Characterization of capsule expression of both MSSA/MRSA isolates from various body sites from colonization and infections cases

Abstract

Background: Staphylococcus aureus is a major cause of nosocomial and community-acquired infections. Despite the predominance of two capsular polysaccharides, types 5 and 8, on the surface of clinical isolates, molecular and epidemiological variations have been reported among various geographical areas in addition to various clinical outcomes of S. aureus increasing the challenge for preventive and control measurements against staphylococcal infections and diseases.

Materials and Methods: A total of 315 S. aureus isolates of various clinical conditions comprised both hospital and community-acquired infections between 2009 and 2011 in Amman and Gaza were studied. All S. aureus clinical isolates included in the present study have been investigated by PCR method to determine the distribution and diversity of capsule polysaccharide expression.

Results: Most of the clinical isolates (90%) expressed either capsular polysaccharide genotypes CP5 (37%) and CP8 (53%), whereas 10% were nontypeable by PCR. Significant predominance of CP8 genotype was reported in clinical isolates of S. aureus from infections in comparison to those which are commonly colonising various normal body sites.
Introduction

Staphylococcus aureus continues to be a major pathogen causing a wide spectrum of infections with high morbidity and mortality (1). Moreover, increased concern on this pathogen emerged from its resistance against virtually all antimicrobial agents available in hospitals and communities (2). The adaptation of S. aureus to the environment has been marked by the acquisition of methicillin-resistant S. aureus (MRSA) and the emergence of multidrug resistance, making the new strategies to manage staphylococcal infections of major importance in clinical practice. Our understanding of virulence and epidemiological behaviour of this species has been greatly enhanced by sequencing of the genomes of several S. aureus strains. For example, the identification of new non-protein coding RNA genes and the presence of mutations within several metabolic genes have been shown to be involved in phenotypic switching and antibiotic resistance of S. aureus (3,4).

S. aureus is equipped with various virulence factors, including surface-associated adhesins, cytotoxins, superantigens, exoenzymes, and capsular polysaccharides, which contribute to the pathogenesis of staphylococcal infections (5). The capsular polysaccharide is a cell wall bacterial component that protects bacterium from phagocytosis and enhances microbial virulence (6). Approximately 90% of S. aureus isolates produce one of 11 capsular polysaccharides, nevertheless, only two types, type 5 and type 8, are clinically relevant since they are predominant among clinical infection isolates of various geographic origins (7-9). The importance and relevance of these capsule types and their role in septic shock were confirmed by the development of a conjugate vaccine, StaphVAX study (10). In addition, the formation of biofilms in S. aureus is enhanced by augmenting adhesiveness due to both intercellular polysaccharide adhesion and capsular polysaccharides (11).

The emergence of resistance to antibiotics through the formation of permeability barrier to antimicrobial agents has been reported in S. aureus and other bacteria (12,13). For example, it has been shown that vancomycin-intermediate resistant S. aureus (VISA) strains produce a distinctly thickened cell wall (14). Few studies were carried out to investigate the potential role of S. aureus polysaccharide capsule as a permeability barrier to several antimicrobial agents (15-16). Generally, although the production of thick capsules in certain serotypes of S. aureus showed a permeability barrier potential to common biocides and affected the susceptibility of S. aureus to some antibiotics, the results are still controversial. Due to the lack of epidemiological and clinical...
data of capsule genotypes of *S. aureus*, this study is conducted to characterize the capsule genotypes of *S. aureus* isolates from diverse clinical diseases and normal colonization in the Middle East region. Capsular genotypes were tested by PCR method using primers specific to type 5 and type 8 capsules. Moreover, data analysis was conducted to determine the relative prevalence of capsule genotypes and their relationship to the nature of disease and/or susceptibility to antibiotics in *S. aureus*.

