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 occurres 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* (SCC*mec*) type IVa in MRSA USA300 strain as the *ACME*-SCC*mec* 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 plats. 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-arc*A 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.

Table 1
The sequence of primers used for PCR

Primer	Primer sequence (5´→3´)	TmºC	Cycle no.	Size(bp)
mecA	F:5 - AGAAATGACTGAACGTCC – 3	49	35	305
	R:5 - ATTCCACATTGTTTCGGTC - 3			
ACME-*arc*A	F: 5'- CTAGGTGCATAAATGTACGTG -3	49	35	577
	R: 5- CCAGAAGTACGCGAGAAC – 3'			
PVL	F: 5- AGGTAAAATGTCTGGACATG-3	49	35	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 (FigureS1A). *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 (FigureS1C), 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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Figures

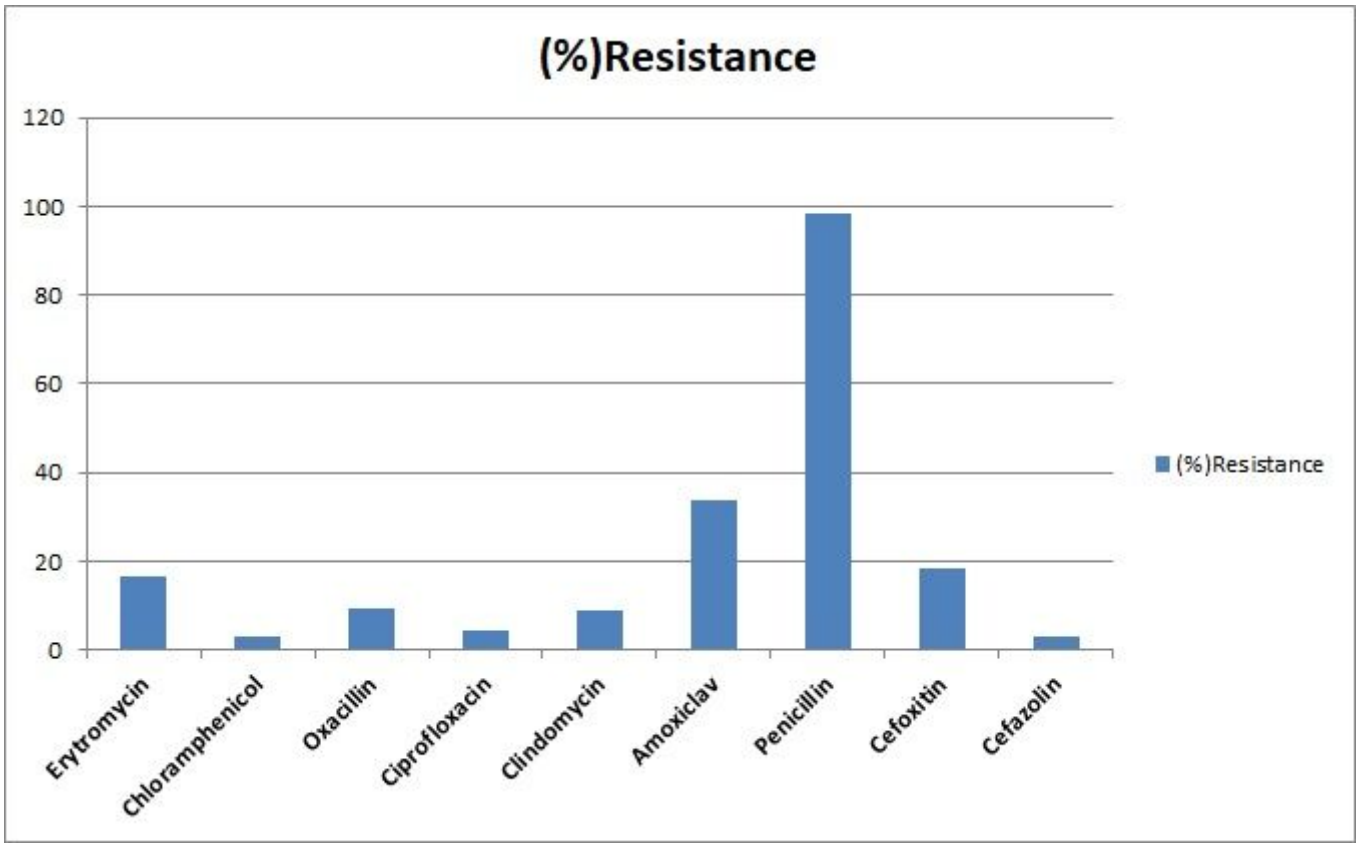

Figure 1

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

Supplementary Files

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- FigureS1.jpg