Journal of Current Biomedical Reports jcbior.com
Volume 1, Number 1, 2020

Original research

Molecular characterization of Panton-Valentine Leukocidin positive *Staphylococcus aureus* isolates obtained from clinical samples in Isfahan, Iran

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Abstract

Staphylococcus aureus is one of the main significant human pathogens which can produce various toxins such as Panton-Valentine Leukocidin (PVL) which is known as a prominent toxin associated with *S. aureus* infections. PVL-positive strains can cause a wide variety of skin, soft tissue, necrotizing pneumonia, fasciitis and life-threatening infections. Therefore, the aim of this study was evaluating the molecular characteristics of PVL-positive strains such as the presence of *mecA*, SCC*mec* types, *agr* types and exfoliative toxin genes. In this study, a total of 152 *S. aureus* strains were collected from clinical samples of patients who referred to Isfahan's Alzahra hospital (Iran). The isolates were confirmed phenotypically by conventional methods and then PVL-positive isolates were identified by PCR molecular test. Thereafter, antibiotic resistance pattern, *agr* groups (I, II, III, and IV), exfoliative toxins (*eta* and *etb*), *mecA* gene and *SCCmec* various types were carried out. Totally, 52 (34.2%) of strains were positive for PVL. Six PVL-positive strains harbored *mecA* gene, one strain had SCC*mec* I, and 5 strains SCC*mec* type IV. The highest ratio of *agr* groups belonged to group (I) and the (*eta)* gene was also detected in 18 isolates. The PVL-positive *S. aureus* strains can cause more serious infections, so identification of the genetic characteristics and antibiotic resistance monitoring of these strains is necessary.

Keywords: *Staphylococcus aureus*, PVL, Antibiotic resistance, Virulence genes

1. Introduction

Staphylococcus aureus is one of the most important nosocomial pathogens which can cause a wide range of various clinical infections. Virulence factors and toxins produced by this bacterium are responsible for several infections in which, Pantone Valentine Leukocidin (PVL) and exfoliative toxins are the most relevant. The PVL was first isolated from furuncles in 1936 [1]. This toxin is composed of two subunits, S and F which have synergistic activity by lukS-PV and lukF-PV genes [2, 3]. Injection of purified PVL, induces the liberating of histamine from basophilic granulocytes, chemotactic

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Received: May, 28, 2020
Accepted: June, 9, 2020

factors (leukotriene B4 & IL-8), oxygen metabolites of human neutrophilic granulocytes and enzymes [β-glucuronidase and lysozyme) [4].

In a mouse infection model, secreted PVL promotes tissue invasion, inflammatory response and necrotizing pneumonia, via mechanisms, including up-regulation of protein A and other adhesions [5]. The accessory gene regulatory system *(agr)* is responsible for regulating the growth, colonization, the expression of exoenzymes, toxins, surface proteins and other virulence factors [6, 7]. This system also regulates the metabolic pathways of organisms as a contributing growth factor [8]. Although most human clinical *S. aureus* isolates are *agr* positive, while several reports indicate that the *agr*-defective mutants have been isolated from infected patients [8]. In the last two decades, the emergence of antibiotic-resistant strains, especially beta-lactam resistant has increased the clinical significance of *S. aureus*. The first case of methicillin-resistant *S. aureus* was described in the UK [9]. Currently, the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in some regions is about 72 up to 90% [10]. The *mecA* gene, which is responsible for resistance to methicillin is carrying by a mobile genetic element, staphylococcal cassette chromosome mec, (SCC*mec*) [9]. Cassette Chromosome Recombinase (ccr) is another important part of SCC*mec*, which is involved in the insertion and exertion of SCC*mec* elements [11]. Until now, by combining different classes of mec and ccr, at least 11 various types of SCC*mec* elements have been identified [12]. Type I, II and III are mainly associated with hospital-acquired MRSA (HA-MRSA), and types IV and V with Community-acquired MRSA (CA-MRSA) [13].

Recently, PVL-positive *S. aureus* strains (PVL-SA) have attracted much attention, because of the critical role of PVL in clinical infections and emerging antibiotic-resistant strains. Therefore, the aim of this study was evaluating the molecular characteristics of PVL-positive strains such as the presence of *mecA*, SCC*mec* types, *agr* types and exfoliative toxin genes.

2. Materials and Method

2.1. Isolates collection and identification

A total of non-duplicate 152 *S. aureus* strains were collected from different specimens such as abscess (28), wound (66), respiratory tract infection (17), osteomyelitis (4), urine (16), a catheter (7) and bacteremia (14) from teaching hospitals of Isfahan, Iran. Isolates were confirmed by phenotypic tests (Gram staining, colony morphology on blood agar, mannitol salt agar, catalase, coagulase and DNase) and finally were stored at -20 ◦C in brain heart infusion broth (BHI) containing (12-15%) glycerol.

