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A REVIEW ON EMERGENCE OF ANTIBIOTIC RESISTANT *Staphylococcus aureus* AND ROLE OF CHITOSAN NANOPARTICLE IN DRUG DELIVERY

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ABSTRACT

Staphylococcus aureus is a most common pathogen in hospital and community acquired disease that causes a wide range of infection such as skin and soft tissue infection to life threatening disease like respiratory tract infection, musculoskeletal infection, endocarditis and urinary tract infection. More than 90% of Staphylococcus strains are resistant to penicillin. In 1961 *S. aureus* developed resistance to methicillin (MRSA), invalidating almost all antibiotics, including the most potent *β*-lactams. Vancomycin, a glycopeptide antibiotic, was used for the treatment of MRSA in 1980. Vancomycin inhibits the bio-synthesis of peptidoglycan and the assembly of NAM-NAG-polypeptide into the growing peptidoglycan chain. Vancomycin resistant *S. aureus* (VRSA) first appeared in the USA in 2002. Increasing resistance of *S. aureus* to last line of drug i.e., vancomycin highlights the need for either the development of new and novel antibiotics or the improvement of efficacy of established antibiotics by the development of new agents capable of enhancing antibiotic activity. A Chitosan nanoparticle delivers anti-infective drugs such as antibacterial, antiviral, antifungal and antiparasitic drugs. In the present review the antibiotic emergence of *Staphylococcus aureus*, formulation techniques of chitosan nanoparticles and different biological profits of chitosan nanoparticles are discussed.

Keywords: *Staphylococcus aureus*, drug resistance, chitosan, vancomycin, drug delivery.

1. INTRODUCTION

Staphylococcus aureus is a bacterium that belongs to the family of *Staphylococcaceae*. The bacteria form part of the normal flora of the skin, intestine, upper respiratory tract and vagina (Lowy F, 1998). *Staphylococcus aureus* can become pathogenic when conditions such as pH, temperature and nutrient availability are altered and become favourable for overgrowth (Mims C et al., 2004). The pathogenicity of *S. aureus* is determined by the production of toxins, such as the 33-kd protein-alpha toxin, exfoliatin A, exfoliatin B and Panton-Valentine leukocidin (PVL) toxins (Lowy F, 1998). These toxins can be harmful to the host and cause skin diseases (carbuncles, boils, folliculitis and impetigo) and other complications, such as endocarditis, meningitis as well as toxic shock syndrome (TSS) (Mims C et al., 2004). *S. aureus* is an important mammalian pathogen that has long been recognized for its propensity to cause serious and invasive diseases. In 1878, Koch first noted that different diseases were caused by Gram-positive cocci depending on whether they formed pairs, chains or clusters. The *staphylococci* were initially identified as grape-like clusters of bacteria isolated from the pus of

human abscesses by Ogeston in 1881 (Ogeston A, 1881). In 1884, Rosenbach differentiated species of *staphylococci* based on pigmentation. The disease-causing *Staphylococcus aureus* produced a golden yellow pigment, whereas the non-disease causing strain was generally white (Rosenbach F, 1884).

1.1 Epidemiology of *Staphylococcus aureu*

In healthy individuals, the carrier rate of *S. aureus* range between 15% to 35% with a risk of 38% of individuals developing infection followed by a further 3% risk of infection when colonized with methicillin-susceptible *Staphylococcus aureus* (MSSA) (File T, 2008). Certain groups of individuals are more susceptible to *S. aureus* colonization than others including health-care workers, nursing home inhabitants, prison inmates, military recruits and children (Ben-David Det al., 2008; Ho P et al., 2008). In a study, conducted in 2007 by the University of the Witwatersrand and the University Hospital of Geneva, health-care workers accounted for 93% of personnel to patient transmission of methicillin-resistant *S. aureus* (MRSA) (Albrich W and Harbath S, 2008). Previously several outbreaks have been reported in Northern-Taiwan in 1997 that suggested MRSA transmission associated with health-care workers, including surgeons (Wang J et al., 2001). Grundmann and colleagues (2006), reported a prevalence of > 50% in countries such as Singapore (1993-1997), Japan (1999-2000) and Colombia (2001-2002) while countries with a prevalence of 25% to 50% included South Africa (1993-1997), Brazil (2001), Australia (2003), Mexico and United States. The lowest prevalence of less than 1% were found in Norway, Sweden and Iceland (1993-1997) (Grundmann H et al., 2006). In 2007, a prevalence of more than 50% of MRSA strains isolated from Cyprus, Egypt, Jordan and Malta was reported by Borg and colleagues (2007). This high prevalence was attributed to overcrowding and poor hand-hygiene facilities in the hospitals (Borg M et al., 2007).

1.2 *Staphylococcus aureus* carriage and disease

Staphylococcus aureus is found as a commensal organism on the squamous epithelium of the anterior nares up to 20% of the population at any one time, however, it has been estimated that *S. aureus* can transiently colonize up to 60% of the human population (Foster T, 2004). *S. aureus* can cause a wide range of infections ranging from minor skin abscesses to more serious invasive diseases. *S. aureus* commonly causes boils, carbuncles, furuncles and impetigo, but after gaining access to the blood, may also be a major cause of endocarditis, osteomyelitis, pneumonia, toxic shock syndrome and septicemia (Lowy F, 1998). Many invasive staphylococcal infections are correlated with nasal carriage of infecting strains. Although immune compromised patients may be at greater risk for developing an invasive staphylococcal infection, healthy individuals may be also susceptible, especially if they are carriers (Peacock S et al., 2001).

1.3 *Staphylococcus aureus* and oxidative stress

S. aureus expresses a wide array of secreted and cell surface associated virulence factors to help evade immune responses (Foster T, 2005). S. *aureus* were able to survive within phagocytic cells both in polymorphonuclear leukocytes (PMN) and monocytes (Steigbigel R et al., 1974). In order to survive and induce infection, pathogenic bacteria have to cope with their changing environment, as well as continuous attacks of the host anti-microbial defense system (Dryla A et al., 2003). Recent review discussed the molecular mechanism of stress antagonizing activity in the survival of bacteria (Vorob'eva L, 2004). The generation and release of toxic reactive oxygen species by phagocytic cells is thought to be an important component of the host's immunity against bacterial infection (Miller R and Britigan B, 1997). Reactive oxygen intermediates are part of the oxygen dependent bactericidal mechanisms that the phagocytic cell employs (Chance B et al., 1979). After engulfment of bacteria by professional phagocytes the induction of highly microbicidal reactive oxygen metabolites during the oxidative burst occurs, resulting in killing (Beaman L and Beaman B, 1984). Monocyte derived macrophages produce a large amount of hydrogen peroxide (H_2O_2) in response to heat killed *S.aureus* (Komuro I et al., 2001). Catalase has been suggested to protect *S. aureus* (Mandell G, 1975). However, the exact mechanism by which bacteria combat oxidative burst during phagocytosis to enable intracellular survival remains unclear. Other studies have indicated

