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Short communication

Genotyping of community-associated methicillin resistant *Staphylococcus aureus* (CA-MRSA) in a tertiary care centre in Mysore, South India: ST2371-SCC*mec* IV emerges as the major clone

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ABSTRACT

The burden of community-associated methicillin resistant *Staphylococcus aureus* (CA-MRSA) is on the rise in population and clinical settings on account of the adaptability and virulence traits of this pathogen. We characterized 45 non-duplicate CA-MRSA strains implicated mainly in skin and soft tissue infections (SSTIs) in a tertiary care hospital in Mysore, South India. All the isolates were genotyped by staphylococcal cassette chromosome *mec* (SCC*mec*) typing, staphylococcal protein A (*spa*) typing, accessory gene regulator (*agr*) typing, and multi-locus sequence typing (MLST). Four sequence types (STs) belonging to three major clonal complexes (CCs) were identified among the isolates: CC22 (ST2371 and ST22), CC1 (ST772) and CC8 (ST8). The majority (53.3%) of the isolates was of the genotype ST2371-t852-SCC*mec* IV [sequence type-*spa* type-SCC*mec* type], followed by ST22-t852-SCC*mec* IV (22.2%), ST772-t657-SCC*mec* V (13.3%) and ST8-t008-SCC*mec* IV (11.1%). ST237I, a single locus variant of ST22 (EMRSA-15 clone), has not been reported previously from any of the Asian countries. Our study also documents for the first time, the appearance of ST8-SCC*mec* IV (USA300) strains in India. Representative strains of the STs were further analyzed by pulsed field gel electrophoresis (PFGE). *agr* typing detected type I or II alleles in the majority of the isolates. All the isolates were positive for the leukotoxin gene, *pvl* (Panton–Valentine leukocidin) and the staphylococcal enterotoxin gene cluster, *egc*. Interestingly, multidrug resistance (resistance to $\geqslant$3 classes of non-beta-lactam antibiotics) was observed in 77.8% ($n = 35$) of the isolates. The highest (75.5%) resistance was recorded for ciprofloxacin, followed by erythromycin (53.3%), and quinupristin–dalfopristin (51.1%). Inducible clindamycin-resistance was identified in 37.7% of the isolates and it was attributed to the presence of *erm*(A), *erm*(C) and a combination of *erm*(A) and *erm*(C) genes. Isolates which showed a phenotypic pattern of M^R/L^S (macrolide-resistance/lincosamide-sensitivity) harbored the *msr*(A) gene. In conclusion, we report a high rate of multidrug resistance among Indian strains of CA-MRSA and the emergence of the lineages ST2371 and ST8 in India.

1. Introduction

Staphylococcus aureus is an extremely adaptable human pathogen equipped with many virulence determinants. The epidemiological success of this pathogen is partly attributed to its remarkable ability to acquire resistance to newer antibiotics (Chambers and DeLeo, 2009). In particular, methicillin resistant *S. aureus* (MRSA) emerged as a global pathogen in both hospital and community settings. Methicillin-resistance dates back to 1960s and it is a classical terminology that implies resistance to all beta-lactam antibiotics, except the recently introduced anti-MRSA cephalosporins such as ceftobiprole (El Solh, 2009). Resistance to methicillin is conferred by the *mec*A gene carried on a mobile genetic element known as staphylococcal cassette chromosome *mec* (SCC*mec*) (Deurenberg and Stobberingh, 2008). *mec*A codes for an altered penicillin binding protein, PBP2a with reduced affinity to beta-lactams.

MRSA causes a range of illnesses, from wound infections to life threatening bacteremia, ventilator-associated pneumonia, sepsis, etc. Initially, MRSA was confined to hospitals infecting mainly patients with health care associated risk factors such as weakened immune system, surgery, presence of indwelling medical devices, etc. Many of these hospital-associated MRSA (HA-MRSA) clones continue to be endemic in hospitals across the globe. The

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epidemiology of MRSA infections has changed considerably since the 1990s with the emergence of a new lineage of MRSA namely community-associated MRSA (CA-MRSA) which has a competitive advantage over HA-MRSA to persist in the community. CA-MRSA is evolved by the acquisition of the *mec*A gene by methicillin-susceptible *S. aureus* (MSSA) prevailed and circulated in the community (Uhlemann et al., 2014).

In contrast to HA-MRSA, strains of CA-MRSA frequently infect healthy and young individuals who do not have any predisposing risk factors for MRSA infection (Otter and French, 2010). Clinical presentations of CA-MRSA are usually skin and soft tissue infections, but also fatal diseases such as necrotizing pneumonia have been associated with this pathogen. CA-MRSA harbors shorter SCC*mec* elements of types IV or V which confer less fitness cost compared to other SCC*mec* types (Otto, 2013). CA-MRSA strains usually remain susceptible to non-beta-lactam antibiotics and often carry the *pvl* gene that encodes Panton–Valentine leukocidin (PVL), a phage borne leukotoxin (Okuma et al., 2002; Vandenesch et al., 2003). Though PVL has been proven to cause excessive inflammatory response and tissue damage (Konig et al., 1995), its role as a virulence factor in CA-MRSA infections remains controversial. Apart from PVL, the majority of CA-MRSA produces genome encoded toxins such as phenol soluble modulins (PSM) and alpha toxin which possess proinflammatory and cytolytic activities (Li et al., 2010).

