The Pathogenesis of Staphylococcus aureus Infection

Anthony R. Dal Nogare, MD

Medical Grand Rounds

March 23, 2007

This is to acknowledge that Dr. Dal Nogare has no financial interests or other relationships with commercial concerns related directly to this program and will not be discussing off-label uses in this presentation.

Introduction

By any measure Staphylococcus aureus (S. aureus) has been an extraordinarily successful nosocomial pathogen over the past twenty years. It is the microbe most often isolated from ventilator-associated pneumonia patients, the second commonest bloodstream isolate (after Staphylococcus epidermidis, a closely related species), and is a frequent cause of endocarditis, osteomyelitis, and device-related infections of artificial joints, pacemaker wires, and vascular grafts. Coincident with its increasing prevalence has been the spread of antibiotic resistant S. aureus strains, particularly methicillin-resistant S. aureus (MRSA). Initially a hospital problem, MRSA has now disseminated widely into the community and now community-acquired MRSA (CAMRSA) has appeared. Thus MRSA must now be considered a possible pathogen in community and hospital acquired sepsis, skin/soft tissue infection, and pneumonia.

The increasing prevalence of MRSA has had two major effects - vancomycin use has skyrocketed and expensive, time consuming infection control measures, designed to identify MRSA colonized patients and prevent transmission to non-colonized patients and hospital workers, are now commonplace in most hospitals.Excessive vancomycin use has predictably led to the emergence of vancomycin resistant S. aureus. This review will concentrate on the molecular mechanisms utilized by S. aureus to colonize and infect humans. The topics reviewed will include skin and catheter colonization, virulence factors important for disease, and antibiotic resistance. Pathogenic mechanisms with potential for novel anti-staphylococcal therapies will be emphasized.

Colonization

S. aureus normally exists as a harmless commensal bacteria colonizing the skin/anterior nares of many healthy individuals. Staphylococcal colonization depends on a number of bacterial cell-surface proteins projecting from the peptidoglycan cell wall; some belong to the MSCRAMM group (microbial-surface components recognizing adhesive matrix molecules) which includes bacterial adhesins for collagen, elastin, fibronectin, and fibrinogen(1). S. aureus is adept at colonizing the surface of plastic tubes and other devices which, by disrupting normal anatomic barriers, allow bacterial entry into the blood, lungs, and other organs. S. aureus can switch from a colonizing phenotype to a virulent, disease producing mode by decreasing cell surface adhesins and upregulating synthesis of secreted virulence factors. When this occurs in a colonized patient serious Staphylococcal disease often ensues. Thus colonization is usually the essential first step leading to a Staphylococcal infection.

Skin colonization – Colonization of skin, perineum, and anterior nares is common. S. aureus adheres particularly well to keratinized epithelial cells in the anterior nares and nasal cultures obtained from from healthy outpatients show that 20% of people are persistently colonized (usually with the same strain over time), 30% carry S. aureus intermittently, and 50% are never colonized . In vitro, nasal epithelial cells obtained from chronic carriers exhibit increased Staphlococcal adherence and non-carriers clear inoculated S. aureus from the nose but carriers do not; these observations suggest

important, presumably genetic, differences in S. aureus epithelial cell ligands but the identity of these ligand(s) is not known (2).

Four bacterial cell surface adhesins, three of which belong to the MSCRAMM family, are known to mediate epithelial cell adherence and colonization. Clumping factor B binds to cytokeratin type 10. Bacterial collagen and fibronectin binding proteins adhere to these interstitial matrix molecules but only when the overlying epithelium is disrupted and they become exposed. Teichoic acid, a complex polymer of ribitol phosphate, N-acetylglucosamine, and D-alanine, is bound to cell wall peptidoglycan and plays a major role. S. aureus mutants lacking an enzyme (UDP-N-acetylglucosamine transferase) essential for teichoic acid synthesis, in contrast to teichoic acid positive Staphylococci, are unable to colonize cotton rat nares and adhere minimally to primary culture human nasal epithelial cells. As shown on Figure 1, the Δ tagO mutants adhere poorly to both nasal epithelial cells and A549 cells, a pulmonary epithelial cell line (3). Cell wall teichoic acid also promotes S. aureus adherence to endothelial cells and virulence in a rabbit endocarditis model (4). The nasal epithelial cell ligand recognized by teichoic acid has not been identified.

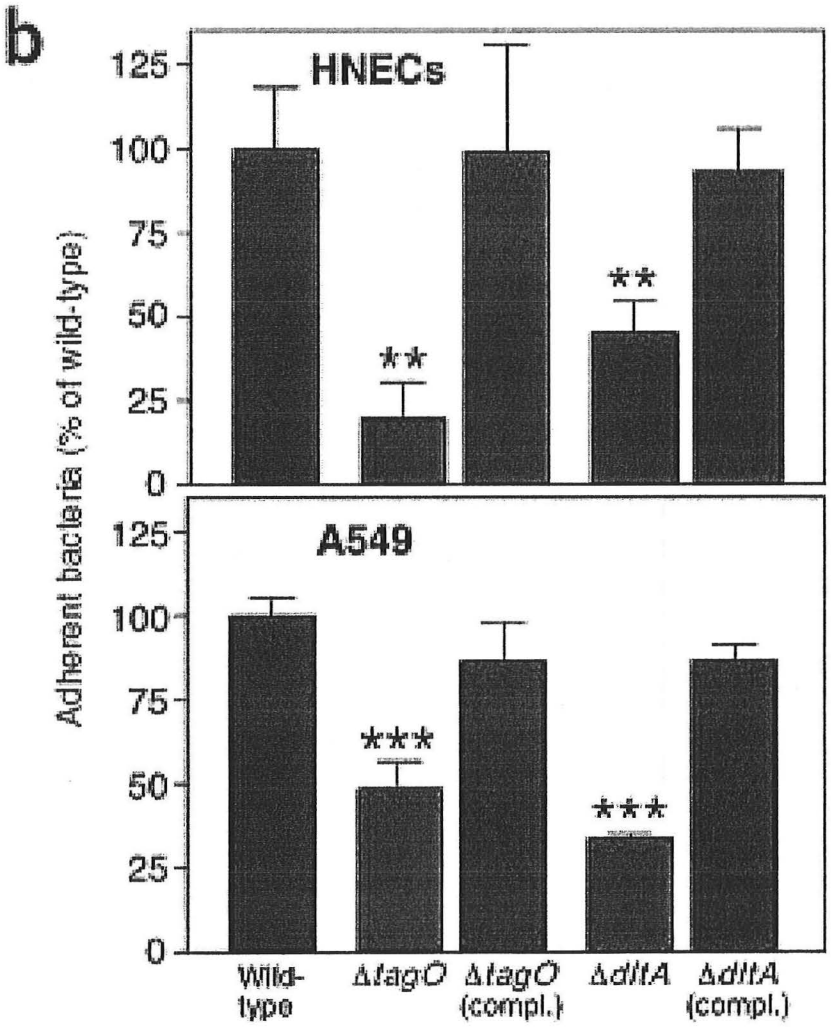


Figure 1. Adherence of teichoic acid deficient (ΔtagO) S. aureus to epithelial cells. Ref. 3

Many clinical studies have shown that nasal colonization at the time of hospital admission confers a 3-12 fold increased risk of S.aureus bacteremia, often from the identical colonizing strain(2). Retrospective investigation of patients with S.aureus bacteremia have demonstrated the same strain (by PFGE pattern) present in the nares of 80% (5). These sorts of observations have led to attempts at de-colonizing patients with mupirocin, a topical antistaphlococcal antibiotic to which most S. aureus is susceptible. Unfortunately topical mupirocin does not significantly reduce S.aureus disease in hospitalized patients (6). Mupirocin is ineffective for several reasons. Many S. aureus infections occur in patients who are not colonized at the time of admission, and mupirocin does not clear bacteria from the skin and perineum, two common ccolonization sites. Even when mupirocin is combined with chlorhexidene body washes only about 25% of patients are successfully decolonized for their entire hosptal stay (7). Due to both its lack of efficacy and concerns about S. aureus becoming mupirocin resistant with heavy use most authorities do not recommend it (8,9).

Device Colonization and Biofilm Growth – The ability of S. aureus to adhere to plastic surfaces is a distinct advantage facilitating infection of instrumented hospitalized patients. After insertion, the outer intravenous catheter surface is rapidly coated with the plasma proteins fibrinogen and fibronectin. S. aureus clumping factor A and B binds to fibrinogen/fibrin, and staphylococcal fibronectin binding protein A recognizes fibronectin; both are MSCRAMM family adhesins expressed during exponential growth. In vitro, coating unused catheters with nanogram amounts of either substance promotes S.aureus adherence, and experiments with used catheters, removed from patients, suggest that catheter-absorbed fibronectin is mainly responsible for staphylococcal adherence (10-12). Within days of intial colonization S. aureus begins secreting an extracellular polysaccharide (PIA, polysaccharide intercellular adhesin) which is a linear homopolymer of B1,6-N-acetylglucosamine and is often called a slime layer or biofilm. S. epidermidis, closely related to S.aureus but less virulent, secretes an identical PIA and often colonizes intravenous catheters. The ability to grow within a biofilm is advantageous for bacteria colonizing inanimate surfaces;the relevance of biofilms to what are called device-related infections has only recently been appreciated(13,14). PIA secretion is controlled by the ica gene locus and is maximal during initial growth when bacterial density is low and the agr global virulence regulon is inactive (15).

Persuasive evidence of biofilms' relevance comes from scanning electron microscopy of catheters removed from patients. Virtually all catheters (Figure 2) and endotracheal tubes (figure 3) removed after four days show S. aureus growing in a thick biofilm matrix(16). From 60-80% of S. aureus removed from infected catheters produce PIA in vitro, as do most S. aureus colonizing endotracheal tubes (17,18). Data obtained from a murine catheter infection model show that S. sureus produces PIA within four days of colonizing a catheter, and PIA positive strains grow faster on the in vivo catheter surfaces (15). Ica mutant S. epidermidis, unable to make PIA, are avirulent in animal catheter infection models; 75% of infected catheters are sterile 8 days after innoculation and few of the ica mutant infected animals develop bacteremia or distant metastatic abscesses (19).

