

SHORT COMMUNICATION

***Staphylococcus aureus* isolates belonging to different multilocus sequence types present specific virulence gene profiles**

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Received 7 February 2012; revised 15 March 2012; accepted 15 March 2012.
Final version published online 16 April 2012.

DOI: 10.1111/j.1574-695X.2012.00958.x

Editor: Peter Timms

Keywords

Staphylococcus aureus; SCCmec; PFGE; MLST; virulence genes.

Abstract

To characterize 73 *Staphylococcus aureus* isolates from infections in an orthopedic hospital in Rio de Janeiro, we investigated the SCCmec types, the clonality by pulsed field gel electrophoresis and multilocus sequence typing, and the presence of virulence genes. Twenty-eight (38.3%) methicillin-resistant (16 SCCmec type IV and 12 type III) isolates were detected. Most (83.5%) of the isolates were included in five lineages: sequence type (ST) 239 (SCCmecIII), 1, 5, 30, and 1462 (SCCmecIV and/or methicillin-susceptible isolates). Virulence genes *fnbB*, *bbp*, and *pvl* were related to STs 239, 30, and 30/1462, respectively. Isolates from STs 1, 5, and 30 presented specific virulence profiles, irrespective of methicillin resistance.

Although *Staphylococcus aureus* remains as the most important hospital pathogen isolated in many countries (Deurenberg & Stobberingh, 2008), a change in its epidemiology has been observed with the emergence of new clones in substitution to other well established ones (Schuenck *et al.*, 2009). In a teaching hospital located in Rio de Janeiro, where methicillin-resistant *S. aureus* (MRSA) strains belonging to the Brazilian epidemic clone (BEC)/sequence type (ST) 239/SCCmecIII are frequently isolated, the emergence of type IV isolates from different lineages was also observed (Schuenck *et al.*, 2009). Some authors have shown that type IV MRSA as well as methicillin susceptible *S. aureus* (MSSA) isolates from different lineages can carry specific virulence and/or resistance genes (Diep *et al.*, 2006; Deurenberg & Stobberingh, 2009; McCarthy & Lindsay, 2010). To characterize *S. aureus* isolates from infections in an orthopedic hospital, we investigated the presence of *pvl*, *icaA*, and adhesins encoding

genes and biofilm production in different lineages determined by pulsed field gel electrophoresis (PFGE), multilocus sequence typing (MLST), and SCCmec typing.

The *S. aureus* isolates were obtained from the Instituto Nacional de Traumatologia e Ortopedia (INTO) located in Rio de Janeiro, Brazil, between August 2005 and September 2007. The isolates were collected consecutively from all patients that presented *S. aureus* infections in this period. The INTO has 144 beds, including 15 for intensive care. Among 73 *S. aureus* isolates, 43.8% were from prostheses infections, 28.8% from surgical sites, 13.7% from bacteremia, 9.6% from osteomyelitis and 4.1% from other sites. The isolates were characterized as *S. aureus* by Microscan – AutoScanTM4 (Siemens, München, Germany) and by detection of coagulase enzyme production. PCR was performed to detect *mecA* and SCCmec types (Milheiro *et al.*, 2007). As our isolates were recovered from an orthopedic hospital, we looked for some

virulence genes that could have been involved in hematogenous osteomyelitis or implant infections by *S. aureus* (Tristan et al., 2003; Montanaro et al., 2011). These genes were *bbp* (bone sialo-binding protein), *cna* (collagen-binding protein), *ebpS* (elastin-binding protein), *fnbB* (fibronectin-binding protein B) (Tristan et al., 2003), *fnbA* (fibronectin-binding protein A) (Peacock et al., 2002), and *icaA*, which codified an enzyme involved in biofilm formation (Arciola et al., 2001). The *lukS*-PV and *lukF*-PV genes, which encode the Panton-Valentine leukocidin (PVL), were also investigated (Lina et al., 1999). For biofilm production, the isolates were classified as previously described (Stepanović et al., 2007). All isolates were typed by PFGE (Vivoni et al., 2006). The MLST method was used for pulsotypes detected among predominant PFGE types (Enright et al., 2000).

