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EVALUATION OF SPA GENE FREQUENCY IN CLINICAL ISOLATES OF STAPHYLOCOCCUS AUREUS WITH PENTON VALENTINE LOCOCIDIN GENE

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ABSTRACT

Background and Purpose: Staphylococcus aureus is one of the important factors in the development of hospital infections. Staphylococcus aureus spa gene encodes the protein A, which is the surface protein of Staphylococcus aureus. This protein, in addition to the bacterial virulence factor, is also used to determine the specific identity of the bacterium. The aim of this study was to evaluate the frequency of spa gene in clinical isolates of Staphylococcus aureus with Valentine's Lactocidin Pantone gene.

Materials and Methods: This cross-sectional study was performed on 135 samples of Staphylococcus aureus isolated from blood culture, throat swabs, urine, nose and other parts of Imam Khomeini Hospital in Behshahr. Staphylococcus aureus isolates were confirmed by various biochemical and microbiological tests according to the brochure. In order to evaluate the antibiotic susceptibility pattern of Staphylococcus aureus isolates, diffusion method was used according to NCCLS. The frequency of spa gene and pvl gene in Staphylococcus aureus isolates was investigated by PCR method.

Results: Out of 135 clinical samples, 38 isolates of Staphylococcus aureus were identified. Phenotypic evaluation of the antibiotic resistance pattern of Staphylococcus aureus strains showed that 11 isolates (30%) were resistant to oxacillin and 32 (62%) isolates were susceptible to oxacillin. Also, 17 isolates (64%) were resistant to mupirocin and 17 (46%) isolates were susceptible to mupirocin. The PCR results showed that out of 38 clinical isolates of Staphylococcus aureus, 30 isolates had the spa gene and 18 isolates possessing the pvl gene. The results also showed that 55% pvl gene isolates had a spa gene and 45% lacked the spa gene.

Conclusion: Considering the high frequency of spa and pvl genes among Staphylococcus aureus-resistant methicillin strains, as well as the severe and deadly causes of these diseases, early diagnosis and appropriate treatment for preventing disease progression should be considered. .

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Introduction

Staphylococcus aureus is known to be the most important pathogens of human pathogens and is one of the main causes of hospital infections. Infections caused by this bacterium are continuous and frequent in hospitalized patients [1]. Infections caused by this bacterium are mainly ulcers espiratory tract secretions and skin [2]; On the other hand, pediatric care, surgery, chemotherapy and intensive care units are important for Staphylococcus aureus infections [3]. In the past 50 years, this bacterium has undergone a lot of genetic changes. Since this genome is flexible, its pathogenic and drug-resistant strains have grown. Staphylococcus aureus causes a wide range of diseases such as endocarditis osteomyelitis, food poisoning, pneumonia, toxic shock syndrome, boils or dill, soft tissue infection and skin scaling syndrome in humans which can be transmitted through direct contact or through objects [4, 5]. Antibiotic resistance in this bacterium is controlled by

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chromosomes and plasmids [6]. Over and over-the-counter use of antibiotics over time will increase resistance and reduce the sensitivity of bacteria to various antibiotics [7]. Staphylococcus aureus is able to produce various toxins including toxin alpha, beta, gamma, delta and leukocyidine.

In recent years, the ability to produce leukocyte toxin has been of interest to researchers [8]. Because Penton Toxin Valentine Leukocyidine (pvll) is a hemolytic exotoxin that increases the permeability of the cell membrane and thereby lactation of leukocytes and tissue necrosis [9]. Staphylococcal Lactoxin family members include: Panton Valentine Leukocide (PVL), (LUKE LUKD) LUHE / D, Hemolysin3 (HIgA HIgB HIgC) and LUKM (LUKM PV LUKF PV LUKF PV) [10]. Pantone Valentine leukocyidine is an exotoxin that has two components of the protein S (33KDa) and F (34 KDa) and belongs to the barrel 2 family of transfusion membranes [11] which are controlled by the luk F PV genes and Luk S PV. Protein S and F are separated from each other by electrophoresis and each of them alone is inactive, both of which are antigenic and can be converted to toxoid [9, 12]. Pantonus valentin leukocyidine through the formation of pores in neutrophils leads to the introduction of cations into them and ultimately causes them to degrade [13, 14]. Staphylococcus aureus PVL strains have high virulence and are associated with furanchol, skin abscesses and severe necrotic infections [15].. The main objective of staphylococcal leukotoxins is human (polymorphonuclear) polysaccharide cells, monocytes and lymphocytes. This gene is found in Staphylococcus strains sensitive and resistant to methicillin [16]. Pantone Valentine leukocyidine is an exotoxin-forming pore and staphylococcus aureus carrying the PVL gene can cause a serious problem. The gene is present in Staphylococcus aureus strains susceptible to and susceptible to methicillin.

