

# Comparison of Hypervariable Region (HVR) of *mecA* Gene in *Staphylococcus aureus* Isolated From Nasal Carriers and Clinical Samples

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**Background:** Methicillin Resistant *Staphylococcus aureus* (MRSA) is a common major human pathogen that causes hospital-acquired infections. Characterization and typing of the staphylococcal cassette chromosome has led to a better understanding of MRSA infection cycle in hospital. The *mecA*-associated hypervariable region size classifies MRSA isolates that colonized in nasal carriers.

**Objectives:** The aim of this study was to compare the genetic background of hypervariable region (HVR) of *mecA* gene in *S. aureus* isolated from nasal carriers and clinical samples.

**Materials and Methods:** A cross sectional study was performed on 261 nasal swabs collected from healthy health care workers (HCW) and 109 clinical samples from Tehran university hospitals. All the *S. aureus* isolates were identified by biochemical tests (Coagulase, Catalase, Manitol fermentation, and DNase tests). *S. aureus* isolates were investigated for the variability of HVR of *mecA* gene by PCR method.

**Results:** Among 261 collected nasal swabs, 70 (27%) were *S. aureus*. Of these, 29(41%) isolates were resistant to Oxacillin and 32 (46%) of those had *mec*-HVR gene. The polymerase chain reaction (PCR) products showed five different patterns of HVR. Also among 109 clinical samples, 52 (48%) of them were *S. aureus*. Of these, 40 (77%) were resistant to Oxacillin and 45 (87%) of them carried the *mec*-HVR gene. The PCR products showed 11 different patterns of HVR.

**Conclusions:** Molecular typing of MRSA isolates by HVR amplification has shown a high diversity among the strains and can be used as a basis for tracking the contaminations and the source of hospital infections from staff to patients and vice versa.

**Keywords:** *Staphylococcus aureus*; Carrier State; Cross Infection

## 1. Background

*Staphylococcus aureus* is one of the most frequent human pathogens that produces a wide range of infections (1). The pathogen is resistant to most of common antibiotics and thus has caused many problems in treatment of diseases. A notable example in this regard is the emergence of Methicillin resistant *S. aureus* (MRSA), which was reported for the first time just one year after the introduction of Methicillin to the clinical practice. MRSA is an important hospital pathogen, and is responsible for development of various infections such as skin and soft tissue infections, pneumonia, bacteremia and catheter-related infections (2, 3).

Resistance against Methicillin in *S. aureus* is due to a large mobile genetic element, called Staphylococcus cassette chromosome *mec* (SCC*mec*). The 20 - 50 kb region of *mec* DNA contains *mecA* gene, which encodes modified Penicillin binding protein (PBP2A) with lower affinity to beta-lactam antibiotics (4). The genomic distance of *mecA* and IS431 (insertion sequence 431) is very variable and is called the hypervariable region (HVR), which is consisted of exact repeated units of the 40 bp length. The variability

of the region is due to the high polymorphism observed in isolates of *S. aureus* (4, 5). The adjunct IS sequences, transposones, and the *mec* DNA can be considered as the hot spots, which include the antibiotic resistance genes (6).

It seems that the staphylococcal carriers play an important role in pathogenesis of the infections caused by *S. aureus*. The asymptomatic colonized patients and hospital staff are the main sources of MRSA in hospital environment. Health care workers (HCW) are considered as the link between patients. Screening of the carriers of MRSA is necessary for control of nosocomial infections (7).

The risk of carrier colonization with this pathogen and infections in diabetic patients, IV drug abusers, hemodialysis and surgical patients, and those with immune system deficiency are noteworthy. Considering the genetic variation of MRSA and the high rate of mortality of the patients and the high costs of treatment of the infections, the control of these infections should be accompanied with molecular typing methods. An ideal molecular technique should be rapid, easy to perform, and interpret. Moreover, the test should have a reasonable differentia-

### Implication for health policy/practice/research/medical education:

*Staphylococcus aureus* persists in nasal canal of hospital health workers and can be transferred to the hospitalized patients. Determination of nasal colonized MRSA and clinical isolates based on *mecA* gene variation might be useful to investigate the cycle of infections in patients and health care workers.

