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VanA and *MecA* Genes in *Staphylococcus aureus* Isolates in North-Eastern Iran

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Abstract

The increasing number of methicillin-resistant *Staphylococcus aureus* (MRSA) infections strains is a global health threat. Vancomycin is one of the very limited options in treating such infections. The emergence of vancomycin-resistant *S. aureus* (VRSA) is therefore a great concern in clinical settings. During recent years, the incidence of vancomycin-intermediate *S. aureus* (VISA) and vancomycin-resistant *S. aureus* has increased in various parts of the world, which have been identified based on criteria defined by the Clinical and Laboratory Standards Institute (CLSI). We have recently shown a high resistance rate to methicillin in *S. aureus* isolates from two main university hospitals in northeastern Iran. Here we expanded the study to reveal the frequency of *vanA* and *mecA* genes in the isolated MRSA strains. We selected 45 MRSA isolates, which were shown phenotypically methicilin- resistant to further genotypic investigation of the *mecA* and *vanA* genes. DNA was extracted from bacterial suspension and *mecA* and *vanA* genes were identified using PCR technique. The majority of MRSA isolates, 42 out of 45 (93%), were positive for the *mecA* gene. None of the MRSA isolates were positive for the *vanA* gene.

The *mecA* gene is frequently circulating among phenotypically identified MRSA isolates, which confirms the phenotypically resistant strains and explains the resistance mechanism. The high frequency of circulating *mecA* gene highlights the need for policies to overcome the MRSA problem in clinical settings. Though none of the isolates showed vancomycin-resistance based on phenotypic tests, we also evaluated the isolates for possible *vanA* gene positivity and none of the isolates were shown to be positive for the *vanA* gene.

Key words: *Staphylococcus aureus*, methicillin, vancomycin, resistance gene

Резюме

Нарастващият брой инфекции с метицилин-резистентни *Staphylococcus aureus* (MRSA) представлява глобален здравен риск. Една от малкото възможности за третиране на тези инфекции е с ванкомицин. Поради това появата на ванкомицин-резистентни *S. aureus* (VRSA) е сериозен проблем в клиничната практика. През последните години в различни части на света се увеличи честотата на щамове *S. aureus* с междинна чувствителност към ванкомицин (VISA) и на ванкомицин-резистентни *S. aureus*, което е доказано на базата на критериите, дефинирани от Института по клинични и лабораторни стандарти (CLSI). Наскоро ние показахме високо ниво на резистентността при *S. aureus*, изолирани в двете основни университетски болници в североизточен Иран. В настоящата работа изследването е разширено с цел доказване на честотата на гените *vanA* и *mecA* при изолираните щамове MRSA. Избрахме 45 изолирани щама MRSA, които фенотипно показват резистентност към метицилин, за по-нататъшно изследване на гените *mecA* и *vanA*. От бактериални суспензии екстрахирахме ДНК и идентифицирахме гените *mecA* и *vanA* чрез метода PCR. Повечето изолати MRSA, 42 от общо 45 (93%) бяха позитивни за гена *mecA*.

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Нито един от изолираните MRSA не беше позитивен за гена *vanA*.

Генът *mecA* често циркулира между изолатите, идентифицирани фенотипно като MRSA, което потвърждава тяхната фенотипна резистентност и обяснява механизма на резистентността. Високата честота на гена *mecA* подчертава необходимостта за политики, насочени към преодоляване на проблема с MRSA в клинични обстоятелства. Въпреки че при фенотипните тестове нито един от изолатите не показа резистентност към ванкомицин, ние направихме проверка и за наличието на *vanA* гена, но нито един от изолатите не беше позитивен за този ген.

Introduction

Staphylococcus aureus is the most important human pathogen among the genus of *Staphylococcus*. *S. aureus* pathogenicity can cause a wide range of illnesses, from skin infections to severe conditions, such as sepsis, endocarditis, osteomyelitis, pneumonia, etc. (Harris *et al.,* 2002; Plata *et al*., 2009). This bacterium is the main cause of hospital and community-acquired infections (Plata *et al*., 2009; Al-Obeid *et al.,* 2010). Owing to high morbidity and increasing resistance against a wide range of antibacterial drugs, this bacterium has become one of the major public health concerns in all clinical settings worldwide.

Methicillin was introduced in the late 1950s as a good choice to treat life-threatening *S. aureus* infections, however, the widespread usage of this antibiotic caused numerous methicillin-resistant *S.aureus* reports (Tong *et al.,* 2012). The increasing number of MRSA strains in hospitals and communities, and more importantly, the emergence of multidrug-resistant (MDR) MRSA, led to the use of vancomycin in the treatment of MRSA infections (Tiwari *et al*., 2009; David and Daum, 2010).

