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INVITED REVIEW

Molecular mechanisms of *Staphylococcus aureus* nasopharyngeal colonization

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SUMMARY

Staphylococcus aureus **is responsible for a wide range of different infections ranging in severity from mild to fatal. However, it primarily exists as a commensal organism in a number of different anatomical sites including the nasopharynx. Although colonization itself is a harmless state, colonized individuals are at risk of endogenous infection when *S. aureus* enters otherwise sterile sites via wounds or indwelling medical devices. As such, studies of colonization may identify important targets for vaccines or other prophylactic approaches. Colonization is a dynamic process; *S. aureus* must attach to host surfaces, overcome immune components and compete with other commensal microbes. This occurs via a number of surface-attached and secreted proteins and other factors such as wall teichoic acid. In addition, colonizing *S. aureus* must constantly replicate to maintain its niche and exclude other strains. These myriad interactions provide a strong selective pressure for the maintenance or enhancement of mechanisms of adhesion, invasion and immune evasion. The evolutionary implications of this may explain why *S. aureus* is such a capable pathogen because many of the proteins involved in colonization have also been identified as virulence factors. This review describes the diverse molecular mechanisms used by *S. aureus* to colonize the host and discusses how the pressures that have selected for these may have led to its virulence.**

INTRODUCTION

Staphylococcus aureus is an important opportunistic human pathogen and leading cause of a wide variety of disease in humans and animals. It is the aetiological agent of a large burden of morbidity and mortality globally, in both hospital and community settings (Diekema *et al.*, 2001). Antibiotic resistance in *S. aureus* is a major clinical problem, in particular, methicillin-resistant *S. aureus*. In response, infection control measures have been enhanced to prevent the spread of this naturally ubiquitous organism, which lives as a commensal of approximately 20% of the population persistently and approximately 60% intermittently (Kluytmans *et al.*, 1997). Carriage of *S. aureus* is a significant risk factor for infection and, as such, studies of colonization provide a potential route to disease prevention (von Eiff *et al.*, 2001). Although there is some evidence that certain *S. aureus* genotypes are more virulent than others, it is generally accepted that all colonizing strains are potentially infectious (discussed by van Belkum *et al.*, 2009a).

The ability of any microorganism to colonize its host is the result of a complex set of interactions. A microbe must first come into contact with the tissues

and then adhere to receptors. It must be able to overcome host defences and then propagate in its new niche. Here, we review some of the current knowledge of the molecular mechanisms by which *S. aureus* colonizes the human host. In addition to discussing mechanisms by which the pathogen can adhere to tissues, the implications of this are considered for both its persistence and capacity to invade host cells. Furthermore, the ability of the bacterium to resist innate defences will be discussed. Last, we will consider whether the selective pressures encountered during colonization have driven *S. aureus* to become a potent pathogen.

STAPHYLOCOCCUS AUREUS COLONIZATION OF THE RESPIRATORY TRACT

The anterior nares are considered to be the primary ecological niche of colonizing *S. aureus*. Typically, three carriage patterns are believed to occur in healthy individuals: non-carriage, intermittent carriage and persistent carriage (Kluytmans *et al.*, 1997). Persistent carriers have higher *S. aureus* load than intermittent carriers, but because of differences in sampling protocols it is possible that individuals labelled non-carriers might in fact be intermittent carriers, or simply carry *S. aureus* at levels around the limit of detection. Indeed, work by van Belkum *et al.* (2009b) suggests that intermittent and non-carriers may essentially comprise the same group because of a similar inability to support *S. aureus* colonization and low levels of anti-staphylococcal antibodies.

