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Short Communication

Hospital-associated methicillin-resistant *Staphylococcus aureus* carrying the PVL gene outbreak in a Public Hospital in Rio de Janeiro, Brazil

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Abstract

Hospital associated methicillin-resist *Staphylococcus aureus* has long been associated to outbreaks in the hospital environment. In this work, we investigated an outbreak of Hospital associated methicillin-resist *Staphylococcus aureus* carrying the Panton-Valentine leukocidin gene, which occurred in a large community hospital in Rio de Janeiro, Brazil.

Key words: methicillin-resistant *Staphylococcus aureus,* nasal colonization, genotypes, PVL.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is associated with hospital infections worldwide (Chambers and Deleo, 2009). The resistance is encoded by the *mecA* gene, located in a staphylococcal cassette chromosome (SCC*mec*) (Deresinski, 2005; Katayama *et al.*, 2000). So far, eleven types of SCC*mec* (I-XI) have been described: types I, II, III and VIII are typically associated to hospital infections; while types IV, V, VI and VII to community infections (Chambers and Deleo, 2009; International Working Group on the Staphylococcal Cassette Chromosome elements, 2011; Li *et al.*, 2011; Milheirico *et al.*, 2007). These latter four types, usually present in Community associated MRSA (CA-MRSA), are the most frequently found isolates from patients lacking exposure to a hospital environment for more than one year (Chen *et al.*, 2009; Deurenberg and Stobberingh, 2008).

Nasal colonization of *S. aureus* increases risk for infection in both healthcare and community settings (Elston and Barlow, 2009). Within this latter environment, colonization by *S. aureus* SCC*mec* type IV has increased (Reinert *et al.*, 2008; Schuenck *et al.*, 2009). Despite numerous preventive measures, a clear correlation still exists between the carriage of *S. aureus* by health care workers and the development of *S. aureus* infections in surgical wounds of patients (Webb *et al.*, 2009).

Methicillin-resistant *Staphylococcus aureus* has been the most prevalent pathogen of surgical infections in Brazil. Phenotypic and molecular approaches have elucidated the major features of the MRSA Brazilian endemic clone (BEC) - antimicrobial multiresistant strains bearing a SCC*mec* type IIIA cassette and usually Panton-Valentine leukocydin negative.

The genes of Panton-Valentine leukocidin (PVL), *luk*S-PV and *luk*F-PV, are usually associated to *Staphylococcus aureus* infections (Genestier, *et al.*, 2005; Lo and Wang, 2011). They are inserted into its chromosome by the phage ϕPVL (Deresinski, 2005). The PVL gene is also associated to community skin infections and necrotizing pneumonia (Deurenberg and Stobberingh, 2008; Genestier, *et al.*, 2005; Lina *et al.*, 1999; Obed *et al.*, 2006). In this report, we describe an outbreak of PVL positive HA-MRSA at a General Hospital in Rio de Janeiro. Furthermore, we define the phenotypic and molecular characteristics of the isolates collected.

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Bacterial isolates from eighty subjects from a General Hospital regularly submitted to the surveillance program of the Hospital Infection Control Committee (HICC) were used in this study (April 2007).

Samples were collected during the first two days of patients' admission using a sterile swab rotated in the anterior vestibule of both nares and cultured by directly inoculating onto a blood agar plate (Plast Labor, Rio de Janeiro, RJ, Brazil). Cultures were further streaked for isolation of single, clonal colonies to grow in liquid cultures to perform species-specific phenotypic analyzes (Shrestha *et al.*, 2009).

Isolates were prepared to antimicrobial susceptibility testing according to CLSI guideline (Clinical and Laboratory Standards Institute, 2011) and applied to a Vitek 2 system using a GPS-651 card (BioMèrieux, Brazil) for processing in a Vitek 120 reader-incubator. The standard stains *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* 25923 were used as susceptibility testing controls.

Presence of the *mecA* gene was confirmed in all methicillin resistant isolates by PCR, as described elsewhere (Oliveira and de Lencastre, 2002).

Methicillin-resistant *S. aureus* were typed by pulsed-field gel electrophoresis (PFGE) as previously described by McDougal *et al.* (2003). SCC*mec* type was determined by multiplex PCR procedure according to Oliveira and de Lencastre (2002). Presence of Panton-Valentine leukocidin (PVL) genes was assessed by PCR in all *S. aureus* isolates, as reported by Lina *et al.* (1999).