that production of catalase correlates to virulence of *S. aureus* and other microorganism (Buchmeirer N et al., 1995). It was proposed that Cu-Zn SOD could offer an important advantage in survival within host cells to bacteria expressing high levels of these enzymes (Battistoni A et al., 2000). The interaction of *S. aureus* with murine macrophages and the contribution of catalase and SOD in intracellular persistence of *S. aureus* within murine macrophages during in vitro infection was reported (Das D et al., 2008). Previous studies have shown that both bovine and mammary epithelial cells and human endothelial cells internalize *S. aureus* and subsequently undergo apoptosis. However, the intracellular fate of *S. aureus* (i.e. dead or live) is not clear from these studies (Bayles K et al., 1998). Using green fluorescent *S. aureus,* it was demonstrated clearly that internalized *S. aureus* is not a passive bystander rather replicates actively inside pulmonary epithelial cells and induces apoptosis (Kahl B et al., 2000). Further studies are needed to understand the molecular mechanisms by which *S. aureus* replicates intracellularly and induces apoptosis.

1.4 Oxidative stress and inflammation

Reactive oxygen species (ROS) are associated with the inflammatory response and frequently they contribute to the tissue damaging effects of inflammatory reactions (Leiro J et al., 2004). Previously, inflammation was recognized as a simple allergic reaction, but now, considered to underline pathophysiology of a much broader spectrum of disease. At sites of inflammation, multiple inflammatory cells, including eosinophils, neutrophils and macrophages, are capable of generating ROS, which can contribute to development of various diseases (Nagata M, 2005). IL-1, IL-6, IL-12, TNF-α are the pro-inflammatory cytokines (Th1 cytokines) and IL-10, IL-13, TGF-β and SLPI (secretary leukocyte protease inhibitor) are the anti-inflammatory cytokines (Th2 cytokines). The pro-inflammatory cytokines act as a transcriptional activator. The inducible isozyme cyclooxygenase 2 (COX-2), and inducible nitric oxide synthase (iNOS) are the two important compoment and mediator of inflammatory reaction and their activity are regulated by redox status of the cell. COX-2 can also regulate the expression of p38. NF-κB and AP-1, transcription factor, which are activated by the redox status of the cell and can control the COX-2 expression (Schreck R and Baeuerle P, 1994). COX-2, on the other hand, is induced by many pro-inflammatory stimuli, including cytokines and bacterial lipopolysaccharide (LPS) (Maier et al., 1990; Mitchell et al., 1993) in cells in vitro and at the site of inflammation in vivo. COX-2 is believed to be responsible for the production of pro-inflammatory prostanoids in various models of inflammation (Chan C et al., 1995). The antioxidants are reducing the inflammatory reactions, expression of COX-2 and iNOS levels, and oxidative stress in the inflammatory cells. Hence, alteration of inflammatory response in relation to *Staphylococcus aureus* induced oxidative stress may take vast importance. Previous studies reported that the presence of TNF- α, whether of host or pathogen origin, was required for the intracellular control of growth and killing of the organisms (Bekkar L et al., 2001). Upon stimulation by *S. aureus* and its products, macrophages are known to synthesize and release the pro inflammatory cytokines TNF- α and IL-6 (Abdelnour A and Tarkowski A, 1993). Previous studies have also reported, the TNF- α and IL-6 has detrimental effects on septic arthritis (Hultgren O et al., 1998). The initial bacterial exposure might have led the circulatory macrophages to release excessive amount of TNF- α and IL-6 to inhibit the bacterial growth inside. It might have played a pivotal role in host defense systems (Das D et al., 2008). Pathogenic bacteria and other infectious agents can stimulate macrophages or monocytes directly, initiating a release of pro-inflammatory cytokines to sustain inflammation and the immunological response. Tumor necrosis factor (TNF- α), interleukin 1 (IL-1), IL-6 and IL-8, are biologically active peptides produced by phagocytic macrophages, polymorphonuclear leukocytes (PMNs), eosinophils, and monocytes induced by the pathogen organisms, endotoxin and other stimuli (Ives T et al., 2003). Further studies are needed to observe whether the intracellularly survived *S. aureus* could potentiate inflammatory response in *in-vivo* condition under the influence of locally released cytokines or its correlation with the anti-oxidant enzymes were not clearly understood.

