Methicillin-Resistant *Staphylococcus aureus* as a Threat to Public Health: a Cellular Approach

Harrison G. Holcomb[2], Kevin J. Durbin[1], Margaret Cho[2], Kyu J. Choi[1], Nellie D. Darling[2], & Allan D. Angerio, PhD[1]

Department of Human Science[1]
Department of International Health[2]
Georgetown University
School of Nursing and Health Studies

Abstract

Bacterial resistance to antibiotics is a significant issue in both healthcare and community settings, causing increases in morbidity and mortality across the population. Bacterial resistance can be induced through random genetic mutations, the improper use of antibiotics, or the uptake of plasmid DNA from foreign cells. Any bacterium can evolve resistance. Recently, methicillin-resistant *Staphylococcus aureus* (MRSA) has become a focus of public health concern due to its increased virulence and resistance to an increasingly broad spectrum of antibiotics.

MSRA can be a nosocomial infection, acquired through extended hospital stays or the use of invasive devices. It can also be acquired in the community, with outbreaks generally occurring in schools, gyms and other crowded settings. Community-acquired infections with MRSA (CA-MRSA) typically colonize either the nasal passages or the skin. MRSA usually presents with various cutaneous disorders or soft tissue infection, occasionally leading to acute systemic infections. It can also penetrate bone tissues, causing osteomyelitis. On a cellular level, MRSA secretes a variety of virulence factors, including Panton-Valentine Leukocidin (PVL), a toxin targeting leukocytes for lysis. MRSA has acquired a variety of immune evasion strategies including the production of superantigens, the formation of biofilms, and the invasion of leukocytes.

Treatment of MRSA is based on the severity of infection, but generally requires the use of a combination of therapies. Several staphylococcal vaccine models have shown promise as effective tools for the management of MRSA, including a genetic vaccine, and one that uses surface proteins to elicit a cell specific immune response.

Overview

Resistance

Resistance refers to the ability of a microorganism to survive the effects of drugs or other treatment, including antiseptics and debriding agents. In bacteria, resistance evolves through naturally occurring genetic mutation and the process of natural selection. Mutations can occur in many parts of the genome, leading to the various patterns of drug resistance that exist today. For example, a mutation in a chromosomal gene of one organism may "reduce the pathogen's ability to transport a particular antibiotic into the cell," while a mutation in another gene may "alter the intracellular target protein for an antibiotic molecule, reducing its inhibitory effects" (1, 351). The mutations that cause such antibiotic resistance frequently occur during conjugation, the "direct transfer of genetic material between two temporarily joined bacterial

cells" (1). Conjugation only occurs in the presence of the F factor, a piece of DNA that enables a bacterium to form sex pili. The F factors either exist within a segment of DNA or a plasmid, defined by Campbell as, "a small circular, self-replicating DNA molecule separate from the bacterial chromosome" (1). The transfer of a resistant, (R) plasmid, from one bacterial cell to another via conjugation can occur between distinct species of bacteria, as observed by *Weigel et al*. (2). Thus, conjugation is a significant cause of the rapid evolution of resistance in a bacterial colony.

Through natural selection, a bacterium with an antibiotic-resistant gene mutation will survive and pass this mutated trait to its offspring. With each following generation there is an increase in the number of different resistant strains that exist. The increased prevalence of resistant strains has made it extremely difficult to treat patients for these bacterial infections. However, bacterial genetics do not provide the complete explanation; human behavior has contributed significantly to the growing cases of resistance. Excess usage of antibiotics has expedited the development of methicillin resistance. Other factors contributing to increased resistance include: unnecessary prescriptions, improper use of antibiotics due to patient noncompliance, and the overuse of antibiotics in livestock used for food. Controlling these human factors is critical to containing the spread of resistance.

An example of antibiotic resistance developed through the uptake of plasmid DNA is *Staphylococcus aureus*. *Staphylococcus aureus* becomes resistant when a plasmid encoding the mecA gene is integrated into the genome. The mecA gene encodes a protein known as Penicillin Binding Protein 2a (PBP2a), which renders the active molecules of methicillin, or any other beta-lactam antibiotic, ineffectual (3). This plasmid can be acquired through conjugation of *Staphylococcus aureus* with a different, non-pathogenic species of staphylococcus, Staphylococcus sciuri as observed by Severin et al. The capacity of *Staphylococcus aureus* to acquire plasmids from other species dramatically increases the velocity at which the bacterium can change, making it even more challenging to the healthcare community.