In many cases, there is a relationship between the Pantone Valentine leukocytene secreted by Staphylococcus aureus and the development of diseases such as skin infections, coccyx and reversible abscesses [16]. Valentine's penton leukocidin is associated with [1]CA MRSA strains but LUKS / LUKF PV genes can be transported by [2]MSSA strains [13]. On the other hand, the genotype of Staphylococcus aureus strains due to the role played by differentiation of isolates has shown that another gene that can be used to detect the origin of the infection and control the contamination caused by this bacterium is a spa gene. This gene is one of the distinguishing factors that has a short-chain X polymorphism [17]. Spa gene encodes Protein A, which is the surface protein of Staphylococcus aureus, in addition to being a bacterial virulence factor, it is also used to identify the specific identity of Staphylococcus aureus. [18,, 19]. Therefore, according to the above study the present study intends to use a molecular method to evaluate the frequency of spa gene in clinical isolates of Staphylococcus aureus with the Pantone Valentine leukocidin gene.

Methods

Sampling

In a descriptive-analytical study with a simple random sampling 135 samples were taken from Imam Hospital during the first 7 months from March 2014 to September 2013, the clinical samples (blood, nose, swab throat, urine were collected from Behshahr Imam Khomeini Hospital and transferred to the Microbiology Laboratory.

Cultivation and isolation

The specimens were prepared and transferred to Platelets containing Blad Agar Steril (Merck, Germany) and prepared tabletop culture Plates were incubated at 37 ° C for 48 hours, From colonies grown on Blag Agar medium repeated isolation of pure colonies was performed sequentially.

Identification of isolates

After isolating each isolate, morphological tests such as hot dyeing and biochemical tests such as catalase test, coagulase test DNase test and test for fermentation of sugar of mannitol isolated from colonies were identified.

Molecular identification

DNA extraction by phenol chloroform

In order to extract 24-hour culture DNA from each of the bacterial colonies the microbial suspension was incubated at 10 ° C with Blag Agar (Merck, Germany) with Vertex and then incubated for 24 hours at 37 ° C. Centrifuges were carried out at 14000 rpm for 10 minutes and the supernatant was discarded and added one ml of Iraizol buffer and 200 µl of chloroform. Microtip at 10000 rpm for 8 minutes centrifugation, after the formation of the two phases, a transient transparent phase containing DNA was transferred slowly to another sterilized microtipoil and stored in a 20 ° C freezer (12).

Polymerase chain reaction

A primer designed from the srRNA region of 16 27F (3 'AGAGTTTGATCMTGGCTCAG 5') and 1992R (3 'GGTTACCTTGTTACGACTT 5') was performed (13). The PCR reaction was carried out in 25 µl volumes, each PCR reaction contained 200 µM dNTP, 10 µmol of each primer, 1.5 µmol / L of MgCl2, 0.5 µg of Taq enzyme and 50 ng DNA pattern. The PCR reaction in thermosecler device was performed as follows: A 10-minute cycle at 95 ° C (initial denaturation) followed by 32 cycles of 60 seconds at 95 ° C, a binding phase of 30 seconds at 60 ° C and a 2-minute extension at 72 ° C finally a 5-minute cycle at 72 ° C. PCR products were examined for the presence of genes by

[1] Community- acquired-Methicillin-resistant Staphylococcus aureus
[2] Methicillin-susceptible Staphylococcus aureus

electrophoresis on 1% agarose gel and after staining with ethidium bromide. Also sequencing of isolated microorganisms was performed using Forward 27F primer.

Antibiotic test by diffusion method

Staphylococcus aureus isolates were isolated separately. Subsequently, the MacFarland half-opacity was prepared from each according to CLLST or NCCLS instructions at a concentration equal to half McFarland bacteria were grown on a culture medium of Muller Hinton Agar. Then with the help of sterile pins antibiotic oxacillin or mupirocin discs (6 mm in diameter) were placed in equal distances and incubated for 24 hours at 37 ° C. The antibacterial properties of the bacteria and the diameter of the non-growth holes around the discs were measured and the results were reported as sensitive, semi-sensitive and resistant.

