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Communication

Lack of correlation between X region *spa* polymorphism and virulence of methicillin resistant and methicillin sensitive *Staphylococcus aureus* strains

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Staphylococcus aureus **is an etiological factor of severe infections in both hospital and ambulatory environments. As methicillin resistant** ***Staphylococcus aureus*** **strains spread quickly across healthcare centers resulting in life-threatening infections with increased mortality, they are considered more virulent than MSSA strains. Protein A, encoded by the** ***spa*** **gene, is one of the virulence factors involved in the staphylococcal pathogenesis. It has been suggested that the number of 24-bp tandem repeat units along the X region of the *spa* gene correlates with the virulence level of the strains. The current work analyzed the relationships between the virulence of MRSA and MSSA strains with region X polymorphism. No obvious correlation was observed.**

Keywords: polymorphism, protein A, *spa*, virulence

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INTRODUCTION

Staphylococcal infections, especially those caused by methicillin-resistant *Staphylococcus aureus* strains (MRSA), are a long-lasting problem of contemporary medicine concerning hospital as well as ambulatory environment (Gosbell, 2005; Deurenberg *et al.*, 2007). Particularly in the case of HA-MRSA strains (hospital acquired-MRSA) a wide distribution of the strains and therapeutically difficult infections connected with increased mortality are observed (Melzer *et al.*, 2003). Moreover, the MRSA strains have a higher ability to cause epidemic and outbreak infections than methicillin-sensitive *S. aureus* strains (MSSA) (Ladhani & Garbash, 2005; Kurlenda *et al.*, 2009).

Protein A is a peptidoglycan-bound surface protein of the *S. aureus* cell wall (Lee *et al.*, 2004; Voyich *et al.*, 2005). It belongs to a group of virulence factors produced during the first stages of infection. Characterized by an ability to bound the Fc fragment of IgG, protein A prevents bacterial opsonization and phagocytosis. It is encoded by a 2-kb *spa* gene. The C-terminal end of the protein includes a sequence required for cell wall binding. The Fc-binding region is localized at the N-terminal segment. The corresponding part of the *spa* gene comprises five 160-bp repeats. The *spa* gene includes a polymorphic sequence, named X region, of a variable number (from 3 to 15) of tandemly repeated 24-bp units (Frenay *et al.*, 1996). Using of PCR-based methods, the number of the repetitive units has been determined among MRSA strains characterized with different virulence level. Strains assigned as more virulent were found to have more than seven repeat units within the X region. Such a correlation is thought to result from the thesis stating that the longer the X region is the more precise and stronger is the binding of the encoded protein A to the Fc fragment of IgG. The stronger binding would in turn lead to more effective defense against the host immunological system (Frenay *et al.*, 1996).

Region X polymorphism is widely used as a basis for genotyping methods, the discriminatory power of which allows the recognition of small differences among genetically related strains and enables effective epidemiological investigation (Frenay *et al.*, 1994).

The current work is an attempt at evaluation if any differences in the number of repeat units within the X region could be observed among MRSA and MSSA strains characterized with different virulence levels.

MATERIALS AND METHODS

Bacterial isolates. One hundred and seventy-six clinical *S. aureus* strains (94 MRSA and 82 MSSA) isolated through seven years were used. The isolates were characterized by Gram-staining and ability to produce coagulase and clumping factor using Slidex Staph Plus (BioMerieux, France). Additionally, the species was identified using the biochemical identification system ID 32 Staph (BioMerieux, France) and PCR-based detection of *S. aureus*-specific *nuc* gene (Tang *et al.*, 2008). Resistance to methicillin and other antibiotics was determined using a disk-diffusion method (CLSI standards), latex test detecting PBP2a protein (Staphytect Plus, OXOID, USA) and PCR-based *mecA* gene detection (Bignardi *et al.*, 1996). The strains were isolated from patients hospitalized in different hospital Wards as follows**:** Orthopedic Ward, Surgery Ward, Intensive Care Unit (ICU), Dermatological Ward, Pediatric and Neonatal Ward, Internal Medicine Ward, and Laryngological Ward (Table 1). Of those strains 31% were isolated from surveillance culture and 69% from patients with local infections, bacteremia

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Abbreviations: HA-MRSA, hospital associated methicillin resistant *Staphylococcus aureus*; MRSA, methicillin resistant *Staphylococcus aureus*; MSSA, methicillin sensitive *Staphylococcus aureus*.