1.5 Oxidative stress and apoptosis

Apoptosis or programmed cell death is essential for the normal functioning and survival of most multicellular organisms. The morphological and biochemical characteristics of apoptosis, however, are highly conserved during the evolution. Recent studies have demonstrated that, reactive oxygen species (ROS) and the resulting oxidative stress play a pivotal role in apoptosis. Furthermore, at intermediate concentrations, it induces necrotic cell death (Renz A et al., 2001). Apoptosis can be divided into three non-distinct phases: an induction phase, an effector phase, and a degradation phase. The induction phase depends on death-inducing signals to stimulate pro-apoptotic signal transduction cascades. Some of these death-inducing signals include reactive oxygen and nitrogen intermediates, TNF-α, ceramide, over activation of Ca^{2+} ion pathways, and Bcl-2 family proteins such as Bax and Bad (Sohal R et al., 1995). In phase two, the effector phase, the cell becomes committed to dye by the action of a key regulator, that is, death domain activation on the cell surface, nuclear activators (such as p53), endoplasmic reticulum pathways, or activation of mitochondrial-induced pathways (release of cytochrome C or apoptosis-inducing factors). The degradation phase involves both cytoplasmic and nuclear events. In the cytoplasm, a complex cascade of protein cleaving enzymes called caspases (cysteine proteases) becomes activated. In the nucleus, the nuclear envelops breaks down, endonucleases are activated, causing DNA fragmentation; and the chromatin condenses. Finally, the cell is fragmented into apoptotic bodies and phagocytosed by surrounding cells (Medvedev Z, 1990). Antioxidant can block or delay the apoptosis pathway. Bcl-2, an endogenously produced protein has been shown to prevent cells from dying of apoptosis apparently by an anti-oxidative mechanism. Taken together ROS, and the resulting cellular redox changes, can be part of signal transduction pathway during apoptosis. During mitochondrial dysfunction, several essential players of apoptosis, including pro-caspase, cytochrome C, apoptosis induced-factor (AIF), and apoptotic protease-activating factor-1 (Apaf-1) are released into the cytosol. The multimeric complex formation of cytochrome C, Apaf-1, and caspase 9 activated downstream caspases leading to apoptotic cell death. Death receptors (CD95/Fas/APO-1; TNF receptor-1) ligation leads to the recruitment of the adapter molecule Fas activated death domain (FADD) and pro-caspase-8 into a death-inducing signaling complex (Bantel H et al., 2001). All the three functional phases of apoptosis are under the influence of regulatory controls. Thus increasing evidences provide support the oxidative stress and apoptosis are closely linked physiological phenomena, and are implicated in pathophysiology of some of the chronic diseases including AIDS, autoimmunity, cancer, diabetes mellitus, and ischemia of heart and brain (Simon H et al., 2000). Staphylococcal infections are typically associated with death of tissue, and evidence suggests intracellular bacteria are capable of inducing apoptosis. *S. aureus*-mediated apoptosis has been reported in epithelial cells (Bayles K et al., 1998; Kahl B et al., 2000; Wesson C et al., 2000), keratinocytes (Nuzzo I et al., 2000), endothelial cells (Menzies B and Kourteva.I, 2000), and osteoblasts (Tucker K et al., 2000). Wesson et al. (Wesson C et al., 2000) demonstrated host caspases-8 and caspase-3 to play a role in *S. aureus*-induced apoptosis, and caspase-8 is known to be associated with apoptosis triggered by engagement of death receptors (Thornberry N and Lazebnik Y, 1998). Alpha toxin (α-toxin), a major cytotoxin of *S. aureus*, is an effective inducer of apoptosis in Jurkat T cells. Induction of apoptosis by α -toxin was independent of death receptor signaling and was mediated via the mitochondrial pathway, which involved the activation of caspase-9 and caspase-3 as well as the post-mitochondrial activation of caspase-8 (Bantel H et al., 2001).

2. Antibiotics

Antibiotics are hailed as the greatest medicinal triumph of the 20th Century. Before their discovery, there was little available to help a patient once they had become infected. The earlier development of vaccination had introduced immunity to some diseases and sterilization had helped to reduce the chance of infection from surgery. With the subsequent formation of germ theory and the work identifying the role of specific bacteria in the diseases anthrax and tuberculosis, the search for a cure began (Pasteur L et al., 1881; Kaufmann S and Schaible U, 2005). In 1929, nearly 250 years after van

Leuwenhoek first discovered bacteria, Fleming noted that the growths of bacteria could be inhibited by the presence of a mould, *Penicilium notatum*. This effect was caused by a metabolic product from the mould that was interacting with the staphylococcal culture (Fleming A, 1929). Penicillin was the first of the family of β-lactam antibacterials that now form the largest share of the antibacterial market.

2.1 Treatment and prevention of *S. aureus* infections

Penicillin is still the main drug of choice for staphylococcal infections as long as the isolate is sensitive to it (Kowalski R et al., 2003). In patients with histories of a delayed-type penicillin allergy a cephalosporin, such as cefazolin or cephalothin can be administered as an alternative choice of treatment. A semisynthetic penicillin, such as methicillin, is indicated for patients with β-lactamase producing staphylococcal isolates (Lowy F, 1998). Patients who have an MRSA infection are treated with a glycopeptide known as vancomycin. Vancomycin is the empirical drug of choice for the treatment of MRSA (Michel M and Gutmann L, 1997). Patients who are intolerable to vancomycin are treated with a fluoroquinolone (ciprofloxacin); lincosamide (clindamycin); tetracycline (minocycline) or trimethoprim-sulfamethoxazole, which is also known as co-trimoxazole (Lowy F, 1998).

Novel quinolones, such as ciprofloxacin with increased antistaphyloccal activity are available but their use may become limited due to the rapid development of resistance during therapy (Lowy F, 1998). Several antimicrobial agents with activity against MRSA are currently evaluated and include: (i) oritavancin, a semisynthetic glycopeptide (Guay D, 2004); (ii) tigecycline, a monocycline derivative (Guay, 2004) and (iii) DW286, a fluoroquinolone (Kim M et al., 2003). Amongst these three antibiotics, tigecycline has been approved by the Food and Drug administration (FDA) in June 2005 (Stein G and Craig W, 2006).

Recently, an evaluation of glycosylated polyacrylate nanoparticles showed to have *in vitro* activities against methicillin-resistant *S. aureus* and *Bacillus anthracis* (Abeylath S et al., 2007). Other recent investigative drugs include, silver nano particles, oleanolic acid from extracted *Salvia officinalis* (Sage leaves) (Yuan W et al., 2008). Two novel antibiotics, neocitreamicins I and II, isolated from a fermentation broth of a *Nocardia* strain have shown to have *in vitro* activity against *S. aureus* and vancomycin-resistant *Enterococcus faecalis* (VRE) (Peoples A et al., 2008). Accurate empirical therapy against *S. aureus* infections would be an important step towards the reduction of the development of resistance in the different strains (Lowy F, 1998).

2.2 Mechanism of action of antibiotics

Antibiotics work in variety of ways (Figure 1). Some antimicrobial agents inhibit bacterial cell wall synthesis. These agents include β-lactam compounds such as penicillins (e.g. penicillin G, ampicillin and methicillin), cephalosporins and carbapenems, as well as monolactams and β-lactamase inhibitors. β-lactams inhibit the final stage of murein synthesis. This, by some undetermined mechanism, triggers murein hydrolases to lyse the cell. A related group of antibiotics that prevent a different step in cell wall synthesis are the glycopeptides, vancomycin and teicoplanin. Other agents have an antibacterial effect by inhibiting protein synthesis. Representatives of this group include the aminoglycosides, tetracyclines, macrolides and chloramphenicol which interfere with ribosome function. In addition, there are antibiotics that inhibit DNA synthesis, including quinolones, fluoroquinolones and sulfonamides (Normark B and Normark S, 2002).

