ORIGINAL ARTICLE

PVL toxin-producing methicillin-resistant *Staphylococcus aureus* (MRSA) are predominant in a tertiary-care metropolitan teaching hospital

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ABSTRACT

Background: There is increasing evidence that the clones of Panton-Valentine Leukocidin toxin, (PVLT)-producing methicillin-resistant *Staphylococcus aureus* (MRSA) are replacing toxin non-producing methicillin-resistant *Staphylococcus aureus*) in healthcare settings. Our study sought to characterize clinical isolates of MRSA and the prevalence of PVL toxin producing MRSA in our tertiary healthcare center in the United States during a one-year period.

Methods: A total of 5,497 clinical samples submitted to microbiology laboratory were processed for presumptive identification of MRSA with further confirmation by polymerase chain reaction (PCR) for the identification of *mecA*, *Staphylococcal* chromosome cassette *mec* (SCC*mec*) type, and Panton-Valentine Leukocidin Toxin (PVLT) gene. The antibiotyping was performed using VITEK® 2 system, and disk diffusion method, and data graphed using Microsoft Office program.

Results: Of *Staphylococcus aureus* isolates 52.2% (n=617) were MRSA. The prevalence of MRSA was higher within the 40-64 year old age bracket (~50%). Panton-Valentine Leukocidin Toxin was identified in 60% of SCC*mec* Type IV positive MRSA isolates and 28% of SCC*mec* Type II positive MRSA isolates; but the isolates were susceptible to vancomycin and rifampicin.

Conclusion: Our findings suggest a high prevalence of PVL toxin-producing isolates of MRSA, and thus adding an increasing risk of virulent infection.

KEY WORDS

Staphylococcus aureus, methicillin-resistance *Staphylococcus aureus*, MRSA, heathcare-acquired MRSA, HA-MRSA, community-acquired MRSA, CA-MRSA, Panton-Valentine Leukocidin toxin, PVL toxin, mecA, *Staphylococcal* chromosome cassette *mec*, SCC*mec*, skin and soft tissue infection.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a Gram-positive pathogen, causing illness in both healthy and immunocompromised patients, and leading to high morbidity and mortality (1). MRSA cause both, hospital- and community-acquired infections. Hospital acquired (HA)-MRSA strains cause nosocomial infections, and are associated with distinct molecular features and predisposition factors than community-acquired (CA)- MRSA strains. Usually CA-MRSA strains are more virulent and likely to infect those without predisposition factors found associated with HA-MRSA (2).

CA-MRSA, particularly the Panton-Valentine Leukocidin Toxin (PLVT)-producing strain leads to invasive infections, often in the soft tissue, such as boils and abscesses (3). CA-MRSA can be distinguished from HA-MRSA by tissue tropism and the size of the *Staphylococcal* chromosome cassette *mec* (SCC*mec*) (1). This genetic element carries the *mecA* gene encoding resistance to beta-lactam antibiotics. There are five known SCC*mec* cassettes with type I-III being associated with HA-MRSA. Type I, II, and III cassettes are traditionally larger and indicating gene transfer of additional drug resistance markers located withinin the cassette, giving HA-MRSA, the phenotype with a multi-drug resistant makeup (4). SCC*mec* type IV and V are associated with CA-MRSA and are significantly smaller in size, which usually do not confer MDR phenotypes, but appears to have resulted in increased mobility, and hence greater potential for horizontal spread to the species of diverse genetic background (4). CA-MRSA infections can still be susceptible to clindamycin, rifampin, levofloxacin, and vancomycin (5). Recent USA studies showed an increased incidence of CA-MRSA (about 21%) since 2011, and involved healthcare-associated infections (5). This is alarming for a number of reasons. The ability of the microbe to transfer the type IV SCC*mec* cassette so readily alludes to the idea that the resistant phenotype may be on the horizon. This is also disconcerting because research into the CA-MRSA phenotype is still in its infancy and will require extensive studies

to determine resistance and spreading patterns. The spread of CA-MRSA into the hospital setting will place compromised patients in an even more dangerous predicament.

