaptations including: enhanced growth at 42°C (core body temperature of chickens) thought due to poultry associated genes SAAV_0062 and SAAV_0064; inhibition of neutrophil activation and chemotaxis with a suggested role in poultry dermatitis; increased lysis of chicken erythrocytes; and loss of genes involved in human pathogenesis. Typical poultry manifestations of *S. aureus* infection include dermatitis, pododermatitis, chondronecrosis and osteomyelitis [81,82]. (C) Rabbits. CC121 is a human lineage thought to have transferred to domesticated rabbits in the last 40 years through adaptations resulting from non-synonymous mutations in the core gene *dltB*. The mechanism by which the C338A SNP causes infection is unknown, but *dltB* is hypothesised to have O-acyltransferase enzymatic activity and potential role in cell signalling. Furthermore, rabbits have increased susceptibility to toxin mediated cytolysis. Typical manifestations of infection in rabbits include abscesses, mastitis, pododermatitis and septicaemia [81,83,84]. (D) CC398 is a multi-host lineage, thought to have originated in humans then transmitted to pigs, in which it gained methicillin resistance and lost the Type I Restriction Modification System (RMS) carried on vSaβ, predisposing the lineage to the acquisition of MGEs and assisting adaptation to multiple host species [81,85,86].

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