Table 1. The origin of analyzed strains

Strain's origin	Number (%) of strains	
	MSSA	MRSA
Local infection (wound)	51 (92.7)	46 (69.7)
Generalized infection (blood)	4 (7.3)	7 (10.6)
Bronchial tree		12 (18.2)
Catheters		1 (1.5)
Total	55 (67.1)	66 (70.2)
Anterior nostrils (carriers)	27 (32.9)	28 (29.8)
Total	82 (100)	94 (100)

and generalized infections. The studied isolates were collected according to the rule that one strain was isolated from one patient. No multiple strain isolation was performed. The antimicrobial susceptibility patterns were determined for the MRSA strains including the standard panel of antibiotics: gentamycin, erthromycin, clindamycin, ciprofloxacin, trimethoprim/sulfamethoxazole, and vancomycin. All tested strains were only susceptible to trimethoprim/sulfamethoxazole and vancomycin, and were *mecA* gene-positive. The epidemiological relatedness was analyzed with the RAPD method. Ten RAPD patterns were identified among the collected MRSA strains. RAPD pattern A, 51%; pattern B, 16%; the remaining 33% belonged to other eight RAPD types. In the case of the MSSA strains, 15 different RAPD patterns were determined and no dominant group was observed. When additional typing methods were used (*coa* and *spa* gene polymorphisms), 25 different groups could be identified among the MRSA collection and 61 groups among the MSSA strains.

***spa* gene polymorphism.** The polymorphic region of the *spa* gene was amplified by PCR with primers located near the variable region as described by Sabat *et al.* (2003). The primer sequences were as follows: SpaF 5'-CAAGCACCAAAAGAGGAA-3' and SpaR: 5'-CACCAGGTTTAACGACAT-3' (Frenay *et al.,* 1996). The PCR products were analyzed on a 3% agarose gel.

RESULTS

Among the studied *S. aureus* strains the majority of MRSA strains had 13 repeat units within the X region of the *spa* gene (66.8% of MRSA strains isolated from infected patients and 50% of MRSA strains isolated from carriers) (Table 2). In the case of MSSA strains the distribution of strains with a particular number of tandem units was more complex (Table 3). Strains with 11 or 12 repeat units were found to be the most common (20.7% and 26.8%, respectively). Within the group of strains isolated from infected patients, strains with 11 or 12 tandem units constituted 18.2% and 21.8%, respectively; in the case of surveillance cultures: 26.0% and 37.0%, respectively. In general, strains with more than seven units were dominant in the studied *S. aureus* collection. Among the MRSA strains they accounted for 96.8% of all strains (95.3% from infected patients, 100% from carriers) and in the case of MSSA strains for 86.6% (81.8% and 96.3% from infections and surveillance cultures, respectively).

Table 2. *spa* typing and number of MRSA isolates detected for each type

Amplicon size (bp)	Number of repeats	Number of isolates (%)	
		Infections	Carriers
210±10	7	3 (4.7)	
230±10	8	8 (12.2)	12 (42.9)
320±10	12	4 (6.1)	2 (7.1)
350±10	13	44 (66.8)	14 (50.0)
370±10	14	7 (10.2)	
Total		66 (100)	28 (100)

Table 3. *spa* typing and number of MSSA isolates detected for each type

Amplicon size (bp)	Number of repeats	Number of isolates (%)	
		Infections	Carriers
170±10	5		1 (3.7)
190±10	6	4 (7.3)	
210±10	7	6 (11.0)	
230±10	8	5 (9.0)	3 (11.1)
260±10	9	5 (9.0)	1 (3.7)
280±10	10	7 (12.7)	3 (11.1)
300±10	11	10 (18.2)	7 (26.0)
320±10	12	12 (21.8)	10 (37.0)
350±10	13	6 (11.0)	2 (7.4)
Total		55 (100)	27 (100)