Our study sought to characterize the prevalence of HA-MRSA and CA-MRSA in a tertiary healthcare center in the United States over a one-year period.

MATERIALS AND METHODS

The samples included in this study were submitted to clinical microbiology laboratory between June 2011 and June 2012, to determine the prevalence of *S. aureus* infection and the prevalence of SCC*mec* types I – III (CA-MRSA) versus SCC*mec* types IV and V (HA-MRSA) in the sample collected from the patients at Hahnemann University Hospital, an urban teaching facility in Philadelphia, Pennsylvania. Glycerol stocks of isolates were revived in Tryptocase Soy Broth (TSB), an antibiogram performed using VITEK® 2 system, and disk diffusion method for retrospective analyses of phenotypically pre-identified MRSA, as per recommendation by the Clinical Laboratory Standards Institute (CLSI) (2). American Type Culture Collection (ATCC) MRSA strains of USA-300 (BAA 1680) and USA-400 (BAA 1683) were included as positive reference strains of CA-MRSA (2).

Isolates were tested for the presence of the *mecA* cassette (SCCmec) by Polymerase Chain Reaction (PCR) for confirmation of MRSA. The isolates were also tested for the presence of the PLVT gene, and for the type of SCC**mec** cassette each isolate was carrying. To isolate bacterial DNA, the isolates were grown overnight at 37˚C in TSB. Cells were pelleted, washed, and subjected to DNA isolation as per instructions of the manufacturer (DNeasy Blood & Tissue Kit, Qiagen, Germantown, MD). DNA concentration of each isolate sample was determined and subjected to PCR using well-established primers for *mecA* and *PLVT* (6, 7). Multiplex PCR was performed to determine the type of SCCmec present in the isolates (8). The data was analyzed and graphed using Microsoft Office Program.

RESULTS

Out of 5,497 isolates, 617 were identified as *Staphylococcus aureus*; of which 322 (52%) were identified as MRSA based on the presence of the mecA gene. Approximately 50% of the MRSA positive samples prevalence lied primarily within the 40-64 years old age bracket, followed by the patients of less than 40 years (27%) and above 64 years (23%) of age groups, infants had contributed about 1% of total MRSA isolates.

The antibiogram demonstrated decreased susceptibility to erythromycin, penicillin, and levofloxacin. A majority of the isolates were susceptible to rifampicin, gentamycin, tetracycline and co-trimoxazole. Oxacillin was included as a control for testing methicillin resistance. All tested isolates were susceptible to vancomycin (Figure 1).

Figure 2 is a graphical presentation, and demonstrates findings of molecular characterization of the isolates. The isolates were positive for *mecA* gene. We determined that 283 (87.6%) of the tested MRSA isolates were PVL positive. SCC*mec* cassettes data demonstrated that none of the isolates contained a type I or type III cassette and 126 (39.1%) had a type II cassette, the

Figure 1. Antibiogram of MRSA isolates. The isolates demonstrated relatively low susceptibility to erythromycin, penicillin and levofloxacin. Isolates were prominently less resistant to tetracycline, gentamycin, rifampicin and bactrin. All isolates tested were notably susceptible to vancomycin.