MRSA

Staphylococcus aureus is a gram-positive bacterium of the family Staphylococcaceae. It can be identified by its distinctive yellow-gold pigmentation. The rapid increase of SA strains resistant to methicillin and other antibiotics, from a mere 2% of isolates in 1974 to 64% in 2004 (see Figure 1), has attracted much attention due to the high rates of human morbidity and mortality associated with these infections (4). Human infections of *Staphylococcus aureus* usually affect people who are under hospital care, or who have recently left a hospital setting. Infections that occur while under hospital care, which do not respond to beta-lactam antibiotics, are referred to as nosocomial or hospital-acquired MRSA (HA-MRSA). HA-MRSA typically presents with skin irritation or even more invasive infections in open wounds, the circulation or internal tracks, resulting in acute systemic pathologies. Another form of MRSA is the community-acquired strain (CA-MRSA), which occurs in people who have not been recently hospitalized. This particular strain is "transmitted primarily through direct skin-to-skin contact, but can also be spread through contamination of environmental surfaces such as clothing and towels" (6). There are many risk factors associated with this bacterial infection aside from a compromised immune system due to openings in the skin. These factors could include crowded living conditions, poor hygiene, and close skin-to-skin contact (5). Since CA-MRSA attacks populations outside of the hospital, its target populations usually include "athletes, military recruits, children, Pacific Islanders, Alaskan Natives, Native Americans, men who have sex with men, and prisoners" (5). CA-MRSA most commonly presents with a nonspecific irritation of the skin, and thus is likely widely underreported in compiled epidemiological data. Symptoms such as fevers, chills, and nausea can arise; however, there is a high likelihood that infected persons will not seek

treatment, unless the infection becomes highly invasive, leading to septic shock or bacteremia.

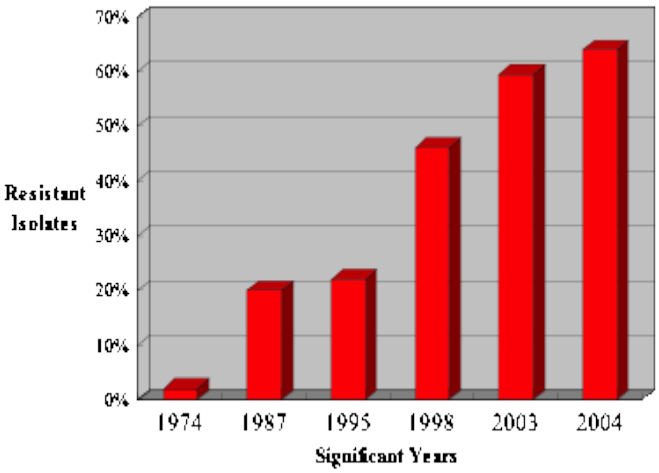

Figure 1
Change in *Staphylococcus aureus* Resistance to Methicillin Over Time
References: Centers for Disease Control and Prevention. S. Aureus and MRSA Surveillance Survey 2007. Available at: http://www.cdc.gov/ncidod/dhqp/ar_mrsa_surveillanceFS.html. Accessed February 19, 2008.

According to the CDC Surveillance Survey from 2007, approximately 2.3 million people in the United States were diagnosed with MRSA. Approximately 86% of these diagnosed with MRSA acquired it nosocomially, whereas 14% were community-acquired cases (4). The emergence of CA-MRSA is concerning because it exposes several large populations to infection that were previously at a very low risk of exposure.

This survey also reported that of the total MRSA cases, 94,000 cases per year become invasive infections and 19,000 resulted in death of the patient. When MRSA's rapid development of a wide spectrum of antibiotic resistance is considered, in addition to its complex and often acute clinical presentation, it is clear that management of MRSA, both in healthcare settings and in the community, is a priority for healthcare providers.

CA-MRSA and Mechanisms of Pathogenesis

CA-MRSA's primary mechanism for pathogenesis is the release of a variety of protein-based virulence factors encoded throughout its genome. These toxins often cause significant morbidity and even death of the patient. One class of toxins produced is the exotoxins, which are produced by most strains of CA-MRSA. Exotoxins cause generalized necrosis in the tissue in which they are released. Necrosis occurs very rapidly, and can lead to the formation of abscesses or sepsis in invasive MRSA.