However, reports of MRSA isolates with reduced susceptibility to vancomycin raised the first alarms about vancomycin-resistant *S. aureus (VRSA)* (Périchon and Courvalin, 2009; Jacob and DiazGranados, 2013). One strain with reduced susceptibility to vancomycin was first reported from Japan in 1996 (Hiramatsu, 2001; David and Daum, 2010). Based on interpretive criteria defined by the Clinical and Laboratory Standards Institute (CLSI), the vancomycin minimum inhibitory concentration (MIC) result reported for the new isolate was in the intermediate range (8μg/mL) (Saderi *et al*., 2005). Shortly after, in 2002, the first clinical vancomycin-resistant *S. aureus* (MIC ≥32μg/mL) strain was isolated in Michigan, USA (Dezfulian *et al*., 2012; Hiramatsu *et al*., 2014). Afterwards, several reports of vancomycin-intermediate *S. aureus* (VISA) and VRSA from different parts of the world provoked a growing concern about the success of vancomycin therapy in critical staphylococcal infections (Howden *et al*., 2010; Azimian *et al.,* 2012). Two main resistance mechanisms have been proposed: i) thickened and poorly cross-linked cell wall for vancomycin intermediate-resistant *S. aureus*, ii) activity of *van A* operon. This operon acquired from *Enterococcus* spp results in high-level resistance and defines vancomycin-resistant *S. aureus* (Hiramatsu, 2001; Périchon and Courvalin, 2009; 2012; Tarai *et al.,* 2013).

The higher incidence of MRSA in different parts of the world may lead to a higher frequency of prescribing vancomycin, which in turn may cause emergence of VRSA. We have recently shown a high resistance rate to methicillin in *S. aureus* isolated in two main university hospitals in northeastern Iran (Rahimipour *et al*., 2015). Here we expand the study to reveal the frequency of *vanA* and *mecA* genes in these isolates.

Material and Methods

Bacterial isolates

The isolates were obtained from clinical samples as described before (Rahimipour *et al*., 2015). We selected 45 MRSA isolates which were previously shown phenotypically methicilin-resistant based on E test and MIC determination. The strains were subjected to further genotypic investigation of the *mecA* and *vanA* genes. Though none of the isolates showed vancomycin resistance in phenotypic study, we also evaluated the isolates for possible *vanA* gene positivity.

DNA extraction

DNA was extracted from bacterial suspension using a DNA extraction kit (Genomic DNA isolation kit VI, DENAzist Asia/Mashhad, Iran) according to manufacturer's instructions.

Primers

We used two primer pairs to detect the *mecA* and *vanA* genes as described before (Azimian *et al.,* 2012). The forward (F) and reverse (R) primers were as follows: F1: 5'AGAAGATGGTATGTGGAAGTTAG3'and R1: 5'ATGTATGTGCGATTGTATTGC3' and F2: 5'GGCAAGTCAGGTGAAGATG3' and R2: 5'ATCAAGCGGTCAATCAGTTC3' for *mecA* and *vanA* genes, respectively.

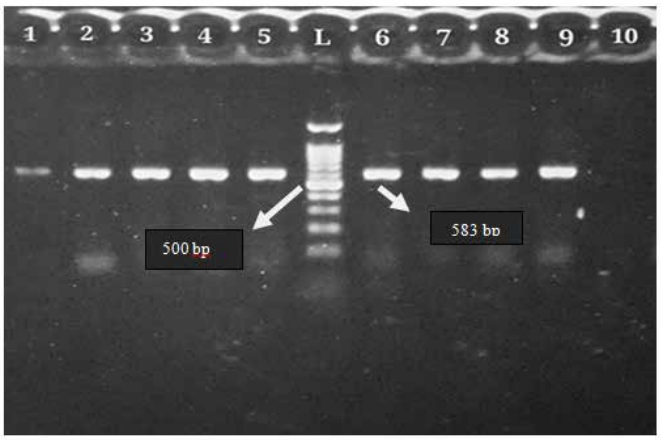

Fig. 1. *MecA* gene pattern of agarose gel (1.5%) electrophoresis. Lane 1-8 clinical strains, Lane 9 positive control, Lane 10 negative control, Lane L DNA marker.