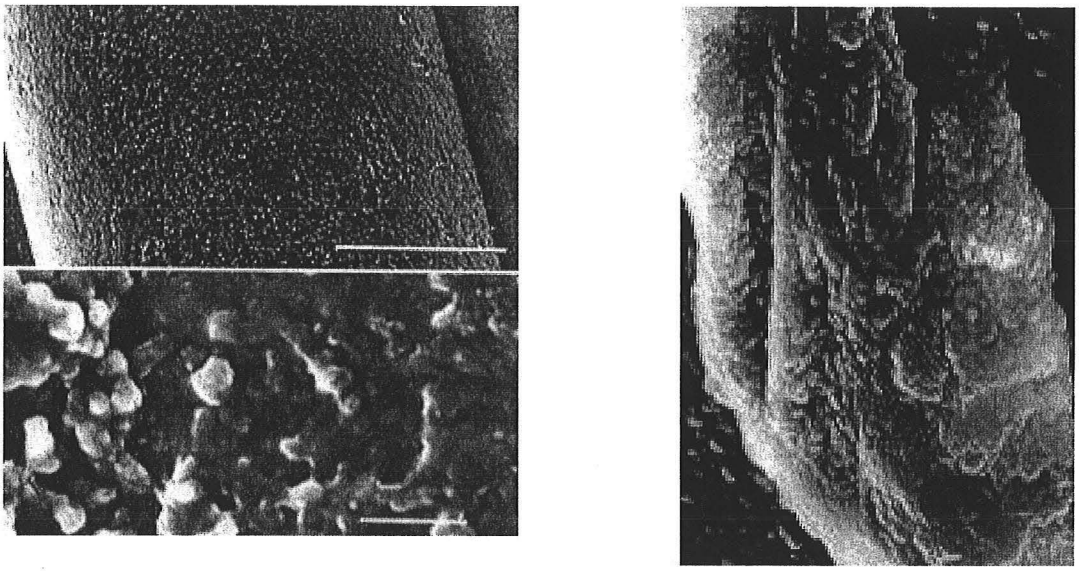


Figure 2. Bacteria within biofilms on medical devices – intravascular catheter (left) and an endotracheal tube (right). Ref. 16

Growth within a biofilm offers three major advantages to S. aureus:
Reduced PMN phagocytosis and killing - Microscopic observations have shown that PMNs can enter biofilms but seem paralyzed and unable to ingest S.aureus within the PIA matrix (20).
Antibiotic resistance - The MBC (minimal bactericidal concentration) of antibiotics is often 1,000 times higher when S. aureus are grown in a biofilm, compared to the same bacterial strain grown in suspension. This relative resistance is not due to impaired diffusion of antibiotics into biofilms; rather it reflects a gradient of bacterial metabolism within the three dimensional biofilm structure, in which bacteria deepest in the film, away from the biofilm's external surface, are growing very slowly and are thus resistant to antibiotics targeting metabolically active bacteria (21,22). An important implication is that S. aureus antibiotic susceptibility, determined in clinical laboratories on bacteria grow in suspension, is not relevant for biofilm infections. Even prolonged courses of antibiotic therapy fail to sterilize biofilms and will select for antibiotic-resistance.
Biofilm emboli - In a clever in vitro model of a catheter surface infection, with flow of culture medium across the catheter, S. aureus quickly forms a biofilm, grows, and emboli of biofilm-encased bacteria break off within 48 hours. Most of the emboli contain 10 to 100 bacteria but some very large ones, containing up to 10^7 CFU S. aureus, were observed (23,24). The MBC of S. aureus in bioemboli was 4 fold increased. It is easy to imagine the clinical significance of bioemboli breaking off from intravenous catheters and seeding distant organs or getting aerosolized off endotracheal tubes into the lungs.

Quorum Sensing and Virulence

Colonized patients are not sick. It is illustrative to compare two closely related Staphylococcus species - S. epidermidis and S.aureus- which are equally adept at chronic, biofilm-mediated colonization of medical devices, but only S. aureus causes disease. Their genomes are very similar except S. aureus contains about thirty extra genes, many coding for secreted or cell-surface proteins which have been linked to virulence(25-28). Understanding how these relatively few virulence genes are regulated

is key to understanding how S. aureus shifts gears and changes from a commensal bacteria to a pathogen. Quorum sensing is one mechanism used by S. aureus to activate many physically unconnected virulence genes in a coordinated fashion and simultaneously turn off colonizing genes. Quorum sensing works because S. aureus can monitor its immediate environs and sense the number of neighboring bacteria; when bacterial density at a colonized site reaches a critical level virulence genes are activated enabling a program of bacterial detachment, tissue invasion, and disease(29-34).

The accessory gene regulation (agr) pathway is the best characterized global virulence regulatory system of S.aureus (fig. 4)

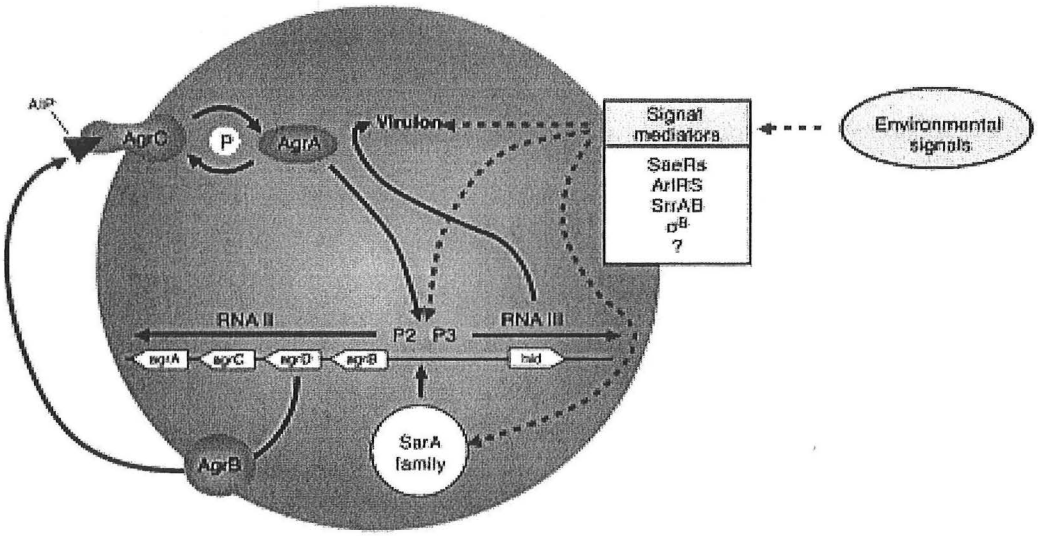


Figure 4. An overview of S. aureus quorum sensing pathways. Ref. 29

The total agr locus consists of two transcription units, RNA II and RNA III, and their corresponding P2 and P3 promoters. The RNA II unit is constitutively expressed and contains four genes, agr A, B, C and D. The protein product of agrD is the actual secreted signal used to sense local bacterial population density; it is called an autoinducing peptide (AIP). AIPs contain 7-9 amino acids and a thiolactone ring and are classified into four groups based on structural differences, as shown on figure 4 (35,36).

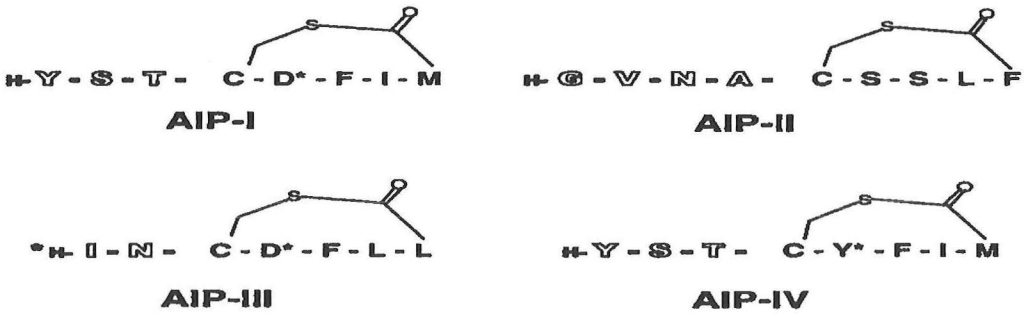


Figure. 5. Secreted AIPs used for quorum sensing. Ref. 35

The other three components of RNA II function as follows- AgrB processes and exports AIPs out into the surrounding mileu, AgrC is a transmembrane sensor which responds to AIP by phosphorylating agrA which then increases transcription of the RNAII unit. Thus RNA II functions as an autocatalytic positive feedback loop coding for both the signal (AIP) and its sensor (agrC and A). At some point, when enough AIP has accumulated due to large numbers of S.aureus, agrC and A activate the P3 promoter, switching on production of a regulatory, noncoding RNA species called RNAIII which has a unique 14 hairpin bend structure. RNAIII acts directly to stabilize mRNA and affects transcription of numerous genes (37,38). Table 1 lists some of the staphylococcal products regulated by RNAIII.

Table 1. Global Regulation by agr

Increased by agr/RNAIII	Decreased by agr/RNAIII
α,β,γ hemolysins	Protein A
Proteases	Polysaccharide intercellular adhesin
Staphylokinase	Fibronectin Binding Proteins
Capsular polysaccharides	Clumping Factor A, B
Toxic Shock Syndrome Toxin 1	

RNA III decreases adherence/colonization proteins and increases secreted virulence factors.The agr system thus acts as a proximal switch triggering staphylococcal virulence; agr activation enbles S. aureus to move away from crowded, nutrient-depleted colonized sites to uncrowded, nutrient rich areas. This concept is shown in schematic form on figure 6