2.2. Antibiotic resistance pattern

Antimicrobial susceptibility testing was performed using Gentamicin, Tetracycline, Ciprofloxacin, Cefoxitin, Rifampin, Trimethoprim/Sulfamethoxazole, Ampicillin and Clindamycin disks (HiMedia) according to CLSI standard guidelines [14]. *S. aureus* ATCC 25923 and ATCC 33591 were used as negative and positive controls.

2.3. DNA extraction

The DNA was extracted using a genomic DNA purification kit (Fermentas, K0512) according to the manufacturer protocol recommendation.

2.4. PCR Molecular test

The PCR reactions were performed with specific primers that are shown in Table 1.The program of amplification were as follow: initial denaturation at 95 °C for 3 minutes, followed by 35 cycles of denaturation at 95 °C for 45 seconds, primer annealing based on references for 30-45 seconds, extension at 72 °C for 45 seconds, and a final extension at 72 °C for 7 minutes. The homology sequence of *agr* and exfoliative products were sequenced and confirmed with the BLAST tools.

3. Results

Various specimens were collected during the sampling period of time. The highest number of strains was belonged to wound 66 (43.4%) and abscess 28 (18.4%) samples. Antimicrobial resistance patterns were determined by disk diffusion method. The most isolates were resistant to tetracycline (56.2%), clindamycin (35.6%) and ciprofloxacin (35.6%) whereas, there was a low resistance to gentamicin (Table 2). By using PCR method, 52 isolates (34.2%) harbored *pvl* gene. All PVL-SA were investigated for the presence of *mecA*, SCC*mec* types, exfoliative toxin and accessory regulatory genes. Among the PVL-SA, 18 (34.6%) strains harbored *eta*, 5 (9.6%) *etb,* and 2 (3.8%) strains carried both *eta* and *etb* genes.

Table1. The specific primers for amplification of *pvl*, *eta*, *etb*, *agr*, *mecA* and SCC*mec* type

Target	Primer	Sequence (5'-3')	Annealing temperature	Reference
pvl	F	ATCATTAGGTAAAATGTCTGCACATGATCCA	51	[15]
	R	GCATCAASTGTATTGGATAGCCAAAAGC		
eta	F	GCAGGTGTTGATTTAGCATT	52	[16]
	R	AGATGTCCCTATTTTTGCTG		
etb	F	ACAAGCAAAAGAATACAGCG	53	
	R	GTTTTTGGCTGCTTCTCTTG		
agr	pan F	ATGCACATGGTGCACATGC	55	[17]
	I R	GTCACAAGTACTATAAGCTGCGAT		
	II R	TATTACTAATTGAAAAGTGCCATAGC		
	III R	GTAATGTAATAGCTTGTATAATAATACCCAG		
	IV R	CGATAATGCCGTAATACCCG		
mecA	F	ACTGCTATCCACCCTCAAAC	55	[18]
	R	CTGGTGAAGTTGTAATCTGG		
SCC*mec*	β F	ATTGCCTTGATAATAGCCYTCT	52	[17]
	α3 R	TAAAGGCATCAATGCACAAACACT		
	ccrC F	CGTCTATTACAAGATGTTAAGGATAAT		
	ccrC R	CCTTTATAGACTGGATTATTCAAAATAT		
	1272 F	GCCACTCATAACATATGGAA		
	1272 R	CATCCGAGTGAAACCCAAA		
	5RmecA F	TATACCAAACCCGACAACTAC		
	5R431	R CGGCTACAGTGATAACATCC		

Table 2. Antibiotic resistance patterns of *S. aureus* isolates

Antibiotics	No. (%) of resistant strains Total No. =52
Gentamycin	2 (3.8)
Ciprofloxacin	5 (9.6)
Ampicillin	3 (5.75)
Clindamycin	6 (11.5)
Tetracycline	15 (28.8)
Co-trimoxazole	4 (7.6)
Rifampicin	4 (7.6)

On the other hand, the *agr* I, II, III, IV were recognized in 39 (75%), 3 (5.7%), 7 (13.5%) and 3 (5.8%) isolates respectively. However, 46 (88.5%) strains were MSSA and 6 (11.5%) strains were MRSA. Finally, SCC*mec* types I and IV, were determined in one and five.

