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The emergence of vancomycin-resistant *Staphylococcus aureus* in an intensive care unit in Kerman, Iran

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Summary Methicillin-resistant *Staphylococcus aureus* (MRSA) is a global threat to public health. This study is the first report of the emergence of vancomycin-resistant MRSA in Kerman, Iran. During a period of 15 months, a total of 205 clinical isolates of *S. aureus* were collected from three university hospitals affiliated with the Kerman University of Medical Science, Kerman, Iran. Screening of methicillin and vancomycin resistance was carried out by phenotypic methods. The resistance and virulence genes of vancomycin-resistant isolates were detected by polymerase chain reaction (PCR). Staphylococcal cassette chromosome *mec* (SCC*mec*) and *spa* typing were used for molecular typing of vancomycin-resistant isolates. Two *S. aureus* isolates were considered vancomycin-resistant by phenotypic and genotypic methods. Both isolates showed a minimum inhibitory concentration (MIC) ≥ 64 µg/ml and belonged to SCC*mec* III and *spa* type t030. Finding vancomycin-resistant *S. aureus* (VRSA) isolates represents a serious problem. More stringent infection control policies are recommended to prevent transmission of such life-threatening isolates in the hospital setting.

Keywords *Staphylococcus aureus* · Methicillin-resistant *Staphylococcus aureus* (MRSA) · Vancomycin-resistant *Staphylococcus aureus* (VRSA) · Staphylococcal cassette chromosome *mec* (SCCmec) · *Spa* type

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Aufkommen von vancomycinresistentem *Staphylococcus aureus* auf einer Intensivstation im iranischen Kerman

Zusammenfassung Der methicillinresistente *Staphylococcus aureus* (MRSA) ist eine weltweite Bedrohung der öffentlichen Gesundheit. Die vorliegende Arbeit stellt den ersten Bericht über das Aufkommen vancomycinresistenter MRSA in Kerman, Iran, dar. Innerhalb von 15 Monaten wurde insgesamt 205 klinische Isolate von *S. aureus* aus 3 Universitätskliniken gesammelt, die der Medizinischen Fakultät der Universität Kerman angeschlossen sind. Das Screening auf Methicillin- und Vancomycinresistenz wurde anhand phänotypischer Verfahren durchgeführt. Die Resistenz- und Virulenzgene vancomycinresistenter Isolate wurden mittels Polymerasekettenreaktion („polymerase chain reaction", PCR) nachgewiesen. Die Staphylococcal-Cassette-Chromosome-*mec*(SCC*mec*)- und -*spa*-Typisierung wurden zur molekularen Typisierung vancomycinresistenter Isolate eingesetzt. Anhand phänotypischer und genotypischer Verfahren wurden 2 *S.-aureus*-Isolate als vancomycinresistent angesehen. Beide Isolate zeigten eine minimale Hemmkonzentration („minimum inhibitory concentration", MIC) ≥ 64 µg/ml und gehörten zum SCC*mec*-III- und -*spa*-Typ t030. Der Befund vancomycinresistenter *S.-aureus*(VRSA)-Isolate stellt ein ernstes Problem dar. Strengere Strategien für die Infektionsüberwachung werden zur Prävention der Übertragung derartiger lebensbedrohlicher Isolate in Krankenhäusern empfohlen.

Schlüsselwörter *Staphylococcus aureus* · Methicillinresistenter *Staphylococcus aureus* (MRSA) · Vancomycin-resistenter *Staphylococcus aureus* (VRSA) · Staphylokokken-Kassetten-Chromosom *mec* (SCCmec) · *spa*-Typ

