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Short Communication

Coexistence of virulence genes in methicillin-resistant *Staphylococcus aureus* clinical isolates

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Abstract

Introduction: The pathogenic versatility of *Staphylococcus aureus* is attributed to various virulence genes, including enterotoxins and hemolysins. **Methods:** Here, the virulence genes in 177 nosocomial MRSA strains in Porto Alegre, Brazil were detected by PCR. **Results:** The overall prevalence rates were as follows: *sea*, 4.5%; *pvl*, 18.6%; *tst*, 27.7%; *hla*, 87.6%; and *hld*, 90.4%. No strain contained all tested genes. However, there was frequent coexistence of *tst* with *pvl* and *hla* with *hld* (40.7% and 26.6%, respectively). **Conclusions:** Horizontal transfer of virulence genes is very common in *S. aureus*, as suggested by the frequent coexistence of several virulence genes.

Keywords: Coexistence of virulence genes. MRSA. Virulence genes.

Virulence and antimicrobial resistance are two characteristics that do not always coexist in bacteria, since both are associated with large metabolic burdens, resulting in decreased fitness[1]. However, if the microorganism harbors the genetic capacity allowing it to produce either the determinants of antimicrobial resistance or virulence factors in specific situations, if a host demonstrate weakness, then both might be concomitantly expressed. *Staphylococcus aureus* has several resistance mechanisms and several virulence genes that promote adhesion to host cells, tissue invasion, and escape from the immune system, to establish a chronic infection[2].

Methicillin resistance is the most prevalent resistance acquired by *S. aureus*, and it is highly disseminated worldwide. As an opportunistic microorganism, *S. aureus* takes advantage of its existence as both a commensal and a pathogen to survive in the host, since selective pressures promote adaptation. Moreover, when living with other commensal bacteria, such as *Staphylococcus epidermidis* and *Enterococcus*, *S. aureus* may acquire additional resistance or virulence characteristics[1,3]. Because many of these genes are encoded on mobile genetic elements, such as plasmids or prophages, they can be transmitted between strains by horizontal transfer[4]. In addition to its antimicrobial resistance arsenal, there are several virulence genes that make *S. aureus* such a versatile pathogen. The most frequent virulence genes encode toxins such as enterotoxins, Panton-Valentine leukocidin, and toxic shock syndrome toxin, and hemolysins such as alpha-, beta-, and delta-hemolysins. The expression of these virulence factors is coordinated by quorum-sensing activity, a cell-communication system that controls gene expression in response to population density[5,6].

In this study, we report the coexistence of quorum-sensing regulated virulence genes among a collection of methicillin-resistant *Staphylococcus aureus* (MRSA) strains obtained from hospitals in Porto Alegre, Brazil. Our findings provide insight into the pathogenesis and evolution of MRSA.

This cross-sectional observational study was conducted with 177 healthcare-associated methicillin-resistant *Staphylococcus aureus* (HA-MRSA) strains recovered between 2012 and 2014 in Laboratories from *Hospital Mãe de Deus* (HMD) and *Hospital Nossa Senhora da Conceição* (HNSC). They were isolated from the respiratory tract (72/177; 40.7%), blood (40/177; 22.6%), skin and soft tissue (39/177; 22.0%), bone and connective tissue (10/177; 5.6%), medical devices (9/177; 5.1%), urine (5/177; 2.8%), and others sites (2/177; 1.1%). The identity of the isolates was confirmed using conventional methods, such as Gram staining, catalase activity, and plasma coagulase production. Methicillin resistance was verified by conventional polymerase chain reaction (PCR) for *mecA* and by the Kirby-Bauer method using cefoxitin (DME, São Paulo, Brazil), according to Clinical and Laboratory Standard Institute (CLSI) guidelines[7].

Deoxyribonucleic acid (DNA) was extracted from MRSA isolates grown for 24h using Chelex® 100 (Bio-Rad, Richmond, CA) and Proteinase K (Sigma-Aldrich, Poole, UK) and was subjected to qualitative PCR. The alpha-hemolysin (*hla);* delta-hemolysin (*hld*); staphylococcal enterotoxin type A (*sea)*; Panton-Valentine leucocidin (*pvl*), and toxic shock syndrome toxin-1 (*tst*) virulence genes were detected according