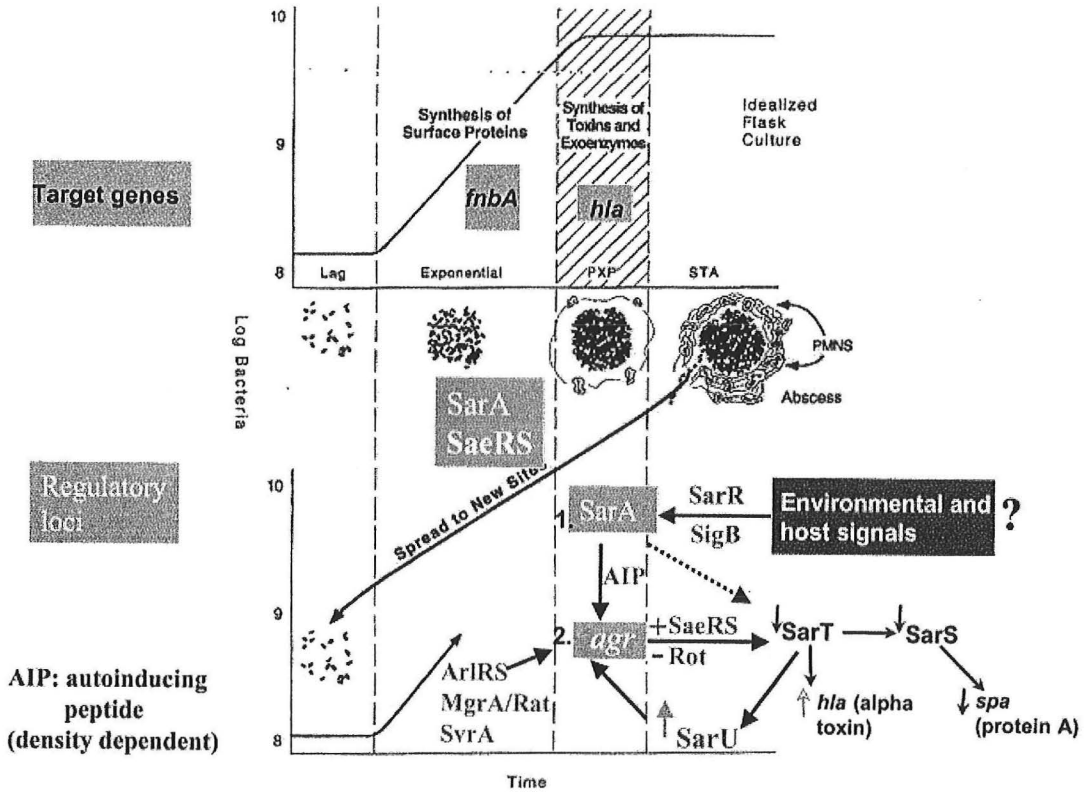


Figure 6. Overview of agr quorum sensing. Ref. 34

Numerous experiments in animal models of arthritis, peritonitis, endocarditis, and pneumonia have shown that agr negative S. aureus mutants are less virulent. For example, in a murine pneumonia model mortality was zero and 41% of the mice were bacteremic during agr negative pneumonia, compared to 30% mortality and 100% bacteremia from a wild type, agr positive S. aureus infection (39). Biofilm/PIA production is reduced in agr positive clinical isolates and is increased in agr negative S.aureus (40). In addition to local crowding at colonization sites there are two other agr triggers relevant to S.aureus infections. Clumping factor A is a bacterial surface protein which binds fibrinogen, resulting in clumping, increased local AIP concentrations, and agr activation; in vivo, host fibrinogen acts as a virulence factor in murine peritonitis and soft tissue abscess models, and fibrinogen depletion is protective(41). Uptake of S. aureus by epithelial cells also activates agr;within three hours of entry RNAIII levels increase, followed by hemolysin production, damage to the endosomal compartment enclosing the bacteria, bacterial proliferation, and finally epithelial cell death(42).

Interference with quorum sensing should reduce virulence and two investigators have shown positive results with such an approach. Salicylic acid, the major metabolite of aspirin, decreases agr activity indirectly through its effect on the regulatory factor sig B. Salicylic acid reduced the size and number of vegetations, and the number of metastatic renal abscesses, in an animal endocarditis model(43,44). As shown on figure 5 AIPs are classified into four groups based on different peptide sequences and thiolactone rings. Individual S. aureus strains only secrete AIPs of one class, and AIPs of one group inhibit agr C receptor signaling of the other 3 AIP groups.Thus chemically synthesized AIPs can be used to block agr-mediated virulence; results are shown on figure 7.

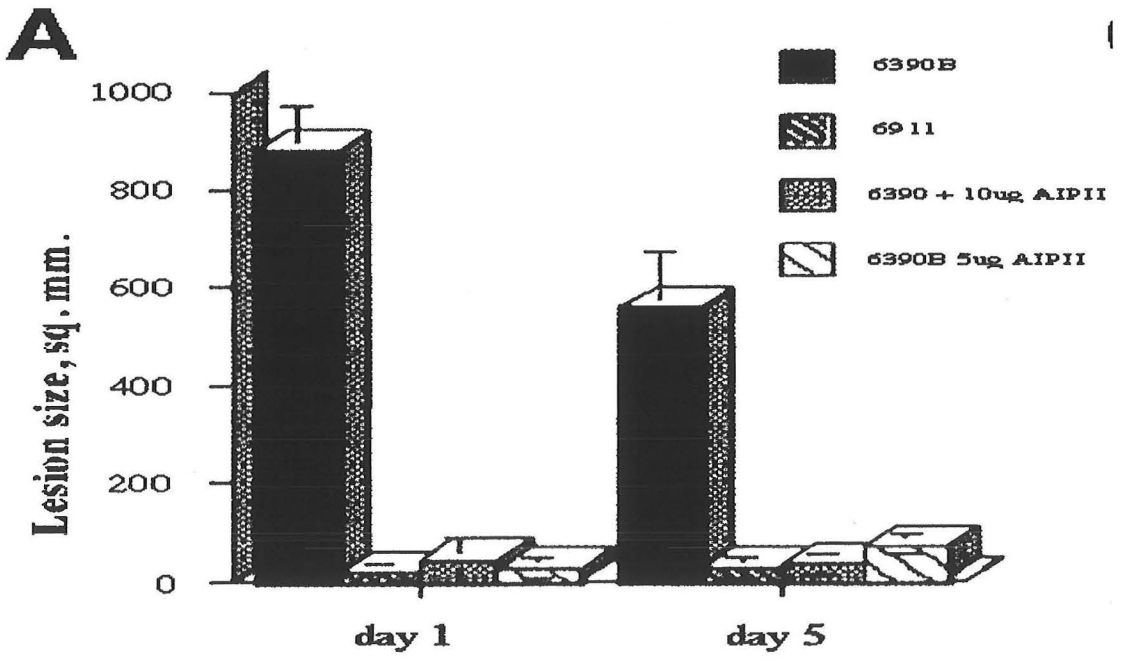


Figure 7. Effect of AIP inhibition on abscess formation. Ref. 45

Subcutaneous injection into mice of a S. aureus strain secreting a group I AIP (strain 6390B) resulted in large abscesses, but adding ug amounts of a group II AIP to the bacteria completely prevented abscess formation (45). In contrast to antibiotic therapy, interfering with quorum sensing/agr activation is very appealing because it has the potential to prevent disease without killing bacteria and thus should exert less selection pressure for drug resistant mutations. However, a potential downside to inhibiting agr signalling is increased biofilm production.

Virulence Factors

The following list of factors is not comprehensive; for information on other factors the interested reader can consult these references (46,47). Many virulence factor genes reside in variable portions of the staphylococcal genome such as bacteriophages, pathogenicity islands, chromosomal cassettes, and plasmids; these elements are all horizontally transferable which facilitates rapid movement of virulence genes between S. aureus strains and even between other bacterial species such as Enterococci and S.epidermidis (25). Thus the plasticity of S. aureus' genome may itself be considered a virulence factor promoting rapid adaptation and spread of disease-producing genes.

Capsule –Although there are eleven different capsular serotypes only four have been biochemically defined. The majority (80%) of clinical isolates produce either type 5 or 8 capsular polysaccharide, which are very similar. Both are immunogenic and most people have anti-capsule antibodies. Capsule production is positively regulated by agr and the capsule has three effects:

Decreased phagocytosis by PMNs - Encapsulated bacteria are cleared less well, presumably because opsonins such as C3b and immunoglobulins, which recognise and bind cell wall components, are unable to do so when a thick capsule surrounds the cell wall (48).

Decreased adherence to host cells - The ability of bacterial cell wall adhesins such as clumping Factor A and fibronectin binding protein A is impaired when a capsule is present, facilitating bacterial detachment from colonized sites and tissue invasion.

Abscess formation – Clinically S. aureus is well known for causing abscesses. Both Type 5 and 8 polysaccharides are homopolymers consisting of a zwitterionic trisaccharide unit in which negatively charged carboxyl groups on N-acetylmannosaminuronic acid alternate with positively charged N-acetyl fucosamine. Experiments with a rat intraabdominal abscess model have demonstrated that purified capsular polysaccharide alone, without any bacteria, causes abscesses; the AD_{50} (amount causing abscesses in 50% of) of type 8 polysaccharide is only 0.33 micrograms. A zwitterionic charge structure is essential for abscess formation, since reducing the carboxyl group or neutralizing the positively charged acetyl group increases the AD_{50} dramatically, to 240 micrograms and 184 micrograms respectively (49). Capsular polysaccharide type 8 is also immunogenic, and prior immunization prevents abscess formation following a subsequent live bacterial challenge. Interestingly, the capsule polysaccharide of another abscess-forming bacteria, Bacteroides fragilis, is also a zwitterion.

Iron – All bacteria require 0.4-4.0 uM iron for growth; this is well above the amount of free iron in humans and thus obtaining iron is a priority for pathogenic bacteria. Mammals sequester iron – 80% of total iron is inside red blood cells, 10% is in muscle myoglobin, and 6% is bound to transferrin – and inflammatory cytokines rapidly upregulate a liver protein (hepicidin) which lowers circulating iron even more by reducing intestinal iron absorption and retaining iron in reticuloendothelial cells. Thus humans try to limit iron during bacterial infections and successful pathogens must obtain free iron from sequestered locations (50).

S. aureus possesses an elaborate set of genes, called the iron regulated surface determinant (ISD) system, consisting of 5 transcriptional units which, as the name implies, are regulated by iron concentration within bacteria. The ISD components include the following:

1. Cell wall anchored surface proteins (ISD A,B,C,H) which bind heme
2. Sortase (SrtB) anchors the cell wall proteins
3. Membrane transporter (Isd D,E,F) transports heme inside S. aureus
4. Two cytoplasmic proteins (Isd G,I) which degrade heme brought inside S. aureus, releasing free iron for use by the bacteria.

In conjunction with its complement of secreted hemolysins, which rupture red blood cells, the ISD system represents a complete program for getting iron from its most abundant source, hemoglobin. In vitro, S.sureus actually prefers heme-iron to transferrin-iron as its iron source. S. aureus knockouts lacking critical ISD genes are much less virulent in animal models of infection; for example, 96 hours after intravenous administration of ISD transporter deficient bacteria, infected mice were less ill and had far fewer renal abscesses and viable bacteria(51,52). The abiltiy to get iron from red blood cells using the ISD system is unique to S.aureus and partially explains its proclivity for bloodstream infections.

Fibronectin Binding Proteins (FNBPs) - > 90% of clinical S.aureus isolates express FNBPs. Fibronectin is a large, "sticky" molecule present in connective tissue and in large (300-400 ug/ml) amounts in blood; the role of plama fibronectin, absorbed to intravenous catheters, in staphylococcal catheter colonization has been discussed. FNBPs recognise and bind to fibronectins' N-terminal area, distant from fibronectins' RGD (arginine-glycine-aspartic acid) sequence; integrins on epithelial and endothelial cells recognise and bind the RGD motif. Both influenzae A infection and endotracheal tubes damage bronchial epithelium, exposing underlying fibronectin and thus promoting bacterial adherence and colonization(53). Recent experiments have delineated another role for FNBP-fibronectin binding which mediates bacterial entry into epithelial and endothelial cells (54-56). When fibronectin-coated S. aureus encounters epithelial or endothelial cells the interaction between fibronectins' RGD domain and cell surafce integrins causes integrin clustering and activates focal adhesion kinases (FAKs) within the cell; the kinases triggers actin cytoskeletal rearrangment, cell surface ruffling and then bacterial ingestion. By this mechanism S. aureus enters a protected environment where it can evade the infected host's immune system, persist, and even proliferate (figure 8).