A number of different factors are believed to be responsible for the different carriage states, including host factors and the composition of the resident microbiota (Peacock *et al.*, 2001; Van Belkum *et al.*, 2009a; Wos-Oxley *et al.*, 2010). Recent work has identified haemoglobin in nasal secretions as crucial for colonization (Pynnonen *et al.*, 2011). Haemoglobin was found to have an inhibitory effect on the quorum sensing system *agr*, suggesting that it may act as an environmental cue for colonizing strains (Pynnonen *et al.*, 2011). Colonization typically involves a single strain of *S. aureus*, which changes only infrequently over time, indicating that once established, *S. aureus* is able to exclude competing strains (Sakwinska *et al.*, 2010).

While nasal carriage has been studied intensively, research is revealing that presence of the pathogen in other parts of the upper respiratory tract may also prove to be important for the establishment of disease. For example, pharyngeal colonization has been reported to be more prevalent than nasal colonization, although other studies contradict this (Nilsson & Ripa, 2006; Mertz *et al.*, 2007). Certainly, screening of both sites is significantly more sensitive than just the nares (Mertz *et al.*, 2007). It appears that pharyngeal colonization can occur in isolation, rather than as a result of seeding from the nasal cavity, particularly in younger individuals without exposure to healthcare settings (Mertz *et al.*, 2007, 2009).

Oro-pharyngeal colonization by *S. aureus* appears to be associated with ventilator-associated pneumonia (Berdal *et al.*, 2007) and may be the primary source for lung infection of individuals with cystic fibrosis (Ridder-Schaphorn *et al.*, 2007). *S. aureus* is also believed to be an important pathogen in recurrent tonsillitis, persisting within host tissues between episodes of symptomatic infection (Zautner *et al.*, 2010).

COLONIZATION IS A DYNAMIC PROCESS INVOLVING MULTIPLE HOST–PATHOGEN INTERACTIONS

Colonization of the mucosae by *S. aureus* is a complicated process that involves a number of different host and bacterial factors. In addition to attaching to host surfaces, *S. aureus* must compete with other microbes, overcome host immune factors and establish itself in a persistent state (Fig. 1).

Attachment of *S. aureus* to host surfaces is multi-factorial

Despite the potential importance of oro-pharyngeal colonization by *S. aureus*, very little is known of the molecular mechanisms by which this occurs. By contrast, a number of recent studies have shed light on how *S. aureus* attaches to the nasal epithelium and have identified targets for prophylactic vaccines aimed at preventing colonization.

Several different *S. aureus* surface proteins that play a role in colonization have been identified using various models (Table 1). Of these the best characterized is ClfB, which binds human type 1 cytokeratin 10 found on the surface of human nasal cells (O'Brien *et al.*, 2002). Strains of *S. aureus* that are

deficient in ClfB showed reduced attachment to desquamated nasal cells, whereas heterologous *Lactococcus lactis* cells expressing ClfB were able to attach (O'Brien *et al.*, 2002). This work was extended using a murine model of nasal colonization, which showed that an isogenic Δ*clfB* mutant failed to colonize as well as the wild-type (WT) strain (Schaffer *et al.*, 2006). In support of these findings, immunization of mice with recombinant ClfB significantly reduced but did not eliminate *S. aureus* colonization (Schaffer *et al.*, 2006).

In addition to studies *in vitro* and in animals, ClfB has been shown to be important in human models of nasal colonization. A WT strain and isogenic Δ*clfB* mutant were administered either separately or together to human volunteers and persistence was measured. Wild-type *S. aureus* persisted for longer and outcompeted the Δ*clfB* strain, confirming an important role for ClfB in human nasal colonization (Wertheim *et al.*, 2008).

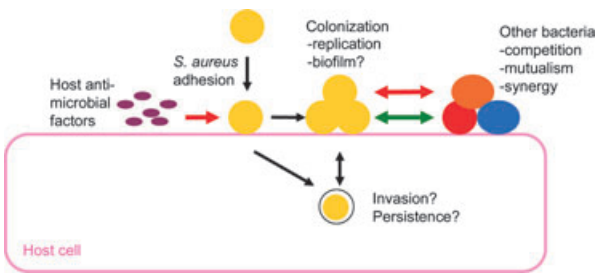

Figure 1 *Staphylococcus aureus* colonization is a multi-factorial process, involving interactions with host cells, immune components and other bacteria.