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tion ability and repeatability (8). PCR based methods that are related to gene variation are currently highlighted. One of these methods is the amplification of HVR using PCR method.

## 2. Objectives

The aim of the study was to evaluate the genetic pattern of *S. aureus* isolated from hospital carriers, and comparing them with clinical specimens using HVR PCR amplification.

## 3. Materials and Methods

### 3.1. Sample Collection and *S. aureus* Identification

Collectively 261 nasal swabs of healthy HCW and 109 clinical samples were obtained from five Tehran University of Medical Sciences hospitals (I, II, III, IV, V) and (IV, V), respectively. The samples were transferred to microbiology laboratory of School of Public Health and sub-

cultured on (5%) blood agar at 37°C overnight. Then all of the *S. aureus* isolates were confirmed by biochemical tests (Coagulase, Catalase, Mannitol fermentation on Mannitol salt agar culture and DNase tests) (9).

### 3.2. Antibiotic Susceptibility

Susceptibility to antibiotic was determined by using agar disc diffusion method on Muller Hinton agar medium containing (2%) NaCl, and Oxacillin disc (MAST, UK) and all plates were incubated at 35°C overnight (10).

### 3.3. Genotype Analysis

#### 3.3.1. DNA Extraction

DNA was isolated by using genomic DNA extraction kit (Bioneer Inc., South Korea) as recommended by the manufacture, with the modification that 5 mM 1.5M lyso-staphin was added to bacterial suspension. Finally the genomic DNA extraction was used as the template for PCR.

**Table 1.** The Frequency of *S. aureus* and HVR Type Among Health Care Staff of Five Hospitals Studied

Hospital No. of HCWs <sup>a</sup>	<i>S. aureus</i> Nasal Isolates, No. (%)	HVR Variability Based on Hospital Wards <sup>b</sup>	Oxacillin Resistance, No. (%)
<b>I (n = 43)</b>	14 (31.8)	H1: [1] (Internal)	5 (35.7)
		H2: [2] (Internal)	
		H6: [3] (Internal)	
		H10: [1] (Internal)	
<b>II (n = 70)</b>	19 (27.1)	H5: [2] (Orthopedic)	5 (26.3)
		H6: [2] (Orthopedic), [1] (Emergency)	
<b>III (n = 79)</b>	15 (19)	H1: [1] (Neurology);	6 (40)
		H5: [2] (Surgery)	
		H6: [1] (Surgery), [1] (Dialysis), [1] (Neurology)	
<b>IV (n = 34)</b>	9 (26.5)	H5: [2] (Internal)	
		H6: [2] (Neurology)	
<b>V (n = 35)</b>	13 (37.1)	H5: [2] (Dialysis), [2] (ICU) <sup>a</sup> , [2] (Transplant)	
		H6: [2] (ICU), [2] (Transplant)	
<b>Total (n = 261)</b>	<b>70 (26.8)</b>	<b>32</b>	

<sup>a</sup> Abbreviations: HCW, health care workers; ICU, Intensive care unit

<sup>b</sup> Number of HVR type

#### 3.3.2. PCR Analysis

A collection of *S. aureus* isolates, were screened for the presence of HVR *mecA* gene. The following primers were used to amplify fragments of mobile elements associated with the *mecA* region (11).

HVR1: (5'-ACT ATT CCC TCA GGC GTC C-3')

HVR2: (5'-GGA GTT AAT CTA CGT CTC ATC-3')

In this study *S. aureus* strain COL (a fully sequenced MRSA strains) was used as positive control and distilled

water as a negative control for PCR. PCR reaction was performed in Ependorf thermocycler at a final volume of 50  $\mu$ L containing, 10  $\mu$ L of 10x PCR Buffer, 3  $\mu$ L of  $MgCl_2$  (10 mM), 0.2 mM dNTP mix, 20  $\rho$ M of each primer, 1U of Taq polymerase and 4  $\mu$ L of template DNA.

Amplification was carried out as follow: denaturation at 94°C for 3 minutes (first denaturation) and repeated in 30 cycles according to the following program: denaturation at 94°C for 45 seconds, annealing at 55°C for 30 seconds and extension at 72°C for 90 seconds and the final extension at 72°C for 10 minutes to complete partial polymerization (11).

