CHAPTER-1

INTRODUCTION

Introduction

"The excursions of the Staphylococcus into disease production seem to be aberrant activities outside the main stream of its existence."

R. Williams 1963

Introduction

S. aureus is Gram positive, non-motile and non-spore forming bacteria belonging to family Staphylococcaceae (Thakker *et al.,* 1998). *S. aureus* is part of the normal flora and can be found in the anterior nares as well as on the skin, axilla, perineum, and pharynx. It has been estimated that 25–35% of healthy humans in the general community have *S. aureus* in their anterior nares (Grundmann *et al.*, 2006). It is now thought that ~20% of population almost always carries *S. aureus*, another 20% rarely carries *S. aureus*, and the remaining 60% of the population is intermittently colonized (Kluytmans *et al.*, 1997).

S. aureus is often found as a commensal on the skin, skin glands and mucous membranes particularly in the nose of healthy individuals (Plata *et al.*, 2009). It is a versatile human pathogen causing infections ranging from relatively mild skin and soft tissue infections to life threatening sepsis, pneumonia, osteomyelitis, endocarditis as well as toxin mediated syndromes such as toxic shock syndrome and food poisoning (Shittu *et al.,* 2011). *S. aureus* is usually a harmless colonizer of about one third of healthy humans and is most likely found in the nares. Nasal carriage of *S. aureus* has been closely associated with *Staphylococcal* disease (Von Eiff *et al.,* 2001). Colonization increases the risk of subsequent infection since those with *S. aureus* infections are usually infected with their colonizing strain (Gordon and Lowy, 2008).

Methicillin-resistant *S. aureus* (MRSA) was first identified over 50 years ago and has become a major nosocomial and community pathogen. MRSA strains are *S. aureus* that have a mecA gene which codes for a unique penicillin-binding protein that has decreased affinity for β-lactams. This protein allows for cell growth in the presence of penicillins and other β-lactam antibiotics, which are the antibiotics of choice for *Staphylococcal* skin and soft tissue infections (Eady and Cove, 2003; Grundmann *et al.,* 2006). Over the last decade community-acquired MRSA (CA-MRSA) causing primarily skin and soft tissue infections has emerged as

a major cause of disease in the general population with no health care exposure or known classical risk factors for MRSA infections around the world (King *et al.,* 2006). CA-MRSA strains initially from community patients are now entering the hospital environments to become the predominant nosocomial MRSA isolates (Popovich *et al.,* 2008).

S. aureus and MRSA can be transmitted from people-to-people, from fomites-to-people, and from air-to-people (Huang *et al.,* 2006). People colonized or infected with *S. aureus* or MRSA shed into their environments contaminating surfaces and fomites at concentrations sufficient for survival for extended periods of time in the environment which should allow for transfer to skin, clothing, and other fomites (Boyce *et al.,* 1997; Otter *et al.,* 2011).

The contamination of inanimate surfaces by *Staphylococci* is probably driven by several factors:-

i) the ability of *Staphylococci* to attach to surfaces (e.g. biofilm formation) (von Eiff *et al.,* 2002),

ii) the ubiquity of *Staphylococci* in animal and human hosts and their shedding/transmission to surfaces (Martins and Cunha, 2007; Kloos and Bannerman, 1994; Guyot and Layer, 2006; Boyce, 2007),

iii) The absence of awareness of proper hygiene and/or decontamination (Scott and Bloomfield, 1990; Neely and Maley, 2000; Guyot and Layer, 2006),

iv) The type of material that constitutes the surface (Neely and Maley, 2000; von Eiff *et al.,* 2002); and,

v) Environmental conditions that affect the surface (e.g. presence of organic material, exposure to light, etc.) (Vuong and Otto, 2002)

Understanding the composition of the community surviving on contaminated surfaces is important, as survival of *Staphylococci* on surfaces can promote the spread of these

pathogens to patients or susceptible individuals in the community, increasing the risk of infection (Neely and Maley, 2000).

Staphylococci may also exist in food products of animal origin or those that are handled directly by humans (Jay *et al.,* 2005). So far, MRSA have been isolated from food producing animals (Vanderhaegen *et al.,* 2010) and foods of animal origin, such as meat products from cattle and pigs (van Loo *et al.,* 2007), milk and dairy products (Normanno *et al.,* 2007). Methicillin Resistance *S. aureus* has been associated with food through contamination from humans (Kluytmans *et al.,* 1995). The occurrence of pathogenic bacteria in milk and milk products can cause severe health hazards to people as they are highly susceptible to variety of microorganism because of high nutritive value and complex chemical composition (Soomro *et al.,* 2003).

S. aureus produces small colony variants (SCV) which have different phenotypic and pathogenic characters. These variants cause persistent as well as recurrent infections several years later after the initial infection (Proctor *et al.,* 2006). The SCVs are defective in their electron transport pathways. These variants generally form non-hemolytic, non-pigmented small colonies on solid media (Kaneko and Kamio, 2004). Also, they are less virulent, but due to slow growth and reduced cell wall synthesis they can tolerate more β-lactam antibiotics than their wild type parents. The low membrane potential of SCVs makes them resistant to antibiotics of aminoglycoside group (Proctor *et al.,* 2006).