There is compelling evidence that the multi-functional iron-regulated surface determinant protein A (IsdA) is also a key component of nasal colonization. IsdA is expressed under iron-limited conditions and binds a number of different substrates including fibronectin, fibrinogen, and several proteins associated with the cell envelope of desquamated nasal epithelial cells including loricrin, involucrin and cytokeratin 10 (Clarke *et al.*, 2004, 2009). In keeping with this, *S. aureus* grown under iron-limited conditions bound more strongly to desquamated human nasal epithelial cells than bacteria grown in abundant iron (Clarke *et al.*, 2004). The importance of IsdA to colonization has been demonstrated in a cotton rat colonization model, where an IsdA-deficient mutant was defective for colonization compared with WT (Clarke *et al.*, 2006).

As is the case for ClfB, IsdA is an attractive candidate antigen for a colonization-blocking vaccine. Inoculation of cotton rats with recombinant IsdA significantly inhibited subsequent *S. aureus* colonization (Clarke *et al.*, 2006). Furthermore, analysis of 150 individuals revealed that healthy non-carriers had significantly higher titres of antibody against IsdA than healthy carriers, suggesting that anti-IsdA antibodies might inhibit human colonization (Clarke *et al.*, 2006).

Table 1 Summary of the evidence for a role in colonization of selected *Staphylococcus aureus* adhesins

Adhesin	Protein binding	Cell binding	Animal model	Human studies	References
ClfB	Binds type I cytokeratin	Binds DNECs	Colonization by a Δ*clfB* mutant strain significantly reduced compared with wild-type. Immunization of mice with rClfB significantly inhibited colonization	Colonization by a Δ*clfB* mutant significantly reduced compared with wild-type	O'Brien *et al.* (2002); Schaffer *et al.* (2006); Wertheim *et al.* (2008)
IsdA	Binds DNEC-associated cell envelope proteins	Binds DNECs	Colonization by a Δ*isdA* mutant strain significantly reduced compared with wild-type. Immunization of mice with rIsdA significantly inhibited colonization	Elevated anti-*isdA* antisera in non-carriers compared with carriers	Clarke *et al.* (2004, 2006, 2009)
WTA		Binds HNECs	Essential for colonization of cotton rat nasal cavities		Weidenmaier *et al.* (2004)
SasG	?	Binds DNECs			Roche *et al.* (2003)
SdrC	?	Binds DNECs			Corrigan *et al.* (2009)
SdrD	?	Binds DNECs			Corrigan *et al.* (2009)

DNEC, desquamated nasal epithelial cells; HNEC, human nasal epithelial cells; WTA, wall teichoic acid.

In addition to proteinaceous adhesins, an *S. aureus* Δ*tagO* mutant lacking wall teichoic acid (WTA) has been shown to be unable to colonize the nasal cavities of cotton rats (Weidenmaier *et al.*, 2004). Disruption of WTA biosynthesis may have profound effects on cell-surface architecture, but the Δ*tagO* mutant does not appear to be deficient in fibronectin or fibrinogen binding, suggesting that WTA deficiency does not reduce surface display of SrtA-anchored adhesins (Weidenmaier *et al.*, 2005). Furthermore, WTA appears to directly interact with host surfaces because pre-incubation of human cells with WTA inhibited *S. aureus* adhesion (Weidenmaier *et al.*, 2004). Conversely, coating latex beads with WTA rendered them able to attach to human cells (Weidenmaier *et al.*, 2004).

Although it appears that ClfB, IsdA and WTA are required for colonization, several other adhesins have been shown to bind desquamated nasal epithelial cells including SasG, SdrC and SdrD, underlining the multi-factorial nature of *S. aureus*–host interactions (Roche *et al.*, 2003; Corrigan *et al.*, 2009). It is therefore possible that selection against ClfB or IsdA (e.g. via a vaccine), may result in escape mutants that employ these other adhesins in colonization.

