Classification of Methicillin-Resistant *Staphylococcus aureus* Strains in Cystic Fibrosis Patients

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INTRODUCTION

Staphylococcus aureus is a normal part of the flora of the skin, mucous membranes, urogenital tract, and alimentary tract of people. Occasionally, *S. aureus* results in infection as opposed to simple colonization. Most *S. aureus* are susceptible to a variety of antimicrobial drugs. However, a far more serious type of infection caused by methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged. MRSA is resistant to most commonly used antimicrobial drugs, including all beta lactam type antimicrobials such as ampicillin, amoxicillin, and cephalosporins. For many years, MRSA infection was primarily a nosocomial infection or hospital-acquired MRSA (HA-MRSA). In the last decade, MRSA infections are increasingly identified outside the hospital setting. Now community-acquired (CA-MRSA) strains account for many cases of MRSA infection.

Cystic Fibrosis (CF) is a genetic disease that usually results in death due to recurrent and persistent pneumonia. Although infection with *S. aureus* is not the major cause of morbidity and mortality in CF patients, *S. aureus* infections are common. Infection with MRSA is potentially far more serious than infection with methicillin susceptible *S. aureus*. Interestingly, no studies to date have systematically evaluated the prevalence of HA-MRSA versus CA-MRSA in patients with CF. We intend to characterize MRSA isolated from the respiratory tract of patients with CF. Molecular methods will determine if these MRSA are HA or CA.

Companion animals are occasionally colonized or infected with MRSA, and have rarely been shown to share an identical MRSA strain with their owners. Eventually, we hope to determine if CF patients share an identical *S. aureus* or MRSA bacterium with their household pet.

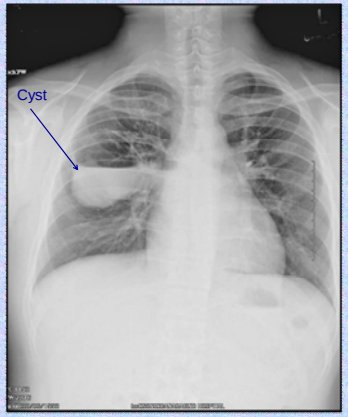

Fig. 1 Radiograph of a patient with lung abscess (necrotizing pneumonia) typically caused by CA-MRSA USA-300 strains in an otherwise normal patient

METHODS

Bacterial isolates were collected from sputum or airway lavage of 88 CF patients over the course of 7 months (2/21/07-9/19/07). *Staphylococcus aureus* were classified as MRSA based on phenotypic resistance to oxacillin. MRSA were further classified by determining their in-vitro susceptibility to clindamycin. If a *S. aureus* was sensitive to clindamycin it was presumptively labeled as CA-MRSA, whereas if it was resistant to clindamycin, it was labeled as HA-MRSA. This method of classification is not always accurate as it relies solely on expression of a given phenotype and may misclassify pathogens.

Genotypic characterization of isolates is more specific than phenotypic classification. In this study, 3 genotypic methods will be used to further classify the isolates that were presumptively identified as HA-MRSA or CA-MRSA. Polymerase chain reaction (PCR) will be used to amplify the *mec*A gene, panton valentine leukocidin (PVL) gene, and a portion of the *spa* gene. The *mec*A gene identifies the isolates as MRSA. Some isolates of *S. aureus* may posses the *mec*A gene, but fail to express the gene in vitro. Hence, detection of the *mec*A is more sensitive and specific for identification of MRSA than antimicrobial susceptibility testing. The *spa* gene will be sequenced to provide a spa-type that will classify types of HA-MRSA and CA-MRSA based on sequence homology with a published database (e.g., USA 100 is HA-MRSA while USA 300 is CA-MRSA). Panton valentine leukocidin is a virulence factor related to some of the severe clinical symptoms of infection with MRSA and is most often found in a specific subtype of CA-MRSA, termed USA 300.