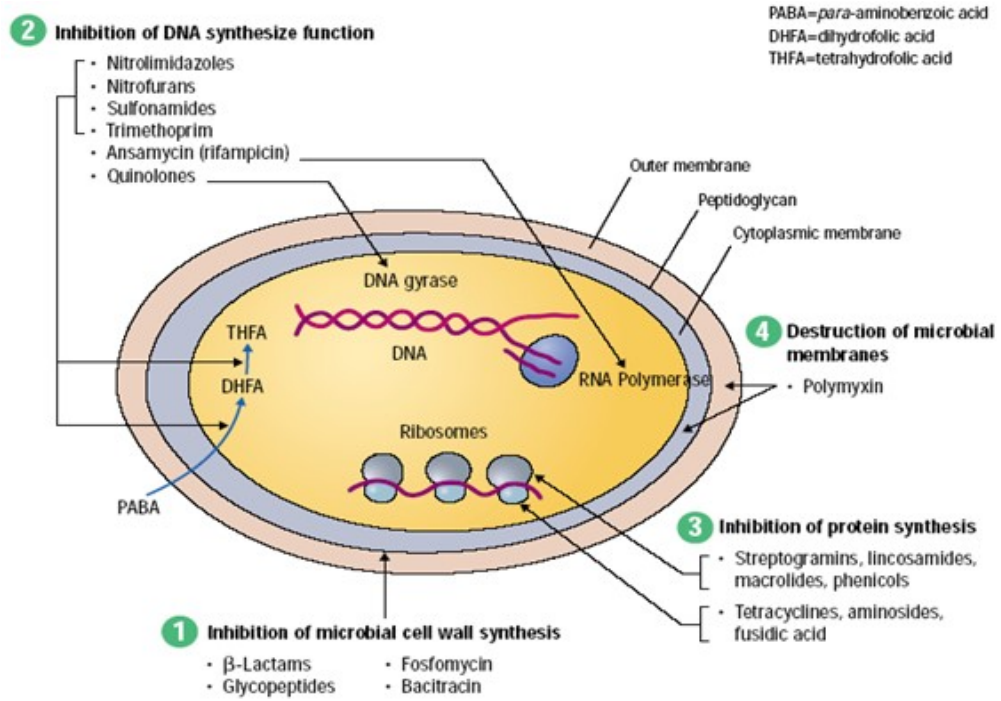

Figure 1: ***Mechanism of action of antibiotics.***

2.3 Appearance of antibiotic resistance

The belief of overcome the infectious disease did not last long: the discovery of antibiotics made them widely available, which only accelerated the appearance of antibiotic resistance, devaluing them. This is the aptly named "antibiotic paradox". The appearance of resistance should have been foreseen. The biochemical warfare between micro-organisms dates back much further than any human intervention, and these organisms had developed defences during that time against the very weapons. The truth is shown no more clearly than in the history of penicillin. Within a decade of antibiotic use, resistance was already being observed, first in hospitals and later in the community (Palumbi S, 2001). As time has progressed, more antibiotics have succumbed to the rising levels of resistance with the effectiveness of most antibiotics dropping. By 1993, the dosages for these antibiotics had increased from 10 to 100-fold beyond the dosage when they were first introduced (Neu H, 1994).

With the appearance of MRSA, the problem has only grown further. While at present MRSA is mostly confined within hospitals and the care community, the risk of community-acquired infection is very real and something leading to much worry within both the medical profession and general public.

2.4 Mechanism of antibiotic resistance

In recent years, antibiotics resistant bacteria have become a great concern to the medicals community. There has been amerced increase in the number of species that have acquired resistance to antibiotics (Table 1). Mechanisms of antimicrobial resistance can be classified into four main groups (Figure 2).

Table 1: ***Some representative antibiotics, their modes of action and mechanisms of resistance.***

Category	Some members	Mode of action	Major mechanisms of resistance
β-Lactams	Penicillins, Cephalosporins, Cefotaximes, Carbapenems	Inhibition of cell-wall synthesis	Cleavage by β-lactamases, ESBLs, CTX-mases, Carbapenemases, altered PBPs
Aminoglycosides	Streptomycin, Gentamycin, Tobramycin, Amikacin	Inhibition of protein synthesis	Enzymatic modification, efflux, ribisomal mutations, 16S rRNA methylation
Quinolones	Ciprofloxacin, Ofloxacin, Norfloxacin	Inhibition of DNA replication	Efflux, modification, target mutations
Glycopeptides	Vancomycin	Inhibition of cell-wall synthesis	Altered cell walls, efflux
Tetracyclines	Tetracycline	Inhibition of translation	Mainly efflux
Rifamycins	Rifampin (Rifampicin)	Inhibition of transcription	Altered β-subunit of RNA polymerase
Streptogramins	Virginiamycins, Quinupristin, Dalfopristin	Inhibition of cell-wall synthesis	Enzymatic cleavage, modification, efflux
Oxazolidinones	Linezolid	Inhibition of formation of 70S ribosomal complex	Mutations in 23S rRNA genes followed by gene conversion

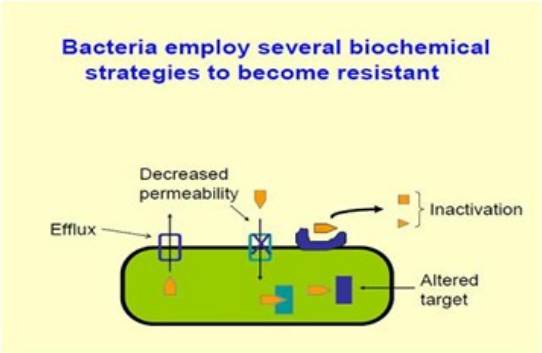

Figure 2: ***Biochemical mechanisms of antibacterial resistance.***

2.4.1 ***Enzymatic inactivation***

Antibiotic modification results in the antibiotic being unable to act on its target. For example β-lactam antibiotics may be destroyed enzymatically by β-lactamases that cleave
the β-lactam ring. These enzymes are widespread among many bacterial species (Gram positive and Gram negative) and show varied degrees of inhibition by β-lactam inhibitors such as clavulanic acid (Livermore D, 1995). Most β-lactamases act to some degree against both penicillins and cephalosporins; others are more specific, cephalosporinases (e.g. AmpC enzyme found in *Enterobacter* spp) or penicillinases (e.g. *S. aureus* penicillinase).

2.4.2 ***Permeability barrier :*** Protection of the target from antibiotic action by preventing the antibiotic from entering the cell or acquiring the ability to pump out the antibiotic. β-lactam antibiotics gain access to Gram negative bacteria through porins. Lack of the specific D2 porin in *P. aeruginosa* results in imipenem resistance. This mechanism is also seen with low level resistance to fluoroquinolones and aminoglycosides. Increased efflux via an energy-requiring transport pump is a mechanism for resistance to tetracyclines and is encoded by a wide range of related genes.

2.4.3 ***Altered targets***

Alteration of the target so that it is no longer recognized by the antibiotic. Most strains of *S. pneumoniae* are highly susceptible to both penicillins and cephalosporins but can acquire DNA from other bacteria which changes the enzyme responsible for

cell wall synthesis. As a result the bacteria develop a low affinity for penicillins and hence become resistant (Garcia-Bustos J and Tomasz A, 1990).

