# INTRODUCTION

# Detailed Contents



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# 1. INTRODUCTION

***Staphylococcus aureus*** is a well known pathogen of man and animals (Sandel *et al.*, 2003; Cucarella *et al.*, 2004; Salasia *et al.*, 2004; Dar *et al.*, 2006; and Orrett, 2008). Despite the development of antimicrobial agents the staphylococcal infection still remains an important cause of morbidity and mortality (Mathur *et al.*, 2000; Coast *et al.*, 2003; Kim *et al.*, 2003; Sabour *et al.*, 2004; and Haveri *et al.*, 2008). The spectrum of disease produced by this organism varies from pyogenic infections to toxin-mediated phenomenon (Shanson, 1981). It also causes serious infections in man including deep-seated abscesses, bacteraemia, endocarditis, osteomyelitis, food poisoning etc. (Brakstad, 1992). The main reservoirs of infection are infected cases and carriers who spread infection in a hospital or in a community through droplets in air or through fomites. Carriage in nose plays an important role in its epidemiology and pathogenesis (Lowy, 2002).

In animals too it causes a wide spectrum of diseases like intramammary infections in lactating animals (Waage *et al.*, 1999; and Cucarella *et al.*, 2004) sporadic septicaemia in pigs, abscesses, synovitis, arthritis, osteomyelitis, dermatitis in poultry and ducks (Jones *et al.*, 1990). Bovine mastitis is one of the most important bacterial diseases in dairy cattle throughout the world, and it is responsible for great economical losses to milk producers as well as to the milk processing industries (Martin *et al.*, 2003 and Emery *et al.*, 2006) and also poses antimicrobial resistance threats (Haveri *et al.*, 2008; and Defra, 2008) in veterinary medicine. The ability of *Staphylococci* to produce coagulase, an enzyme that clots plasma was first reported by Loeb in 1903. However, there is no convincing evidence that coagulase is

directly involved in pathogenicity. It has been proposed that the coagulase may inhibit phagocytosis and protect the cocci from antibacterial substances in tissue fluids by laying down a fibrin barrier around them and walling off the lesions. Its production is the principal criterion used by the clinical microbiologists for the identification of *S. aureus* isolates from human infection.

A novel typing method for *Staphylococcus aureus* based on polymerase chain reaction amplification of the variable region of the coagulase gene followed by *AluI* restriction fragment length polymorphism (RFLP) was developed (Goh *et al.*, 1992).

*S. aureus* strains produce an extra cellular "Thermostable nuclease" (Tnase) a protein with a molecular mass of 17,000 KDa and is considered as a virulence marker. The *nuc* gene is widely employed as the target gene for specific detection of *S. aureus* (Wilson *et al.*, 1991; Kim *et al.*, 2001; Tamarapu *et al.*, 2001; and Ramesh *et al.*, 2002). Jasper (1973) found a close correlation between thermostable nuclease and coagulase production. PCR has the potential for the rapid diagnosis of *Staphylococcous aureus* infection.

Balban *et al.*, (2000) reported that food borne disease has a major impact on public health. In a study of food poisoning in England, the most prevalent contaminated foods were ham (75%), poultry or their products, other contaminated food products including fish and shellfish (7%) and milk products (8%). Contamination occurred most often at homes followed by restaurants and food stores (Wieneke *et al.*, 1993). The milk and dairy products are probably the types of foods most frequently implicated in food poisoning outbreaks (Zehren and Zehreen, 1968; Troller, 1976; DeBuysen *et al.*, 1985;

Genigeorgis, 1989; Leploute, 1994; Adesiyun *et al.*, 1998; and Asao *et al.*, 2003). A variety of exoproteins produced by *S. aureus* cause disease in the mammalian host (Salasia *et al.*, 2004). The most notable virulence factors associated with *S. aureus* are enterotoxins (Dinges *et al.*, 2000). Contamination of food with *S aureus* during storage could lead to the production of enterotoxin. This intoxication resulting from the ingestion of food containing preformed heat stable enterotoxins results in acute disease known as Staphylococcal food poisoning (Gilmour and Harvey, 1990 and Su and Wong, 1997).