Out of 80 patients, 16 (16/80 - 20%) were nasal culture-positive for *S. aureus*, while 13 (13/80 - 16%) were nasal culture-positive for MRSA. MRSA were considered Hospital-Associated because they matched the expected hospital SCC*mec* profile (SCC*mec* type III). All *S. aureus* samples were positive for detection of the PVL gene. Experiments (PVL typing) were carried out in triplicates.

A PFGE was performed on the 16 positive *S. aureus* samples. According to the PFGE profile, the samples were classified in 6 groups (Figure 1). Nine were classified as group A, two were classified as group B, one was classified as group D, one was classified as group E, one was classified as group F, one was classified as group G and one was not classified in any group, being confirmed as MSSA.

Out of 16 isolates, 13 were positive for the *mec*A gene, all 16 were positive for the PVL gene and all MRSA samples presented the SCC*mec* type III (data not shown).

This study reports a prevalence of 81.25% of MRSA among all *S. aureus* collected within a two month time period at a General Hospital in Rio de Janeiro, Brazil. The genotyping and phenotyping of these isolates suggest that all can be classified as Hospital-Associated. The PVL gene was detected in all sixteen *S. aureus* isolates. This is consistent with the observation of isolates collected from another hospital in Brazil (Schuenck *et al.*, 2009; Souza *et al.*, 2009).

Although the PVL gene is usually associated with community-acquired samples, we detected the PVL gene in SCCmec type III (HA-MRSA) samples. There are few studies for surveillance of PVL in this type of Staphyloccocal Cassette Chromossome. However, Mimica *et al.* (2011) published a study where they have found four SCCmec type IV and four SCCmec type III isolates among hospital inpatients with cystic fibrosis and none of them carried the PVL gene.

The presence of this gene in isolates obtained in a hospital setting is a major concern. MRSA isolates that carry the PVL gene are more pathogenic and present a higher morbidity (Diep *et al.*, 2004; Genestier *et al.*, 2005;

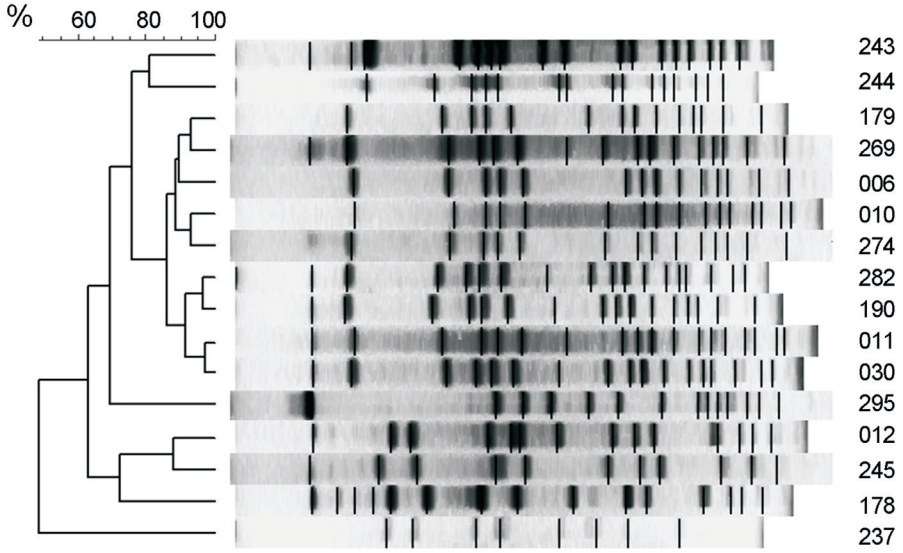

Figure 1 - Dendrogram of PFGE containing all the *S. aureus* samples analyzed. The percentage at the upper left designates the relatedness between samples' genome. The numbers in the right identify the sample's PFGE.

Mimica *et al.*, 2011; Obed *et al.*, 2006; Souza *et al.*, 2009). This report suggests that a molecular surveillance for PVL positive SCCmec type III samples should be implemented.

The results herein obtained show that although many preventive measures are being taken, the hospital environment is still one major risk factor for *S. aureus* colonization and infections (Lee *et al.*, 2011). Also, there is the possibility of hospital samples acquiring extra virulence factors, which are usually present in community samples, such as the PVL gene.

This study corroborates the importance of an active surveillance in hospitals, once *S. aureus* has been acquiring resistance to several kinds of antimicrobials, and the incorrect treatment scheme with these drugs seems to be directly related to their acquirement of resistance (Dancer, 2008).

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