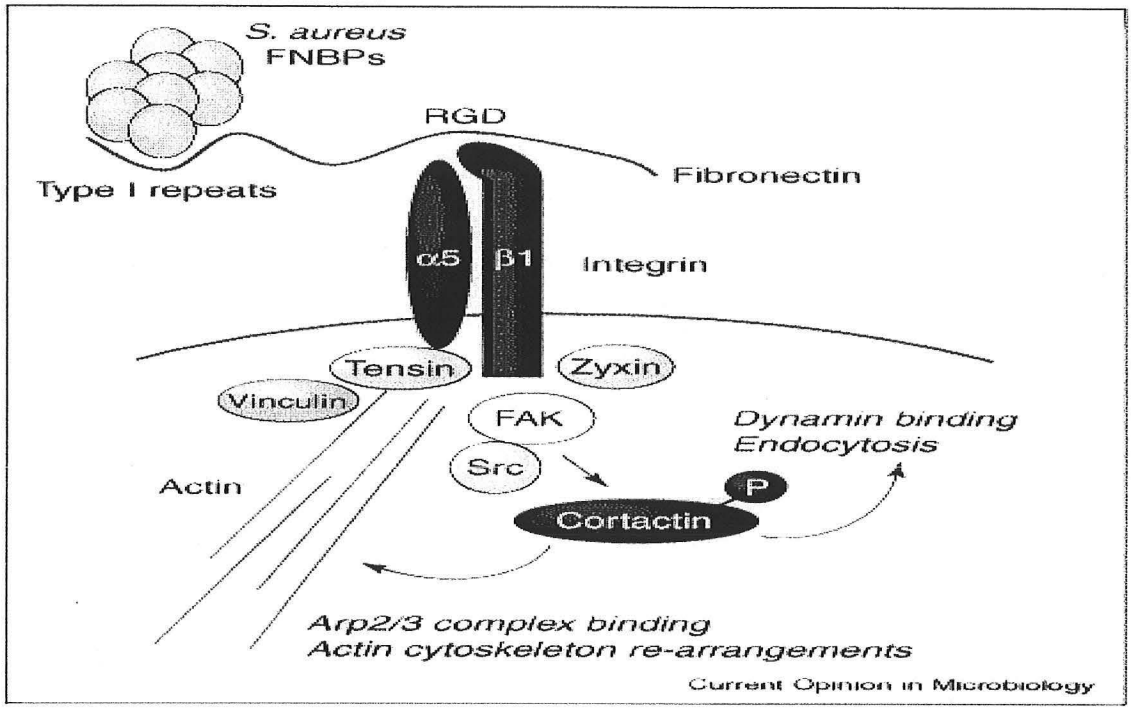
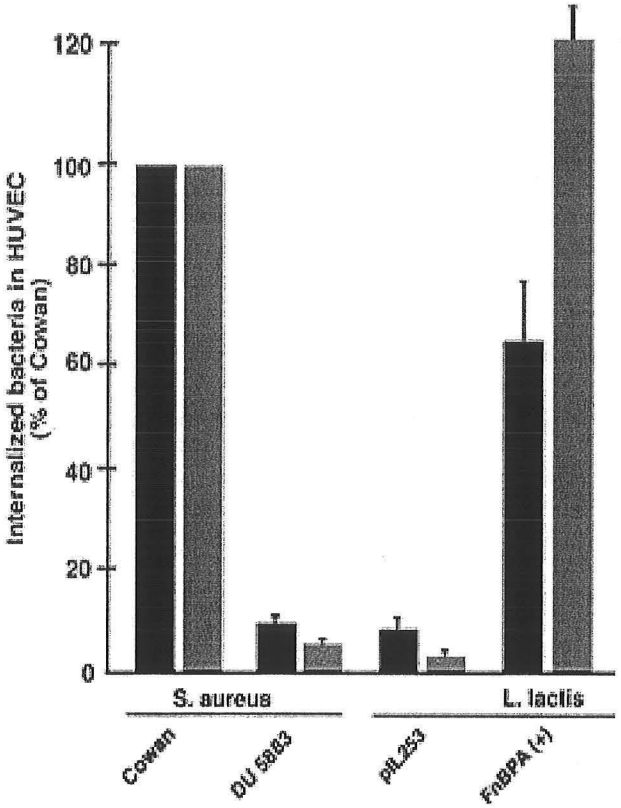


Figure 8. Fibronectin binding allows bacterial entry into host cells. Ref. 55

In vivo evidence for FNBPs importance comes from an animal endocarditis model using Lactococcus lactis. These normally avirulent bacteria were engineered to express FNBP A, which has binding sites for both fibronectin and fibrinogen. FNBP positive Lactococci easily entered human endothelial cells (figure 9) and, after intravenous injection, FNBP positive bacteria produced valve vegetations and large numbers of viable bacteria were present inside aortic endothelial cells (figures 9, 10) (57). FNBP positive bacteria increased in number over three days and caused more severe disease. Uptake of S.aureus into endothelial cells is followed by bacterial proliferation and endothelial apoptosis (58). In addition to endothelial cells S. aureus can enter, replicate within, and cause apoptosis of pulmonary epithelial cells in vitro, and microscopy of nasal biopsies obtained from patients with chronic staphylococcal rhinosinusitis shows large numbers of bacteria within epithelial cells (59,60). Bacteria phagocytosed by PMNs are usually killed but recent evidence suggests that S.aureus can survive inside phagocytic cells; sarA, a global regulon similar to agr, is rapidly activated after phagocytosis and in turn upregulates antioxidants (SOD, catalase) and leukocidins which are protective. SarA positive bacteria, compared to sarA negative controls, can escape from the PMN phagosome, proliferate, and damage PMNs within hours of their ingestion (61,62). These results show that the traditional concept of S. aureus as an extracellulear pathogen is incorrect; it is adept at getting into, and thriving, within many different host cells. An intracellular niche is advantageous since it facilitates immune evasion, decreases bacterial antibiotic exposure, and potentiates chronic and persistent infections (63). Interestingly S. aureus, exposed to subinhibitory concentrations of fluoroquinolone antibiotics, increases FNBP expression (64).



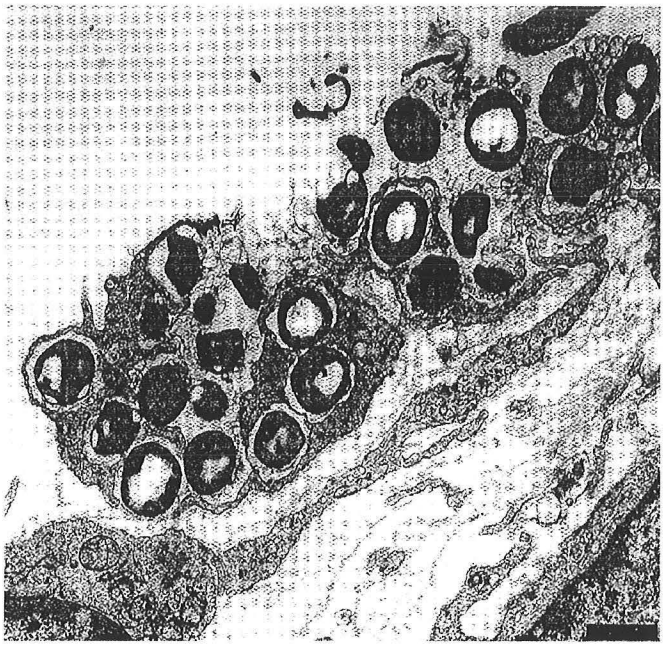


Figure 9 (left). Endothelial cell uptake of FNBP positive Lactococcus lactis. Ref. 57
Figure 10 (right). EM photos of FNBP positive bacteria inside aortic endothelial cells. Ref.57

Toxic Shock Syndrome Toxin 1 (TSST-1) – Also upregulated by agr, TSST-1 belongs to the pyrogenic toxin superantigen family (PTSAG) which includes the Staphylococcal enterotoxins. Compared to the other PTSAGs TSST-1 is unique in its ability to diffuse across mucous membranes, which likely accounts for the observation that toxic shock syndrome often occurs in patients who are either only colonized or have trivial staphylococcal skin infections. Although 20% of clinical isolates have the TSST-1 gene the toxic shock syndrome is relatively rare; toxic shock patients usually present with fever, hypotension, organ failures, and a diffuse erythematous rash. Desquamation of the palms and soles after 7 days is characteristic (65,66). The molecular basis for the toxins effect is the folowing-TSST-1 binds to the invariant region of MHC class II receptors on antigen presenting cells and to the variable region of the T cell receptors β chain. From 5 to 30% of a patients T cells can become activated as a result, leading to a massive outpouring of TH1 type cytokines such as TNFα, IL-2, and IL-6 (figure 11). The resulting cytokine storm is, in essence, septic shock and results from tiny amounts of toxin.

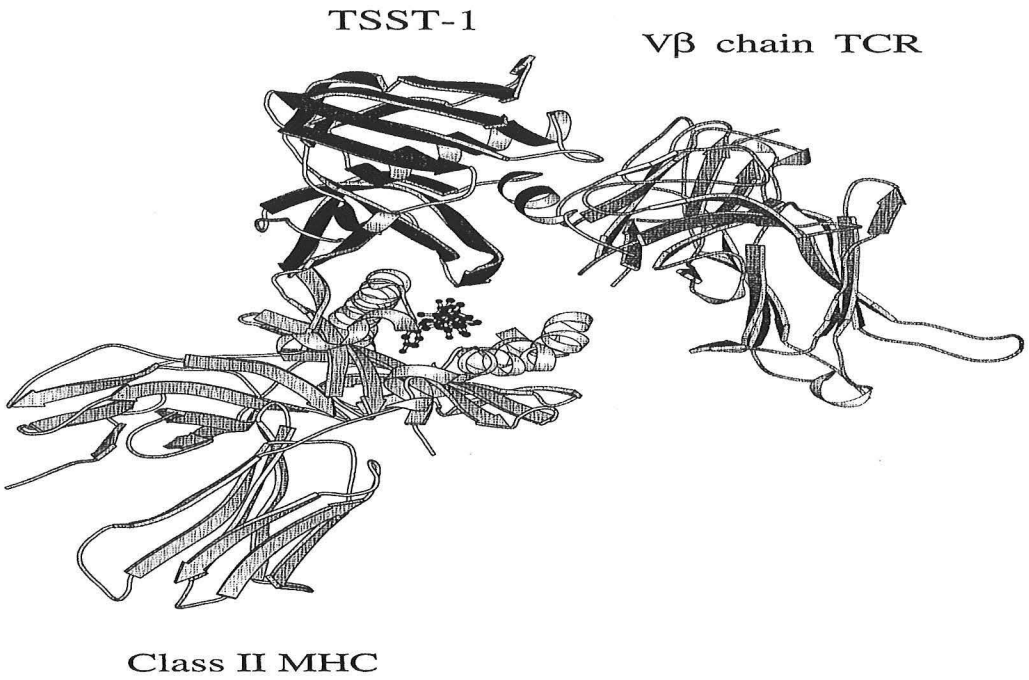


Figure 11. Mechanism of TSST-1 T cell activation. Ref. 65

Panton-valentine Leukocidin (PVL) – Classed as a γ hemolysin, PVL is a pore forming, two component toxin which lyses PMNs, macrophages, and monocytes(67). The clinical characteristics of PVL positive S. aureus pneumonia were described in a series of 16 casese from France and included extensive lung necrosis, leukopenia, hemoptysis, tracheal ulcers, and a 75% mortality(68). Until recently PVL positive S.aureus were uncommon, present in only 3% of clinical isolates, but virually all of the recent USA CAMRSA isolates are PVL positive and often cause extensive skin/soft tissue necrosis. Recent experiments demonstrate that low (5nM) PVL concentrations cause PMN apoptosis due to mitochondrial damage, whereas higher PVL concentrations (200 nM) lyse the PMN cell membrane, explaining the association between PVL positive S. aureus infection and leukopenia (69). In a murine pneumonia model PVL positive bacteria were more lethal and caused much more inflammation and lung necrosis; instillation of purified PVL alone, without bacteria, was sufficient to cause severe pulmonary disease (70).