A highly virulent exotoxin associated with CA-MRSA is Panton-Valentine Leukocidin (PVL), encoded on the PVL gene (8). According to Boubaker K. et al. in Emerging Infectious Diseases, PVL is "a bicomponent cytotoxin encoded by two contiguous and cotranscribed genes carried on a bacteriophage" (7). PVL is a pore-forming toxin, meaning it kills the host cells by stabbing holes in their membranes. These holes allow essential elements, like amino acids, to flow out of the cytoplasm disrupting the cellular reactions (9). PVL causes leukocyte destruction, as well as tissue necrosis. (4, 7). Patients afflicted with CA-MRSA encoded with the PVL gene tend to die quickly, often within 72 hours (13). CA-MRSA encoded for PVL is more likely to become invasive, leading to acute systemic conditions such as bacteremia, severe sepsis and necrotizing fasciitis (8). According to the Wisconsin Medical Journal, PVL was identified in approximately 85% of the strains of CA-MRSA that caused necrotizing pneumonia, an inflammation of the lungs (14).

Strains of CA-MRSA have also been found to secrete Phenol-soluble modulins (PSMs), a group of polypeptide compounds that have been associated with both proinflammatory effects and with the shift of MRSA from chronic to aggressive infection. These modulins are important in eliciting an inflammatory response, triggering the production of cytokines by human cells (18). Furthermore, when PSMs are released into the host's system, the protein causes the lysis of cells by forming pores in the cell membrane (15). According to Otto M. et al. at the National

Institute of Allergy and Infectious Diseases, CA-MRSA is very effective in its control of PSMs, upregulating production to trigger detachment of cells from the MRSA biofilm when the immune system is most vulnerable to attack. PSMs are downregulated when the immune system is strong, encouraging the infection to become quiescent and form a stable biofilm (17). The ability to regulate PSM production, and thus the nature of the infection, based on immune system response is one of the major causes of the severe virulence and continued existence of the community acquired strain of MRSA.

CA-MRSA has developed several highly effective immune evasion strategies. The strains of CA-MRSA most frequently associated with invasive infections in the United States all produce high levels of staphylococcal enterotoxin c (SEC), a pyrogenic superantigenic exotoxin, according to *Dong-Liang Hu et al*. (19). SEC co-litigates between the major histocompatibility complex class II (MHC II) molecules on the surface of antigen presenting cells and the receptor sites on T-lymphocytes. Once the SEC has bound to the receptor sites of T-lymphocytes, there is a massive production of T-1 helper cells, which then release huge quantities of cytokines, especially the tumor necrosis factor α. This leads leading to a crushing systemic inflammation characterized by fever, rashes, sepsis, shock, or the demise of the patient.

If the initial wave of inflammation is survived, the result is frequently a generalized anergy of T cells. Choi and Schwartz describe anergy as, "a hyporesponsive state that a lymphocyte can assume following an encounter with antigen. This state is to be distinguished from programmed cell death by a half life of greater than several days and from immunoregulation and suppression by the intrinsic nature of the biochemical alteration" (21). The result of this anergy is that the MRSA is allowed to multiply, and move about the body entirely unchecked by antibodies, while the massive inflammatory response significantly weakens the host, using up clotting factors and reducing the efficacy of enzymes.

The second technique used by CA-MRSA, the invasion and production of toxins inside leukocytes, not only evades the cell-mediated immune response through the release of Panton-Valentine Leukocidin, but also serves to spread the infection. MRSA has been shown to invade neutrophils in order to produce proteins, in a method similar to the Trojan horse, wherein the neutrophil conceals the bacterial activity. In their analysis of CA-MRSA exoproteins, Burlak et al. found the production of virulence-associated proteins Aureolysin, α-hemolysin chain G, V8 protease and Cysteine Protease Precursor increased after the cell was phagocytized by human polymorphonuclear leucocytes (PMN). In addition, they observed that over time, the proteins were distributed across the entire PMN, not simply the phagocytic vacuole (22). This capacity to remain metabolically active inside PMNs significantly enhances the efficacy of CA-MRSA since it enables it to produce critical proteins with complete protection from all immune defenses. These proteins are then released when PVL, a pore-forming toxin that has been shown to have an affinity for granulocytes, lyses the host cell (22). Recent research conducted by *Kubica et al*. suggests that CA-MRSA is also able to survive for four to five days in many other types of phagocytic immune cells, including fibroblasts, osteoblasts, and macrophages, creating a highly mobile reservoir of disease (23). This mobility not only increases the risk of multi-system infections in highly invasive cases, it also increases the risk of an initially superficial infection becoming invasive, as granulocytes responding to the cutaneous infection then move into the bloodstream and release the bacteria.