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to a previously described method[8], with some modifications. The PCR mixture contained 2.75mM $MgCl_2$, 0.2mM each deoxyribonucleotide triphosphate, 1× Taq buffer, 1.25U of Taq DNA polymerase, 0.5µM primers, and 1.8µl of DNA template in a total volume of 15µl. The thermal cycler program used for *sea*, *hla*, *hld*, and *tst* was as follows: an initial denaturation step at 94°C for 5 min, followed by 30 cycles of denaturation at 95°C for 30 sec, annealing at 50°C (*tst*), 52°C (*sea*), or 58°C (*hla*, *hld*) for 45 sec, and extension at 72°C for 1 min, and a final extension at 72°C for 7 min. The thermal cycler program used for *pvl* was as follows: an initial denaturation step at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 15 sec, annealing at 53°C for 15 sec, and extension at 72°C for 1 min, and a final extension at 72°C for 10 min. The amplification was performed in a LifePro Thermal Cycler (Hangzhou Bioer Technology Co. Ltd., Hangzhou, China) with the following primers: GSEAR-1 5′-GGTTATCAATGTGCGGGTGG-3′ and GSEAR-2 5′-CGGCACTTTTTTCTCTTCGG-3′ for *sea* (102bp); HLA-1 5′-CTGATTACTATCCAAGAAATTCGATTG-3′ and HLA-2 5′-CTTTCCAGCCTACTTTTTTATCAGT-3′ for *hla* (209bp); HLD-1 5′-AAGAATTTTTATCTTAATTAAGGAAGGAGTG-3′ and 5′-TTAGTGAATTTGTTCACTGTGTCGA-3′ for *hld* (111bp); GTSSTR-1 5′-ACCCCTGTTCCCTTATCATC-3′ and GTSSTR-2 5′-TTTTCAGTATTTGTAACGCC-3′ for *tst* (326bp); and PVL-1 5′-ATCATTAGGTAAAATGTCTGGACATGATCCA-3′ and PVL-2 5′-GCATCAAGTGTATTGGATAGCAAAAGC-3′ for *pvl* (443bp). *S. aureus* N315 (*hla* and *hld*), *S. aureus* JCSC 4469 (*sea* and *tst*), and *S. aureus* ATCC 14458 (*pvl*) were included as control strains.

The overall prevalence rates of the virulence genes among the HA-MRSA isolates were as follows: *sea*: 4.5%; *pvl*: 18.6%; *tst*: 27.7%; *hla*: 87.6%; and *hld*: 90.4%. The distribution pattern is presented in **Figure 1**. The respiratory tract (40.7%) and blood (22.6%) were the major MRSA recovery sites, which were followed by the minor sites.

In this study, the coexistence of the most prevalent virulence genes, *sea*, *hla*, *hld*, *pvl*, and *tst*, was investigated in several MRSA clinical isolates. The most common coexistence was *hla* + *hld* (40.7%), followed by *tst* + *hla* + *hld* (26.6%) and *pvl* + *hla* + *hld* (13.6%). The least frequent were *hla* + *hld* + *sea* (1.7%), *hla* + *hld* + *sea* + *pvl* (1.7%), *hla* + *hld* + *pvl* + *tst* (0.6%), and *hla* + *hld* + *sea* + *tst* (0.6%). Despite these low prevalence rates, 49 out of 49 *tst*-positive isolates and 7 out of 8 *sea*-positive isolates contained at least one other virulence gene.

Some MRSA isolates (45%) recovered from blood cultures contained both *hla* and *hld*, and 92.5% that contained both genes, harbored another gene. In 10 isolates, no virulence gene was found, and no strain contained all genes.