Sortase – Ten Staphylococcal surface proteins, including proteins responsible for fibrinogen binding (Clumping factor A and B), fibronectin binding (FNBPs A and B), and neutralization of IgG (protein A, which inhibits antibody-mediated opsonization) are covalently attached to cell wall peptidoglycan by an enzyme called sortase. Sortase deficient S. aureus mutants cannot adhere to fibrinogen or fibronectin in vitro; in vivo, renal abscess formation after intravenous innoculation is markedly decreased and there is a significant, 100 fold attentuation in lethality (71). Since sortase is essential for attaching many virulence proteins to the cell wall but is not necessary for bacterial viability it is an attractive target for drug development (72)

Exfoliative Toxins A and B (ETA, ETB) - These toxins cause the Staphylococcal scalded skin sydrome, which occurs primarily in children and in chronically ill adults

with renal failure. Both toxins are proteases which specifically cleave Desmoglein 1, an epidermal adhesion molecule; loss of desmoglein 1 leads to the characteristic skin pathology of midepidermal cleavage with minimal inflammation. The scalded skin sydrome does not involve mucous membranes because desmoglein 3, which is resistant to the staphylococcal protease, maintains mucous membrane epithelial integrity (73,74).

Antibiotic resistance – A characteristic of S.aureus which undoubtedly contributes to its rising prevalence is its ability to rapidly develop resistance to virtually any antibiotic. This is well illustrated by the initial report of penicillin resistant S. aureus in 1941, within one year after penicillins' first clinical use. S. aureus easily acquires antibiotic resistance genes via horizontal transfer of chromosomal DNA and by acquiring antibiotic resistance plasmids (75).

Methicillin resistant S. aureus (MRSA)- In 1961, within 6 months of the introduction of the semi-synthetic penicillin methicillin, the first MRSA were detected. Methicillin resistance is due to an altered penicillin-binding protein called PBP2a which is carried on the mec gene; the mec gene itself is within a larger mobile genetic element called the Staphylococcal cassette chromosome or SCC. Five types of SCCmec have been described; types I, IV, and V carry no additional antibiotic resistance genes apart from mec, whereas mec types II and III carry genes for resistance to multiple other antibiotics plus methicillin(76,77). The mec type can be used to trace clonal spread of S. aureus. For example, most hospital-acquired MRSA are SCCmec types I, II, or III, whereas community-acquired MRSA are type IV . Extensive horizontal transfer of SCCmec genes can occur both within S. aureus strains and between S. aureus and S. epidermidis, providing a pathway for acquisition of antibiotic resistance genes from normal skin flora. MRSA prevalence remained at a relatively low level until the late 1980's when it begin to increase dramatically; in many hospitals and communities >90% of S.aureus isolates are now MRSA. Prior exposure to cephalosporins or fluoroquinolones has been strongly linked epidemiologically to MRSA prevalence and heavy use of these two classes of antibiotics over the past 15 years has undoubtly contributed to current MRSA prevalence. The fluoroquinolone story is illustrative- fluoroquinolones are excreted in sweat but only in low concentrations (20 - 38% of simultaneous plasma levels) (78). S. epidermidis, obtained from the skin of antibiotic-naïve volunteers, is sensitive to both fluoroquinolones and methicillin, but after just seven days of fluroquinolone administration bacteria developed resisitance to both (79). Why fluroquinolone therapy selects for methicillin resistance is not well understood, but most fluroquinolone resistant S. aureus and S. epidermidis are also methicillin resistant and there is a strong epidemiologic association between hospital fluoroquinolone use and MRSA prevalence (figure 12) (80).

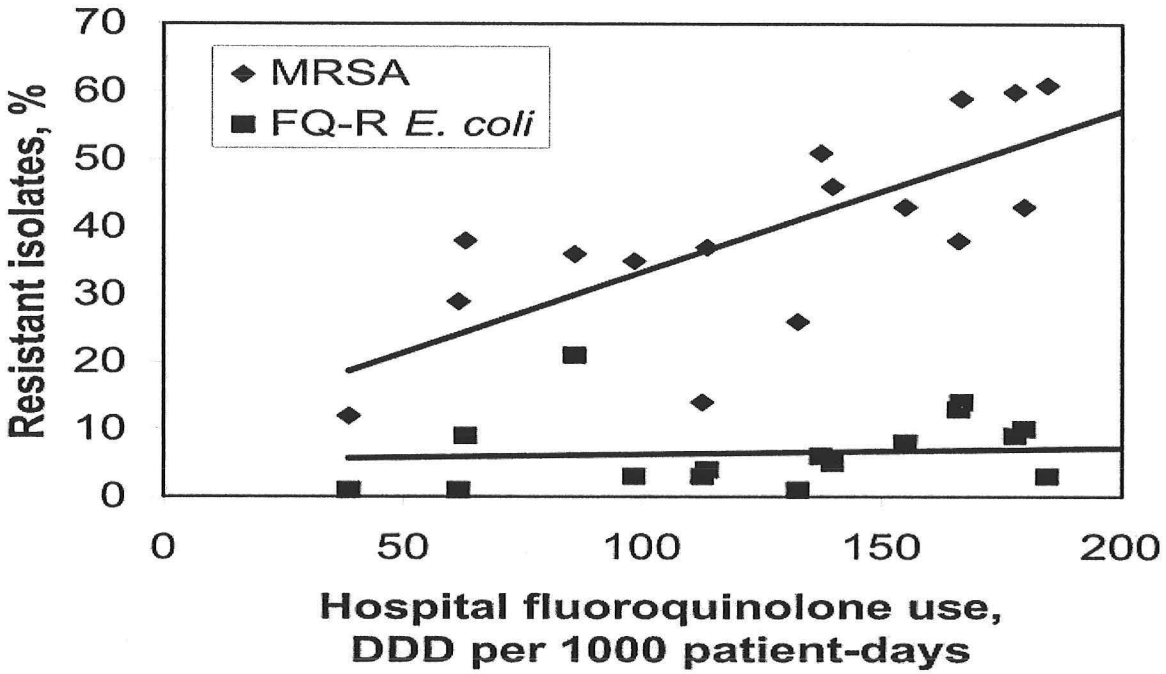


Figure 12. Association between fluoroquinolone use and MRSA prevalence in 17 US hospitals. Ref. 80

Until recently MRSA was largely limited to hospitals but that situation has changed dramatically with the emergence of community-acquired MRSA (CAMRSA).First described in Minnesota in 2000, CAMRSA has spread rapidly and widely to the point where 60% of skin infections seen in emergency rooms all across the US are due to CAMRSA (81,82). Most isolates are clonally derived from the USA 300 strain; they produce the Panton-Valentine leukotoxin, carry the SCCmec type IV (and thus are methicilin resistant but sensitive to a number of antibiotics including trimethoprim/sulfa, clindamycin, rifampin, and tetracycline), and tend to produce severe necrotizing infections (83,84).

Glycopeptide-intermediate resistant S.aureus (GISA) –In 1999 two US GISA cases were reported; both occurred in patients who had received vancomycin for months, often in the presence of fomites such as peritoneal dialysis catheters. The biofilm-protected MRSA were able to persist in the presence of vancomycin, developed increased vancomycin MICs of 8-16 ug/ml, and had thickened, fuzzy cell walls (85). GISA strains synthesize increased amounts of cell wall peptidoglycan containing large amounts of free D-alanine residues; since vancomycin binds to free D-alanine groups the excess cell wall material thus acts as a sink, trapping vancomycin and preventing it from inhibiting peptidoglycan cell wall synthesis at usual bactericidal vancomycin concentrations.

Vancomycin-resistant S. aureus (VRSA) – The most recent, and potentially most serious, type of resistance was first noted in 2002. The mechanism of frank vancomycin resistance is acquisition of the VanA gene from vancomycin-resistant enterococci. The vanA gene product is an altered cell wall peptidoglycan precursor which lacks the terminal D-alanine to which vancomycin binds. Vancomycin thus becomes completely ineffectual and VRSA are totally resistant, with vancomycin mic values > 1,000 ug/ml. Typically VRSA strains have emerged when patients, who were colonized with both MRSA and

enterococci, received prolonged courses of vancomycin, and the enterococcus exchanged a plasmid containing the enterococcal vanA gene with MRSA (86). Fortunately VRSA has not spread widely and to date only four isolates have been described in the US (87).

Prevention of S. aureus Infections

Vaccination – A commercially available vaccine (StaphVax) exists and has been tested in a group of 1,798 dialysis patients, a population chosen due to their high incidence (about 4%/year) of staphylococcal bacteremia. The vaccine consists of type 5 and 8 capsular polysaccharide congugated to an adjuvant; 892 patients received vaccine, 906 placebo, and they were followed for 54 weeks. S. aureus bacteremia was the measured outcome. Vaccination afforded minimal protection for the first three weeks, followed by a 57% reduction in Staphylococcal bacteremias between weeks 3 and 40. After week forty the vaccine lost efficacy and bacteremia rates were similar in both groups. These characteristics make it unlikely that this particular vaccine will be widely used in either a general outpatient population or in acutely ill, hosptalized patients. (88).