2.4.4 *Metabolic bypass*

Acquisition of an alternative metabolic pathway, bypassing the antibiotic's site of action. The alternative penicillin binding protein (PBP2a) produced by MRSA is an example of this. The protein is encoded by the *mecA* gene and because PBP2a is not inhibited by antibiotics such as flucloxacillin the cell continues to synthesise peptidoglycan and hence has a structurally sound cell wall (Michel M and Gutmann L, 1997). A further example of this type of resistance is shown by vancomycin resistant bacteria (Leclerq R and Courvalin P, 1997). In enterococci sensitive to vancomycin the normal target of vancomycin is a cell wall precursor that contains a pentapeptide that has a D-alanine terminus to which the vancomycin binds, preventing further cell wall synthesis. If an enterococcus acquires the *vanA* gene cluster it can make an alternative cell wall precursor ending in D-alanine-D-lactate to which vancomycin does not bind.

2.5 Emergence of antibiotic resistance in *Staphylococcus aureus*

Staphylococcus aureus, a major cause of potentially life-threatening infections acquired in healthcare and community settings, has developed resistance to most classes of antimicrobial agents (Figure 3). Penicillin was the first choice of antibiotics to treat staphylococcal infection. In 1944, by destroying the penicillin by penicillinase (beta-lactamase), *S. aureus* become resistant to penicillin (Kirby W, 1944). Most *S. aureus* strains (≥90%) are resistant to penicillin (Neu H, 1992). Methicillin, a semisynthetic penicillins was used to treat Penicillin Resistant *Staphylococcus aureus* but resistance finally emerge in 1962 (Livermore D, 2001; Lowy F, 1998). Methicillin-resistance in *S. aureus* is mediated by the presence of penicillin-binding protein 2a (PBP-2a) which is expressed by an exogenous gene, *mecA*. This gene is carried by a genetic element, designated as staphylococcal cassette chromosome *mec (SCCmec)*, which is inserted near the chromosomal origin of replication (Hiramatsu K, 2001). High prevalence of methicillin resistant *Staphylococcus aureus* (MRSA) in hospitals has been reported from many states of India (Rajaduraipandi K et al., 2006). Methicillin resistance among *S. aureus* isolates has reached phenomenal proportions in Indian hospitals, with some cities reporting 70% of the strains to be resistant to methicillin (Anupurba S et al., 2003). Vancomycin continues to be an important antimicrobial agent for treatment of Methicillin Resistant *Staphylococcus aureus* (MRSA) infections but resistance finally emerges. In 1996, a *S. aureus* strain with intermediate resistance to vancomycin (VISA) (vancomycin MIC= 8 µg/ml) was first isolated from a patient in Japan (Hiramatsu K et al., 1997). Shortly afterward, VISA strains were isolated in USA, Europe and other Asian countries (Hamilton-Miller J, 2002), arousing considerable concern regarding the emergence of *S. aureus* strains for which there will be no effective therapy. Characterization of these VISA strains indicates that the mechanisms of resistance are complex and involve changes in cell wall content and composition (Avison M et al., 2002).

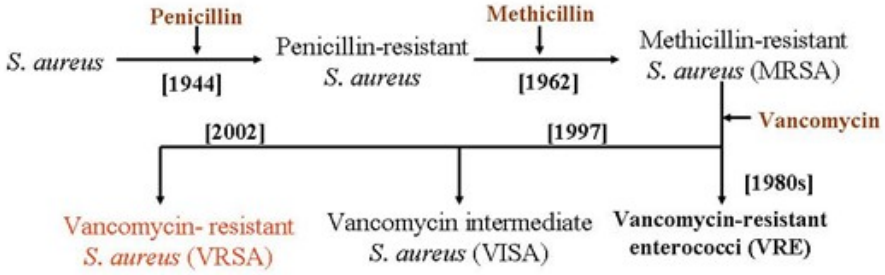

Figure 3: ***Evaluation of antibiotic resistance in Staphylococcus aureus.***

In June 2002, the world's first reported clinical infection due to *S. aureus* with high resistance to vancomycin (VRSA) (vancomycin MIC>128 µg/ml) was diagnosed in a patient in the USA (Sievert D et al., 2002). This isolate contain the vanA genes from enterococci and the methicillin-resistance gene mecA. The possible emergence and dissemination of VRSA strains is a serious health threat and makes it absolutely necessary to optimize prevention strategies and fast detection methods. Till today only five VRSA have been found all over the world, first in USA in 2002 (Sievert D et al., 2002), second in Michigun in 2002 (Chang S et al., 2003), third in Pennsylvania in 2002 (Tenover F et al., 2004), fourth in New York in 2004 (Kacica M, 2004), fifth in New York in 2005 (Perichon B and Courvalin P, 2006), and the sixth in Kolkata (India) in 2005 (Saha B et al., 2008).

2.6 Drug of last resort

Due to the nature of MRSA, when a patient is unfortunate to acquire an infection, the resulting treatment inevitably involves the use of vancomycin. As such, vancomycin is thought of as a drug of last resort. While vancomycin is currently an effective treatment in the battle against MRSA, past experience has shown us that this should not lead to complacency and with the increasing use of vancomycin in the treatment of such super-bugs the appearance of vancomycin resistance is only to be accelerated. To compound these fears, vancomycin resistance is already well documented in vancomycin-resistant enteriococci (VRE), and has been know about for many years (Uttley A et al., 1988). This, and the emergence of MRSA with resistance to vancomycin (VRSA) in a small number of isolated cases, is definitely cause for alarm. If new antibiotics cannot be found then such infections will once again be without cure and the consequences deadly. As such, the current situation of looking for new antibiotics or ways to circumvent the current methods of resistance employed by bacteria has lead to the comparison to an arms race, with the hope that we can develop the cures faster than the bacteria evolve.

3. Vancomycin

Vancomycin was isolated in 1953 by Edmund Kornfeld from a soil sample collected from the interior jungles of Borneo by a missionary. It is a glycopeptide antibiotic (Figure 4). It is used in the prophylaxis and treatment of infections caused by Gram-positive bacteria. It has traditionally been reserved as a drug of "last resort", used only after treatment with other antibiotics had failed, although the emergence of vancomycin-resistant organisms is increasingly being displaced from this role by linezolid and daptomycin. The organism that produced it was eventually named *Amycolatopsis* orientalis. The original indication for vancomycin was for the treatment of penicillin-resistant *Staphylococcus aureus*. The compound was initially labelled compound 05865, but was eventually given the generic name, vancomycin (Levine D, 2006). Eli Lilly first marketed vancomycin hydrochloride under the trade name Vancocin (Moellering R, 2006) and as COVANC (India, Marketed by Nucleus).