A third immune evasion strategy employed by CA-MRSA is the formation of biofilms. *Brady et al*. defined biofilms as, "microbial derived sessile community and typified by cells that are attached to a substratum, interface, or each other, are embedded in a

matrix of extracellular polymeric substance, and exhibit an altered phenotype with regard to growth, gene expression, and protein production" (24). Biofilms are effective immune evasion mechanisms because the biofilm produces a different catalog of antigens, which means that host cells sensitized to CA-MRSA in its planktonic (free floating) form will not immediately target the biofilm. Furthermore, there is the potential that bacteria from the biofilm will break off and enter circulation, leading to a more severe infection. *Staphylococcus aureus*' capacity to form biofilms is dependent on its ability to modulate genetic expression based on environmental conditions. This relies on a two component signal transduction system regulated by the genes *agr* and *arlRS*, as demonstrated by *in vitro* experiments conducted by*Toledo-Arana A. et al*. (25). This makes MRSA resistant to humoral immune secretions such as complement. This combination of biofilm virulence enhancement and highly effective immune evasion is another example of the close association of increased morbidity and resistance in MRSA's genome.

Treatment of MRSA

Treatment for MRSA is based on the severity of the infection. The severity of an infection with MRSA can be divided into four stages. (See Figure 2) In the case of superficial colonization of a wound, without any signs of soft tissue infection, the regular debridement of the wound with Hibciclens (chlorhexidine gluconate), a chemical antiseptic bactericidal to gram-positive microbes (26), combined with a dressing containing silver saccharinate has been effective in clearing infection. In a small, open study of nine hospitalized patients with MRSA, Leak found that treatment with ACTISORB Silver 220 around the wound entry site eradicated MRSA and the majority of wounds improved in one week (27, 28). Despite the small sample size, the dramatic results of the study strongly suggests, that when combined with careful monitoring, silver based dressings are effective in the treatment of superficial MRSA.

The second degree of infection, the case of superficial skin and soft tissue infection or cellulitis, should be treated with local wound cleansing and debridment, and often requires oral antibiotic therapy for ten days in order to resolve the infection. A cocktail of antibiotics including trimethoprim-sulfamethoxazole, Minocycline or doxycycline, and rifampin, have been shown to be effective. Rifampin should not be used as monotherapy due to rapid emergence of resistance. If treatment with this cocktail fails, Zyvox (linezolid) should be considered. Zyvox is an antibiotic of the oxazolidinone class. However, use of Zyvox for greater than ten days requires close monitoring for myelosuppression, including anemia, leucopoenia, pancytopenia, and thrombocytopenia (26).

In the third degree of infection, a soft tissue infection, which has not entered the blood or bone tissue, the course of treatment differs based on whether the infection is nosocomial or community acquired. In both community acquired and nosocomial MRSA, aggressive debridement of necrotic and infected skin and subcutaneous tissue is essential to the success of any antibiotic therapy. Monitoring of peripheral vascular sufficiency (adequate oxygen perfusion to the extremities) is important. In the case of CA-MRSA, the patient will require treatment with antibiotics for at least ten days, but drug choice may vary depending on severity of infection and clinical response. Noscomial MRSA is treated by placing the patient on a two to three week course of Zyvox. If the infection requires greater than three weeks of antibiotic therapy, the patients are often placed on intravenous vancomycin, which requires weekly monitoring of kidney function because of the high risk of toxicity, resulting in hearing loss, renal damage, and thrombocytopenia. In patients suffering from renal disease and the elderly, Zyvox may be used in lieu of vancomycin.