Both WTA and proteinaceous adhesins have been implicated in adhesion to nasal cells or associated proteins but it is likely that these play different roles during colonization. Analysis of gene expression over a 10-day colonization experiment in rats indicated that different adhesins appear to be expressed at different times. Specifically, genes governing WTA synthesis were important during the early phase of colonization, whereas *clfB* and *isdA* were upregulated later. As such, it seems that WTA is important for initial host–pathogen interactions, whereas ClfB and IsdA are important for maintaining attachment and colonization (Burian *et al.*, 2010a,b). In support of this, Weidenmaier *et al.* (2008) showed that a WTA-deficient *S. aureus* mutant strain failed to colonize the nasal cavity of cotton rats for even short periods, whereas an isogenic Δ*srtA* mutant managed to colonize for 6 days but was eradicated after 14 days. Given the apparent functional redundancy within the adhesin repertoire it is likely that any colonization-blocking vaccine would need to include several antigens, including ClfB and IsdA, to be fully effective in humans.

Is cellular invasion important for colonization?

Although originally considered to be an extracellular bacterium, *S. aureus* is in fact able to enter a diverse range of host cell types (Sinha *et al.*, 1999). Invasion is mediated by the interaction of staphylococcal fibronectin-binding proteins (FnBPs) with host cell $\alpha_5\beta_1$ integrins via a fibronectin bridge (Sinha *et al.*, 1999). FnBPs consist of an N-terminal domain that is followed by 10 (FnBPB) or 11 (FnBPA) non-identical fibronectin-binding repeats (FnBRs) with either high-affinity or low-affinity for fibronectin, and the SrtA recognition motif LPXTG (Meenan *et al.*, 2007). Recent work has shown that invasion of keratinocyte cells is dependent upon the presence of multiple high-affinity FnBRs within FnBPA (Edwards *et al.*, 2011). Although triggered by bacterial FnBPs, bacterial entry is entirely mediated by the host cell and involves actin rearrangement (Sinha *et al.*, 1999). A role for FnBPA in colonization is supported by transcriptomic analysis of colonizing strains in humans, which have shown that *fnbA* is strongly expressed by colonizing *S. aureus* (Burian *et al.*, 2010a,b).

Although the significance of cellular invasion in colonization or infection is unclear, it has been hypothesized to provide a protected niche, away from host immune surveillance and antimicrobial peptides, and so serve as a reservoir for recurrent colonization or infection. This hypothesis is supported by the presence of intracellular *S. aureus* both within nasal epithelial cells recovered from asymptomatic patients after treatment for recurrent rhinosinusitis and from the tonsils of patients with recurrent tonsillitis (Clement *et al.*, 2005; Zautner *et al.*, 2010). Hence, it is likely that intracellular *S. aureus* provides a protected reservoir for reinfection after therapy. However, it is not known whether invasion of host cells is a normal part of the colonization process.

Staphylococcus aureus overcomes multiple elements of the host immune system

External surfaces such as human mucosae and skin are coated in a cocktail of antimicrobial peptides and fatty acids that deter invading microorganisms. To successfully colonize a niche, any microorganism must be able to resist the actions of host defences. As *S. aureus* inhabits multiple niches, often within the same host, it can be presumed that the combination

of antimicrobial molecules faced by the pathogen differs between sites and therefore a requirement exists for a combination of multiple resistance factors. This probably explains why *S. aureus* deploys such a broad range of strategies to overcome host immune components.