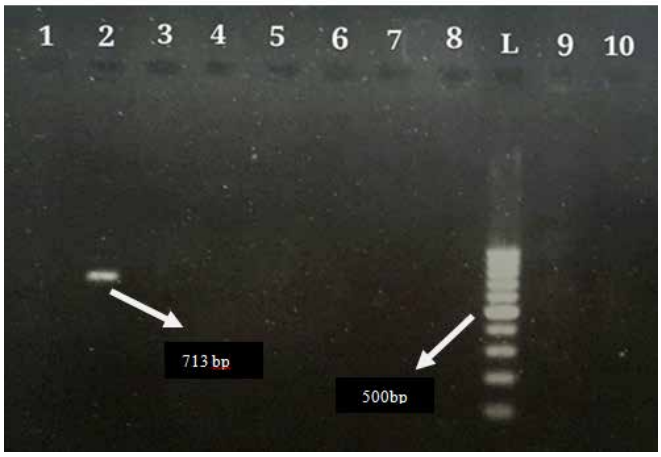

Fig. 2. *Van A* gene pattern of agarose gel (1.5%) electrophoresis. Lane 1 negative control, Lane 2 positive control, Lane 3-10 clinical strains, Lane L DNA marker

PCR reaction conditions

The PCR reaction was optimized by applying concentration and temperature gradients. Finally a 25 µl reaction consisted of: 5 µl template DNA, 0.2 µl DNA polymerase (Takapouzist, Tehran), 2 µl primer (100 Pmol), 0.5 µl dNTPs (200µM), 2 µl MgCl2 (1.5mM), 2.5 µl PCR buffer (10X) and 12.8 µl DDW. The final PCR program was determined using gradient PCR optimization. The final program was set at the following conditions: 5 min at 94°C for initial denaturation, followed by 35 cycles consisting of denaturation at 94°C for 1 min for *mecA* and 1 min for *van A*, annealing at 45°C for 1 min for *mecA* and at 46°C for 1 min for *van A*, and extension at 72°C for 1 min for *mecA* and 90 s min for *vanA*, with a final extension step at 72°C for 5 min. Next, the PCR products were subjected to 1% agar gel electrophoresis. The gels were stained and visualized with an UviDoc system. The PCR products were finally sent for DNA sequencing with the above mentioned primers.

Results

A 583 bp fragment corresponding to *mecA* and a 713bp for *vanA* were observed on DNA gel electrophoresis of PCR products (Fig. 1, Fig. 2, respectively). Among all MRSA isolates, 42 out of 45 (93%) were positive for the *mecA* gene. None of the MRSA isolates were positive for the *vanA* gene.

The sequenced PCR products were aligned with the sequences of the *mecA* gene using Nucleotide BLAST (Basic Local Alignment Search Tool) available in NCBI database and a homology of >96.% was observed.

Discussion

MRSA infections are considered as a main concern in hospital settings all around the world. Among these infections, MRSA bacteremia has a higher mortality rate (almost double) than methicillin-susceptible *S. aureus* (MSSA) bacteremia (Moise-Broder et al., 2004). It has been also reported that MRSA isolates are mostly multidrug-resistance (Sharif *et al*., 2013; Tiwari *et al.,* 2009). Similarly, the majority of our MRSA strains were multidrug-resistant to other antibiotics including penicillin, gentamicin, clindamycin and erythromycin (Rahimipour *et al.*, 2015). In this regard, one could imagine that VRSA strains tend to have simultaneous resistance against a large number of other antibiotics, resulting in a narrow treatment option and higher morbidity and mortality (Thati *et al.,* 2011).

In our geographic region, we have recently reported that 45 out of a total of 122 strains (36.88%) were MRSA strains (Rahimipour *et al.,* 2015).

The genetic mechanisms of methicillin and vancomycin resistance in MRSA and VRSA are related to *mecA* and *vanA* genes, respectively. According to previous studies, the *vanA* gene can be easily transferred from *Enterococci* to *S. aureus* (Périchon and Courvalin, 2009). In this study, 42 isolates contained the *mecA* gene, but all strains were negative for the *vanA* gene. The high frequency of circulating *mecA* gene among *S. aureus* strains alarms for possible emergence of VRSA in upcoming years. This underscores the need for careful strategies for

managing such possible health system problem. During recent decades, the increasing prevalence of methicillin-resistant *S. aureus* in many parts has resulted in a dramatically increased use of vancomycin (Tiwari *et al*., 2009; Rao and Prabhakar, 2011). Such prescriptions should be used with utter care. For example, suitable vancomycin dosing to ensure complete destruction of bacteria has been emphasized. Additionally, the use of combination therapy against MRSA should not be ignored (Shahriar *et al*., 2012).

To summarize, based on the present study and previous published studies *mecA* gene is widely detected in *S. aureus* strains, which may lead to increased treatment with vancomycin and ultimately may result in emergence of VRSA strains. Therefore, an urgent response is essential to restrain further spread and emergence of resistant strains. With this regard, a precise protocol on proper antibiotic use is needed in all clinical settings and agents such as vancomycin should be used in particular conditions, when it is absolutely necessary.

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