S. aureus and MRSA isolates from patients with CF were stored at -80ºC. MRSA isolates were cultured on blood agar plates and one or two colonies of each sample were mixed with 95µl of TE buffer. PCR will be performed to amplify the *mec*A, PVL, and *spa* genes using specific primers for these gene regions in a multiplex PCR reaction [3,4,7]. PCR product will be run on a horizontal gels and photographed. The PCR product from the original PCR tubes will be purified using the PureLink™ PCR Purification Kit (Invitrogen). The purified DNA will be sent for sequencing with the *spa* specific sequencing primers at the UM DNA core facility. After sequencing is completed, we will compare the sequence with known *spa* sequences. The data will be presented using descriptive characterization.

EXPECTED OUTCOME

It is likely that the outcome of the molecular characterization will be similar to that of the distribution based on phenotypic classification. Because twice as many samples are likely to be HA as compared to CA, the presence of PVL virulence factor will be rare. It is impossible to predict which specific USA type will account for most isolates.

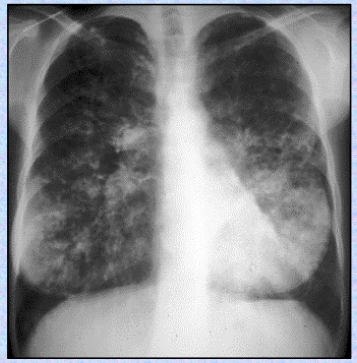

Fig. 2 Radiograph of the lungs of a patient with CF

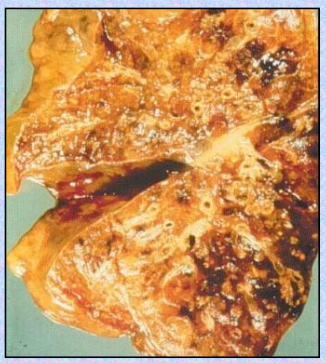

Fig. 4 Post mortem lung of a CF patient filled with abscesses and necrotic tissue. CF is caused by a defective chloride channel which leads to abnormal fluid secretion across tissues. Respiratory tissues fill with thickened mucus and are susceptible to repeated and persistent bacterial infection.

FUTURE DIRECTIONS

In the future, pets in households of CF patients colonized or infected with MRSA will be evaluated. Looking at these same patients might be difficult to achieve especially if they are no longer infected or colonized. Hence, a prospective study design will be used to sample people and their pets.

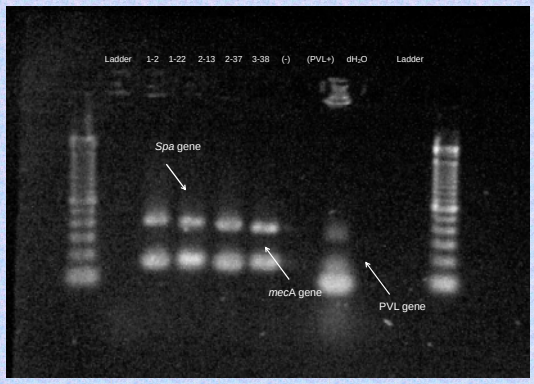

Fig. 3 Gel electrophoresis of 5 MRSA isolates. DNA ladders are shown, as are + and – controls. One MRSA isolate (1-2) failed to amplify. Four isolates possess mecA and spa genes, but none of the isolates shown in this gel possess the PVL gene.

Distribution of *S. aureus* Isolates

Total # of Patients	Total # of S. aureus Isolates	Average # of Samples Per Patient	Total # of MRSA + Isolates	Total HA-MRSA Isolates	Total CA-MRSA Isolates
88	170	1.93	72	50	22

Table 1.1 Distribution of *S. aureus* isolates recovered from sputum or airway lavage from patients with CF over a 7 month period. Characterization of HA or CA MRSA is based on clindamycin susceptibility.

SOURCES

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