#### 4. Results

In this study on 261 HCW nasal swab samples, 70 (27%) were found to be *S. aureus*. Of these, 29 (41%) were MRSA and 32 (46%) had *mec*-HVR gene. The proportion of MRSA among nasal carriers isolates from different hospitals varied from 26% to 62%. The highest incidence of *S. aureus*

belonged to V hospital (37.1%) and also the highest incidence of MRSA belonged to that hospital (61.5%). Molecular typing of the 70 *S. aureus* from nasal carrier isolates by HVR-PCR illustrated five HVR types: HVR 1 (350 bp), HVR 2 (400 bp), HVR 5 (550 bp), HVR 6 (600 bp) and HVR 10 (800 bp). The hospital wards that these types obtained were internal, orthopedic, emergency, surgery, dialysis, neurology, ICU, and transplantation wards (Table 1).

Different HVR types obtained from eight mentioned wards collected from nasal carriers has been distributed, Internal (1, 2, 5, 6, 10), Orthopedic (5, 6), Emergency (6), Neurology (2, 6), Surgery (5, 6), Dialysis (5, 6), Transplant (5, 6) and ICU (5, 6). The greater variations of HVR types were found in internal ward (1, 2, 5, 6, 10) (Table 1).

Also among 109 clinical samples collected from patients of two hospitals, 52 (47.7%) were *S. aureus*. Of these, 40 isolates (77%) were MRSA and 45 isolates (87%) have *mec*-HVR gene. The highest incidence of *S. aureus* collected from V hospital (83.3%) and also the highest proportion of MRSA was also detected in this hospital (Table 2).

**Table 2.** The Frequency Distribution of *S. aureus* and HVR Type Among Clinical Isolates of Two Studied Hospitals

Hospital No. of HCWs <sup>a</sup>	<i>S. aureus</i> Nasal Isolates, No. (%)	HVR Variability Based on Hospital Wards <sup>b</sup>	Oxacillin Resistance, No. (%)
<b>IV (56)</b>	22 (39.2)		15 (68.2)
		H2: [2] (Neurology)	
		H3: [1] (NICU) <sup>b</sup> , [1] (PICU) <sup>c</sup>	
		H4: [1] (PICU)	
		H5: [1] (NICU)	
		H6: [1] (NICU)	
		H7: [2] (PICU); H8: [1] (NICU), [1] (Internal)	
		H9: [1] (PICU), [1] (NICU), [1] (Internal)	
		H10: [2] (PICU), [2] (NICU)	
		H11: [2] (PICU)	
<b>V (53)</b>	30 (56.6)		25 (83.3)
		H1: [1] (Surgery); H4: [1] (ICU), [1] (Neurology)	
		H5: [2] (ICU), [1] (Emergency), [1] (Surgery)	
		H6: [2] (ICU)	
		H7: [1] (Neurology)	
		H8: [1] (ICU), [1] (Emergency)	
		H9: [1] (Orthopedic), [1] (ICU), [1] (Emergency)	
		H10: [4] (ICU), [2] (Surgery), [2] (Orthopedic)	
		H11: [2] (Orthopedic), [1] (ICU), [1] (Endocrinology)	
<b>Total (109)</b>	52 (47.7)	45	40 (76.9)

<sup>a</sup> Abbreviations: HCW, health care workers; NICU, Neonatal Intensive Care Unit; PICU, Pediatric Intensive Care Unit

<sup>b</sup> Number of HVR type

Molecular typing of 52 *S. aureus* from clinical isolates by HVR-PCR method have demonstrated the presents of HVR in HVR types. In addition of the types which has been observed in nasal carriers, the types HVR 3 (450 bp), HVR 4 (500 bp), HVR 7 (650 bp), HVR 8 (700 bp), HVR 9 (750 bp) and HVR 11 (850 bp) only are conspicuous in clinical isolates. The hospital wards which these types obtained were surgery, orthopedic, emergency, neurology, ICU, endocrinology, PICU and NICU. Distribution of different HVR types collected from nine wards were as follow: Surgery (1, 5, 10), ICU (4, 5, 6, 8, 9, 10, 11), Neurology (4, 7, 2), Emergency (5, 9), Orthopedic (9, 10, 11), Endocrinology (11), NICU (3, 5, 6, 9, 10), PICU (3, 4, 7, 9, 10, 11) and Internal (8, 9) (Table 2). The variation of HVR types in collected isolated from intensive care units (ICUS) was higher than other wards of hospital.

