INTRODUCTION

Staphylococcus aureus is an important human pathogen capable of causing diseases in the hospital and community settings. It can produce enterotoxin in humans and animals. S. aureus is also known to be the common cause of nosocomial community-acquired infections and surgical wound infections (Kennedy et al., 2008). Numerous states have reported an increased incidence of community-acquired infections caused by multidrug-resistant organisms where reportedly no exposure to antibiotics or health care in the 3 months before symptom onset occurred (Kenneley, 2010; Nicolau and Stein, 2010). CA-MRSA soft tissue infections may clinically present as cellulitis, folliculitis, furuncles, carbuncles, and abscesses (Cohen and Kurzrock, 2004). The infection often begins as a mild superficial infection of the skin, which may look harmless at onset but rapidly develop into large abscesses within 24 to 48 hr. The lesions are often mistaken for spider bites (File, 2007). Although skin and soft tissue infections are more common with CA-MRSA, osteomyelitis, otitis media, respiratory tract, and blood sepsis can occur (Fridkin et al., 2005). CA-MRSA infections have become epidemic in many parts of the world and are responsible for significant morbidity and mortality (Macario, 2010). Several risk factors, such as recent hospitalization or exposure to a health care setting, residence in long-term care facilities, invasive or surgical procedures, and injection drug use, predispose a patient to MRSA acquisition (Salim et al., 2005). Panton-Valentine leukocidin (PVL) is a synergohymenotropic toxin (SHT) (Horts et al., 2010). It is two-component cytolitic toxin epidemiologically linked to CA-MRSA infections, including serious invasive infections caused by the epidemic clone referred to as strain USA300 (Olsen et al., 2010). The traditional identification of bacteria on the basis of phenotypic characteristics is generally not as accurate as identification based on genotypic methods. Comparison of the bacterial 16S rRNA gene sequence has emerged as the preferred method for antibacterial identification and antimicrobial therapies. Methicillin Resistant Staphylococcus aureus (MRSA) isolated from pus samples of clinical patients. Isolated MRSA named as SMKV-1 and SMKV-2 grown properly using with Oxoid CM3 Nutrient agar medium. Genomic DNA from above strains was isolated and 16S rDNA were amplified using suitable primers. The amplified 16S rDNA sequences have been deposited in the GenBank (DQ 306890 and DQ 306891) and selected 16S rDNA sequences of the genus Staphylococcus from the database were used to study the phylogenetic relationship with the present MRSA isolates. mecA gene appeared on 310 bp region on agarose gel electrophoresis. In the present study we found that the MRSA isolates were 100% similar to GenBank strains.

MATERIALS AND METHODS

Two strains of Methicillin Resistant Staphylococcus aureus (MRSA) named as SMKV-1 and SMKV-2 were isolated from
the pus samples of the patients using OXOID CM3 Nutrient agar medium supplemented with 0.04% (wt/vol) KH₂PO₄ and 0.24% (wt/vol) Na₂HPO₄·12 H₂O (pH 6.8). The pellets from actively grown cultures (1.5mL) were obtained by centrifugation at 13,000 rpm for five minutes and washed with TE buffer (10 mM Tris-HCl; 1 mM EDTA, pH 8.0). The cell pellets were then dissolved in 200 µL of TE. Cell lysis was obtained at 37°C after treatment with lysozyme (2mg/mL; final concentration) for 30 min and using SDS (1%). The lysate was extracted twice with phenol/chloroform followed by two extractions with chloroform/isooamyl alcohol (24:1). After adding 1/10 volume of sodium acetate (3 M, pH 5.2) and 0.5 volume of isopropanol, the supernatant was incubated at -20°C for 30 min. The DNA was then sedimented by centrifugation at 13000 rpm for 20 min and the resulting pellet was washed with 70% ethanol and dried under vacuum. The DNA pellets were then dissolved in 100µL TE and used as template for PCR amplification of 16S rDNA sequence (Pitcher et al., 1989).

For the amplification of 16S rDNA, 100µL reaction mixture contained 200 µM dNTP (Perkin-Elmer Product, France), 0.1 µM concentration of forward and reverse primers (27f: 5’-GAGTTTGATCTGCGATCAG-3’ and 1525r: 5’-AGAAGGAGGTGCATTGCGAC-3’), 357f (5’-TACGGGAGGCAGCAG-3’), 907r (5’-CCGTCAATTTCATTTGAGTTT-3’), 1114f (5’-AGC(C/A)GCCGCGGTAAT(T/A)C-3’), 519r (5’-AGC(C/A)GCCGCGGAAT(T/A)T-3’), and 803f (5’-ATTAGATACCCGTGTACG-3’). The reaction mixture contained 1× PCR buffer (Perkin-Elmer Product, France), 1.5 mM MgCl₂, 2.5 U of Taq polymerase (Qiagen) and 10 µL of template DNA. An initial denaturing step of 95°C for 10 min was followed by 30 cycles of amplification (1 min at 94°C, 1 min at 55°C and 1 min at 72°C) and a final extension step at 72°C for 10 min. DNA was amplified with RT-PCR and amplified DNA was checked by ethidium bromide. The PCR product was purified using NucleoTrap PCR extraction kit (Macherey Nagel Product) and sequenced (ABI Prism 377 DNA sequencer, Applied Biosystems) using the primers, 27f (5’-GAGTTTGATCTGCGATCAG-3’) and 1525r: 5’-AGAAGGAGGTGCATTGCGAC-3’ (Shrestha et al., 2002), 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM MgCl₂, 2.5 U of Taq polymerase (Qiagen) and 10 µL of template DNA. An initial denaturing step of 95°C for 10 min was followed by 30 cycles of amplification (1 min at 94°C, 1 min at 55°C and 2 min at 72°C) and a final extension step at 72°C for 10 min. DNA was amplified with RT-PCR and amplified DNA was checked by ethidium bromide. The PCR product was purified by using NucleoTrap PCR extraction kit (Macherey Nagel Product) and sequenced (ABI Prism 377 DNA sequencer, Applied Biosystems) using the primers, 27f (5’-GAGTTTGATCTGCGATCAG-3’) and 1525r: 5’-AGAAGGAGGTGCATTGCGAC-3’ (Shrestha et al., 2002). For the phylogenetic analysis, related 16S rRNA gene sequences from the Genbank database were downloaded and aligned using ClustalW (Thompson et al., 1994). An evolutionary tree for the datasets was prepared from the neighbor-joining method of Saitou and Nei (1987). Performing bootstrap analysis of the evolutionary divergence, 1000 bp resembling was used to assess the stability of relationships.