Reduced Antibiotic Use – As previously noted, heavy cephalosporin and fluoroquinolone use drove drove the spread of MRSA, and now heavy use of vancomycin has led to problems with vancomycin-resistant enterococci, GISA, and VRSA. In 1995 the CDC issued guidelines for reducing vancomycin use. The gudielines stress that vancomycin should not be used to treat colonization and discourage empiric use when no microbiologic evidence of S. aureus exists, but these recommendations are widely ignored (89).During a four year period at Parkland Hospital, from 2003 through 2006, vancomycin use increased 2.6 fold. During the same period the number of serious S. aureus infections, as measured by positive blood cultures, did not increase -297 positive blood cultures in 2004, 282 in 2006 (data coutesy of Paul Southern,MD). Extensive vancomycin use will inexorably lead to decreased vancomycin susceptibility and this can be measured as MIC creep; in one large Los Angeles teaching hospital, the percentage of MRSA isolates with MICs of < 0.5 ug/ml decreased from 80% in 2000 to 29% in 2004, while MRSA isolates having MICs of 1 ug/ml increased from 20 to 70% during that same time period (Table 2)(90).

Table 2. Vancomycin MIC Trends

Year	# of S.aureus	% of S.aureus with MICs of:	
		< 0.5	1.0
2000	945	80	20
2001	1026	81	19
2002	1317	65	35
2003	1297	60	39
2004	1418	29	70

Similar data comes from the multi-continent SENTRY collection of S.aureus clinical isolates. 15% of S. aureus collected between 1999 and 2003 were vancomycin tolerant, as defined by an MBC/MIC ratio > 32 (91).If these trends continue, and especially if GISA and VRSA become prevalent, vancomycin will lose efficacy. Although effective

alternatives to vancomycin such as linezolid and daptomycin are available Staphylococcal resistance to these two new agents has already been described.

It is difficult to obtain accurate data on US antibiotic use, but such information does exist for European countries and has been collected since 1997. The ESAC (European Surveillance of Antimicrobial Consumption) database contains accurate, standardized information from 32 countries measuring outpatient antibiotic use, expressed as daily defined dose, for all antibiotics as well as for individual drug classes. Over the five years of data collection antibiotic consumption has steadily increased. Figure 13 shows total outpatient antibiotic use by country during 2003. As can be appreciated antibiotic use varied by a factor of 3.2 from low use countries such as the Netherlands and Denmark to high use countries including France and Greece.Even more variability was seen when individual classes of antibiotics were compared; for example fluoroquinolone use varied by a factor of 12! Significant seasonal variation in antibiotic use was seen as well, with winter month peaks, suggesting excessive antibiotic prescribing for viral respiratory tract infections (92,93). Heavy antibiotic use in European countries correlates with the prevalence of antibiotic resistance (94). Since it seems unlikely that excessive deaths due to untreated bacterial infections are occurring in the low use countries, these patterns of variation strongly suggest that most outpatient antibiotic use is unnecessary.

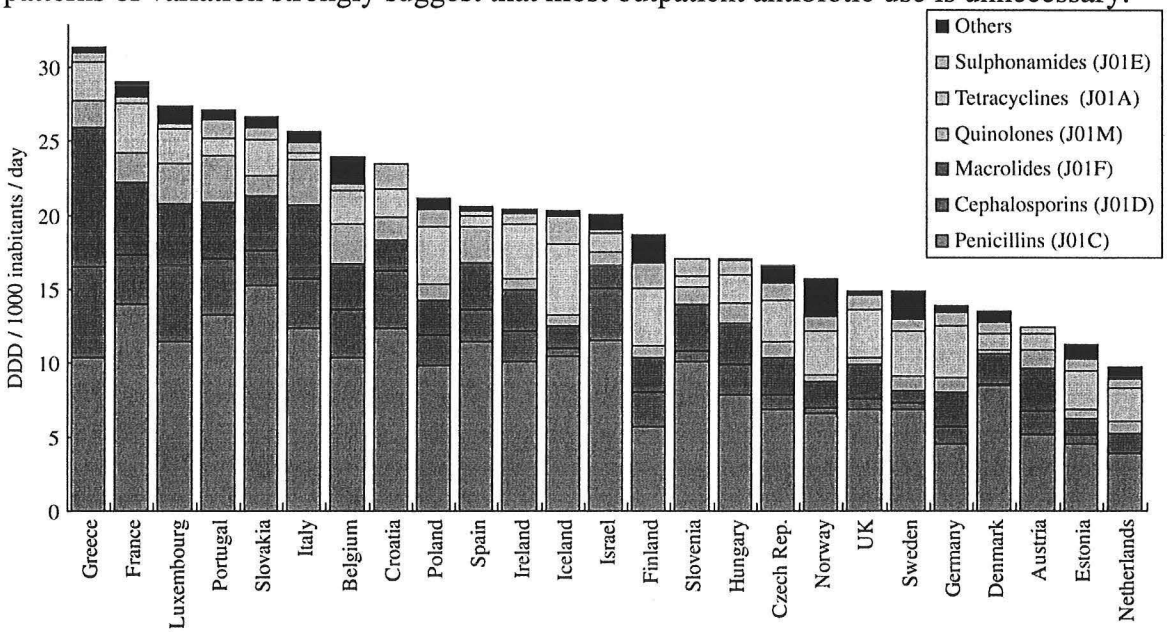


Figure 13. Total outpatient antibiotic use by country , 2003. Ref.92

Given the marked variations in cephalosporin and fluoroquinolone use shown on figure 13 is not surprising that MRSA prevalence is very low (<3%) in the Netherlands and high in France and Greece. Whether this sort of data will lead to reductions in excessive antibiotic use is problematic at this time.

Intravascular Catheters – Most catheter-related S. aureus bacteremia occurs after bacteria colonizing the outer catheter surface enter the bloodstream; colonizing bacteria originate either from patients skin or are introduced by health care workers hands.The use of chlorohexidene skin washes at the catheter entry site is superior to other types of local antiseptics. Both antiseptic substances such as chlorohexidene and antistaphylococcal

antibiotics can be bonded to central venous catheters or incorporated into soft cuffs placed at the catheter entry site, and randomized trials have shown significant reductions in catheter-associated bacteremia when coated catheters are used (95,96).

Table 2. Coated catheters and Bacteremia Risk Reduction. Ref. 95

Technology	# of Trials	Relative Risk	P Value
1st generation chlorohexidene-silver	15	0.65	0.01
minocycline-Rifampin	1	0.00	0.02
silver iontophoretic	4	0.40	0.01
2nd generation chlorohexidene-silver	1	0.34	0.60
chlorohexidene sponge (Biopatch)	1	0.37	0.01

A recent report has shown that a low-tech approach, focused on systematically applying known principles of excellent catheter care in intensive care units, is even more effective than novel catheters. Over 100 Michigan intensive care units participated in a two year trial. After a baseline period (catheter-related bacteremia rate of 2.7 bacteremias/1,000 central line days) an intervention was performed at each ICU and bacteremia rates measured over the ensuing 18 months. The intervention consisted of measures to reduce ventilator-associated pneumonias, use of chlorohexidene for skin preparation and hand washing, full sterile technique during catheter insertion, preferred use of the subclavian site, and daily reminders, usually during patient care rounds, that unnecessary central venous catheters should be removed. Catheter-related bacteremias fell to zero for the entire 18 months of the study. These dramatic results show that relatively simple interventions, if rigorously applied to daily patient care, can markedly improve outcomes (97).

Noninvasive ventilation (NIV) – Ventilator-associated pneumonias are often caused by S.aureus and lead to significant morbidity and mortality. NIV is an alternative mode of mechanical ventilation which removes the endotracheal tube and substitutes a face mask, covering the nose and mouth, as the ventilator to patient connection. Many types of respiratory failure have been successfully managed with NIV and several prospective, randomized trials have compared peumonia rates for conventional mechanical ventilation vs NIV; on average, NIV patients have a 74% reduction in ventilator-associated pneumonia (VAP) (98-100). The absence of a biofilm-encrusted endotracheal tube, reduced sedation, less time in the intensive care unit environment, and the ability to sit upright likely all account for the observed decrease in VAPs.

1. Hauck, C., Agerer, F., Muenzner, P., et al, Cellular adhesion molecules as targets for bacterial infection; **European Journal of Cell Biology**, Vol. 85, Pg. 235-242, 2006.
2. Wertheim, H., Melles, D., Vos, M., The role of nasal carriage in Staphylococcus aureus infections; **Infection**, Vol. 5, Pg 751-762, 2005.
3. Weidenmaier, C., Kokai-Kun, J., Kristian, S., et al., Role of Teichoic Acids in Staphyylococcus aureus nasal colonization, a major risk factor in nosocomial infections; **Nature Medicine**, Vol. 10, No. 3., Pg 243-245, 2004.
4. Weidenmaier, C., Peschel, A., Xiong, Y-Q., et.al., Lack of Wall Teichoic Acids in Staphylococcus aureus Leads to Reduced Interactions with Endothelial Cells and to Attenuated Virulence in a Rabbit Model of Endocarditis, **Journal of Infectious Diseases**, Vol. 191, Pg. 1771-1777, 2005.
5. Eiff, C., Becker, K., Machka, K., et al, Nasal Carriage as a source of Staphylococcus Aureus Bacteremia; **New England Journal of Medicine,** Vol. 344, No. 1, Pg 11-16, 2001.
6. Perl, T., Cullen, J., Wenzel, R., et al., Intranasal Mupirocin to Prevent Postoperative Staphylococcus Aureus Infections; **New England Journal of Medicine**, Vol. 346, No. 24, Pg. 1871-1877, 2002.
7. Harbarth, S., Dharan, S., Liassine, N., Randomized, Placebo-Controlled, Double-Blind Trial To Evaluate the Efficacy of Mupirocin for Eradicating Carriage of Methicillin-Resistant Staphylococcus aureus; **Antimicrobial Agents And Chemotherapy**, Vol. 43, No. 6, Pg. 1412-1416, 1999.
8. Marshall, C., Wesselingh, S., McDonald, M., et al., Control of endemic MRSA-what is the evidence? A personal view; **Journal of Hospital Infection**, Vol. 56, Pg. 253-268, 2004.
9. Laupland, K., Conly, J., Treatment of Staphylococcus aureus Colonization and Prophylaxis for Infection with Topical Intranasal Mupirocin: An Evidence-Based Review; **Clinical Infectious Diseases**, Vol. 37, Pg. 933-938, 2003.
10. Vaudaux, P., Francois, P., Proctor, R., et al., Use of Adhesion-Defective Mutants of Staphylococcus aureus To Define the Role of Specific Plasma Proteins in Promoting Bacterial Adhesion to Canine Arteriovenous Shunts; **Infection and Immunity,** Vol. 63, No. 2, Pg. 585-590, 1995.
11. Vaudaux, P., Pittet, Didier, Haeberli, A., et al, Fibronectin Is More Active than Fibrin or Fibrinogen in Promoting Staphylococcus aureus Adherence to Inserted Intravascular Catheters; **The Journal of Infectious Diseases** Vol. 167, Pg 633-641, 1993.
12. Vaudaux, P., Pittet, D, Haeberli, A., et al; Host Factors Selectively Increase Staphylococcal Adherence on Inserted Catheters: A Role for Fibronectin and Fibrinogen or Fibrin; **The Journal of Infectious Diseases**, Vol. 160, No. 5, Pg 865-873, 1989.
13. Costerton, W., Veeh, R., Shirtliff, M., et al., The Application of biofilm science to the study and control of chronic bacterial infections; **The Journal of Clinical Investigation,** Vol. 112, No. 10, Pg 1466-1477, 2003.
14. Gotz, F., Staphylococcus and biofilms; **Molecular Microbiology**, Vol. 43, No. 6, Pg. 1367-1378, 2002.
15. Fluckiger, U., Ulrich, M., Steinhuber, A., et al., Biofilm Formation, icaADBC Transcription, and Polysaccharide Intercellular Adhesin Synthesis by Staphylococci in a Device-Related Infection Model; **Infection and Immunity**, Vol. 73, No. 3, Pg. 1811-1819, 2005.
16. Marrie, T., Costerton, J.W., Scanning and Transmission Electron Microscopy of In Situ Bacterial Colonization of Intravenous and Intraarterial Catheters; **Journal of Clinical Microbiology**, Vol. 19, No. 5, Pg. 687-693, 1984.
17. Arciola, C., Baldassarri, L., Montanaro, L., Presence of icaA and icaD Genes and Slime Production in a Collection of Staphylococcal Strains from Catheter-Associated Infections; **Journal of Clinical Microbiology,** Vol. 39, No. 6, Pg. 2151-2156, 2001.
18. Adair, C., Gorman, S., Feron, B., Implications of endotracheal tube biofilm for ventilator-associated pneumonia; **Intensive Care Medicine,** Vol.25, Pg. 1072-1076, 1999.
19. Rupp, Mark., Fey, P., Heilmann, C., et al., Characterization of the Importance of Staphylococcus epidermidis Autolysin and Polysaccharide Intercellular Adhesin in the Pathogenesis of Intravascular Catheter-Associated Infection in a Rat Model; **The Journal of Infectious Diseases**, Vol. 183, Pg. 1038-1042, 2001.

