The Prevalence of ACME-arcA and PVL Genes Among Staphylococcus Aureus Isolates in a Student Population from North-West of Iran

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Research note

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Abstract

Objectives: Staphylococcus aureus (S. aureus) is the most prevalent cause of skin infections, especially in colonized individuals. Panton–Valentine leukocidin (PVL) and Arginine catabolic mobile element (ACME) are known as the most common virulence factors of S. aureus. This cross-sectional study was conducted to examine the prevalence of ACME-arcA and PVL genes among S.aureus isolates in the student population. Nasal swab samples were randomly collected from 400 healthy students from Tabriz, Iran. The antibiotic resistance pattern of S.aureus isolates was examined by the disk diffusion method. The presence of ACME-arcA, PVL, and mecA genes was detected by PCR reaction.

Results: overall, 15% (60/400) students were nasal carriage of S. aureus and 2.75 % (11/400) were MRSA carriage. The frequency of mecA, ACME-arcA, and PVL genes was 54.54% (36/60), 46.66% (28/60), and 16.66% (10/60) respectively. The prevalence of ACME-arcA and PVL genes was independent of gender ($P =0.142$, $P=0.337$, respectively). A notable association was observed between the existence of ACME-arcA gene and the frequency of mecA gene ($P<0.05$), while the incidence of PVL was independent on mecA. These findings highlight the necessity of monitoring nasal carriers in a healthy community to prevent subsequent infections.

Introduction

Staphylococcus aureus (S. aureus) is the most prevalent cause of skin infections, especially in colonized individuals [1–3]. The S. aureus colonization is not often the leading cause of infection, but may act as the main reservoir of clinical infections in the carriage persons [4]. Based on reports, S.aureus colonization occurs in 30 to 50% of the healthy population[5, 6].

At present, the incidence of Methicillin- resistant S. aureus (MRSA) strains has become problematic in the clinical settings. The resistance to methicillin and beta-lactam antibiotics is related to the existence of mecA, a gene encoding low-affinity penicillin -binding protein (PBP2a) [7]. Although, MRSA colonization is considered as the leading cause of subsequent infections, the escalating MRSA is related to the occurrence of factors promoting colonization such as Panton–Valentine leukocidin (PVL) and arginine catabolic mobile element (ACME) [8]. In this respect, S.aureus pathogenesis is dependent on virulence factors such as PVL and ACME that facilitate adherence and attachment of pathogen to host cells [9].

ACME was first identified downstream of the cassette chromosome mec (SCCmec) type IVa in MRSA USA300 strain as the ACME-SCCmec composite island [10]. This gene is known as a factor enhancing colonization of S.aureus in the skin and mucous membranes, which act through neutralization of acidic pH and enhancement of the acid tolerance of pathogen. As regards, antimicrobial fatty acids and low pH can protect human skin against bacterial pathogens [11]. Moreover, PVL as a pore -forming toxin plays a crucial role in the occurrence of skin and soft tissue infections in the community- associated (CA-MRSA) stains so that it is known as a diagnostic marker of community- acquired (CA) strains[12].
This study was aimed to examine the frequency of ACME-arcA and PVL genes in the nasal carriage of S.aureus among students.

**Methods**

**Bacteria Identification**

A total of 400 students aged 16 to 17 years from high schools (mean age: 16.5) of Tabriz city e participated in a cross-sectional study from January 1, 2018 to March 1, 2018. The healthy students without previous antibiotic consumption (during the last three months) were included in this study. The nasal swab samples were randomly obtained from students and transferred into tryptic soy broth media and incubated overnight at 37 °C. The S. aureus isolates were recognized via conventional biochemical and microbiological[13].