The 73 *S. aureus* isolates were distributed among 15 PFGE lineages (A to P) (Table 1), but 61 (83.6%) of them were included in only five lineages (A, B, C, D, and E) (Table 2). However, only the lineages B (ST5/USA800, 20 isolates), C (ST1/USA400, 17), and E [ST30/Ocean Southwest Pacific Clone (OSPC), 4] comprised MSSA and type IV MRSA isolates. These isolates showed the same virulence gene profiles in each PFGE type. There was no relationship between lineages and/or virulence profiles and types of infection, because these characteristics were equally distributed among infections related and not related to prostheses ($P > 0.05$).

According to Diep & Otto (2008), the emergence of the MRSA lineages around the world may be related to the successful conversion of certain strains of MSSA by the acquisition of *SCCmec*. In our study, *S. aureus* isolates from the STs 1, 5, and 30 had similar clonality and virulence characteristics (Table 2), irrespective of their methicillin susceptibility. Furthermore, these lineages have recently emerged in Brazilian hospitals (Vivoni et al., 2006; Schuenck et al., 2009). These facts can indicate that these lineages present more ability to acquire this cassette. However, further studies involving more *S. aureus* isolates from other regions are necessary.

In our study, there were some virulence genes that were more related to some MRSA lineages prevalent in Brazilian hospitals, such as the *bbp* gene that was only detected in OSPC/ST30 isolates (9.6% of 73 MRSA isolates) (Table 1). Reva et al. (2009) analyzed *S. aureus* isolates from several STs obtained from different countries and found the *bbp* gene only in four lineages, including the ST30. The *pvl* genes are also commonly found in a few lineages, such as STs 8, 30, 22, and 59 isolates (Diep et al., 2006; Reva et al., 2009). In the present study, we verified that only isolates from ST30 and a few isolates from ST1462 (9.6% of total) carried this gene. Furthermore, ST30 isolates also carried the virulence genes *icaA*, *cna*, *fnbA*, and *ebpS*. It is possible that a large number of virulence genes carried by *S. aureus* ST30 isolates, including the *pvl* genes that are related to invasive infections (Ellington et al., 2010), are responsible for the high severity of infections caused by this type of lineage. However, further investigations are necessary to confirm these findings.

We found the *cna* gene in the majority of lineages (67.1%), except among USA800/ST5 isolates (Table 1). Some authors have found this gene in some lineages of *S. aureus*, such as STs 1 and 30, but they did not find it in the ST5 lineage (Deurenberg & Stobberingh, 2009; Reva et al., 2009; McCarthy & Lindsay, 2010), confirming our findings. Thus, to distinguish these three lineages, that are more frequently found in Brazilian hospitals, we could consider the presence of the *cna* gene only among STs 1 and 30 isolates.

The majority of the isolates presented the *fnbA* (87.7%) and *ebpS* (72.6%) genes (Table 1). However, among the MRSA isolates, only the BEC/ST239/*SCCmec*III did not present the *ebpS* gene. On the other hand, the *fnbB* gene was found only in this lineage (16.4%). Booth et al. (2001) found this gene in 20% of isolates that belonged to the same lineage, while Nienaber et al. (2011) analyzing *S. aureus* isolates from different countries detected the *fnbB* gene in < 14% of them. Amaral et al. (2005) showed that this Brazilian lineage presented more

Table 1. General characteristics of 73 *Staphylococcus aureus* isolates from an orthopedic hospital located in Rio de Janeiro, Brazil, between August 2005 and September 2007