Small, cationic host antimicrobial peptides, such as defensins and cathelicidin, disrupt bacterial membranes. To reach their target, hydrophobic and electrostatic interactions take place. Addition of D-alanine residues to teichoic acids, membrane protein MprF and cell wall protein IsdA all reduce the net negative charge of *S. aureus* (Peschel *et al.*, 1999, 2001; Clarke *et al.*, 2007). Furthermore, IsdA renders the bacterium less hydrophobic, conferring resistance not only to antimicrobial peptides, but also bactericidal lipids, and promotes *S. aureus* survival on human skin (Clarke *et al.*, 2007). In addition to these activities, IsdA can bind bactericidal lactoferrin (Clarke & Foster, 2008), the predominant anti-*S. aureus* protein in the human nose. The antibiotic activity of lactoferrin against bacteria has been recognized for many years and was attributed to iron sequestration until the irreversible inhibition of *Streptococcus mutans in vitro* was found to be independent of iron deprivation. Apolactoferrin has been shown to have multiple enzymatic activities, but serine protease is responsible for its anti-*S. aureus* activity. In binding to apolactoferrin, IsdA acts as a protease inhibitor and reduces its ability to kill the pathogen (Clarke & Foster, 2008).

In addition to resistance to host innate defences via modification of the cellular envelope, *S. aureus* employs a number of extracellular proteins that can degrade antimicrobial peptides. Staphylokinase, a bacteriophage-encoded enzyme expressed by some *S. aureus* strains, binds defensins and inhibits their activity (Jin *et al.*, 2004). Similarly, human cathelicidin LL-37 is readily degraded by aureolysin, a metalloprotease produced by *S. aureus* (Sieprawska-Lupa *et al.*, 2004).

As described for adhesins, some evasins appear to be required at different stages of colonization. The β-haemolysin converting bacteriophages encode the immune evasion cluster, which includes chemotaxis inhibitory protein, staphylococcal complement inhibitor and staphylokinase. Whereas 90% of colonizing strains from persistent carriers encode the immune evasion cluster it appears to be completely unnecessary during the early stages of colonization (Verkaik *et al.*, 2011). In addition to the innate arm of the immune system, humoral immunity also appears to be an important aspect of *S. aureus* colonization. Persistent adult carriers had higher antibody titres than non-carriers against the *S. aureus* toxins toxic shock syndrome toxin and staphylococcal enterotoxin A, as well as the adhesins ClfA and ClfB, indicating that these proteins are expressed during colonization (Verkaik *et al.*, 2009). Although these antibodies clearly do not prevent colonization they may explain why carriers suffer less severe *S. aureus* bacteraemia than non-carriers (Verkaik *et al.*, 2009).

Colonizing *S. aureus* must constantly replicate

The microbiota of the human nasal cavity appears to be reasonably stable, differing only slightly over weeks or months (Frank *et al.*, 2010). In keeping with this, introduction of *S. aureus* into the nasal cavities of neonatal cotton rats revealed that the numbers of *S. aureus* colony-forming units appeared to reach a plateau and subsequently remain constant over time, regardless of the size of the inoculum (Margolis *et al.*, 2010). This might suggest a situation akin to stationary phase in broth culture, but transcriptomic analyses of colonizing *S. aureus in vivo* indicate otherwise.

Staphylococcus aureus in stationary phase typically switches on its quorum-sensing accessory gene regulator (*agr)* system leading to downregulation of surface proteins and induction of cytolytic toxins and protease production (Recsei *et al.*, 1986). This facilitates bacterial spread to new locations and generates sources of nutrition such as damaged tissue. Analysis of *agr* expression within the human nasal cavity found it to be very weak and in keeping with this, genes encoding the cytolytic toxins *hla*, *psm* and *blhB* were also poorly expressed (Burian *et al.*, 2010a,b). This, in combination with high-level expression of surface proteins, is indicative of exponential growth and implies that there are high rates of turnover among colonizing *S. aureus*. This is further supported by the high-level expression of genes involved in cell wall biosynthesis such as WalKR (Burian *et al.*, 2010a,b). Significant numbers of *S. aureus* and other organisms are lost through shedding of nasal epithelial cells and mucous flow. This means that individuals with low levels of colonizing *S. aureus* are more likely to eliminate the pathogen

than individuals with higher colonizing loads (Sakwinska *et al.*, 2010). Replication must therefore occur constantly to enable colonization of the newly exposed surfaces and outcompete other *S. aureus* strains (Sakwinska *et al.*, 2010), which requires high-level expression of surface proteins. This has evolutionary implications for bacteria within the nasal cavity; a consistently high turnover rate of colonizing *S. aureus* provides ample opportunity for the selection of spontaneous mutants with enhanced ability to replicate, attach, overcome immune factors or outcompete other organisms.