**Antibiotic Susceptibility Testing**

The antibiotic resistance pattern of S.aureus isolates was examined using the disk diffusion method based on the Clinical Laboratory Standards Institute (CLSI) guidelines [14]. Antibiotic disks used were including amoxicillin/clavulanic acid (20/10 µg), chloramphenicol (30 µg), cefazolin (30 µg), penicillin (6 µg), erythromycin (15 µg), novobiocin(5 µg), oxacillin(1 µg), clindamycin(2 µg), ciprofloxacin(5 µg), cefoxitin(30 µg). The antibiotic discs were produced by Biomaxima, Poland. Cefoxitin (30 µg). To perform antibiotic susceptibility tests, the bacterial concentrations 0.5 McFarland were used for the inoculation of Muller-Hinton agar plates. The inoculated plates containing the antibiotic disks were incubated overnight at 37 °C.

**Primer Designing**

The design of specific primers for ACME-arcA, PVL, and meca genes were performed on the S. aureus genome sequence available in the Gene Bank database using Gene Runner software. Primer-BLAST was used to confirm the specificity of designed primers.

**PCR amplification for detection of meca, ACME-arcA and PVL positive genes**

The bacterial DNA was extracted from isolates by the boiling method through TE buffer (10 mM Tris, 1 mM EDTA). The quality and quantity of DNA were assessed by the ratio of the absorbance at 260 nm and 280 nm wavelength. After that, PCR reaction was carried out to detect meca, ACME-arcA and PVL genes using specific primers (Table 1) in a 25-µL reaction for 35 cycles (94 °C for 1 min, 49 °C for 1 min, 72 °C for 1 min) after an initial denaturation at 94 °C for 4 min. The final extension was carried out at
72 °C for 5 min). PCR products were visualized by %1 agarose gel electrophoresis. For further validation, PCR products were analyzed by sequencing.

| Primer | Primer sequence (5´→3´) | Tm°C | Cycle no. | Size(bp) |
|--------|--------------------------|------|-----------|----------|
| mecA   | F: 5′- AGAAATGACTGAACGTCC − 3 ′ | 49   | 35        | 305      |
|        | R: 5 ′- ATTCCACATTGTTTCGGTC - 3 ′ |      |           |          |
| ACME-arcA | F: 5′-CTAGGTGCATAAATGTACGTG -3 | 49   | 35        | 577      |
|        | R: 5- CCAGAAGTACGCGAGAAC − 3′ |      |           |          |
| PVL    | F: 5-AGGTAAATGTCTGGACATG-3 |      |           | 427      |

Statistical analysis:

Statistical analysis was performed by SPSS version 16. Demographic and clinical variables were compared by Chi-square test (p < 0.05).

Results:

Antimicrobial Susceptibility

Out of 400 students, 60 (15%) S. aureus strains were isolated that 9.5% (38/400) of the isolates were related to male students and 5.5% (22/400) of the isolates were from female students. Based on statistical analysis results, the prevalence of S.aureus among students was dependent on gender (p = 0.025, p < 0.05) (Table 2). Also, the highest resistance rate was against penicillin antibiotics (98.33%). Totally, 31.66% (19/60) of the S. aureus isolates were multiple drug resistance (MDR) based on resistance to 3 or more classes of antibiotics and 18.33% (11/60) of the isolates were resistant to methicillin (MRSA nasal carriage) which overall 2.75% (11/400) of the students were MRSA nasal carriage (Fig. 1).

Table 2
The frequency of MRSA isolates and the mecA, ACME-arcA, and PVL genes in a student population.

| Study group (n = 400) | S.aureus | MRSA | mecA | ACME-arcA | PVL | ACME/PVL |
|----------------------|----------|------|------|-----------|-----|----------|
| Male (n = 200)       | 38       | 6    | 19   | 15        | 5   | 4        |
| Female (n = 200)     | 22       | 5    | 17   | 13        | 5   | 3        |
| p.value              | P = 0.025 | P = 0.503 | P = 0.68 | P = 0.142 | P = 0.337 | P = 0.717 |
Identification of *mecA*, *ACME-arcA* and *PVL* positive isolates