<i>S. aureus</i> (no. of isolates)	PFGE type (no. of isolates)	% of virulence genes						
		<i>cna</i>	<i>fnbA</i>	<i>fnbB</i>	<i>bbp</i>	<i>ebpS</i>	<i>icaA</i>	<i>pvl</i>
MRSA								
SCCmec III (12)	A (12)	100	100	100	0	0	100	0
SCCmec IV (16)	B (6); C (9); E (1)	62.5	100	0	6.2	100	100	6.2
MSSA (45)	B (14); C (8); D (8); E (3); F (2); G (2); H (1); I (1); J (1); L (1); M (1); N (1); O (1); P (1)	60	80	0	13.3	82.2	100	13.3
Total (73)		67.1	87.7	16.4	9.6	72.6	100	9.6

Table 2. Characteristics of 61 *Staphylococcus aureus* (MRSA and MSSA) belonging to five predominant lineages

ST	PFGE type/ clonality	Susceptibility to methicillin (no. of isolates)	SCCmec type	Biofilm production (no. of isolates)				Virulence genes profile							Total of isolates
				+++	++	+	–	<i>cna</i>	<i>fnbA</i>	<i>fnbB</i>	<i>bbp</i>	<i>ebpS</i>	<i>icaA</i>	<i>pvl</i>	
1	C/USA400	MRSA (9)	IV	0	7	2	0	+	+	–	–	+	+	–	9
		MSSA (8)	na	2	4	2	0	+	+	–	–	+	+	–	7
								–	+	–	–	+	+	–	1
5	B/USA800	MRSA (6)	IV	0	5	1	0	–	+	–	–	+	+	–	6
		MSSA (14)	na	1	11	1	1	–	+	–	–	+	+	–	13
								+	+	–	–	+	+	–	1
30	E/OSPC	MRSA (1)	IV	0	1	0	0	+	+	–	+	+	+	+	1
		MSSA (3)	na	0	3	0	0	+	+	–	+	+	+	+	2
								+	+	–	+	+	+	–	1
239	A/BEC	MRSA (12)	III	5*	5	1	1	+	+	+	–	–	+	–	12
1462	D/nd	MSSA (8)	na	0	8	0	0	+	–	–	–	–	+	+	3
								+	–	–	–	–	+	–	1
								+	–	–	–	+	+	–	1
								+	+	–	–	+	+	–	1
								+	+	–	–	–	+	+	1
								+	+	–	–	–	+	–	1

+++; strong biofilm producer; ++, moderate biofilm producer; +, weak biofilm producer; –, nonbiofilm producer; na, not applicable; nd, not determined.

*BEC was associated with strong biofilm formation by relative risk (RR > 4) calculated with 95% confidence interval and showed moderate positive association by Phi coefficient (0.41).

ability to bind, persist, and invade epithelial cells *in vitro* than other types and related this ability to the biofilm formation. In addition, Sinha *et al.* (1999) showed that *S. aureus* fibronectin-binding proteins can act as invasins. In our study, the BEC isolates were associated with strong biofilm formation (RR > 4; Phi coefficient 0.41). Moreover, BEC/ST239 was the only lineage that carried both *fnbA* and *fnbB* genes, and to our knowledge, this is the first report of this finding. These features may contribute to the spread and maintenance of this lineage in Brazilian hospitals.

In summary, we showed that *S. aureus* belonging to STs 1, 5, and 30, irrespective of their methicillin susceptibility, presented specific virulence gene profiles. This indicates the ability of certain lineages to acquire the *mec* cassette providing a selective advantage that would allow their global dissemination. Moreover, the virulence genes *fnbB*, *bbp*, and *pvl* were related to the STs 239, 30, and 30/1462, respectively, and can be considered possible markers of these lineages.

Acknowledgements

This study was supported by Brazilian grants from Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coor-

denação de Aperfeiçoamento Pessoal de Nível Superior (CAPES), Fundação Universitária José Bonifácio (FUJB), and Programa de Núcleos de Excelência (PRONEX). We have no disclosures or conflicts of interest to report.

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