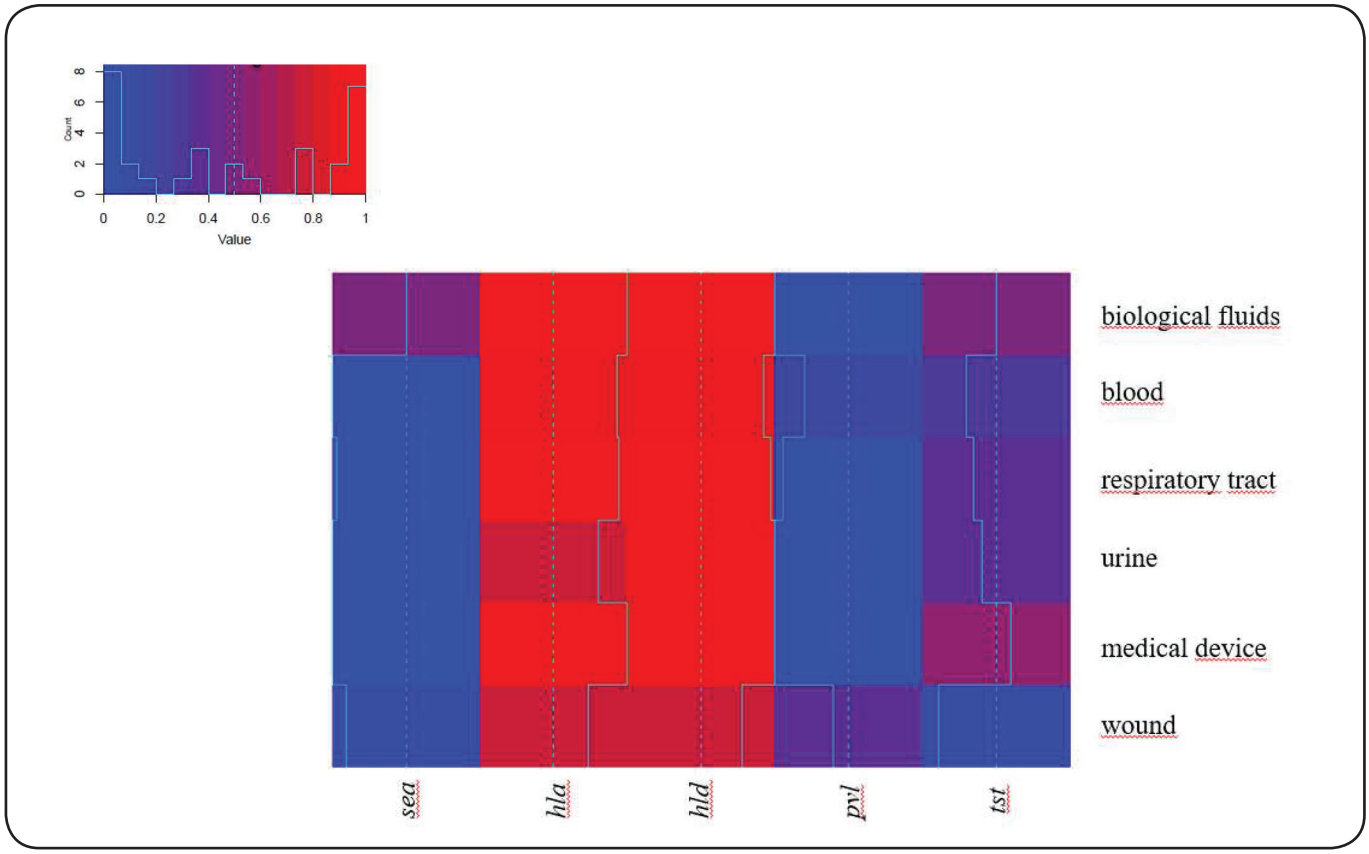

FIGURE 1: Heatmap showing the distribution of virulence genes among 177 MRSA clinical isolates, according to infection site. ***sea:*** staphylococcal enterotoxin type A; ***hla;*** alpha-hemolysin; ***hld;*** delta-hemolysin; ***pvl*:** Panton-Valentine leucocidin; ***tst:*** toxic shock syndrome toxin; **MRSA:** methicillin-resistant *Staphylococcus aureus.*

Staphylococcus aureus is a dangerous and versatile pathogen that can cause a multitude of different diseases. Most frequently, it causes infections of the skin and respiratory tract[4]. *S. aureus* secretes a group of peptides that can damage the host cell plasma membrane, such as pore-forming toxins like hemolysins[9]. The vast majority of *S. aureus* strains harbors all four hemolysin genes, *hla*, *hlb*, *hld*, and *hlg*. It is notable that the two hemolysin genes we tested in this study showed the highest coexistence, highlighting their importance to virulence.

Toxic shock syndrome toxin-1(TSST-1) is a potent superantigen, and it is the most common cause of toxic shock syndrome. It is produced exclusively by *S. aureus*, and approximately 20% of natural isolates are TSST-1 producers[10]. In this study, 27.7% of the MRSA isolates harbored the *tst* gene, which is inserted into a pathogenic island[11], which is widely assumed to be a mobile element. Therefore, TSST-1 can be transmitted through horizontal transfer, which is responsible for its spread among *S. aureus* strains.

The Panton-Valentine leucocidin (PVL) toxin in *S. aureus* is responsible for the destruction of polymorphonuclear and mononuclear cells, through necrosis and apoptosis, which usually causes skin or soft tissue infections and necrotizing pneumonia[12]. In our study, *pvl* was detected in 18.6% of the HA-MRSA strains, as was observed in previous reports[13,14]. The increasing prevalence of *pvl* in HA-MRSA is likely because of the presence of infective PVL phages[13].

In summary, horizontal transfer of virulence genes is very common in *S. aureus*, which is suggested by the considerable coexistence of several virulence genes in this study.

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Conflict of interest

The authors declare that there is no conflict of interest.

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