**Materials and Methods**

**Collection and characterization of clinical isolates**

In the present study, a total of 315 clinical isolates of *S. aureus* were collected between 2009 and 2011 from three hospitals in both Amman (Jordan university hospital, Islamic hospital) and Gaza (Shifa hospital). Isolation and identification of *S. aureus* was performed on the basis of routine procedures used in the clinical microbiology laboratory. Subcultures of single colonies of homogeneous size and pigmentation from primary isolation agar plates were performed with Trypticase soy agar and Mannitol salt agar. Homogeneous colonies from subculture plates were frozen in brain heart infusion-glycerol (20%) until further use. Presumptive identification of *S. aureus* was based on colony morphology, Gram staining, catalase test, coagulase activity on rabbit plasma. All isolates were confirmed as *S. aureus* by the presence of the encoding thermonuclease gene (*nuc* gene) (17). Amplification of this gene was also used as a quality control for DNA extraction of all study isolates (18). The genomic DNA was extracted from an overnight culture of *S. aureus* using E.Z.N.A.® Bacterial DNA Kit Omega bio-tik (Norcross, GA, USA). Susceptibility to methicillin was determined according to CLSI recommendation using the standard cefoxitin (30 μg) disk diffusion method. Suggestive susceptibility testing results of methicillin-resistant *S. aureus* was subsequently confirmed by *mecA*-specific PCR (18).

**Detection of capsular genotype by PCR method**

Genomic DNA of *S. aureus* was used as a template for PCR amplification with primers Cap5 F (5’-GAA AGT GAA CGA TTA GTA GAA – 3’) and Cap5 R (5’-GTA CGA AGC GTT TTG ATA GTT-3’) for the gene of CP5 and Cap8 F (5’- GTG GGA TTT TTG TAG CTT TT – 3’) and Cap8 R (5’- CGC CTC GCT ATA TGA ACT AT -3’) for the gene of CP8. Amplification was carried out on a PE-9600 thermocycler (Perkin-ElmerCorp., Norwalk, CT) under the following conditions: an initial 10-min denaturation step at 94°C, followed by 30 cycles of 30 s of denaturation at 94°C, 30 s of annealing at 45°C, and 2 min of extension at 72°C; with a final extension step at 72°C for 5 min. PCR products were analysed by electrophoresis on ethidium bromide-stained 2% agarose gels. The sizes of the amplicons were 532bp for CP5 and 437bp for CP8, respectively (20).

**Statistical analysis**

Chi-squared test was used to compare distribution of capsular polysaccharide types 5 and 8 and non-typeable strains among different groups using SPSS software version 17.0 (SPSS, Inc., Chicago, IL). P values <0.05 were considered significant.

**Results**

A total of 315 clinical *S. aureus* isolates from different hospitals in the region were analysed for the genetic and epidemiologic characteristics of the capsule expression specifically for the genotypes 5 and 8 of which most the human *S. aureus* isolates belong to. These isolates were obtained from various
body sites representing different clinical outcomes associated with \textit{S. aureus} infections. Among these strains, 225 (71%) were methicillin-sensitive \textit{S. aureus} (MSSA) and 90 were MRSA (29%). The isolates were categorized as symptomatic infection associated 183 (58%) and 132 (42%) asymptomatic carriers or colonization. The proportions of the strains in regards to their origin were closely distributed with very few samples from cases of pneumonia (3.5%) and bacteremia (5.7%).

The molecular characterization of the isolates revealed that the majority of the clinical strains (90%) are harbouring capsule genes compared to only 10% of the strains that did not show the presence of positive capsule gene by PCR.CP5 comprised 37% of the cases while 53% of all of the isolates were of CP 8 (Fig 1). The predominance of CP8 was observed in both the infection and colonization groups. Interestingly, the percentage of the nontypeable (NT) strains was significantly higher in the colonization group (19%) in comparison to the infection group (3%) (Fig 2).