RESULTS AND DISCUSSION

The first taxonomic description of the genus Staphylococcus is classified based on colony pigmentation, a key factor used to differentiate the opportunistic pathogen S. aureus, which causes high morbidity and mortality, and other staphylococci generally, considered being harmless commensals or saprophytic bacteria. Using a variety of morphological criteria and physiological and biochemical tests, it soon became obvious that more staphylococcal species are associated with humans and animal species than had been recognized so far (Kloos and Schleifer, 1975). In the present study, the 16S rDNA sequences of the MRSA isolates SMKV-1 and SMKV-2 were deposited in the GenBank (NCBI, USA) with the accession numbers DQ306890 and DQ306891 respectively. The results showed 100% sequence similarity with S. aureus strain MSA476 (BX571857) and Staphylococcus sub sp. aureus MRSA252 (BX571857) followed by 99.86% similarity with S. aureus (L36472), 99.79% similarity with S. aureus (X68417), 99.65% with S. aureus (X70648), 98.59 with Staphylococcus sp. H780 (AB177644) (Fig. 1). The 16S rDNA sequence results were comparable with the findings of Saitou and Nei (1987) study coincides with the present study. They reported that the S. aureus genomes were composed of a complex mixture of genes, many of which seem to have been acquired by lateral gene transfer. Gurtler and Barrie (1995) reported that the sequence conservation of the 16S rDNA region is higher than the 16S-23S spacer region as a stable and direct indicator of the evolutionary divergence of S. aureus strains. As per the report of Garrity and Holt (2001), the recent results of molecular phylogenetic classification, it was proposed to reclassify the genus Staphylococcus into a family “Staphylococcaceae”. Differentiation up to the species level may also have substantial consequences for the management of patients (Jones et al., 2002). While conventional differentiation schemes based on physiological and biochemical tests are relatively cumbersome and time-consuming and require various approaches. The commercial “rapid” identification systems share the problems of failure to identify commonly encountered bacteria, uselessness in identifying uncommon isolates, and lack of adequate strains in the accompanying databases (Renneberg et al., 1995; Spanu et al., 2003). Furthermore, commercial systems may provide ambiguous results, presenting two or more species for identification with a comparable safety level. The techniques based on amplification of 16S rRNA genes for comparing bacterial communities are now widely used in microbial ecology, but calibration of these techniques with traditional tools, such as cultivation, has been conspicuously absent (Dunbar et al., 1999). The presence of mecA gene allows a bacterium to be resistant to antibiotics such as Methicillin, Penicillin, and other penicillin-like antibiotics. The mecA gene does not allow the ring like structure of penicillin-like antibiotics to attack the enzymes in the cell wall of the bacterium, thus the penicillin cannot enter the bacterium and destroy it (Sendi et al., 2005). A study carried out by Banerjee et al. (2010) reported the genome resequencing of MRSA strain, CRB, revealed that it differs from its parent by five single-nucleotide polymorphisms in three genes. More specifically, the genes which are encoding PBP4, a low-molecular-weight penicillin-binding protein, GdpP, a predicted signaling protein, and AcrB, a cation multidrug efflux transporter. CRB displayed resistance to a variety of β-lactams but was hyper susceptible to cefoxitin. In this present study, two MRSA isolates SMKV-1 and SMKV-2 are positive for mecA.
A gene (Fig. 2) which was confirmed by PCR analysis. Anvari and Safarimotlagh (2010) recently reported the rapid detection of methicillin-resistant staphylococci by multiplex PCR. The development of molecular typing methods has enabled the tracking of different strains of *S. aureus*. This may lead to better control of outbreak strains. A greater understanding of how the staphylococci evolve, especially due to the acquisition of mobile genetic elements encoding resistance and virulence genes helps to identify new outbreak strains and may even prevent their emergence (Lindsay et al., 2006).

**ACKNOWLEDGMENTS**

The authors are acknowledged to their family members for their constant encouragement and support. They are thankful to the research scholars of Microbiology department, Bharathidasan University for their technical support.

**REFERENCES**


