Examination of virulence genes in nasal isolates and community-acquired strains of methicillin-resistant *Staphylococcus aureus*

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ABSTRACT

Community-acquired strains of *Staphylococcus aureus* (CASA) infections have emerged as a health problem in Wisconsin, particularly those CASA strains that are resistant to the antibiotic methicillin (CA-MRSA). Bacteria such as *S. aureus* create products that allow the organism to infect a host called virulence factors. There are potentially numerous virulence factors that could contribute to CA-MRSA strains causing infections in humans, but little is presently known about whether these strains carry the genes for the potential virulence factors. In order to ascertain the genetic differences between CA-MRSA strains and hospital-acquired *S. aureus* strains or natural *S. aureus* isolates from the nasal passages of healthy people, a PCR-based analysis was used to screen for the presence of the adherence genes *clfA* and *fnbA* and *pvl*, which codes for a leukocidin. The analysis of the *clfA* gene indicated that 27/36 CA-MRSA strains tested positive and 26/45 nasal isolates tested positive. The *fnbA* gene yielded 37/45 positive nasal isolates and 31/36 positive CA-MRSA strains. For the *pvl* gene, 0/40 nasal isolates tested positive, but 29/36 CA-MRSA tested positive. These results indicate that the *clfA* and *fnbA* genes appear to be conserved across strains of *S. aureus*. On the other hand, the acquisition of the *pvl* gene may be part of a virulence gene repertoire that allowed CA-MRSA strains to emerge and cause disease in humans.

INTRODUCTION

Staphylococcus aureus is a normal inhabitant of various parts of the human body, residing on the skin and in hair follicles, in the throat, and within nasal membranes of healthy individuals. However, it is also an opportunistic disease-causing organism responsible for a variety of infections, typically in those with established risk factors such as a recent hospitalization or surgery, use of intravascular devices or catheters, or habitation in a long-term care facility (1). The acquisition of these strains of *S. aureus* within the at-risk group usually occurs in a hospital and are called hospital-acquired *S. aureus*. More than half of these strains are resistant to a commonly used antibiotic called methicillin, which makes treatment options more difficult. Moreover, a new group of *S. aureus* strains have recently emerged that also display a resistance to methicillin, but are instead acquired in the community and are referred to as community-acquired methicillin-resistant *S. aureus* (CA-MRSA) (2).

Cases of CA-MRSA infections in Wisconsin have been increasing over the past twenty years (4). These CA-MRSA strains cause a variety of skin infections and pneumonia. It is uncertain how the transition from normal flora to disease causing organism occurred in the recent past, but now CA-MRSA have become a significant health problem in the United States. To ascertain how this group of *S. aureus* have emerged as a significant health problem, it is important to analyze the genetic make up of the strains that may contribute to the disease causing ability of CA-MRSA, compared to the genetic make up of commensal *S. aureus* nasal isolates residing normally in human nasal passages.

There are many mechanisms or virulence factors that CA-MRSA may use to infect humans. This study examined three of these potential virulence factor genes. The *clfA* gene encodes for a Ser-Asp-rich fibrinogen-binding protein and the *fnbA* gene encodes for a fibronectin binding protein, both which facilitate adherence to the host cell (3). The *pvl* gene encodes for the Panton-Valentine leukocidin which aids in the destruction of white blood cells. This study examined the genomic DNAs of CA-MRSA and nasal isolate strains to determine if these genes were present and therefore contributing to the pathogenicity of the CA-MRSA strains.

MATERIALS AND METHODS

Strains

Strains of CA-MRSA and nasal *S. aureus* isolates from Marshfield Clinic were used in this study. The MW2 strain of *S. aureus*, known to possess the virulence factor genes, was used as a positive control. *Staphylococcus epidermidis* that is known to lack the virulence factor genes was used as a negative control.

DNA extraction

Genomic DNA was extracted utilizing a commercial kit (Edge Biosystems, Gaithersburg, MD) with a lysostaphin digestion (10 min, 37ºC) added at the first step to assist in lysis of the bacteria.

PCR

Oligonucleotide primer pairs between 18 and 24 base pairs in length were commercially synthesized. For the *clfA* gene, the following primers were used : ClfA1 5' TTA CGC AAT CTG ATA GCG CA 3' and ClfA2 5' TGC TTG AAT GAG TTG CC 3'; For the *fnbA* gene, the following primer pairs were used: FnbA1 5' GAG CAG CAT CAG TAT TCT TAG 3' and FnbA2 5' TTT GTG CTT GTA CTG CT3'; *For* the *pvl* gene, the following primers were used: Pvl1 5' ATC ATT AGG TAA TAA AAT GTC TGG ACA TGA TCC A 3' and Pvl2 5' GCA TCA AST GTA TTG GAT AGC AAA AGC 3'. These primers were then standardized to 50 pmol per primer for use in PCRs. Taq polymerase was also used. Both primer pairs utilized identical amplification conditions set at 35 cycles under the following parameters: denatruation for 30 sec at 94ºC, annealing for 1 minute at 55ºC and elongation for 1 minute at 72ºC. The PCR amplification was run in a PE9700 thermal cycler (Perkin Elmer, Wellesley, MA). Two PCR amplification runs for each gene from each strain were performed to ensure reproducibility.

Analysis of PCR results

Following PCR amplification, the PCR products were separated on 1.5% agarose gels and stained with ethidium bromide to visualize the respective DNAs. For each run, a 100 base-pair molecular weight DNA standard (New England Biolabs) was used to verify the proper size for each PCR product. Control lanes were examined to confirm the proper results. If the positive and negative controls for that run worked, then a tally of the number of CA-MRSA strain possessing that virulence factor gene were made.

CONCLUSION

The species *S. aureus* is constantly evolving. In recent years, the emergence of CA-MRSA has become a concern to the healthcare community. Little is known about why these strains of *S. aureus* have become problematic. This study attempted to address this question by looking at three virulence factor genes. *For* the *clfA* gene, 27/36 CA-MRSA strains and 26/45 nasal isolates tested positive; whereas 31/36 CA-MRSA strains and 37/45 nasal isolates tested positive for the *fnbA* gene. This suggests conservation of both the *clfA* and *fnbA* genes across strains of *S. aureus*. It can be expected that attachment mechanisms, such as the fibronectin-binding protein and the Ser-Asp-rich fibrinogen-binding protein are present in normal flora and disease causing strains because they are utilized for adherence to host cells without necessarily contributing to the organisms' pathogenicity (3). However, the *pvl* gene showed 29/36 positive CA-MRSA strains and 0/40 positive nasal isolates. The presence of the Panton-Valentine leukocidin gene in CA-MRSA strains but not commensal *S. aureus* isolates suggests that this gene may be part of a repertoire that allowed CA-MRSA strains to emerge and cause disease in humans (1).

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