Based on PCR results, the *mecA* gene fragment was revealed as a single band of 305 bp in 54.54% (36/60) of the isolates (Figure S1A). *ACME-arcA* gene was identified in 46.66% (28/60) of the isolates as an expected band of 577 bp (Figure S1B). Also, results indicated that 16.66% (10/60) cases were positive for the *PVL* gene (Figure S1C), which among them 11.66% (7/60) of the isolates was positive for both *PVL* and *ACME-arcA* genes. A significant correlation was observed between the presence of the *ACME-arcA* gene and resistance to methicillin (*p* < 0.05), while 90% (9/10) of the *PVL* positive isolates were sensitive to methicillin (*p* < 0.05). According to statistical analysis, the prevalence rate of *ACME-arcA* and *PVL* genes among *S. aureus* isolates was independent gender (*P* = 0.142, *P* = 0.337, respectively) (Table 2).

Sequencing analysis

For confirmation of the accuracy of PCR, *ACME-arcA/PVL* positive isolates were sequenced and then analyzed by NCBI BLAST. Based on results, *ACME-arcA/PVL* positive isolates with 99% identity were related to USA300 strain (Sequence ID: CP027476.1).

Discussion:

In this study, we first surveyed the MRSA carriage rate (2.75%) in healthy students in North West of Iran, which results were indicating a higher rate of MRSA in student than children (1.3%) in IRAN[15].

The outbreak of MRSA in this study was similar to a study performed in Belgium, which 2.1% of non-hospitalized patients were MRSA carriage (16). Our results were almost identical to the MRSA rate in health care workers, 3.4% (7/204) from Western Nepal [17].

However, the MRSA carriage rate in our region was less than the results obtained from farmworkers (8.7%) in Turkey [18].

In addition to, the high resistance to penicillin and ampicillin, the highest resistance rate was observed to amoxicillin/clavulanic acid (33.84%), cefoxitin (18.40%) and erythromycin (16.44%), respectively. These results were not consistent with a similar study done in Nigeria that 25% of cases were resistant to amoxicillin-clavulanic acid and 23% to erythromycin [19]. The difference observed between resistance rate to cefoxitin (11/60), and frequency of *mecA* (36/60) was related to the spread of the cefoxitin/oxacillin susceptible *mecA* positive OS-MRSA isolates in healthy population consistent with previous studies [20, 21].

Based on results, the frequency of the MRSA colonization in this study was dependent on gender consistent with research carried out by Humphreys H, in 2015 regarding higher prevalence of MRSA carriage in men [22]. In a similar survey done in 2019, the outbreak of MRSA in males was more than
female[23]. In the present study, 31.66% of the isolates were positive MDR, similar to a research done in Kashan, IRAN, with a prevalence of 29.3% MDR [24].

According to our findings, 46.66% of the isolates were positive for the ACME-arcA gene, and 16.66% % of the cases were positive for the PVL gene. In this respect, 11.66% of the PVL positive isolates were positive for the ACME-arcA gene. These findings were not consistent with a study done in central IRAN, with a prevalence rate of 17% and 20% for ACME–arcA and PVL genes, respectively [25]. Consistent with the previous researches[26], in this study, there is a significant relationship between the presence of ACME-arcA gene and the frequency of mecA positive strains (MRSA). In contrast 85.71% PVL positive isolates were MSSA indicating lack of association between the occurrence of PVL and rate of MRSA.

**Conclusion:**

The results of this study is indicating the prevalence of ACME positive MRSA strains on a healthy population as the leading cause of skin infections; hence there is an essential need for continuous monitoring of nasal carriers in a healthy community to prevent subsequent infections.

**Limitations:**

However, there were limitations to our study. First, the period of the study was short. Moreover, samples were only obtained from students of high school. Despite these limitations, the incidence of PVL/ACME-arcA positive MRSA isolates indicates the necessity of control of MRSA colonization in a healthy population.

**Abbreviations**

MRSA: methicillin resistant *Staphylococcus (S.) aureus*; PVL: Panton–Valentine leukocidin; ACME: Arginine catabolic mobile element; MDR: multi-drug resistant.