The analysis of the variation of \textit{S. aureus} capsule genotypes among clinical isolates also showed that among the MRSA isolates, 48% of the isolates were CP8 and 46% were CP5. On the other hand, 55% and 34% of the MSSA strains were CP8 and CP5 respectively (Table 1). The statistical analysis of the capsule genotype distribution among MSSA/MRSA strains showed that no significant differences were reported (P> 0.05) and no correlation could be established between the capsule genotypes and the resistance to methicillin.

In relation to the body site and type of lesions, there was also predominance of CP8 in most of the specimens obtained from different types of infection with no significant differences between the genotypes except for genito-urinary specimens, for which CP5 comprised 75% of cases and 25% for CP8 (P<0.05). The highest prevalence of NT isolates was observed in stool samples (22%) and nasal samples (17%) which composed mostly the samples from colonization cases (Table 2).

\textbf{Figure 1}. Distribution of capsule genotypes among \textit{S. aureus} clinical isolates. Capsule genotype 8 (CP8) was significantly higher than genotype 5 (CP5) (P < 0.05). NT: non typeable isolates.
Figure 2. Comparative analysis of the capsule genotypes of S. aureus in relation to colonization or infection. Significant correlation between capsule genotypes and clinical and colonising isolates (P < 0.05). Infection with S. aureus tends to be typeable while colonization is not.

Table 1. Distribution of capsule genotypes among 315 clinical and colonization isolates of MSSA/MRSA

<table>
<thead>
<tr>
<th>Clinical picture</th>
<th>No. of isolates</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MSSA</td>
<td>MRSA</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>Cap5</td>
</tr>
<tr>
<td>Infection</td>
<td>110</td>
<td>37</td>
</tr>
<tr>
<td>Colonization</td>
<td>115</td>
<td>43</td>
</tr>
<tr>
<td>Total</td>
<td>225</td>
<td>80</td>
</tr>
</tbody>
</table>

* NT; nontypeable, NS: not significant

Table 2. Encapsulated and NT strains of S. aureus isolates from different body sites.

<table>
<thead>
<tr>
<th>Sample source</th>
<th>No. (%) of isolates</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Cap5</td>
</tr>
<tr>
<td>Skin and soft tissues</td>
<td>59 (18.7)</td>
<td>19 (32)</td>
</tr>
<tr>
<td>Nasal</td>
<td>83 (26.3)</td>
<td>28 (34)</td>
</tr>
<tr>
<td>Pus</td>
<td>70 (22.3)</td>
<td>25 (36)</td>
</tr>
<tr>
<td>Blood</td>
<td>18 (5.7)</td>
<td>5 (28)</td>
</tr>
<tr>
<td>Respiratory</td>
<td>11 (3.5)</td>
<td>4 (36)</td>
</tr>
<tr>
<td>*Genitourinary</td>
<td>24 (7.6)</td>
<td>18 (75)</td>
</tr>
<tr>
<td>Stool</td>
<td>50 (15.9)</td>
<td>18 (36)</td>
</tr>
<tr>
<td>Total</td>
<td>315</td>
<td>117</td>
</tr>
</tbody>
</table>

* Genitourinary isolates showed significantly higher rates of CP5 than CP8. In cases of Bacteremia, CP8 was significantly higher (P < 0.05)
Discussion