3.1 Pharmacology and Chemistry

Vancomycin is a branched tricyclic glycosylated non-ribosomal peptide. It has multiple chemically distinct rotamers owing to the rotational restriction of some of the bonds. The form present in the drug is the thermodynamically more stable conformer and, therefore has more potent activity. The chemical data of vancomycin is shown in Table 2.

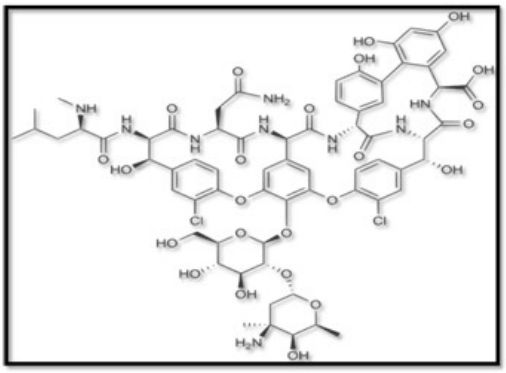

Figure 4: ***Chemical structure of vancomycin.***

Table 2: ***Chemical data of vancomycin.***

Formula	C66H75Cl2N9O24
Mol. mass	1449.3 g.mol-1
Bioavailability	Negligible (oral)
Metabolism	Excreted unchanged
Half life	4–11 hours (adults)
Excretion	Renal

3.2 Mechanism of action of vancomycin

Vancomycin acts by inhibiting proper cell wall synthesis in Gram-positive bacteria. The mechanism inhibited, and various factors related to entering the outer membrane of Gram-negative organisms mean that vancomycin is not active against Gram-negative bacteria. Specifically, vancomycin prevents incorporation of N-acetyl muramic acid (NAM) and N-acetyl glucosamine (NAG)-peptide subunits into the peptidoglycan matrix; which forms the major structural component of Gram-positive cell walls (Figure 5). The large hydrophilic molecule is able to form hydrogen bond interactions with the terminal D-alanyl-D-alanine moieties of the NAM/NAG-peptides. Normally this is a five-point interaction. This binding of vancomycin to the D-Ala-D-Ala prevents the incorporation of the NAM/NAG-peptide subunits into the peptidoglycan matrix (Saha B et al., 2008).

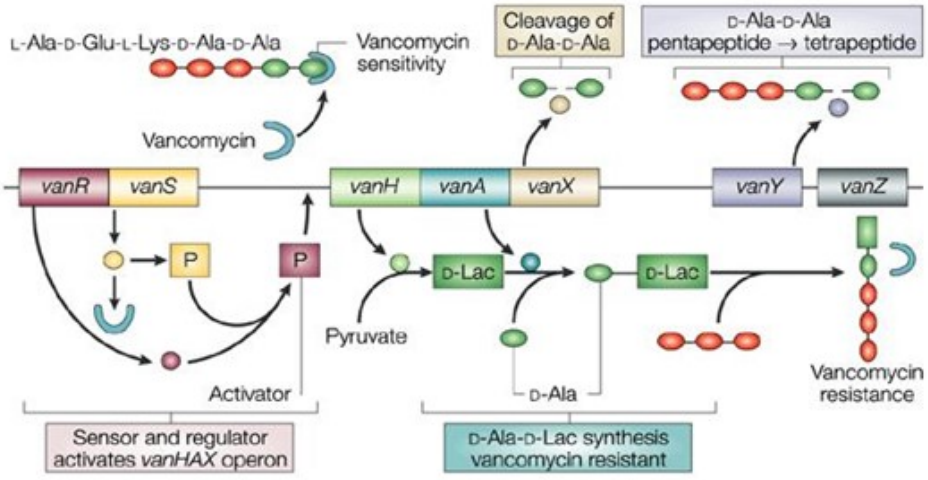

Figure 5: ***Mechanism of action and resistant of Vancomycin***

3.3 Mechanism of resistance

The clinical isolates resistant to vancomycin are characterized by a subtle change in the structure of peptidoglycan termini, as a result of the action of D-alanyl-D-X ligases, which have dual-subsite specificity at their second amino acid-binding site. The switch from D-Ala-D-Ala peptidoglycan termini to D-alanine-D-lactate (D-Ala-D-Lac) results in the loss of crucial hydrogen-bonding interactions, causing a 1,000-fold reduction of vancomycin-binding affinity. Inducible high-level vancomycin resistance in *Enterococcus faecium* BM4147 is associated with the five genes located on the transposable element Tn1546 and is the most common clinical phenotype (Walsh C et al., 1996). A two-component transcriptional-activation system of *VanS* and *VanR* controls the induction of three structural genes *VanH*, *VanA*, and *VanX*. *VanH* is an α-keto acid reductase that converts pyruvate to D-Lac for D-Ala-D-Lac synthesis by the ATP-dependent desipeptide ligase, *VanA* (Bugg T et al., 1991). *VanA* is a 38.5-kDa protein capable of utilizing both hydroxyl acids and D-Ala as substrates with a concomitant switch from ester to peptide bond formation dependent on pH (Park I et al., 1996). The presence of any peptidoglycan chains ending in D-Ala-D-Ala, as a consequence of endogenous peptidoglycan biosynthesis genes, is negated by the action of *VanX*, a Zn^{2+}-dependent D-Ala-D-Ala dipeptidase (Reynolds P et al., 1994). Thus the action of these three genes in concert ensures the production of D-Ala-D-Lac-terminating peptidoglycan chains, which are bound very weakly by the glycopeptide antibiotic vancomycin, rendering the bacteria resistant (Figure 5).

4. Nanotechnology

Nanotechnology is enabling technology that deals with nano-meter sized objects. Nanotechnology is developed at several levels: materials, devices and systems. The nanomaterials level is the most advanced at present, both in scientific knowledge and in commercial applications. A decade ago, nanoparticles were studied because of their size-dependent physical and chemical properties (Murray C et al., 2000). Now they have entered a commercial exploration period (Paull R et al., 2003). Living organisms are built of cells that are typically 10 μm across. However, the cell parts are much smaller and are in the sub-micron size domain. Even smaller are the proteins with a typical size of just 5 nm, which is comparable with the dimensions of smallest man-made nanoparticles. This simple size comparison gives an idea of using nanoparticles as very small probes that would allow us to spy at the cellular machinery without introducing too much interference (Taton T, 2002). Understanding of biological processes on the nanoscale level is a strong driving force behind development of nanotechnology (Whitesides G, 2003). Out of plethora of size-dependant physical properties available to someone who is interested in the practical side of nanomaterials, optical and magnetic effects are the most used for biological applications (Parak W et al., 2003).