In the past, a number of techniques have been developed for detection of Staphylococcal enterotoxins like immunodiffusion (Casman *et al.*, 1969), ELISA (Notermans, 1983) reverse passive latex agglutination (Shingaki *et al.*, 1981) and immunoblotting (Orden *et al.*, 1992). But disadvantages of these methods are that they are slow, relatively insensitive, and are more expensive. In addition, they may give false positive results due to interference by food components and protein A (Orden *et al.*, 1991). Johnson *et al.*, (1991) reported the PCR procedure, which rapidly and specifically detects genes for *staphylococcal* enterotoxins by using synthetic oligonucleotide primers. Polymerase chain reaction offers the possibility of specific amplification of the genes responsible for the SEs production (Saiki, 1988). The studies on detection of enterotoxins from foods of animal origin and clinical specimens are available worldwide but there is paucity of literature on this aspect in India.

The prognosis of patients with *S. aureus* infections was extremely poor before the advent of antibiotics. In early days of antimicrobial era, most infections caused by *S. aureus* were sensitive to

penicillin and other antibiotics. Subsequently strains acquired resistance to previously effective antimicrobials (Duckworth, 1993). They developed resistance to penicillin by virtue of beta-lactamase production, an enzyme, which destroys penicillin. Penicillin-resistant *S. aureus* strains persist in the environment, in carriers and in paramedical staff in hospitals and community (Klimek, 1976; Peacock *et al.,* 1980; Locksley *et al.,* 1982; www.net doctor co. Uk, 2009). The pathogen causes both nosocomial and community acquired infections. At a time when majority of nosocominal *S. aureus* isolates were already resistant to penicillin, the introduction in 1960 of newer penicillinase resistant semi-synthetic penicillin was seen as a major therapeutic breakthrough. These beta-lactamase resistant penicillins include methicillin, oxacillin, nafcillin, cloxacillin, flucloxacillin and others. Unfortunately, just one year later, the first methicillin resistant *S. aureus* (MRSA) was isolated in London (Jevons, 1961). Since then, there have been many reports of MRSA causing various infections throughout the world (Parker and Hewitt, 1970; Locksley *et al.,* 1982; Rao *et al.,* 1990; and Mathur *et al.,* 1994). This form of resistance encompasses all resistant beta-lactam antibiotics including cephalosporins (Sabath, 1982). Beta-lactamase in *S. aureus* can be either intrinsic or chromosomal and is due to the presence of a distinctive gene *mec* A, which encodes penicillin binding proteins (PBPs).

The emergence of antibiotic resistant organisms and their spread in the community has been a subject of increasing concern (WHO report, 1983). *S. aureus* is one of the commonest causes of hospital-acquired infections and the emergence of methicillin-resistant *Staphylococcus aureus* is one of the worst nosocomial hazards. MRSA

can cause infections like pneumonia, postoperative infections, bacteraemia and other infections in community and referral hospitals. MRSA are often resistant to the penicillinase resistant penicillins, tetracyclines, erythromycin, gentamycin, clindamycin, neomycin and trimethoprim. Clones of multidrug-resistant MRSA have become notorious hospital acquired organism causing serious, even fatal, infections in patients admitted for other diseases. In addition to antibiotic resistance, MRSA is often more readily colonized and transmitted. The outbreaks of nosocomial infections are difficult and expensive to control.

It is difficult to identify factors, which contribute to the persistence of MRSA carriage. Excessive antibiotic usage has been incriminated as one of the factors. The transmission and acquisition of MRSA is a multifactorial process depending not only on organism factors but also on host factors. The reservoirs of MRSA are wounds, intravenous catheters, tracheostomies and sites of dermatitis. Some hospital personnel may become chronic carriers.

The present study was therefore carried out to evaluate the phenotypic and genotypic characters of *S. aureus*, which might provide an understanding of the distributation of prevalent *Staphylococcus aureus* clones in India and might help in the development of steps to control *Staphylococcus aureus* infections in man and animals. Moreover, understanding genetic diversity of the organism may have far reaching implications for public health intervention strategies such as tracking the global spread, and understanding the emergence of drug resistance in *Staphylococcus aureus.*

# Aims and Objectives

Following were the aims and objectives of this study:

1. Phenotypic characterization of *Staphylococcus aureus* isolates obtained from clinical and animal-origin food samples.
2. To detect the presence of coagulase, thermostable nuclease and commonly encountered enterotoxin genes in the isolates.
3. Molecular characterization by Coa-PCR-RFLP in clinical and animal-origin isolates.
4. To study any similarity or diversity in isolates obtained from clinical and animal-origin samples.
5. To study the resistance rates and patterns of *Staphylococcus aureus* isolates.