Findings

Morphological

In this study, among the 135 clinical specimens, 38 samples of Staphylococcus aureus were isolated and identified. Disk results for each antibiotic disc were isolated in 38 isolated bacterial isolates. And showed that 17 isolates (46.9%) of the 38 isolates studied in the present study, resistant to Mupirocin antibiotic resistance 11 isolates (30%) were resistant to oxacillin antibiotics and were considered as MRSA. Also oxacillin antibiotics were more susceptible to antibiotic mupirocin (Table 1). the highest diameter of oxalic acid antibody was observed in isolated Staphylococcus aureus isolates from the nose, urine, throat swab and other parts respectively. And the highest diameter of the non-growth pathway of antibiotic mupirocin was observed in isolates isolated from clinical specimens in other parts, urine, nose, and throat swabs respectively.

Table 1. Number and percentage of susceptible, semi-sensitive and resistant samples for Oxacillin and Mupirocin antibiotics

	S	I	R
Oxacillin	23(62%)	3(8%)	11(30%)
Mupirocin	17(46%)	3(8%)	17(46%)

Molecular

After performing the PCR reaction on Staphylococcus aureus specimens and loading the reaction product on 2% agarose gel, Staphylococcus aureus possesses spa and pvl gene in a band of 347 bp. In this study, we tried to examine the presence of two spa and pvl genes in Staphylococcus aureus, the results showed that the number of positive spa samples was higher than negative samples and also the number of negative PVL samples compared to more positive samples. From the 38 strains of Staphylococcus aureus 30 isolates possessed the spa gene and 18 isolates possessing pvl as well as 18 strains had both spa and pvl genes at the same time(Table 2).

Table 2. The number of positive and negative samples for pvl and spa based on sample types

	Nose		Urine		Throat Swab		Blood Culture		Other	
	pvl	spa	pvl	spa	pvl	spa	pvl	spa	pvl	spa
POSITIVE	13	20	1	5	2	2	0	1	2	2
NEGATIVE	12	5	5	1	1	1	1	0	1	1

Discussion

Pantone Valentine Leukocyidine is an exotoxin that is controlled by the luk F PV and Luk S PV genes, protein S and F are separated from each other by electrophoresis, each of which alone is inactive and both of which are toxin antigenic and can be converted to toxoid [9, 12]. The spa gene is characterized by the diversity of the X region for the typing of strains of Staphylococcus aureus. Therefore, it is necessary to identify the infection agent properly by using appropriate diagnostic methods and conducting molecular studies in this regard can be very helpful. It seems that achieving a fast and repeatable method in medical centers will help to quickly diagnose and control strains of the gene spa and other toxins generating the bacterium. In this study, from 38 strains of Staphylococcus aureus, 18 strains were positive for the presence of pvl protein and 30 strains for the presence of a positive spa protein.The most strains of pvl and spa were positive to isolated strains of the nose and in samples isolated from blood culture, no positive PVL strain was reported.

In the present study, the most positive isolates of pvl and spa were respectively nasal, urine, swab and other samples and at the end of the blood culture, the isolates of pvl and spa p were positive for MRSA. In a study by Moodley et al. ,the spa gene was studied in 320 Staphylococcus aureus and 5 patterns of spa were reported [20]. In Iran, many studies have also

been done on strain typing of Staphylococcus aureus. Among these we can mention the study of Iman Eini and his colleagues in 2011. In this study 21 different patterns of spa including two new patterns of t7685 and t7692 were reported [21].More recently, more research is focused on PVL-positive MRSA strains. If positive MSL-positive PVL infections also play an important role in the release of PVL strains. About 60% of all strains of Staphylococcus aureus PVL positive in England in the last five years were sensitive to methicillin [22].

Conclusion

The results of this study showed high resistance of Staphylococcus aureus strains to oxacillin antibiotics and mupirocin antibiotics. Therefore, in order to prevent the increase in resistance to these antibiotics as well as other common antibiotics, unwanted prescription and unnecessary use of antibiotics should be avoided. In order to prevent drug resistance, it is suggested that antibiogram test be used before using them. Therefore, the selection of appropriate antibiotics is important for the correct treatment of staphylococcus aureus infections and prevention of resistance to antibiotics that are effective against the dominant strains. Also due to the high frequency of spa and pvl genes among Staphylococcus aureus-resistant methicillin strains as well as the severe and deadly causes of the disease early detection and appropriate treatment to prevent the progression of the disease should be considered.

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