Does colonization provide the selective pressure for maintenance of virulence factors?

Although *S. aureus* is responsible for a wide range of both superficial and invasive infections, it is primarily a commensal organism. *S. aureus* is not unique in this respect; a number of other invasive pathogens are also members of the commensal nasopharyngeal microbiota, including *Neisseria meningitidis*, *S. pneumoniae* and *Haemophilus influenzae*. All of these organisms encode a range of different adhesins, invasins and evasins that enable them to cause serious invasive infections.

It is likely that superficial skin infections promote *S. aureus* persistence and transmission. By contrast, it is unclear whether invasive infections benefit *S. aureus* or are merely the result of accidental entry of the organism into normally sterile sites. Although *S. aureus* bacteraemia can be fatal, resulting in a dead end for the pathogen, it can also lead to persistent or chronic infections such as osteomyelitis and septic arthritis (Fowler *et al.*, 2003) or abscesses, which may facilitate further spread. As such, it is currently unclear what the net benefit is to the bacterium of causing invasive infections.

The ability of *S. aureus* to cause such diverse invasive infections is the result of its myriad adhesins, invasins, evasins and toxins. However, it seems unlikely that relatively rare invasive infections provide the selective pressure for the maintenance of this broad range of effector molecules. Rather, one plausible explanation for why *S. aureus* is so well equipped to cause infection is that this selective pressure is provided by colonization. In support of this hypothesis, many of the *S. aureus* adhesins, invasins and evasins described above as having a role in colonization have also been identified as being important in invasive infections (Table 2).

One example of this is the presence of multiple FnBRs within FnBPA. The invasion by *S. aureus* of endothelial cells is thought to facilitate bacterial escape from the bloodstream during bacteraemia and promote penetration into surrounding tissues (Edwards *et al.*, 2010). Recent work showed that multiple FnBRs within FnBPA are essential for virulence in a murine bacteraemia model, but it seemed unlikely that such a rare and often rapidly fatal condition would provide selective pressure for the composition of FnBPA functional regions (Edwards *et al.*, 2010). A follow-up study revealed that multiple FnBRs are critical for invasion of keratinocytes. Hence, *S. aureus* interactions with the skin and nasal cavity probably provide the selective pressure for the composition of the FnBR region within FnBPA (Edwards *et al.*, 2011).

Table 2 Comparison of the role of various *Staphylococcus aureus* proteins and other elements in colonization and infection

Element	Role in colonization	Role in infection[1]	References
WTA	Important adhesin in early stages of nasal colonization	Lack of WTA associated with reduced virulence in a rabbit endocarditis model	Weidenmaier *et al.* (2004, 2005)
FnBPA	Multiple FnBRs within FnBPA essential for invasion of keratinocytes	Multiple FnBRs within FnBPA essential for virulence in a murine sepsis model	Edwards *et al.* (2010, 2011)
ClfB	Important adhesin for attachment to nasal epithelial cells	A Δ*clfB* mutant had reduced penetration into kidneys compared with wild-type in a murine bacteraemia model	O'Brien *et al.* (2002); Cheng *et al.* (2009)
IsdA	Multiple roles including adhesion and immune evasion	A Δ*isdA* mutant had reduced penetration into kidneys compared with wild-type in a murine bacteraemia model	Clarke *et al.* (2004, 2007, 2009); Cheng *et al.* (2009)

[1]Selected examples.