## 5. Discussion

In the current study, 70 and 52 isolates of *S. aureus* were evaluated by detecting the HVR of *mecA* in isolates collected from HCW and patients, respectively. Amplification of HVR by PCR resulted in identification of 5 and 11 types in carriers and patients, respectively. In a similar study on typing of clinical isolates of MRSA, 72 isolates were classified according to the results of HVR typing (12). Moreover, seven HVR types were identified, two of which were common in the species of pandemic MRSA and one type had a high prevalence in sporadic species (12).

The HVR typing method was used in another study to type the MRSA isolates obtained from clinical specimens. The primers were similar to those we used in the current study. In the study, four types of HVR (451 bp, 491 bp, 571 bp, and 611 bp) were reported, which were close to types 3, 4, 5, and 6 in our study (6). In another study, by using six classification methods including HVR typing, 183 clinical isolates of MRSA in five HVR types of 400, 450, 500, 550, and 600 bp, which were corresponding to types 2, 3, 4, 5, and 6 were classified (11). Types 2, 5 and 6 were recognized in carriers, while types 2, 3, 4, 5 and 6 were observed in patients. A notable finding was that the highest prevalence and varieties of MRSA types was detected in samples collected from ICU wards.

Considering the above mentioned findings, it can be concluded that typing the clinical isolates of MRSA based on HVR amplification has a higher diversity in our country and can be used as a basis for tracking the contaminations and the source of hospital infections from staff to patients and vice versa (as a cycle). For instance, HVR 5 and 6 types in the current study were isolated from 13 and 12 samples, respectively, both from the carriers and patients. Furthermore, the types 5 and 6 isolated from ICU wards were similar in carriers and patients. These two types were isolated from samples taken from the ICU patients and also the staff working in ICU wards. These types may be circulated in the ward and transmitted from staff to patients and vice versa.

It should be noted that HVR 6 (600 bp) was the most frequent type in all hospitals under evaluation. Also, the type collected from hospital staff with working history of six months to ten years and it seems that the type has a high colonization capability.

The typing of pathogens isolated from carriers using HVR typing method was not observed in similar studies. Also, the number of isolated types from the carriers was less than half of the number of HVR types isolated from the patients. Out of 70 *S. aureus* samples collected from the carriers, 32 samples were positive for *mecA*-HVR and 29 samples were resistant against Methicillin. The results indicated that when the isolates are phenotypically Methicillin resistant, genotypically they are positive for *mecA* gene. This is while some sensitive isolates also have *mecA* gene. The underlying cause of this discrepancy between phenotype and genotype can be pointed the mutations, which leads to palindrome defect in -10 region of *mecA* promoter (13).

Moreover, it was mentioned that the clinical isolates from the ICU wards had shown the highest number of HVR types. The ICU wards, which also include NICU and PICU, are probably appropriate places for epidemic outbreaks of the infections. The highest prevalence of *S. aureus* and MRSA among the patients was observed in V hospital. It was observed that HVR 10 (800 bp) was circulating among the patients hospitalized in ICU ward of V hospital. The lowest number of isolates was detected in both patients and carriers patients in dialysis and transplantation wards in all hospital. In a study carried out in Switzerland HVR typing mentioned as a useful method for exploration of the infection outbreak source, since it has a high specify and in comparison with other typing methods such as PFGE, this is a rapid and inexpensive method (14).

In conclusion, *S. aureus* exists in nares of HCW and can be transferred to the hospitalized patients. Detection of MRSA nasal colonization and clinical isolates based on *mecA* gene variation might be useful for investigation the cycle of infections among patients and health care workers who are continuously and directly in contact with patients. Therefore, the study confirms the significant effect of detecting the carriers, treating and decolonizing them in order to take proper treatment measures and prevent their transmission, mortality and diseases progress.

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## Authors' Contribution

None declared.

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