Table 1 List of primers used in this study

Target gene	Primer name	Oligonucleotide sequence (5′–3′)	Product size (bp)	Reference
nuc	Nuc-F Nuc-R	GCGATTGATGGTGATACGGTT AGCCAAGCCTTGACGAACTAAAGC	279	9
mecA	MECA-F MECA-R	TCCAGATTACAACTTCACCAGG CCACTTCATATCTTGTAACG	162	11
ermA	ErmA-F ErmA-R	TATCTTATCGTTGAGAAGGGATT CTACACTTGGCTTAGGATGAAA	139	11
ermB	ErmB-F ErmB-R	CTATCTGATTGTTGAAGAAGGATT GTTTACTCTTGGTTTAGGATGAAA	142	11
ermC	ErmC-F ErmC-R	AATCGTCAATTCCTGCATGT TAATCGTGGAATACGGGTTTG	297	11
mrs(A/B)	MRS(A/B)-F MRS(A/B)-R	GCAAATGGTGTAGGTAAGACAACT ATCATCATGTGATGTAAACAAAAT	402	11
vanA	VanA-F VanA-R	CATGAATAGAATAAAAGTTGCAATA CCCCTTTAACGCTAATACGATCAA	1030	12
vanB	VanB-F VanB-R	GTGACAAACCGGAGGCGAGGA CCGCCATCCTCCTGCAAAAAA	433	12
pvl	PVL-F PVL-R	ATCATTAGGTAAAATGTCTGGACATGATCCA GCATCAASTGTATTGGATAGCAAAAGC	433	13

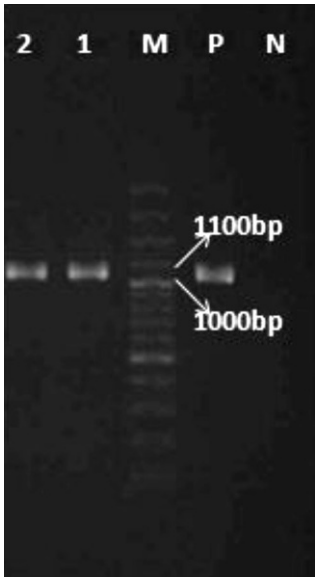

Fig. 1 Agarose gel electrophoresis of PCR-amplified *vanA* gene. *N* negative control, *P* positive control, *1* and *2* positive strains, *M* size marker (100 bp)

Methicillin-resistant *Staphylococcus aureus* (MRSA) is considered as one of the most important multiple drug resistant (MDR) pathogens in intensive care units (ICUs) [1, 2]. Over the past decades, the incidence of MRSA isolates has increased dramatically worldwide [2]. Vancomycin is a drug of choice for the treatment of MDR *S. aureus* infections [3]. Resistance to vancomycin can be linked to the presence of the *vanA* gene or thickening of the bacterial cell wall [4]. Vancomycin resistant *S. aureus* (VRSA) was first reported in the USA in 2002 [5]. By the end of 2015, several VRSA strains had been reported in different countries, which made treatment more complicated [5, 6]. The first VRSA isolate from Iran was reported in Tehran in 2008 [7]. Herein, the authors describe the emergence of the first VRSA isolates from two hospitalized patients in Kerman, southeastern Iran.

Materials and methods

From February 2015 to May 2016, 205 *S. aureus* isolates were collected from patients admitted to three university hospitals affiliated with the Kerman University of Medical science in Kerman, Iran. These isolates were obtained from various samples such as urine, blood, cerebrospinal fluid, wound and respiratory tract. All isolates were identified as *S. aureus* by positive Gram staining, as well as positive tests for catalase, coagulase, DNAse and fermentation of mannitol [8]. Polymerase chain reaction (PCR)-mediated amplification of the *nuc* gene was performed to confirm these isolates genotypically [9].

The antibiotic susceptibility profile of isolates was determined by the disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) recommendations [10]. The following antibiotic disks were employed: gentamicin (10 µg), amikacin (30 µg), erythromycin (15 µg), clindamycin (2 µg), tetracycline (30 µg), ciprofloxacin (5 µg), trimethoprim/sulfamethoxazole (1.25/23.75 µg) and linezolid (30 µg). Screening for MRSA and VRSA isolates was carried out by detection of resistance to a cefoxitin disk (30 µg) and growing on Brain Hearth Infusion agar (BHI; Difco, BD, NJ, USA) containing 6 µg/ml vancomycin (Sigma-Aldrich, MO, USA), respectively. Also, the minimum inhibitory concentration (MIC) of vancomycin was determined using the broth microdilution method. *S. aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212 were used as standard strains in the antimicrobial susceptibility tests.