4.1 Nanoparticles

Nanoparticles are solid colloidal particles with diameters ranging from 1-1000 nm. They consist of macromolecular materials and can be used therapeutically as adjuvant in vaccines or drug carriers in which the active ingredient is dissolved, entrapped, encapsulated, adsorbed or chemically attached. Polymers used to form nanoparticles can be both synthetic and natural polymers. There are two types of nanoparticles depending on the preparation process: nanospheres and nanocapsules (Allemann E et al., 1993). Nanospheres have a monolithic-type structure (matrix) in which drugs are dispersed or adsorbed onto their surfaces; and nanocapsules exhibit a membrane-wall structure and drugs are entrapped in the core or adsorbed onto their exterior (Figure 6).The term “nanoparticles” is adopted because it is often very difficult to unambiguously establish whether these particles are of a matrix or a membrane type.

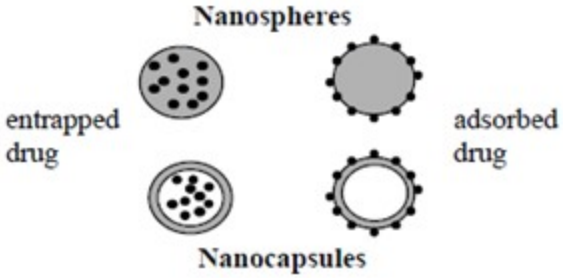

Figure 6: ***Various types of drug loaded nanoparticles (Allemann et al., 1993).***

Nanoparticles not only have potential as drug delivery carriers as they offer non-invasive routes of administration such as oral, nasal and ocular routes, but also show to be good adjuvant for vaccines. Despite these advantages, there is no ideal nanoparticle system available. Most of nanoparticles prepared from water-insoluble polymers are involved heat, organic solvent or high shear force that can be harmful to the drug stability. Moreover, some preparation methods such as emulsion polymerization and solvent evaporation are complex and require a number of preparation steps that are more time and energy consuming. In contrast, water-soluble polymers offer mild and simple preparation methods without the use of organic solvent and high shear force.

4.2 Chitin

Chitin is one of the most abundant organic materials, beings second only to cellulose in the amount produced annually by biosynthesis. It occurs in animals, particularly in crustacea, molluscs and insects, where it is a major constituent of the exoskeleton, and in certain fungi, where it is the principal fibrillar polymer in the cell wall. Chitin has a crystalline structure and it constitutes a network of organized fibres, this structure confers rigidity and resistance to organisms that contain it (Roberts G , 1992). Chitin is poly [β-(1→4)-2-acetamido-2-deoxy-D-glucopyranose], and its idealized structure is shown in Figure 7.

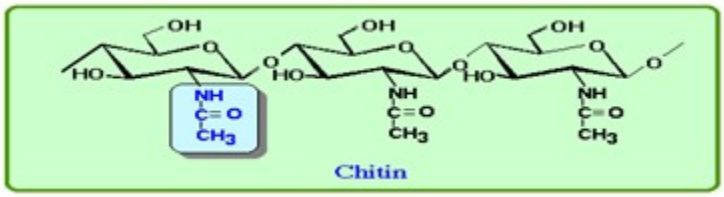

Figure 7: ***Chemical structure of Chitin.***

4.3 Chitosan

Chitosan is a modified natural carbohydrate polymer prepared by the partial N-deacetylation of chitin, a natural biopolymer derived from crustacean shells such as crabs, shrimps and lobsters. Chitosan is also found in some microorganisms, yeast and fungi (Illum L, 1998). The primary unit in the chitin polymer is 2-deoxy-2-(acetyl amino) glucose. These units combined by β-(1,4) glycosidic linkages, forming a long chain linear polymer and its idealized structure is shown in Figure 8. Chitosan is soluble in most organic acidic solutions at pH less than 6.5 including formic, acetic, tartaric, and citric acid (LeHoux J and Grondin F, 1993). It is insoluble in phosphoric and sulfuric acid. Chitosan is available in a wide range of molecular weight and degree of deacetylation. Molecular weight and degree of deacetylation are the main factors affecting the particle size, particles formation and aggregation. Chitosan possesses some ideal properties of polymeric carriers for nanoparticles such as biocompatible, biodegradable, nontoxic, and inexpensive. These properties render chitosan a very attractive material as a drug delivery carrier. In the

last two decades, chitosan nanoparticles have been extensively developed and explored for pharmaceutical applications (Roberts G, 1992).

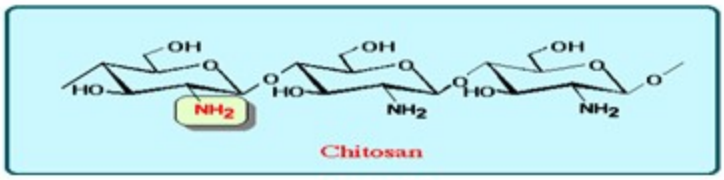

Figure 8: ***Chemical structure of Chitosan.***

4.4 Biological profits of chitosan nanoparticle

4.4.1 Parenteral administration

Nano-sized particles can be administered intravenously because the diameter of the smallest blood capillary is approximately 4 μm. The biodistribution of nanoparticles can vary depending on the size, surface charge and hydrophobicity of the administered particles (Tabata and Ikada, 1988). Particles greater than 100 nm in diameter are rapidly taken up by the reticuloendothelial system (RES) in the liver, spleen, lung and bone marrow, while smaller-sized particles tend to have a prolonged circulation time. Negatively-charged particles are eliminated faster than positively-charged or neutral particles (Tabata Y and Ikada Y, 1988). The most promising drugs that have been extensively studied for delivery by this route are anticancer agents. Following intravenous injection, many nanoparticle systems including chitosan NP exhibited a marked tendency to accumulate in a number of tumors (Kreuter J, 1994). One possible reason for the phenomenon may involve the leakiness of tumor vasculature (Sadzuka Y et al., 1998).