Virulence of *S. aureus* is multifactorial, and attributable to the combined action of virulence determinants such as cell-surface proteins, secreted toxins, and enzymes. The expression of virulence factors is generally regulated in a growth phase dependent manner governed by the accessory gene regulator (*agr*) system (Janzon and Arvidson, 1990). *S.*

aureus possesses a variety of virulence factors. There are two main mechanisms in the pathogenesis- invasion of the host body and production of various toxins.

The totality of the accumulated bacteria and the extracellular slime on a solid surface is referred to as biofilm. Biofilm consists of multilayered cell clusters embedded in a matrix of extracellular polysaccharide, which facilitates the adherence of microorganisms to biomedical surfaces and protect them from host immune system and antimicrobial therapy (O'Gara and Humphreys, 2001). The biofilm formation is of great clinical significance because bacteria in biofilm have increased resistance to environmental stress, host immunological defence and antimicrobial agents (Otto, 2008). Biofilm formation in *S. aureus* is regulated by expression of polysaccharide intracellular adhesin (PIA), which mediates cell to cell adhesion and is the gene product of *icaADBC* (Mathur *et al.,* 2006). These genes are also involved in the resistance of *S. aureus* to various environmental stresses (Gertz *et al.,* 2000; Rachid *et al.,* 2000 and Lim *et al.,* 2004).

A group of exoprotein such as nucleases, lipases, protease and hyluronidase are secreted by all *S. aureus* strains. These proteins convert the local host tissue into nutrients required for their growth. The cytolytic toxin forms pores in the plasma membrane which lead to lysis of the target cell due to leakage of cellular contents (Foster, 2005). This bacterium produces a variety of exoproteins that contribute to its ability to colonize mammary gland (Salasia *et al.,* 2004), with five of them being different membrane-damaging toxins, four hemolysins (alpha-, beta-, gamma- and delta-hemolysin) and leucocidin.

Beta (β) and alpha (α) hemolysins are the most important in pathogenesis of the intra-mammarian infections (Park *et al.,* 2004). α-hemolysin become inserted into eukaryotic membrane and oligomerizes into a β-barrel which forms a pore and cause osmotic cytolysis. This toxin is responsible particularly cytolysis of human platelets and monocytes (Menestrina

et al., 2001). β-hemolysin lysis of red blood cells is only observed after the cells are switched to low temperature, suggesting that the lytic activity of β-hemolysin is not as efficient as that of other hemolysins, atleast toward erythrocytes (Doery et al.,1963).

S. aureus produces *Staphylococcal* enterotoxin (SE) and is responsible for almost all *Staphylococcal* food poisoning (Montville and Matthews, 2008; FDA, 2012). There are several types of SE (A, B, C, D, G, H and I). Enterotoxin A is most commonly associated with *Staphylococcal* food poisoning. Enterotoxins D, E and H, and to a lesser extent B, G and I, have also been associated with *Staphylococcal* food poisoning (Seo and Bohach, 2007; Pinchuk *et al.*, 2010).

Many studies have characterized *S. aureus* and MRSA isolates from individual hospitals or countries and have identified strains that appear to be well adapted to the hospital environment, are established in several hospitals within a country, or have spread internationally (epidemic-MRSA or EMRSA) (Enright *et al.*, 2002). This has allowed a better understanding of the evolution of both *S. aureus* and MRSA over time and the ability to compare the genetic variation in different geographic locations. Such studies are important as the epidemiology and resistance patterns of *S. aureus* show large interregional inconsistency. The emergence of MRSA strains resistant to glycopeptides, as well as the increasing prevalence in the community highlights the need for worldwide epidemiological studies of this pathogen. Therefore the characterization of isolates may provide baseline information needed in establishing effective infection control measures. In fact, certain virulence factors like biofilm etc. can be associated with distinct human diseases strengthens the importance of examination of genes encoding pathogenicity factors. This study therefore seek to investigate

the genetic and phenotypic features of the virulence factors of *S. aureus* isolated from different clinical and environmental sources from South Assam region.

Thus, the aim of this research was to investigate the phenotypic and genotypic characteristics of virulence factors of *S. aureus* isolates from South Assam region in order to gain new insights into the evolution of *S. aureus* strains of both clinical and environmental origin.

The specific objectives of this research were:

i) To isolate of *S. aureus* from clinical and environmental samples and to study the biochemical and molecular characteristics.

ii) To determine the antibiotic susceptibility profile of *S. aureus* isolates.

iii) To investigate various virulence factors like biofilm formation, haemolysin and enterotoxin profile of *S. aureus* isolates.

iv) Co-relation between clinical and environmental isolates.

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