The total genomic DNA of VRSA strains was extracted by Exgene™ Clinic SV (GeneALL, Seoul, Korea)

Table 2 Genetic characteristics and clinical information of two VRSA strains

Strains	Gender	Age	Source	Unit	MIC(μg/ml)	*vanA*	SCC*mec type*	*ermA*	*ermB*	*ermC*	*mrsA/B*	*pvl*	*spa* type
1	Female	76	BAL	ICU	≥64	+	III	+	–	+	–	–	t030
2	Female	66	BAL	ICU	≥64	+	III	–	+	–	–	–	t030

VRSA vancomycin-resistant *S. aureus, BAL* bronchoalveolar lavage

according to manufacturer's guidelines. The oligonucleotide primers used for amplification of the *mecA, vanA, vanB, ermA, ermB, ermC, mrsA/B* and *pvl* genes are listed in Table 1. The PCR amplifications for the above genes were carried out as described previously [11–13]. Finally, SCC*mec* and *spa* typing was performed as described previously [14, 15].

Results

In this study, 100 (48.78%) of the 205 isolates were determined as MRSA by phenotyping methods. Two MRSA isolates were identified as VRSA and both isolates were *vanA* positive (Fig. 1). These isolates were from two women with pneumonia, hospitalized in the same ICU. It is not known exactly how long these women were hospitalized before specimen collection, but it is clear that the specimens had been collected at least 4 days after hospitalization. One of the isolates had been obtained in February 2015 from the bronchial aspirate of a 76-year-old woman with a history of diabetes mellitus and haemodialysis, who was not treated and died. According to our information, this patient had been admitted to the ICU on arrival. The other isolate had been obtained in April 2015 from the bronchial aspirate of a 66-year-old woman with pneumonia. This patient had been transferred from the urology unit (no further data available). Both VRSA isolates were resistant to gentamicin, amikacin, erythromycin, clindamycin, tetracycline, ciprofloxacin, trimethoprim/sulfamethoxazole and cefoxitin, but they were susceptible to linezolid. Genetic characteristics of and clinical information pertaining to these isolates are shown in Table 2.

Discussion

During the past decade, VRSA strains have been reported from different countries such as the USA, Portugal and India [3, 5, 6]. This study is the first report of the emergence of VRSA in the southeast of Iran. In contrast to previously reported VRSA isolates that were susceptible to gentamicin and other antibiotics [2–8], the isolates presented here showed a MDR phenotype. Although MRSA strains with SCC*mec* III and *spa* t030 have been reported from different countries, such as China, Germany, Denmark, Sweden and even other regions in Iran, none of them showed vancomycin resistance [16–19]. VRSA strains with *spa* type t292 (SCC*mec* IV) and t019 (SCC*mec* IV) have been detected in Brazil and the USA, respectively [5, 20]. In 2012, one VRSA with SCC*mec* III, *spa* t037 and *pvl* negative was reported by Azimian et al, in the northeast of Iran [8]. In another study in Iran, one VRSA isolate harbouring *vanA* (MIC: 64 μg/ml) was reported by Aligholi, et al. [7]. Therefore, it seems that the incidence of VRSA in Iran is increasing. In the present report, both VRSA isolates belonged to SCC*mec* III and *spa* type t030, with a different presence of *erm* and *mrsA/B* genes. These findings confirm that these isolates have acquired new resistance genes during persistence in ICU and hospitals. Also, SCC*mec* III is found predominantly in healthcare-associated MRSA isolates and is transferred by person-to-person spread in the hospital. Since no VRSA strain was observed in the authors' subsequent epidemiological studies, it seems these isolates have been not transmitted from patient to other patient, or to healthcare workers.

Conclusion

Since a high rate of MRSA isolates has been reported in Iran, finding VRSA isolates is a serious threat for Iranian hospital settings. Therefore, proper infection-control policies, appropriate antimicrobial agents management and improved awareness of healthcare personnel are needed to prevent the emergence and transmission of VRSA isolates in Iran. Due to the importance of VRSA emergence, these cases were reported to the infection control committee of the affected hospital. As no further VRSA was detected during the next year of specimen collection in this study (until May 2016), it can be concluded that the more strict preventive measures taken to control dissemination of resistant strains in the hospital setting were effective.

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Conflict of interest Y. Fasihi, F. Saffari, S. Mansouri, and D. Kalantar-Neyestanaki declare that they have no competing interests.

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