Recently, researchers have focused their efforts on understanding the molecular mechanisms of virulence of *S. aureus* as an integral part in controlling the dramatic expansion of this pathogenic bacterium and its associated diseases. One of the recent measures regarding this issue is the investigation of the polymorphism of capsule genotypes which have been linked to higher infectious behaviour and host and geographical variations(21,22). This study was conducted to characterize the genotypes of capsule polysaccharide expression of *S. aureus* isolates from diverse clinical conditions associated with this bacterium in the Middle East area. The isolates represented various body site infections and colonization with approximately 29% of the isolates were MRSA confirmed by meca PCR test. In this study, where *S. aureus* is currently the most common pathogen encountered in both community and hospital acquired infections in the region, capsule typing has been performed using molecular based techniques which classified the strains into cap5 genotype, cap 8 genotype and non typeable (NT) genotypes. Our preliminary data reported higher prevalence of capsule harbouring isolates with higher prevalence of CP8 genotypes in both infection and colonizing strains. This is in accordance with most of the studies conducted in different geographical regions where the majority (80%) of the strains that were isolated from human diseases belong to cap5 and cap8 genotypes.In addition, the present study showed a higher prevalence of NT genotypes in the colonization group (19%) compared to clinical infection group (3%).The highest prevalence of NT isolates was observed in samples obtained from colonization cases particularly those from nasal and stool samples. Several studies have been conducted to investigate the virulence potential of NT strains in causing *S. aureus* infections in animals. A study reported that *S. aureus* lacking a capsule was able to persist in the murine mammary gland and the loss of CP5 or CP8 expression may enhance the persistence of staphylococci in the mammary glands of chronically infected hosts(23). In another study, the prevalence of NT strains in bovine mastitis was dramatically higher (86.3%) than CP5 and CP8(24). In contrast, a recent study conducted in the same region reported higher prevalence of CP5 and CP8 among bovine mastitis cases(25). Moreover, it has been reported that NT strains of *S. aureus* were more frequently isolated from patients with chronic osteomyelitis than from those with acute osteomyelitis (9). Differences in proportions of NT and CP5 and CP8 could be explained to the time frame of the studies, and in addition to possible role of the clonal variation among *S. aureus* clinical isolates. Regarding the association of the capsule genotype variation and MRSA/MSSA phenotypes and genotypes, our results reported no statistically significant differences among the CP genotypes of MRSA/MSSA groups, and the distribution of CP genotypes is due to the variation in the sample sources. This is also in agreement with the previously published data(26).In a study conducted by Na’was et al. 43% and 45% of the studied strains (238 MSSA and 16 MRSA)were CP5 and CP8 respectively (27). On the other hand, a study conducted by Verdier et al., 2007 reported strong statistical association between MSSA and cap 8 genotype among a total of 195 *S. aureus* clinical isolates tested (126 MSSA and 69 MRSA)(6). Our results could be explained to the diversity of the bacteria isolates among with in each geographical area. Further molecular characterization using MLST and spa typing should be performed to elucidate whether the lack of association between capsule genotypes and resistance to methicillin is due to the clonal variation among *S. aureus* isolates included in our study. One interesting finding of our study is the higher prevalence of CP5 compared to CP8 in genitourinary tract samples for which more than 75% of
the cases were CP5(< 0.05). We could not explain this result experimentally, however, it could be due to the wider variation of the capsule structure of CP5 and virulence and spectrum of infections associated with the reported capsule genotype variations. In a study conducted by Watts et al., 2005 CP5 and CP8 differed in a number of biological properties, contributed to the relative virulence of CP5 and CP8 in vivo(28). Strains with CP5 showed significantly higher bacteremia in mice and higher in vitro and opsonophagocytic killing. In the light of our findings, CP5 could be targeted as a useful choice in controlling and minimizing the outbreaks of S. aureus infections in neonates and premature infants and reducing the rate of colonization of the pregnancies.

In conclusion, this study shows that the prevalence of S. aureus CP5 and CP8 is very high (90%; P < 0.05) in two Middle East Arab regions. Together with the previously published studies, our results indicate that there are remarkable differences in the prevalence of CP5 and CP8 among strains of S.aureus from different clinical conditions regardless of their nature of resistance to methicillin. Since the vaccination of S. aureus is currently implemented in several countries, our results highlighted the potential benefits of using S. aureus CP5 and CP8 in the vaccine preparation because of the predominance of capsulated strains in our Jordanian area. Further studies are required to investigate the role of S. aureus capsule genotype variation in the virulence and pathogenesis of this highly and commonly human pathogenic microorganism.
References