**Declarations**

**Ethics Committee Approval**

Tabriz University of Clinical Research Ethics Committee, (reference number: IR. TBZMED. REC.1398.448). The swab samples were obtained after written consent with a brief description about the importance of the study to the participants.

**Consent for publication**

Not applicable.
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Author Contributions
Concept – LR, AD; Design – LR; Supervision – AD, LR, BN; Data Collection and, or Processing – RKh, AG; Analysis and, or Interpretation – AD, BN.

Conflict of Interest
The authors have no conflict of interest.

Availability of data and materials
All data generated or analyzed during this study are included in this published article.

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References
1. Li M, Cheung GY, Hu J, Wang D, Joo HS, DeLeo FR, et al. Comparative analysis of virulence and toxin expression of global community-associated methicillin-resistant Staphylococcus aureus strains. Journal of Infectious Diseases 2010;202(12):1866-76.
2. Lin YC, Peterson ML. New insights into the prevention of staphylococcal infections and toxic shock syndrome. Expert review of clinical pharmacology 2010;3(6):753-67.
3. Klein S, Menz MD, Zanger P, Heeg K, Nurjadi D. Increase in the prevalence of Panton-Valentine leukocidin and clonal shift in community-onset methicillin-resistant Staphylococcus aureus causing skin and soft-tissue infections in the Rhine-Neckar Region, Germany, 2012-2016. International journal of antimicrobial agents 2019;53(3):261-7.
4. Davoodabadi F, Mobasherizadeh S, Mostafavizadeh K, Shojaei H, Havaei SA, Koushki AM, et al. Nasal colonization in children with community acquired methicillin-resistant Staphylococcus aureus. Advanced biomedical research 2016;5.
5. Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler VG. Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management. Clinical microbiology
6. Boswihi SS, Udo EE. Methicillin-resistant Staphylococcus aureus: An update on the epidemiology, treatment options and infection control. Current Medicine Research and Practice 2018;8(1):18-24.

7. Milheiriço, Catarina, Hermínia, Tomasz A. MRSA strains carrying the novel mecC gene: full genome sequencing identifies in the genetic background several determinants that modulate the resistant phenotype. Antimicrobial Agents and Chemotherapy 2017;AAC-02500.

8. Hoppe PA, Hanitsch LG, Leistner R, Niebank M, B++hrrer C, von Bernuth H, et al. Periorbital infections and conjunctivitis due to Panton-Valentine Leukocidin (PVL) positive Staphylococcus aureus in children. BMC infectious diseases 2018;18(1):371.

9. Kong EF, Johnson JK, Jabra-Rizk MA. Community-associated methicillin-resistant Staphylococcus aureus: an Enemy amidst Us. PLoS pathogens 2016;12(10):e1005837.

10. Thurlow LR, Joshi GS, Clark JR, Spontak JS, Neely CJ, Maile R, et al. Functional modularity of the arginine catabolic mobile element contributes to the success of USA300 methicillin-resistant Staphylococcus aureus. Cell host & microbe 2013;13(1):100-7.

11. Sabat AJ, Ilczyszyn WM, van Rijen M, Akkerboom V, Sinha B, Kluythmans J, et al. Genome-wide analysis reveals two novel mosaic regions containing an ACME with an identical DNA sequence in the MRSA ST398-t011 and MSSA ST8-t008 isolates. Journal of Antimicrobial Chemotherapy 2015;70(5):1298-302.

12. Niemann S, Bertling A, Brodde MF, Fender AC, Van de Vyver H+, Hussain M, et al. Panton-Valentine Leukocidin associated with S. aureus osteomyelitis activates platelets via neutrophil secretion products. Scientific reports 2018;8(1):2185.

13. Koosha RZ, Hosseini HM, Aghdam EM, Tajandareh SG, Fooladi AAI. Distribution of tsst-1 and mecA Genes in Staphylococcus aureus isolated from clinical specimens. Jundishapur journal of microbiology 2016;9(3).

