Prevalence of Community-Associated Methicillin-Resistant
Staphylococcus aureus in Patients with Cystic Fibrosis

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We prospectively determined the prevalence of community-associated Staphylococcus aureus in a large cystic fibrosis (CF) center between October 2005 and October 2007. We found that 2.7% (19/707) of the CF patients who had cultures during the study period were infected with this organism, representing 14% of the total methicillin-resistant Staphylococcus aureus strains (n = 140) recovered from the patient population during the study period.

Community-associated methicillin-resistant Staphylococcus aureus (CA-MRSA) has recently emerged as an important human pathogen (6, 9, 11, 17). CA-MRSA is characterized by the presence of the mecA gene on a small mobile staphylococcal cassette chromosome (SCC) designated as SCCmec type IV (11). In addition, the CA-MRSA strains harbor unique virulence factors, such as Panton-Valentine leukocidin (PVL) (17). PVL is a two-component pore-forming toxin complex encoded by two cotranscribed genes (lukS-PV and lukF-PV) that cause tissue necrosis and leukocyte destruction (5, 14, 15). One of the most striking features of this pathogen is its association with necrotizing pneumonia (6). S. aureus is typically the first bacterial pulmonary pathogen recognized in cystic fibrosis (CF) patients, resulting in chronic S. aureus infection that can persist for years. The specter of a highly virulent S. aureus strain, which has been reported to cause necrotizing pneumonia in a small number of CF patients (4), is a huge concern.

According to the Cystic Fibrosis Foundation patient registry, there was a significant rise in MRSA infection among CF patients, from 0.1% in 1995 to 18.9% in 2006 (2, 7). A recent 10-year cohort study of over 1,700 children and adults with CF showed that persistent MRSA infection was associated with a more rapid decline in lung function as measured by forced expiratory volume in 1 s (often referred to as “FEV1”) (3). Less rapid decline in lung function as measured by forced expiratory volume in 1 s (often referred to as “FEV1”) (3). Less

We describe our methods and results and discuss the clinical and epidemiological implications of our findings. We conclude with a discussion of the role of CA-MRSA in CF patients and potential strategies to control its spread.

Community-acquired methicillin-resistant Staphylococcus aureus (CA-MRSA) is an important pathogen in the United States, particularly among healthy young adults. CA-MRSA infections are associated with skin and soft tissue infections, bloodstream infections, and necrotizing pneumonia. CA-MRSA is characterized by the presence of the mecA gene on a small mobile staphylococcal cassette chromosome (SCCmec) type IV (11). In addition, the CA-MRSA strains harbor unique virulence factors, such as Panton-Valentine leukocidin (PVL) (17). PVL is a two-component pore-forming toxin complex encoded by two cotranscribed genes (lukS-PV and lukF-PV) that cause tissue necrosis and leukocyte destruction (5, 14, 15). One of the most striking features of this pathogen is its association with necrotizing pneumonia (6).

The purpose of this 2-year prospective study was to determine the frequency with which CA-MRSA is found in our CF population as well as how long it persists and if specific clones of the organism are being spread among our patients. This study also provides the framework for future studies to determine the impact of CA-MRSA on the lung function of CF patients.

(A preliminary report of this work was presented previously by J. S. Goodrich, T. N. Sutton-Shields, J. P. Wedd, M. B. Miller, and P. H. Gilligan at the 107th General Meeting of the American Society for Microbiology, 2007 [8].)

During this longitudinal study at the Clinical Microbiology-Immunology Laboratories at the University of North Carolina Hospitals (Chapel Hill, NC) from 19 October 2005 to 19 October 2007, 5,265 respiratory cultures from 707 CF patients were screened for MRSA. Respiratory samples were plated onto horse blood, colistin nalidixic acid, MacConkey, mannitol salt, and Burkholderia cepacia selective agar plates. Isolates were identified as MRSA based on a positive tube coagulase test or a positive BactiStaph (Remel, Lenexa, KS) latex agglutination test, and susceptibility tests were performed by Kirby-Bauer disk diffusion following CLSI guidelines (1). Of these cultures, 13% (n = 662) contained MRSA. These MRSA-positive cultures were collected from respiratory samples from 140 patients; thus, 20% of CF patients harbor MRSA.

To determine if these MRSA isolates were hospital associated (SCCmec type II and PVL negative) or community associated (SCCmec type IV and PVL positive), molecular characterization was performed. The presence of PVL was assessed using real-time PCR assays targeting pvlS and pvlF (12) and the intergenic region between pvlS and pvlF. The assay was done using 200 nM of the probe (5’FAM-CTC ATG AAA TTA AAG TGA AAG GAC-TAMRA, where FAM is 6-carboxyfluorescein and TAMRA is 6-carboxytetramethylrhodamine) and 900 nM of each primer (forward, 5’ AAC AGA AAT TAC ACA GTT A; reverse, 5’ ATT GAT GTA ACA ACT GAT GAT). Using an ABI 7500 instrument (Applied Biosystems, Foster City, CA), the following cycling parameters were employed: 50°C for 2 min; 95°C for 10 min; 45 cycles of 95°C for 15 s, 55°C for 30 s, and 72°C for 35 s.