20. Leid, J., Shirtliff, M., Costerton, J., et al., Human Leukocytes Adhere to, Penetrate, and Respond to Staphylococcus aureus Biofilms; **Infection and Immunity**, Vol. 70, No. 11, Pg. 6339-6345, 2002.
21. Coquet, L., Junter, G., Jouenne, T., Resistance of artificial biofilms of Pseudomonas aeruginosa to imipenem and tobramycin; **Journal of Antimicrobial Chemotherapy**, Vol. 42, Pg. 755-760, 1998.
22. Xu, K., McFeters, G., Stewart, P., Biofilm resistance to antimicrobial agents; **Microbiology**, Vol. 146, Pg. 547-549, 2000.
23. Stoodley, P., Wilson, S., Hall-Stoodley, L., et al., Growth and Detachment of Cell Clusters from Mature Mixed-Species Biofilms; **Applied and Environmental Microbiology**, Vol. 67, No. 12, Pg. 5608-5613, 2001.
24. Fux, C.A., Wilson, S., Stoodley, P., Detachment Characteristics and Oxacillin Resistance of Staphyloccocus aureus Biofilm Emboli in an In Vitro Catheter Infection Model; **Journal of Bacteriology**, Vol. 186, No. 13, Pg. 4486-4491, July.
25. Holden, M., Feil, E., Lindsay, J., et al, Complete genomes of two clinical Staphylococcus aureus strains: Evidence for the rapid evolution of virulence and drug resistance; **PNAS**, Vol. 101, No. 26, Pg. 9786-9791, 2004.
26. Benton, B., Zhang, J., Bond, S., et al, Large-Scale Identification of Genes Required for Full Virulence of Staphylococcus aureus; **Journal of Bacteriology**, Vol., 186, No. 24, Pg. 8478-8489, 2004.
27. Sibbald, J., Ziebandt, A., Engelmann, S., Mapping the Pathways to Staphylococcal Pathogenesis by Comparative Secretomics; **Microbiology and Molecular Biology Reviews**, Vol. 70, No. 3, Pg. 755-788, 2006.
28. Lindsay, J., Holden, M., Staphylococcus aureus: superbug, super genome?; **Trends in Microbiology,** Vol. 12, No. 8, Pg. 378-385, 2004.
29. Yarwood, J., Schliever, P., Quorum sensing in Staphylococcus infections, **The Journal of Clinical Investigation**, Vol. 112, No. 11, Pg. 1620-1625, 2003.
30. Novick, R., Autoinduction and signal transduction in the regulation of staphylococcal virulence; **Molecular Microbiology,** Vol. 48, No. 6, Pg. 1429-1449, 2003.
31. Federle, M., Bassler, B., Interspecies communication in bacteria; **Journal of Clinical Invest.,** Vol. 112, Pg. 1291-1299, 2003.
32. Donadedian, H., Review: Quorum Sensing and its Relevance to Infectious Diseases; **Journal of Infection**, Vol. 46, Pg. 207-214, 2003.
33. Bronner, S., Monteil, H., Prevost, G., Regulation of virulence determinants in Staphylococcus aureus: complexity and applications; **FEMS Microbiology Reviews**, Vol. 28, Pg. 183-200, 2004.
34. Cheung, A., Bayer, A., Zhang, G., et al., Regulation of virulence determinants in vitro and in vivo in Staphylococcus aureus; **FEMS Immunology and Medical Microbiology**, Vol 40, Pg. 1-9, 2004.
35. Lyon, G., Novick, R., Peptide signaling in Staphylococcus aureus and other Gram-Positive bacteria; **Peptides**, Vol. 25, Pg. 1389-1403, 2004.
36. Ji, G., Beavis, R., Novick, R., Cell density control of staphylococcal virulence mediated by an octapeptide pheromone; **Proc. Natl. Acad. Sci.,** Vol. 92, Pg. 12055-12059, 1995.
37. Geisinger, E., Adhikari, R., Jin, R., et al. Inhibition of rot translation by RNAIII, a key feature of agr function; **Molecular Microbiology**, Vol 61. No. 4, Pg. 1038-1038, 2006.
38. Johansson, J., Cossart, P., RNA-mediated control of virulence gene expression in bacterial pathogens; **Trends in Microbiology**, Vol. 11, No. 6, Pg. 280-285, 2003.
39. Heyer, G., Saba, S., Adamo, R., et al., Staphylococcus aureus agr and sarA Functions Are Required for Invasive Infection but Not Inflammatory Responses in the Lung; **Infection and Immunity**, Vol 70, No. 1, Pg. 127-133, 2002.
40. Vuong, C., Saenz, H., Gotz, F., et al., Impact of the agr Quorum-Sensing System on Adherence to Polystyrene in Staphylococcus aureus; **The Journal of Infectious Diseases** Vol. 182, Pg. 1688-1693, 2000.

41. Rothfork, J., Dessus-Babus, S., Wamel, W., et al., Fibronogen Depletion Attenuates Staphyloccocus aureus Infection by Preventing Density-Dependent Virulence Gene Up-Regulation, **The Journal of Immunology**, Vol. 171, Pg. 5389-5395, 2003.
42. Shompole, S., Henon, K.., Liou, L., et al, Biphasic intracellular expression of Staphylococcus aureus virulence factors and evidence for Agr-mediated diffusion sensing; **Molecular Microbiology**, Vol. 49, No.4, Pg. 919-927, 2003.
43. Kupferwasser, L., Yeaman, M. Nast, C., Salicylic acid attenuates virulence in endovascular infections by targeting global regulatory pathways in Staphylococcus aureus; **The Journal of Clinical Investigation**, Vol. 112, Pg. 222-233, 2003.
44. Herrmann, M. Salicylic acid: an old dog, new tricks, and staphylococcal disease; **Journal of Clinical Investigation**, Vol. 112, Pg. 149-141, 2003.
45. Mayville, P., Ji, G., Beavis, R., Structure-activity analysis of synthetic autoinducing thiolactone peptides from Staphylococcus aureus responsible for virulence; **Proc . Natl. Acad. Sci.**, Vol. 96, Pg. 1218-1223, 1999.
46. Rooijakkers, S., van Kessel, K., van Strijp, J., Staphylococcal innate immune evasion; **Trends in Microbiology**, Vol. 13, No. 12, Pg. 596-601, 2005.
47. Foster, T., Immune Evasion by Staphylococci; **Nature Reviews Microbiology,** Vol 3, Pg. 948-958, 2005.
48. O'Riordan, K., Lee, J., Staphylococcus aureus Capsular Polysaccharides; **Clinical Microbiology Reviews,** Vol. 17, No.1, Pg. 218-234, 2004.
49. Tzianabos, A., Wang, J., Lee, J., Structural rationale for the modulation of abscess formation by Staphylococcus aureus capsular polysaccharides; **PNAS**, Vol. 98, No. 16, Pg. 9365-9370, 2001.
50. Skaar, E., Schneewind, O., Iron-regulated surface determinants (Isd) of Staphylococcus aureus: stealing iron from heme; **Microbes and Infection**, Vol. 6, Pg. 390-397, 2004.
51. Maresso, A., Schneewind, O., Iron acquisition and transport in Staphylococcus aureus; **BioMetals**, Vol. 19, Pg. 193-203, 2006.
52. Skaar, E., Humayun, M., Bae, T., Iron-Source Preference of Staphylococcus aureus Infections; **Science,** Vol. 304, Pg. 1626-1628, 2004.
53. Mongodin, E., Bajolet, O., Cutrona, J., et al, Fibronectin-Binding Proteins of Staphylococcus aureus Are Involved in Adherence to Human Airway Epithelium; **Infection and Immunity,** Vol. 70, No. 2, Pg. 620-630, 2002.
54. Menzies, B., The role of fibronectin binding proteins in the pathogenesis of Staphylococcus aureus infections; **Current Opinion Infectious Disease**, Vol. 16, Pg. 225-229, 2003.
55. Hauck, C., Ohlsen, K., Sticky connections: extracellular matrix protein recognition and integrin-mediated cellular invasion by Staphylococcus aureus; **Current opinion in Microbiology**, Vol. 9, Pg. 5-11, 2006.
56. Schwarz-Linek, U., Hook, M., Potts, J., Fibronectin-binding proteins of Gram-positive cocci; **Microbes and Infection**, Vol. 8, Pg. 2291-2298, 2006.
57. Que, Y., Haefliger, J., Piroth, L., et al., Fibrinogen and fibronectin binding cooperate for valve infection and invasion in Staphylococcus aureus experimental endocarditis; **Journal of Experimental Medicine,** Vol. 201, No. 10, Pg. 1627-1635, 2005.
58. Menzies, B., Kourteva, I., Internalization of Staphylococcus aureus by Endothelial Cells Induces Apoptosis; **Infection and Immunity**, Vol. 66, No. 12, Pg. 5994-5998, 1998.
59. Clement, S., Vandaux, P., Francois, P., et al., Evidence of an Intracellular Reservoir in the Nasal Mucosa of Patients with Recurrent Staphylococcus aureus Rhinosinusitis; **The Journal of Infectious Diseases**, Vol. 192, Pg. 1023-1028, 2005.
60. 60.Kahl, B., Goulian, M., Van Wamel, W., et.al, Staphylococcus aureus RN6390 Replicates and Induces Apoptosis in a Pulmonary Epithelial Cell Line; **Infection and Immunity**, Vol. 68, No. 9, Pg. 5385-5392, 2000.
61. Voyich, J., Braughton, K., Sturdevant, D., et al, Insights into Mechanisms Used by Staphylococcus aureus to Avoid Destruction by Human Neutrophils; **The Journal of Immunology**, Vol. 175, Pg. 3907-3919, 2005.