14. Wayne PA. Clinical and laboratory standards institute. Performance standards for antimicrobial susceptibility testing. 2011.

15. Nikfar R, Shamsizadeh A, Kajbaf TZ, Panah MK, Khaghani S, Moghddam M. Frequency of methicillin-resistant Staphylococcus aureus nasal carriage in healthy children. Iranian journal of microbiology 2015;7(2):67.

16. den Heijer CD, van Bijnen EM, Paget WJ, Pringle M, Goossens H, Bruggeman CA, et al. Prevalence and resistance of commensal Staphylococcus aureus, including meticillin-resistant S aureus, in nine European countries: a cross-sectional study. The Lancet infectious diseases 2013;13(5):409-15.

17. Khanal R, Sah P, Lamichhane P, Lamsal A, Upadhaya S, Pahwa VK. Nasal carriage of methicillin resistant Staphylococcus aureus among health care workers at a tertiary care hospital in Western Nepal. Antimicrobial resistance and infection control 2015;4(1):39.

18. Garipcin M, Seker E. Nasal carriage of methicillin-resistant Staphylococcus aureus in cattle and farm workers in Turkey. Veterinarski arhiv 2015;85(2):117-29.
19. Ugwu MC, Anie CO, Ibezim EC, Esimone CO. Antimicrobial evaluation of methicillin-resistant Staphylococcus aureus nasal carriage amongst healthy students in Agbor, Delta State, Nigeria. Arch Clin Microbiol 2016;7(2):1-4.

20. Zeinalpour Ahrabi S, Rahbarnia L, Dehnad A, Naghili B, Ghaffari Agdam MH, Nazari A. Incidence of Oxacillin-Susceptible mecA-Positive Staphylococcus aureus (OS-MRSA) Isolates and TSST-1 Virulence Factor Among High School Students in Tabriz, Northwest of Iran. Archives of Clinical Infectious Diseases14(4).

21. Saeed K, Ahmad N, Dryden M, Cortes N, Marsh P, Sitjar A, et al. Oxacillin-susceptible methicillin-resistant Staphylococcus aureus (OS-MRSA), a hidden resistant mechanism among clinically significant isolates in the Wessex region/UK. Infection 2014;42(5):843-7.

22. Humphreys H, Fitzpatrick F, Harvey BJ. Gender differences in rates of carriage and bloodstream infection caused by methicillin-resistant Staphylococcus aureus: are they real, do they matter and why? Clinical Infectious Diseases 2015;61(11):1708-14.

23. Garoy EY, Gebreab YB, Achila OO, Tekeste DG, Kesete R, Ghirmay R, et al. Methicillin-Resistant Staphylococcus aureus (MRSA): Prevalence and Antimicrobial Sensitivity Pattern among PatientsGÇöA Multicenter Study in Asmara, Eritrea. Canadian Journal of Infectious Diseases and Medical Microbiology 2019;2019.

24. Erami M, Soltani B, Ardakani AT, Moravveji A, Rezaei MH, Soltani S, et al. Nasal carriage and resistance pattern of multidrug resistant Staphylococcus aureus among healthy children in Kashan, Iran. Iranian Red Crescent Medical Journal 2014;16(9).

25. Fard-Mousavi N, Mosayebi G, Amouzandeh-Nobaveh A, Japouni-Nejad A, Ghaznavi-Rad E. The dynamic of staphylococcus aureus nasal carriage in central Iran. Jundishapur journal of microbiology 2015;8(7).

26. Motamedi H, Abadi SSR, Moosavian SM, Torabi M. The association of Panton-Valentine leukocidin and mecA genes in Methicillin-Resistant Staphylococcus aureus isolates from patients referred to Educational Hospitals in Ahvaz, Iran. Jundishapur journal of microbiology 2015;8(8).

Figures
Figure 1

Antibiotic susceptibility test of the S.aureus isolates in a healthy student population in Tabriz.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- FigureS1.jpg