WTA, wall teichoic acid; FnBR, fibronectin binding repeats.

Colonization also provides selective pressure for proteins that aid immune evasion. Both nasal secretions and saliva contain proteins associated with innate or acquired immunity including complement components, immunoglobulins, lactoferrin, transferrin, lysozyme, mucin and scavenger receptors (Casado *et al.*, 2005; Hu *et al.*, 2005). This provides a strong selective pressure for maintenance of at least some of the vast number of different staphylococcal immune evasion proteins and capsule, which was found to be required for maximal colonization levels in a murine model (Kiser *et al.*, 1999).

Although colonization probably selects for many of the adhesins, invasins and evasins within the *S. aureus* virulence factor arsenal, it does not appear to provide the selective pressure for expression of cytolytic toxins, superantigens and phenol-soluble modulins. As described above, expression of cytolytic α-haemolysin and phenol-soluble modulins by colonizing *S. aureus* is weak (Burian *et al.*, 2010a,b). In keeping with this, *agr* is also expressed at very low levels in the nasal cavity (Burian *et al.*, 2010a,b). Indeed, forced constitutive expression of *agr* in a murine model resulted in reduced levels of colonization (Pynnonen *et al.*, 2011). However, although *agr* dysfunction is not a barrier to transmission and colonization, it is a rare occurrence in *S. aureus* colonizing healthy individuals (Shopsin *et al.*, 2008). This suggests that there is either an unappreciated role for *agr* in colonization or another selective pressure is responsible for maintaining *agr* function among colonizing *S. aureus*.

Although *S. aureus* does not typically colonize the skin, it is a common cause of superficial and soft-tissue infections. These usually mild, often self-limiting infections are strongly linked to the production of cytolytic toxins, phenol-soluble modulins or exfoliative toxins and have been hypothesized to facilitate *S. aureus* transmission (Massey *et al.*, 2006). As such, superficial and soft-tissue infections probably provide the selective pressure for *agr* function and toxin production in *S. aureus* and hence transmission, the vital pre-requisite to colonization. Within a host and those within close physical proximity (e.g. mother–child), maintenance of high adhesiveness and low toxicity is beneficial, allowing continuous colonization. The expanded transmission potential, beyond these close quarters, provided by the ability to switch to a toxic, infection-causing phenotype can explain why high adhesion and low toxicity are maintained.

In summary, colonization probably selects for at least some of the adhesins, invasins and evasins that are important for causing serious invasive infection (Table 2). However, it is unlikely to drive the maintenance of *agr* or genes that encode *S. aureus* toxins, the presence of which are important for transmission, and distinguish *S. aureus* from other nasal-colonizing bacteria. This explains why other successful colonizers of the nasal cavity, such as *Staphylococcus epidermidis*, are apparently less pathogenic than *S. aureus*. The *S. aureus* adhesins, invasins and evasins are important for superficial and invasive infections, but the cytolytic toxins are also crucial (Massey *et al.*, 2006; Otto, 2010). As such, it can be hypothesized that *S. aureus* is able to cause invasive infections because of the combined selective pressures of colonization and superficial skin infection.

Staphylococcus aureus has shown a remarkable ability to adapt to environmental pressures, particularly with respect to its ability to overcome antibiotic therapy and its success within healthcare settings. The requirement of *S. aureus* to constantly adapt to its environment, running to stand still, is unlikely to lead to a less virulent, truly commensal phenotype. Indeed, the emergence of community-associated methicillin-resistant *S. aureus*, able to resist antibiotic therapy and cause severe superficial and soft-tissue infections in otherwise healthy individuals, provides evidence that *S. aureus* continues to evolve enhanced mechanisms of transmission, persistence and virulence (Otto, 2010).

Outstanding questions

How important are each of the different anatomical sites for *S. aureus* colonization, *S. aureus* transmission, and as a source of infection?

What role if any does *agr* play in colonization?

What are the environmental cues for expression of colonization-dependent genes?

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