62. Gresham, H., Lowrance, J., Caver, T., et al., Survival of Staphylococcus aureus Inside Neutrophils Contributes to Infection; **The Journal of Immunology,** Vol. 164, Pg. 3713-3722, 2000.
63. Proctor, R., von Eiff, C., Kahl, B., Small colony variants: a pathogenic form of bacteria that facilitates persistent and recurrent infections; **Microbiology**, Vol. 4, Pg. 295-305, 2006.
64. Bisognano, C., Vaudaux, P., Rohner, P., Induction of Fibronectin-Binding Proteins and Increased Adhesion of Quinolone-Resistant Staphylococcus aureus by Subinhibitory Levels of Ciprofloxacin; **Antimicrobial Agents and Chemotherapy**, Vol, 44, No. 6, Pg. 1428-1437, 2000.
65. Dinges, M., Orwin, P., Schliever, P., Exotoxins of Staphylococcus aureus; **Clinical Microbiology**, Vol. 13, No. 1, Pg. 16-34, 2000.
66. Arad, G., Levy, R., Hillman, D., et al, Superantigen antagonist protects against lethal shock and defines a new domain for T-cell activation; **Nature Medicine**, Vol. 6, No. 4., Pg. 414-421, 2000.
67. Menestrina, G., Serra M., Comai, M., Ion channels and bacterial infection: the case of B-barrel pore-forming protein toxins of Staphylococcus aureus; **FEBS**, Vol. 552, Pg. 54-60, 2003.
68. Gillet, Y., Issartel, B., Vanhems, P., et al., Association between Staphylococcus aureus strains carrying gene for Panton-Valentine leukocidin and highly lethal necrotizing pneumonia in young immunocompetent patients; **The Lancet**, Vol. 359, Pg. 753-759, 2002.
69. Genestier, A., Michallet, M., Prevost, G., et al., Staphylococcus aureus Panton-Valentine leukocidin directly targets mitochondria and induces Bax-independent apoptosis of human neutrophils; **The Journal of Clinical Investigation**, Vol. 114, No. 11, Pg. 3117-3127, 2005.
70. Labandeira-Rey, M., Couzon, F., Boisset, S., et al, Staphylococcus aureus Panton-Valentine Leukocidin Causes Necrotizing Pneumonia; **Science**, Vol. 315, Pg. 1130-1134, 2007.
71. Mazmanian, S., Liu, G., Jensen, E., Staphylococcus aureus sortase mutants defective in the display of surface proteins and in the pathogenesis of animal infections; **PNAS,** Vol. 97, No. 10, Pg. 5510-5515, 2000.
72. Cossart, P., Jonquieres, R., Sortase, a universal target for therapeutic agents against Gram-positive bacteria?; **PNAS**, Vol. 97. No. 10, Pg. 5013-5015.
73. Stanley, J., Amagai, M., Pemphigus, Bullous Impetigo, and the Staphylococcal Scalded-Skin Syndrome; **New England J. Medicine**, Vol. 355, No. 17, Pg. 1800-1810, 2006.
74. Ladhani, S., Understanding the mechanism of action of the exfoliative toxins of Staphylococcus aureus; **FEMS Immunology and Medical Microbiology**, Vol. 39, Pg. 181-189, 2003.
75. Lowy, F., Antimicrobial resistance: the example of Staphylococcus aureus; **The Journal of Clinical Investigation**, Vol. 111, No. 9, Pg. 1265-1273, 2003.
76. Grundmann, H., Aires-de-Sousa, M., Boyce, J., et al., Emergence and resurgence of meticillin-resistant Staphylococcus aureus as a public-health threat; **Lancet,** Vol. 368, Pg. 874-885, 2006.
77. Hanssen, A, Sollid, J., SCCmecin staphylococci: genes on the move; **FEMS Immunol Med Microbiol,** Vol. 46, Pg. 8-20, 2006.
78. Hooper, D., Fluoroquinolone resistance among Gram-positive cocci; **The Lancet** Infectious Diseases, Vol. 2, Pg. 530-538, 2002.
79. Hoiby, N., Jarlov, J., Kemp, M., Excretion of ciprofloxacin in sweat and multiresistant Staphylococcus epidermidis; **Lancet,** Vol. 349, Pg. 167-169, 1997.
80. MacDougall, C., Powell, J., Johnson, C., et al., Hospital and Community Fluoroquinolone Use and Resistance in Staphylococcus aureus and Escherichia coli in 17 US Hospitals; **Clinical Infectious Diseases**, Vol 41, Pg. 435-440, 2005.
81. Fridkin, S., Hagerman, J., Morrison, M., Methicillin-Resistant Staphylococcus aureus Disease in Three Communities; **New England Journal of Medicine**, Vol. 352, No. 14, Pg. 1436-1444. 2005.
82. Moran, G., Krishnadasan, A., Gorwitz, R., et al, Methicillin-Resistant S. aureus Infections among Patients in the Emergency Department; **The New England Journal of Medicine**, Vol. 355, No. 7, Pg. 666-674, 2006.

83. Miller, L., Perdreau-Remington, F., Rieg, G., et al., Necrotizing Fasciitis Caused by Community-Associated Methicillin-Resistant Staphylococcus aureus in Los Angeles; **The New England Journal of Medicine**, Vol. 352, No. 14, Pg. 1445-1454.
84. 84.Chambers, H., Community-Associated MRSA—Resistance and Virulence Converge; **New England Journal of Medicine**, Vol. 352, No. 14, No. 1485-1488, 2005.
85. Smith, T., Pearson, M., Wilcox, K., et al., Emergence of Vancomycin Resistance in Staphylococcus Aureus; **The New England Journal of Medicine**, Vol. 340, No. 7, Pg. 493-502, 1999.
86. Chang, S., Sievert, D., Hageman, J., et al., Infection with Vancomycin-Resistant Staphylococcus aureus Containing the vanA Resistance Gene; **The New England Journal of Medicine**, Vol. 348, Pg. 1342-1347, 2003.
87. Tenover, F., McDonald, L., Vancomycin-resistant staphylococci and enterococci: epidemiology and control; **Current Opinion in Infectious Diseases**, Vol. 18, Pg. 300-305, 2005.
88. Shinefield, H., Black, S., Fattom, A., et al., Use of A Staphylococcus Aureus Conjugate Vaccine in Patients Receiving Hemodialysis; **New England Journal of Medicine**, Vol. 346, No. 7, Pg. 491-496, 2002.
89. Anonymous, Recommendations for Preventing the Spread of Vancomycin Resistance Recommendations of the Hospital Infection Control Practices Advisory Committee HICPAC; **Morbidity and Mortality Weekly** Report, Vol 44(RR12), Pg. 1-13, 1995.
90. Wang, G., Hindler, J., Ward, K., et al., Increased Vancomycin MICs for Staphylococcus aureus Clinical Isolates from a University Hospital during a 5-Year Period; **Journal of Clinical Microbiology**, Vol 44, No. 11, Pg. 3883-3886, 2006.
91. Jones, R., Microbiological Features of Vancomycin in the 21st Century: Minimum Inhibitory Concentration Creep, Bactericidal/Static Activity, and Applied Breakpoints to Predict Clinical Outcomes or Detect Resistant Strains; **Clinical Infectious Diseases,** Vol. 42, Pg. S13-24, 2006.
92. Ferech, M., Coenen, S., Malhotra-Kumar, S., et al., European Surveillance of Antimicrobial Consumption (ESAC): outpatient antibiotic use in Europe; **Journal of Antimicrobial Chemotherapy**, Vol. 58, Pg. 401-407, 2006.
93. Ferech, M., Coenen, S., Malhotra-Kumar, S., et al., European Surveillance of Antimicrobial Consumption (ESAC): outpatient quinolone use in Europe; **Journal of Antimicrobial Chemotherapy**, Vol. 58, Pg. 423-427, 2006.
94. Goossens, H., Ferech, M., Stichele, R., et al, Outpatient antibiotic use in Europe and association with resistance: a cross-national database study; **The Lancet**, Vol. 365, Pg. 579-587, 2005.
95. Rupp, M., Lisco, S., Lipsett, P., et al, Effect of a Second-Generation Venous Catheter Impregnated with Chlorhexidine and Silver Sulfadiazine on Central Catheter-Related Infections; **Annals of Internal Medicine**, Vol. 143, No. 8, Pg 570-580
96. Crnich, C., Maki, D., The Promise of Novel Technology for the Prevention of Intravascular Device-Related Bloodstream Infection. I. Pathogenesis and short-term devices; **Clinical Infections Diseases**, Vol. 34, Pg 1232-1242, 2002.
97. Pronovost, P., Needham, D., Berenholtz, S., et al., An Intervention to Decrease Catheter-Related Bloodstream Infections in the ICU; **The New England Journal of Medicine**, Vol. 355, No. 26, Pg. 2725-2784, 2006.
98. Antonelli, M., Conti, G., Rocco, M., A Comparison of Noninvasive positive-pressure ventilation and conventional mechanical ventilation in patients with acute respiratory failure; **New England Journal of Medicine**, Vol. 339, No. 7, Pg. 429-435, 1998.
99. Brochard, L., Mancebo, J., Wysocki, M., Noninvasive ventilation For Acute Exacerbations Of Chronic Obstructive Pulmonary Disease; **The New England Journal of Medicine**, Vol. 333, No. 13, Pg. 817-822, 1995.
100. Ferrer, M., Esquinas, A., Leon, M, Noninvasive Ventilation in Severe Hpoxemic Respiratory Failure; **American Journal of Respiratory Critical Care Medicine**, Vol. 168, Pg. 1438-1444, 2003.