4. Discussion

S. aureus is colonized in up to 25 percent of healthy people and even more common among those with skin, eye, throat and nasal mucosal surfaces. *S. aureus* is capable to produce various toxins in which, PVL is the most important one. The PVL-SA are more pathogenic in comparison with other strains and also can cause skin and soft tissue infections, but in some cases may lead

to invasive infections including necrotizing hemorrhagic pneumonia [19]. Due to the importance of these strains, evaluation of antibiotic resistance and genetic characteristics of PVL- positive strains could play an important role in determining the health policy, hence, rapid detection and monitoring of PVL-SA is necessary for appropriate treatments and can be helpful in epidemiologic studies. The prevalence of PVL-strains in Iran was reported 18% in 2013, although the PVL-positive strains were distinguished among blood and urine specimens other samples were negative [20]. However, in 2014, Shrestha et al. reported that PVL was produced by 35.6% of *S. aureus* strains and was detected from bacteremia, surgical site, respiratory and urinary tract infection [2].

In the present study, 34.2% of the isolates were PVL-positive and the mentioned gene was isolated from respiratory tract infections, wound infections, abscess and osteomyelitis specimens. In addition, none of the bloodstream and urine samples were harbored the *pvl* gene.

The relation between PVL-SA and skin, soft tissue infections and necrotizing pneumonia has been demonstrated in several studies [2, 3, 5]. Bocchini et al. (2017) reported that there was a correlation between PVL toxin production and the intensity of osteomyelitis [21].

In our study, two PVL-positive strains were isolated from osteomyelitis. The osteomyelitis which is caused by PVL-SA is more complicated and similar to deep venous thrombosis or developed chronic osteomyelitis [21]. On the other hand, the *pvl* gene-positive MSSA contains a larger number of virulence factor genes in comparison to MRSA, although they are susceptible to more antimicrobial agents [22]. Numerous conducted studies have been demonstrated that the prevalence of PVL-positive strains among MSSA is more than MRSA strains [23, 24]. For instance, in two separate studies by Afroz et al. (2008) and Vorobieva et al. (2008), all PVL-positive strains were methicillin-susceptible [23, 24]. The prevalence of PVL-positive strains, among MRSA strains in the UK and Greece, have been reported 1.6% and 45%, respectively. It seems MSSA strains are more involved in the spread of *pvl* genes among *S. aureus* strains.

Karimi et al (2017) in Iran, verified that CA-MRSA harbored SCC*mec* IV-V and *pvl* gene is widely associated with the presence of SCC*mec* IV-V [25]. Moreover, a relation among CA-MRSA, SCC*mec* IV and PVL have been proved in study conducted by Shukla et al. (2004) [26]. Generally, from 40 to 90% of PVL-SA harbor SCC*mec* IV while, only 5% of PVL-positive strains have SCC*mec* I & III [9]. In our study 5 (9.6%) of MRSA-PVL positive strains carried SCC*mec* IV and only 1 (1.9%) had SCC*mec* I.

According to other virulence factors, Jarraud et al. (2000) investigated that each of the *agr* systems is responsible for regulating certain virulence genes and noted that TSST and exfoliative producing strains mainly contain both *agr* group III and IV [27]. Several studies on PVL-SA have shown that these strains do not belong to a specific *agr* group [28, 29].

In our study, 75% of PVL-SA had *agr* group I, while amongst MRSA strains, three strains had *agr* group III and other *agr* groups (I and IV). *agr* group III in some studies is regarded as the main one in MRSA-PVL positive strains [30]. It should be noted that *agr* group I was prevalent in most isolates, *agr* group II was dominant in nasal swabs (30%), *agr* group III was common in respiratory tract infections (26%), and *agr* group IV was higher in cutaneous isolates (12.1%) [31]

Various reported on exfoliative toxin-producing strains have suggested that the *etb* gene rate is lower than *eta* [32]. In a study performed by Koosha et al (2014) in Tehran, 197 isolates of *S. aureus* was investigated. The prevalence of *eta* and *etb* genes was reported (94.4%) and (7.6%), respectively [33]. Though in our study, the prevalence of *eta* gene was (34.6%), whereas *etb* was only (9.6%).

In conclusion, our findings indicate the high prevalence of PVL-SA in clinical samples. Close surveillance of these strains is essential to monitoring their spread and antimicrobial resistance profiles. Several studies show the increasing importance of PVL-SA in nosocomial infections. Therefore, determining the frequency of virulence factors and antibiotic resistance profile can be useful in selecting the right therapeutic strategy. The indiscriminate usage of antibiotics, high ability to exchange genetic elements and various types of resistance plasmids can multiply the importance of these pathogens. Our results emphasized that the susceptibility or resistance of *S. aureus* to antibiotics commonly used in various geographical regions is different. According to the increased resistance of *S. aureus* to antibiotics, continuing control of antibiotic-resistant will prevent the emerging of drug-resistant strains.

Author Contributions

All authors contributed equally to this manuscript, and approved the final version of manuscripts.

Conflict of Interests

The authors declare that they have no conflicts of interest.

Ethical declarations

Not applicable.

Financial Support

This study was supported by Isfahan University of Medical Science (Grant No: 392490).

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