The SCCmec types were identified using a previously published protocol (13).

Isolates from MRSA-positive patients were molecularly analyzed at 2-month intervals to determine the persistence or
acquisition of CA-MRSA. Depending on the cultures collected during the 2-year period, 1 to 10 isolates were screened per patient, resulting in the molecular characterization of 470 isolates. Of these isolates, 10% (n = 47) contained genes for PVL. All PVL-positive isolates harbored SCCmec type IV. PVL-negative MRSA isolates (n = 423) were characterized as follows: 89% (n = 378) contained SCCmec type II, 1% (n = 3) contained SCCmec type III, and 10% (n = 42) harbored SCCmec type IV. Based on either the most frequently isolated or the final isolate from each patient, 14% (n = 19) of the patients screened were positive for MRSA that harbors PVL, while 86% (n = 121) were not positive for PVL. Considering the PVL status and SCCmec type, the majority of the MRSA-positive patients maintained the same organism for the duration of the study with the exception of four patients. Two patients with PVL-negative SCCmec type II MRSA acquired PVL-negative, SCCmec type IV MRSA. One patient had SCCmec type III and acquired SCCmec type II. Finally, one patient initially had a PVL-negative, SCCmec type II MRSA isolate but acquired a PVL-positive, SCCmec type IV MRSA strain. Defining CA-MRSA as those isolates of MRSA that harbor PVL and SCCmec type IV, the prevalence of CA-MRSA among CF patients screened during this 24-month period was 2.7% (19/707).

The persistence of the CA-MRSA infections as chronic or transient was determined. Chronic infection is defined as having three or more cultures containing MRSA during the study period, with the most recent culture still positive. Transient infection is defined as having one or more positive CA-MRSA culture(s) followed by at least three subsequent negative cultures. Of the patients with CA-MRSA infection, 32% (n = 6) were chronically infected, 26% (n = 5) were transiently infected, and the chronicity of infection could not be determined for 42% (n = 8) because too few cultures had been collected. Interestingly, one chronically infected patient had hospital-associated MRSA and acquired CA-MRSA during the study period.

Pulsed-field gel electrophoresis analysis reveals that both MRSA strains USA300 and USA400 entered our CF population before the study. One isolate per culture from each patient with CA-MRSA was analyzed (n = 47), resulting in one to six isolates per patient, depending on the number of cultures obtained. Pulsed-field gel electrophoresis was performed, and the results were interpreted as previously described (10, 16). Banding patterns were compared to prototype USA300 and USA400 clones. Eighty-nine percent (n = 17) of the CA-MRSA-positive patients have USA300 or a closely related clone, 5% (n = 1) have USA400, and 5% (n = 1) have a unique clone. As the majority of the clones are similar to USA300 and USA400, which are commonly found in the general population (10, 17), and a specific unique clone is not found in multiple patients, it is not possible to determine if there was patient-to-patient spread among CF patients.

The emergence of CA-MRSA in the CF population has been described previously (4, 7), but our prospective study following an entire CF clinic population (n = 707) for 24 months has allowed us to examine the epidemiology of this organism longitudinally. Several observations were possible. First, approximately 20% of our CF population harbors MRSA. This number is consistent with the 18.9% national MRSA infection rate among CF patients (2). Like Elizur et al. (4), we found that approximately 10% of the MRSA isolates recovered during our study period were CA-MRSA. We found that persistence of the CA-MRSA clone was similar to that of other MRSA clones in the CF patient population (3). While the source of the clones infecting the CF population is unknown, there is no evidence of the spread of a CA-MRSA clone unique to the CF population in our center.

Acquisition of this potentially virulent strain was an unusual clinical event; it was seen in only 2% (n = 13) of the CF patients during the 2-year study that included 707 patients. Of the patients with PVL-positive strains, 12 had at least two MRSA-negative cultures prior to acquiring a PVL-positive MRSA isolate, and one had a PVL-negative MRSA isolate prior to obtaining a PVL-positive one. Four percent (n = 29) of all CF patients acquired PVL-negative MRSA during the course of the study. Assuming that each patient was infected with only one strain of CA-MRSA at any given time, our data suggest that in the CF lung environment, the USA300 and USA400 clones are no more fit than are other MRSA clones. However, if a patient was colonized simultaneously with multiple strains of MRSA, this may not have been detected in our analysis, as only one isolate was screened per culture.

The importance of CA-MRSA in CF lung disease is uncertain. Elizur et al. (4) described two CF patients with cavitary lesions associated with PVL-positive MRSA. This observation is consistent with other reports of serious pulmonary manifestations of CA-MRSA infection (6). On the other hand, Gikman and colleagues (7) did not find an association between CA-MRSA and either pulmonary exacerbation or necrotizing pneumonia. In the 19 patients seen here with CA-MRSA infection, there were no episodes of necrotizing pneumonia (M. Muhlebach, personal communication). The paucity of data on the clinical outcome of CF patients infected with CA-MRSA suggests a need for a well-controlled, multicenter study to assess the impact of this organism on CF lung disease, which is currently ongoing.

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REFERENCES