The infection enters the fourth stage and becomes invasive, when it is present in the blood, bones, or other organ systems. As MRSA penetrates deeper into tissue, the treatments become markedly less effective. According to the CDC, in 2007 there was a 56.6%

Figure 2
Treatment Guidelines for MRSA
References: FDA Drug Approval Package Printed Labelling: Avagard. Available at http://www.fda.gov/cder/foi/nda/2001/21-074_Avagard.htm Accessed: January 28, 2008., Leak K. PEG site infections: a novel use for Actisorb Silver 220. British Journal of Community Nursing 2002; 7: 321 - 325., Wound Care Service, Doncaster Royal Infirmary 2005.29. Stranger-Jones Y., Bae T., Schneewind O. Vaccine assembly from surface protein of staphylococcus aureus. Proceedings of the National Academy of Science 2006;103(45):16942-16947

mortality rate for persons with stage four infections and a 32.4% mortality rate for those with stage three infections. With increasing severity of infection, treatment becomes more expensive and the frequency of adverse events increases significantly (20). When considered in combination with the increase in prevalence of community acquired MRSA, the large population at risk for infection, and the speed at which resistance is evolving, developing an effective vaccine is a critical next step in addressing the public health threat posed by the disease. Several features of the disease, including its superantigenic toxins SEC and SEB and certain surface proteins, suggest promising options for vaccine development.

Dong-Liang et al. modified the pyrogenic antigenic exotoxin SEC to eliminate its superantigenic capacity while retaining its fundamental structure. When injected into mice, the modified SEC (mSEC) successfully elicited a coordinated cell-mediated immune response, which produced antibodies specific to mSEC. There was also a 0% mortality rate for inoculated mice versus 78% mortality for non-inoculated mice when challenged with MRSA. While further study is needed, these results indicate that such a genetic-type vaccine could offer long-term, broad-spectrum protection (19).

Another vaccine model explored by *Stranger-Jones et al*. is the use of surface proteins IsdA, IsdB, SdrD, and SdrE, to create a vaccine that provides broad-spectrum protection from *Staphylococcus aureus* by eliciting an antibody response. In a trial in mice, the vaccine reduced the bacterial load in homogenized tissue by a

factor of approximately three natural logarithms (29). Recent clinical trials of a conjugate vaccine of this type have been inconclusive, however, a new pentavalent vaccine being developed by Nabi Biopharmaceuticals shows great promise in reducing SA morbidity and mortality (30).

Conclusion

Resistant strains of *Staphylococcus aureus* are a growing threat to public health. In less than forty years, the disease has transformed from a nosocomial infection, affecting those who had almost no immunity, to a disease that can kill a healthy person within 72 hours of infection. It is easily transferred from person to person, via skin-to-skin contact or the sharing of contaminated objects. The bacterium has developed sophisticated immune evasion tactics, which often enable it to spread its virulence factors, like PVL and PSM even more effectively, and make eradication of the infection without treatment very difficult. Conventional treatment for MRSA is growing more expensive and less reliable as the rapidly evolving bacterium becomes resistant to one drug after another. Mortality rates have grown exponentially. In order to effectively manage this emerging epidemic, vaccine development must be pursued. Both the genetic and conventional programs show promise in creating a safe, broadly effective vaccine.

References

1. Campbell, Neil. Biology. Seventh Edition. Neil A. Campbell, Jane B. Reece. San Francisco: Pearson. 2005. 349-351.
2. Weigel L., Donlan R., Shin D., et al. High-level vancomycin-resistant staphylococcus aureus isolates associated with a polymicrobial biofilm. Antimicrobial Agents and Chemotherapy. 2007 51(1): 231-238.
3. Severin A., Wei Wu S., Tabei K., et al. High-level β-lactam resistance and cell wall synthesis catalyzed by the mecA homologue of staphylococcus sciuri introduced into staphylococcus aureus. Journal of Bacteriology 2005; 187(19): 6651-6658.
4. Centers for Disease Control and Prevention. S. Aureus and MRSA Surveillance Survey 2007. Available at: http://www.cdc.gov/ncidod/dhqp/ar_mrsa_surveillanceFS.html Accessed February 19, 2008.
5. Centers for Disease Control and Prevention. Community acquired MRSA: information for the public. Available at http://www.cdc.gov/ncidod/dhqp/ar_mrsa_ca_public.html. Accessed February 19, 2008.
6. Alliance Working for Antibiotic Resistance Education. Health professionals: CA-MRSA. Available at: http://www.aware.md/HealthCareProfessionals/CAMRSA.aspx. Accessed February 18, 2008.
7. Boubaker, K, et al. Panton-Valentine Leukocidin and Staphylococcal Skin Infections in Schoolchildren. Emerging Infectious Diseases. 2004; 10: 121-123.
8. Moroney S. M., Heller L.C., Arbuckle J. et al. Staphylococcal cassette chromosome mec and panton-valentine leukocidin characterization of methicillin-resistant staphylococcus aureus. Journal of Clinical Microbiology. 2006; 45(3): 1019-1021.
9. Wardenburg, J.B. et al. Surface Proteins and Exotoxins Are Required for the Pathogenesis of *Staphylococcus aureus* Pneumonia. Infection and Immunity. 2007; 75: 1040-1043.
10. Napolitano, L.M. Community-acquired methicillin-resistant staphylococcus aureus infections: changes in practice required? Medscape Infectious Diseases. Available at: http://bcbsma.medscape.com/viewarticle/566787_3 Accessed: February 17, 2008.
11. Boyle-Vavra, S., Daum, R. Community-acquired methicillin-resistant *Staphylococcus aureus*: the role of Panton-Valentine Leukocidin. Laboratory Investigation 2007:87 3-9.
12. Gilbert, R.J. Pore-forming Toxins. Cellular and Molecular Life Sciences. 2002; 59: 832.
13. Cool, LC. Deadly Superbugs. Readers Digest. August 2007.
14. Drews, TD. et al. Community-Associated Methicillin-Resistant *Staphylococcus aureus*: Review of an Emerging Public Health Concern. Wisconsin Medical Journal. 2006; 105: 52-56.
15. Mehlin, C, et al. An Inflammatory Polypeptide Complex from Staphylococcus epidermidis: Isolation and Characterization. The Journal of experimental Medicine. 1999; 189: 907-909.