The distinction between CA-MRSA and HA-MRSA is becoming blurred in recent years, with CA-MRSA infiltrating hospitals to become endemic strains by replacing the traditional nosocomial clones over time. Strains of CA-MRSA belong to diverse clonal backgrounds such as ST1-IV (USA400), ST8-IV (USA 300), ST80-IV (European clone), ST59-V (Taiwan clone), ST30-IV (Southwest Pacific clone), etc. (Tristan et al., 2006). USA400, which caused earliest reported cases of CA-MRSA infections in the United States, was gradually replaced by USA300 which now accounts for nosocomial infections too. USA300 is a well adapted community lineage and the major CA-MRSA clone implicated in healthcare associated infections in the United States (Seybold et al., 2006; Popovich et al., 2008). USA300 clone is characterized by the presence of ACME (Arginine Catabolic Mobile Element), a genomic island that has been shown to enhance the fitness of this clone. The ACME gene, *speG* which encodes a spermidine acetyl transferase allows USA300 strains to evade the toxicity of polyamines that are secreted on human skin and helps in colonization and infection (Joshi et al., 2011).

The problem of MRSA has been well recognized in Indian hospitals and community. A nation-wide study by the Indian Network for Surveillance of Antimicrobial Resistance (INSAR) in 2008 and 2009 reported an overall MRSA prevalence of 41% in Indian hospitals (Joshi et al., 2013). In India, ST239-III has been largely responsible for hospital-acquired MRSA infections (Nadig et al., 2006). In recent years, Indian hospitals witnessed a progressive decline of this traditional hospital clone and the emergence of community lineages such as ST22-IV (EMRSA-15) and ST772-V (D'Souza et al., 2010). These are epidemic clones implicated in both hospital- and community-associated infections. A recent study from Bangalore, South India reported other emerging clones such as ST1208 and ST672 (Shambat et al., 2012).

Here we analyzed forty-five MRSA strains collected from outpatients in a tertiary care hospital in Mysore, South India by SCC*mec* typing, *spa* typing, *agr* typing, MLST and PFGE.

2. Material and methods

2.1. Study design

The study was conducted from October 2012 to January 2013 at JSS Hospital, a 1500-bed teaching hospital and research centre located in the city of Mysore, Karnataka State, India. Strains of MRSA obtained from outpatients as a part of routine clinical care were chosen for molecular analysis. A case of MRSA infection was considered as community-associated if MRSA was isolated from an individual with no history of hospitalization or invasive medical procedures in the previous year. Patients were excluded, if they had any of the risk factors for MRSA infection as suggested by CDC (Kallen et al., 2010).

2.2. Identification and susceptibility testing of isolates

The isolates were confirmed as *S. aureus* by Gram staining, catalase test, tube coagulase test and characteristic growth patterns on mannitol salt agar and DNase plates. Methicillin-resistance was confirmed using a cefoxitin disc (30 μg) on Mueller Hinton plates supplemented with 4% NaCl. Susceptibility testing was performed on all the isolates for a panel of 10 antibiotics by the Kirby–Bauer disc diffusion method. $iMLS_B$ (inducible Macrolide–Lincosamide–Streptogramin B) resistance was analyzed by D-zone test. The results were interpreted according to the guidelines by CLSI. Isolates which had shown resistance to at least one agent in three or more classes of non-beta-lactam antibiotics were classified as multidrug resistant (MDR) strains.

2.3. Extraction of genomic DNA

DNA was extracted using the commercial kit, Nucleospin Tissue (Machery-Nagel, Germany) with the addition of lysostaphin (Sigma–Aldrich, USA) to a final concentration of 15 μg/ml.

2.4. SCCmec typing and agr typing

Multiplex PCR was employed for SCC*mec* typing (Milheirico and Oliveira, 2007). Amplification was performed using Accuprime Taq DNA polymerase system (Invitrogen, USA) with the following PCR conditions: denaturation at 95 °C for 15 min followed by 35 cycles of 95 °C for 25 s, 53 °C for 30 s, and 72 °C for 1 min; and a final extension at 72 °C for 7 min. The prototype strains used as controls for typing were *S. aureus* COL (type I), *S. aureus* BK2464 (type II), *S. aureus* ANS46 (type III), *S. aureus* MW2 (type IV), *S. aureus* WIS (type V) and *S. aureus* HDE288 (type VI).

agr typing was performed by multiplex PCR as described by Gilot et al., 2002. The primer set comprised a common forward primer (*Pan*) and reverse primers (*agr1*, *agr2*, *agr3*, and *agr4*) specific for each *agr* group.

2.5. Detection of toxin genes

The *pvl* genes were amplified using the primers (*luk-PV-1* and *luk-PV-2*) and PCR conditions described by Lina et al., 1999. *S. aureus* MW2 was used as the positive control. PCR screening of staphylococcal enterotoxin A (*sea*), toxic shock syndrome toxin (*tsst*) and enterotoxin gene cluster (*egc*) was performed as described previously (Jarraud et al., 1999, 2002).

2.6. Detection of ACME and speG

Detection of ACME, which is characteristic of USA300 was accomplished by screening the isolates for the presence of two ACME genes *arcA* and *opp3AB* using the primer pairs described by Diep et al., 2008a. The ACME-associated *speG* gene was amplified according to Lin et al., 2014.

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