4.4.2 Peroral administration

Delivery of anti-infective such as antibacterial, antiviral, antifungal and antiparasitic drugs, is another common use of nanoparticles (Page-Clisson M et al., 1998). The low therapeutic index of antifungal drugs, short half-life of antivirals and the limited ability of antibiotics to penetrate infected cells in intracellular compartments make them ideal candidates for nanoparticle delivery. The absorption promoting effect of chitosan has been extensively studied by several research groups and found to be due to a combination of mucoadhesion and transient opening of tight junctions in the mucosal cell membrane which have been verified both *in vitro* and *in vivo* (Aspden T et al., 1996). Pan *et al.* reported that hypoglycemic effect was observed in induced diabetic rats after orally administration of chitosan nanoparticles (Pan Y et al., 2002). Furthermore, chitosan can be employed as a coating material for liposomes, micro/nanocapsules to enhance their residence time, thereby improving drug bioavailability (Vila A et al., 2002).

4.4.3 Non-viral gene delivery vectors

Viruses can efficiently transfer genes into cells, concerns such as host immune response, residual pathogenicity, and potential induction of neoplastic growth following insertional mutagenesis have led to the exploration of non-viral gene transfer systems (Otto E et al., 1994). These latter delivery systems are generally considered to be safer since they are typically less immunogenic and lack mutational potential. Chitosan as a promising gene delivery vector was first proposed by Mumper (Mumper R et al., 1995). Park *et al.* developed liver targeted delivery system by preparing galactosylated-chitosan-graft-dextran DNA complexes, as galactose is known as liver targeted delivery (Park I et al., 2000). Similarly, Mao *et al.* prepared transferrin-chitosan-DNA nanoparticles as a targeted drug delivery (Mao H et al., 2001).

4.4.4 Delivery of vaccines

Nanoparticles often exhibit significant adjuvant effects in parenteral vaccine delivery since they may be readily taken up by antigent presenting cells (Kreuter J, 1995). Among the polymers used to form vaccine nanoparticles, chitosan is one of the most

recently explored and extensively studied as prospective vaccine carriers. Furthermore, chitosan has also been widely explored as the application for DNA mucosal vaccines. For instance, a chitosan-based DNA flu vaccine showed high antibody level in mice after intranasal administration (Illum L et al., 2001). Plasmid pCMVArah2 encoding peanut allergen genes were reported successfully incorporated into chitosan NP with good antigen expression and good protection after oral administration in mice (Roy K et al., 1999).

4.4.5 Ocular administration

Nanoparticles have been found to be potential carriers for ocular delivery following the observation that various types of nanoparticles tend to adhere to the ocular epithelial surface (Wood R et al., 1985). The resulting prolonged residence time of nanoparticles leads to a much slower elimination rate compared to conventional ophthalmologic formulations, thereby improving drug bioavailability. As a consequence, nanoparticles have been developed for targeted ophthalmic delivery of anti-inflammatory, antiallergic and beta-blocker drugs (De Campos A et al., 2001). Felt *et al.* found that chitosan solutions prolonged the cornea resident time of antibiotic in rabbits (Felt O et al., 1999). The same effects were also observed employing chitosan NP as demonstrated by De Campos *et al.* that chitosan NP remained attached to the rabbits' cornea and conjunctiva for at least 24 hr (De Campos A et al., 2001). Chitosan also shown to be a low toxic material, ophthalmic formulation based on chitosan exhibited an excellent tolerance after applied chitosan onto the rabbit's corneal surface (Felt O et al., 1999). Beside employing chitosan NP to improve drug transport via ocular, chitosan-coated nanoparticles can also be utilized as it exhibited ability to enhance the corneal penetration (Calvo P et al., 1997).

4.5 Chitosan-as drug delivery vehicles

Chitosan is the most important chitin derivative in terms of application. Chitosan has attracted considerable interest because of its unique combination of properties, such as biocompatibility, biodegradability, metal complexation and antibacterial activity. Chitosan has a variety of current and potential applications in various fields, for example, biotechnology (Mao et al., 2001), pharmaceutics (Illum, 1998), wastewater treatment (Ramnani and Sabharwal, 2006), cosmetics (Majet N and Kumar R, 2000), and food science (Chien P et al., 2007). The antibacterial activity of chitosan has been widely explored (Hong K et al., 2002; Tsai G et al., 2004; Liu N et al., 2006). Penicillin bound acrylare nanoparticles have equipotent in vitro antibacterial properties against methicilin susceptible and methicillin resistance form of *Staphylococcus aureas* and indefinite stability toward beta lactamase (Turos E et al., 2007). Recently it was reported that chitosan nanoparticle coated with Folic acid containing pendant group enhances drug delivary in cancer cell (Sahu S et al., 2010).

5. CONCLUSION

Resistance of *S. aureus* to antibiotics appeared within a few years after the onset of the antibiotic era, and this problem has reached epic proportions owing to overuse and improper use of antibiotics. *S. aureus* resistance to antibiotics currently spans all known classes of natural and synthetic compounds. Increasing resistance of *S. aureus* to last line of drug i.e., vancomycin highlights the need for either the development of new and novel antibiotics or the improvement of efficacy of established antibiotics by the development of new agents capable of enhancing antibiotic activity. Staphylococcal infections are typically associated with death of tissue, and evidence suggests intracellular bacteria are capable of inducing apoptosis. *S. aureus*-mediated apoptosis has been reported in epithelial cells, keratinocytes, endothelial cells, and osteoblasts. A number of chitosan derivates with different modifications have been prepared to improve its antibacterial activity. A Chitosan nanoparticle also delivers anti-infective drugs such as antibacterial, antiviral, antifungal and antiparasitic drugs. In view of antibiotic resistance of *S. aureus*, staphylococcal infections mediated apoptosis in cellular system and important beneficial effects of chitosan nanoparticles, it may be concluded that chitosan derivatives may shows the antibacterial activity against VRSA after binding with vancomycin.

Abbreviations:

COX	: Cycloxygenase
DNA	: Deoxyribonucleic acid
FDA	: Food and Drug administration
H_2O_2	: Hydrogen peroxide
IL	: Interleukin
iNOS	: Inducible nitric oxide synthase
MRSA	: Methicillin resistant *Staphylococcus aureus*
NAM	: N-acetyl muramic acid
NAG	: N-acetyl glucosamine
PBP	: Penicillin binding protein.
PMN	: Polymorphonuclear leukocytes.
PVL	: Panton-Valentine leukocidin.
ROS	: Reactive oxygen species
S. aureus	: *Staphylococcus aureus*
SOD	: Superoxide dismutase
TNF	: Tumor necrosis factor
TSS	: Toxic shock syndrome.
VRE :	: Vancomycin resistant *Enterococcus*
VRSA	: Vancomycin resistant *Staphylococcus aureus*
VSSA	: Vancomycin sensitive *Staphylococcus aureus*

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