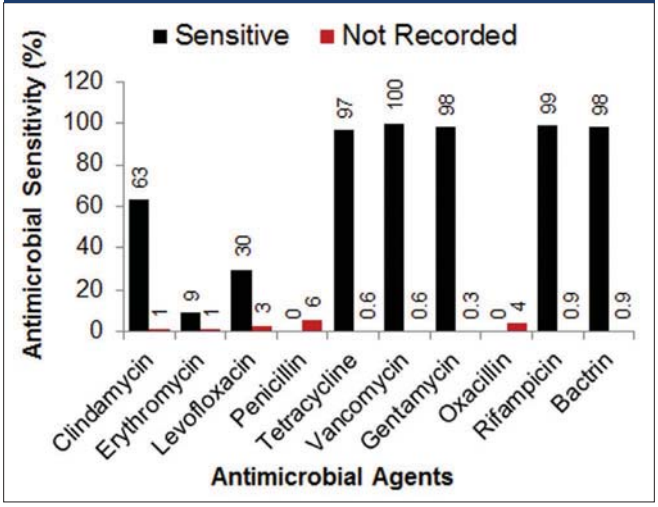
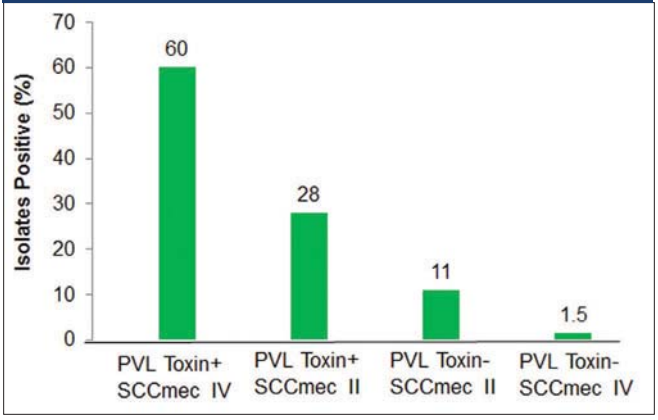


Figure 2. Genetic characterization of MRSA isolates. About 87% and 61% of MRSA isolates were positive for *PVL* toxin gene and SCC*mec* Cassett Type IV, respectively, indicating that vast majority of isolates were having molecular features of community-acquired MRSA (CA-MRSA). The majority of the CA-MRSA isolates were PVL toxin-producers.



prototypical cassettes contained in HA-MRSA. The majority of the isolates (198 of 322; 61.5%) from this study tested positive for type IV, the standard cassette found in CA-MRSA.

In order to correlate isolates of different cassettes types with the presence of PVL toxin gene, we used PCR analysis, and the showed that only 1.5% of the SCC*mec* type IV isolates and 11% of the type II cassette isolates were PVL toxin negative. Sixty percent (193 of 322) of the tested isolates were positive for both SCC*mec* type IV and the PVL toxin while 28% (90 of 322) of the SCCmec isolates that were identified as type II were positive for the PVL toxin. Taken together, this data suggests that this sampling pool is mostly type IV SCC*mec* and PVL positive,

Return to TABLE OF CONTENTS

indicating that a majority of these isolates are having molecular features of community-acquired MRSA.

DISCUSSION

The situation that CA-MRSA would exceed the prevalence of HA-MRSA at this hospital is possible since horizontal spread allows for the simple transfer of resistance markers between microbes (1, 9). The SCC*mec* cassette characteristic of CA-MRSA and encoded with this cassette is the PVL toxin, suggesting that the presence of the leucocyte-toxic gene is favored.

Ninety percent of the isolates tested showed resistance to erythromycin, but only 34% were resistant to clindamycin, suggesting inducible clindamycin resistance. Previous work in our lab from 2008 suggested an increase in prevalence of clindamycin resistance from 2008 to 2011 (10). Interestingly, we found that, when comparing isolates from 2008 to 2011, there was a significant increase in susceptibility to tetracycline, gentamycin, and rifampicin. Although we did not use antibiogram as criteria to differentiate CA-MRSA from HA-MRSA, this finding supports our assumption that CA-MRSA prevalence is on the rise at our hospital as this particular type tends to be more susceptible to non-beta lactams.

The rapid emergence and spread of CA-MRSA also has a negative implication of how easily it can transpose the type IV cassette, and suggests that isolates could easily pick up resistance markers from other strains in the environment (4). MRSA isolates that contain the PVL toxin destroy leucocytes and skin and mucous membrane epithelium and confer increased virulence that can lead to life-threatening infections such as necrotizing hemorrhagic pneumonia with very high mortality rates (3). Therefore, the rates of PVL-positivity in MRSA of 87%, as in this study, are alarming for infectious disease specialists and infection control practitioners.

In conclusion, we suggest that the increased prevalence of CA-MRSA might be due to the small size of the IV cassette; however, there could be other mechanisms at play giving it a genetic advantage. This is one of the rare report detecting PVL toxin gene in majority of the MRSA isolates.

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