DISCUSSION

The MRSA and MSSA strains can cause all types of infections in adults as well as in children (Sola *et al.*, 2002; Huang *et al.*, 2007; Collins, 2007; Kuint *et al.*, 2007; Kurlenda *et al.*, 2007; 2009). The suggested higher virulence of HA-MRSA strains has not been proven and the recent research often presents ambiguous results. Melzer *et al.* (2003) in a study carried out in the years 1995–2000 compared 382 patients with HA-MRSA bacteremia and 433 with MSSA-caused bacteremia. After a preliminary analysis higher mortality was observed in the HA-MRSA group (11.8%) in comparison with MSSA (5.1%). However, after thorough verification of the results, it was revealed that patients from the first group had higher risk factors and after this correction the adjusted mortality was 7.1% and 6.2%, respectively (Melzer *et al.*, 2003). In the analysis of the amount of protein A produced, ability to form capsules and the extent of adherence to the human epithelial cell line Hep-2, no differences were found between MRSA and MSSA (Aathithan *et al.*, 2001). Protein A is thought to be a significant virulence factor as it shields bacteria from the immunological response of the host organism at the first stages of infection. According to Frenay *et al.* (1994) strains with a high number of tandem repeat units are described as more virulent. This is based on the thesis that multiple repetitions within region X result in a longer protein A domain that binds the Fc fragment of IgG. This in turn leads to a more effective binding to IgG facilitating the development of infection (Frenay *et al.*, 1994; Kuzma *et*

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al., 2005). A correlation between the number of tandem repeats and the strain's ability to cause epidemic has also been suggested. Strains with more than seven tandem units are considered epidemic (Frenay *et al.*, 1994). However, the published results concerning such a correlation are often contradictory, especially in the case of MSSA strains. Studies of Kuzma *et al.* (2005) and Jakubczak *et al.* (2007) revealed that among *S. aureus* strains isolated from cows suffering from mastitis, a highly variable number of tandem repeats (from 2 to 11) was observed. El-Sayed *et al.* (2005) analyzed nineteen MSSA strains isolated from birds and found only one with less than seven tandem units. Ambiguous results were obtained by Vimercati *et al.* (2006) who reported that among cows and goats the majority of strains (70%) were those with 8 to 12 repeat units, but in sheep strains with 2–7 units were common (81%). It is assumed that the number of repetitive units below seven correlates with decreased virulence of the strain (Vimercati *et al.,* 2006). However, this was not confirmed in the studies of Nashev *et al.* (2004) who analyzed 20 strains isolated from surveillance culture (anterior nostrils) and revealed the presence of strain with five tandem units in only one case. All other strains (19 strains) had from 8 to 13 repeat units (Nashev *et al.*, 2004). Our results are similar. The majority of MSSA strains studied here had more than seven repetitive units. Only five strains with less than seven units (6 and 5 units, 4.9% and 1.2%, respectively) were observed. Moreover, in the case of MRSA strains no isolates with less than seven repetitions were described. Frenay *et al.* (1994) analyzed MRSA strains isolated from patients suffering from cystic fibrosis and reported the variability of the number of tandem units from 3 to 15. Furthermore, a highly significant correlation (chi-square test [$P<0.005$]) between number of tandem units ≥ 7 and the epidemic character of the strains was proved.

Other studies concerning epidemic strains showed that among *S. aureus* with the profile PFGE 1 (EMRSA 16) 78% had 10–13 tandem units, and among strains with PFGE 5 (EMRSA 15) 90% had 15–17 repetitions (Walker *et al.*, 1998). Our studies do not confirm the thesis mentioned above, as no correlation between the number of tandem units lower than seven and lower virulence was observed. Such strains were isolated from carriers as well as patients suffering from bacteremia and local infections. However, the fact that strains with seven tandem units were not commonly isolated (their percentage within the MRSA collection was 3.2%) could confirm their lower ability to spread and cause epidemic outbreaks in a hospital environment. The same results were obtained in the case of MSSA strains, where 6.1% of the strains had less than seven repeat units and all of them were isolated from non-epidemic patients.

As the *S. aureus* virulence results from synergistic interactions of a variety of toxins and factors, the strains presented in the current communication were additionally analyzed in respect to the *cna, pls, ica* and *emp* genes as well as the ability to produce biofilm *in vitro*. The obtained results have already been published (Grinholc *et al.*, 2007; Kurlenda *et al.*, 2008).

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