16. Fournier, B. et. al. Recognition of *Staphylococcus aureus* by the Innate Immune System. Clinical Microbiology Reviews. 2005; 18: 528.
17. National Institute of Allergy and Infectious Diseases. Press Releases. Available at: http://www3.niaid.nih.gov/news/newsreleases/2007/ca-mrsa.htm. Accessed February 17, 2008.
18. Yao, Y. et al. Genome wide Analysis of Gene Expression in Staphylococcus epidermidis Biofilms: Insights into the Pathophysiology of S. epidermidis Biofilms and the Role of Phenol-Soluble Modulins in Formation of Biofilms. The Journal of Infectious Diseases. 2005; 191:296.
19. Dong-Liang H., Jing-Chun C., Katsuhiko O. et al. A mutant of staphylococcal enterotoxin c devoid of bacterial superantigenic activity elicits a Th-2 immune response for protection against staphylococcus aureus infection. Infection and Immunity 2006; 73(1): 174-180.
20. Klevens R., Morrison M., Nadle J. et al. Invasive methicillin-resistant staphylococcus aureus infections in the united states. Journal of the American Medical Association 2007 298(15):1763-1771.
21. Seeyoung C., Schwartz R.H. Molecular mechanisms for adaptive tolerance and other T-cell anergy models. Seminars in Immunology 2007; 19(3): 140–152.
22. Burlak C., Hammer C. H., Robinson M.A. et al. Global analysis of community associated methicillin resistant staphylococcus aureus exoproteins reveals molecules produced in vitro and during infection. Cellular Microbiology 2007; 9(5): 1172-1190.
23. Kubica M., Guzika K., Koziel J. et al. A potential new pathway for staphylococcus aureus dissemination: the silent survival of s. aureus phagocytized by human monocyte-derived macrophages. PloS ONE 2008; 3(1): e1409.
24. Brady R.A., Leid J.G., Camper A.K. et al. Identification of staphylococcus aureus proteins recognized by the antibody-mediated immune system in response to a biofilm infection. Infection and Immunity 2006; 74(6): 3415-3426.
25. Toledo-Arana A., Merino N., Vergara-Irigaray M., et al. *Staphylococcus aureus* develops an alternative ica - independent biofilm in the absence of a two component system. Journal of Bacteriology 2005; 187(15): 5318-5329.
26. FDA Drug Approval Package Printed Labelling: Avagard. Available at http://www.fda.gov/cder/foi/nda/2001/21-074_Avagard.htm Accessed: January 28, 2008.
27. Leak K. PEG site infections: a novel use for Actisorb Silver 220. British Journal of Community Nursing 2002; 7: 321 - 325.
28. Wound Care Service, Doncaster Royal Infirmary 2005.
29. Stranger-Jones Y., Bae T., Schneewind O. Vaccine assembly from surface protein of staphylococcus aureus. Proceedings of the National Academy of Science 2006;103(45):16942-16947.
30. PentaStaph (Staphylococcal Polysaccharide Conjugate and Toxoid Vaccine) [press release] Rockville, MD Nabi Biopharmaceuticals September 9, 2008.