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High frequency of Panton-Valentine leukocidin in *Staphylococcus aureus* causing pediatric infections in the city of Cartagena-Colombia

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Summary Panton-Valentine leucocidin (PVL) is a pore-forming toxin that has been epidemiologically associated with CA-MRSA infections. However, its role in the pathogenicity of *Staphylococcus aureus* is still unclear. We evaluated the prevalence of PVL-coding genes in methicillin-resistant (MRSA) and methicillin-sensitive (MSSA) isolates that cause infections in pediatric patients in the city of Cartagena, Colombia. We obtained *S. aureus* isolates from patients at the Napoleon Franco Pareja Children's Hospital in Cartagena. Then, we evaluated the presence of the *nuc*, *mecA*, and PVL genes in these isolates by multiplex PCR and determined the antibiotic susceptibility profiles using CLSI standards. We further correlated methicillin susceptibility and the presence of PVL genes with clinical variables. Overall PVL prevalence in *S. aureus* isolates was 73.91%, with a frequency of 80.92% among MRSA isolates and 67.59% among MSSA. We found a correlation between erythromycin resistance and lack of PVL and found that PVL+ cases were more common in older patients. We found a high PVL prevalence in both MRSA and MSSA isolates, in concordance with previous regional reports.

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Introduction

Staphylococcus aureus is a major human pathogen that causes both nosocomial and community-acquired infections, which range from skin and soft tissue infections to osteomyelitis, pneumonia, bacteremia and infections associated with medical devices [1]. Additionally, *S. aureus* behaves as a commensal bacterium, colonizing up to 60% of healthy populations [2]. In Cartagena, Colombia, *S. aureus* colonization ranges from 15.9 to 38.5% [3,4], depending on the study population.

Both colonizing and pathogenic potentials of *S. aureus* are related to the virulence factors carried by circulating strains [5—7]. High prevalence of Panton-Valentine leucocidin (PVL) has been documented in methicillin-resistant isolates of community origin (CA-MRSA) [6]. PVL is a pore-forming leukotoxin composed of two components, S and F, which are encoded by the *Luk*S-PV and *Luk*F-PV genes in the lysogenic phage *phi*SLT [8]. Due to the epidemiological association between PVL and CA-MRSA isolates, many efforts have been directed toward identifying the pathogenic role of this toxin, but the results have been controversial. Several *in vitro* studies have shown that PVL induces cell lysis by forming pores in the membranes of polymorphonuclear cells (PMNs), and it induces apoptosis by interacting with the mitochondrial membrane [9] and activating downstream TLR-2 signaling pathways, leading to an inflammatory response [10] and complement receptor-mediated cytotoxicity [11]. However, animal model studies have failed to demonstrate a pathogenic role for PVL in staphylococcal infections [12,13]. Despite this discrepancy, PVL has been increasingly associated with severe clinical manifestations of *S. aureus* infections [14—16]; thus, more research is still needed to identify subjects at risk for developing severe forms of infection [17].

The prevalence of PVL has not been as well-studied in MSSA isolates as in MRSA isolates [18]. However, it has been determined that the presence of genes encoding PVL varies by region, and there is a low prevalence in countries such as Spain and Portugal and higher prevalence reported in African countries [18] and Argentina [19]. This information is important for regions in which most cases of infections by PVL-positive isolates correspond to MSSA strains [20].

In this study, we evaluated the prevalence of PVL in both MRSA and MSSA *S. aureus* isolates that were obtained from pediatric infection patients in the regional children's hospital, and we correlated the presence of the PVL virulence factor with the type of infection and other clinical variables.

Materials and methods

Design and study population

This was an observational cross-sectional study that was carried out at the Napoleon Franco Pareja Children's Hospital in Cartagena; this is a University-affiliated pediatric hospital of tertiary care that receives patients from rural and urban areas, and it is the regional referral hospital. The ethics committee of the University of Cartagena approved this study. From October 2009 to June 2012, *S. aureus* isolates that caused laboratory-confirmed *S. aureus* infections were analyzed in the microbiology laboratory of the Genetics and Molecular Biology Research Group of the University of Cartagena. One isolate per patient was included in the study, and we reviewed the clinical data for each case to obtain sociodemographic information and infection type. Children's guardians signed an informed consent allowing us to use the information for research.

Bacterial isolates

Only one isolate per case was included in the study; isolates were confirmed by standard microbiological methods. Using the disk diffusion method, antibiotic susceptibility was determined according to CLSI standards for rifampicin (5 μg), clindamycin (2 μg), erythromycin (15 μg), gentamicin (10 μg), and trimethoprim-sulfamethoxazole (1.25/23.75 μg); for vancomycin, the minimum inhibitory concentration was determined using the agar dilution test. The D test was used to detect macrolide-inducible resistance to clindamycin.

DNA extraction and multiplex PCR assay

Genomic DNA from each isolate was extracted using a modification of the method developed by Millar et al. [21], as we have previously described [22]. Genomic DNA from each isolate was used to determine the presence of *nuc*, *mecA*, and *lukS/F*-PV genes by multiplex polymerase chain reaction assay, according to protocols that we have previously described [22]. The PCR products were subjected to electrophoresis in a 1.5% agarose gel, followed by ethidium bromide staining and visualization using UV trans-illumination.

Data analysis

Statistical analysis was performed using the IBM SPSS software for Windows, version 20. According to *mec*A gene presence, the isolates were classified