Molecular epidemiology of the *Staphylococcus aureus* by Rep-PCR method in Sanandaj hospitals

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Abstract: *Staphylococcus aureus*is a major pathogen within hospital and community. The aim of this study was genetic relationship determination in isolates bacteria and dynamic transmission in hospital. Eighty eight *S. aureus*strains isolated from different clinical samples and were characterized by Repetitive extragenic palindromic (Rep–PCR) technique. The received results and the similarity between the strains were determined on the basis of the Jaccard similarity coefficient in the SAHN program of the NTSYS-pc software. The Rep–PCR profile allowed the typing of the 88 isolates into 7 main clusters. In conclusion, our results showed more diversity in *S.aureus* isolates that indicates the low rate of hospital infection in Sanandaj hospitals and the results of the share pattern of especially among ICU, Pediateric and Internal wardsindicate the exist of nosocomial infections in these wards, According to the study that we carried out the greatest resistance was observed to erythromycinAnd for all drives vancomycin-resistant, MIC of vancomycin using E-test strips were placed by all who are sensitive, so that the Disc Diffusions are not reliable at all.

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Introduction

Staphylococcusaureus is a major pathogen within hospital and in the community(1). This pathogen has persisted and is now resurging as an important hospital and community acquired pathogen(2). The development of resistance to a wide range of antibiotics in *S. aureus*is diversified, such as resistance to methicillin that takes the account of *S.aureus*to most β-lactams, macrolides and aminoglycosides(3). Fingerprinting of this bacterium has been detected by pulsed-field gel electrophoresis (PFGE)(4), multilocus sequence typing (MLST)(5, 6), and repetitive extragenic palindromic polymerase chain reaction (rep-PCR). The Rep-PCR principle is based on a solid primer binding to the same chromosomal regions that allow amplification of the region between them. For this purpose, three kinds of Repetitive elements that are typically used including REP (Repetitive extragenicpalyndromic) ERIC (enterobacterial repetitive intergenic consensus) and BOX(7). These sequences can be amplified using a set of primers (8). Rep-PCR provides high potential for epidemiological studies in which a large number of strains of bacteria could be analyzed(9). The rep-PCR confirmed the differential method and a device with the ability to repeat subtype analysis of microbial and microbial epidemiology. This method is favorable due to high sensitivity and fast procedure(10). It is evident that resistance bacteria associated with increased costs of treatment. We did some studies in regard other bacteria in our university in order to clarify the resistance and transmission (11-17). The emergence of antibiotic resistant strains of *Staphylococcus aureus* leads to increased length of hospitalization, medical expenses and mortality. Therefore, one of the main problems in the control of nosocomial infections is determination of infectious and resistant strains. The purpose of this study was identifying the source and spread of resistant strains.

Materials and methods

Bacterial strains and identification

This study included 88 *S. aureus* isolates collected from clinical specimens, hospital environment and hospital staff, 50, 20 and18 cases, respectively, between 2011 and 2012 in Kurdistan University Toohid and Beasat hospitals. Clinical specimens included urine, wound, abscess, blood and cerebrospinal fluid. All isolates were previously identified as *S.aureus* by a standard microbiological procedure(18). Isolates were incubated at 37°C for 24h on blood agar; Single colonies were tested with tube and slide coagulase, catalase and DNase tests and growth on mannitol salt agar. Isolates were confirmed by using PCR of the thermonuclease (nuc) gene as a gold standard(19).

Antibiotic susceptibility testing

Antibiotic sensitivity of the bacteria was tested by the standardized agar diffusion test on Muller-Hinton agar. Sensitivity to antibiotics were determined

by using Kirby-Bauer methods with gentamicin 10µg, vancomycin 30µg, ciprofloxacin 5µg, erythromycin 15µg stratified according to CLSI (Clinical and Laboratory Standards Institute) were tested(20) and for all drives vancomycin-resistant, MIC of vancomycin using E-test strips were placed.

Rep-PCR

DNA template was prepared and purified and stored until needed at -20^{C}(21). The primers used for the REP-PCR reaction were *REP1R-I, 5'-IIIICGICG ICA TCI GGC-3'and REP2-I, 5'ICG ICT TAT CIGGCC TAC-3'* (22). Rep-PCR reactions were performed in 25 µl volumes containing of 1 µl purified DNA, 1 µl of each primer (final concentration 50pmol/µl), 12.5 µl of Master Mix (Applied Biosystem) and 9.5 µl of deionized water. The Rep-PCR amplification conditions for Rep primer were as: the initial denaturation 95°C, 2min, and next 35 cycles consisting of a denaturation step 92°C, 1min; annealing 40°C, 1min; extension 65°C 8min as well as a final extension step 65°C for 8min and storage at 4°C. The Rep-PCR fragments obtained were examined by DNA electrophoresis, using 1.5% agarose gel, 1-kb DNA markers (Fermentas) and applying an electric field of 100 V during 45 minutes (23) and stained for 10 min with a solution containing 0.5 mg of ethidium bromide per ml. Rep-PCR fingerprints of amplified DNA fragments obtained by agarose gel electrophoresis were recorded by analysisof Gel images using an electronic documentation system. The positions of the bands on each lane and each gel were normalized using the 1kb DNA ladder (Fermentas) as an external reference standard. The presence of a given band was coded as 1 and the absence of a given band was coded as 0 in a data matrix and analyzed using the NTSYS-pc software. The similarity between the strains was determined on the basis of the Jaccard similarity. The dendrogram was constructed on the basis of the averaged similarity of the matrix with the use of the algorithm of the Unweighted Pair-Group Method (UPGMA) in the SAHN program of the NTSYS-pc software(24). The nearest neighbour-joining clustering method has been used to show relations between similar groups.

Results

This study results indicated that 88 *S. aureus* strains were resistance to as vancomycin34 (38.4%), ciprofloxacin47 (53.4%), erythromycin 79 (89.7%) and gentamicin64 (72.7%) are shown in Table1, and for all drives vancomycin-resistant, MIC of vancomycin using E-test strips were placed by all who are sensitive in Figure1. The genomic diversity analysis of 88 strains of *S.aureus* has been carried out with the use of the Rep-PCR fingerprinting method with Rep primers. The electrophoretic profiles of the DNA fragments obtained after PCR amplification using specific primers for REP sequences (7). The Rep-PCR profiles allowed the differentiation of the 88 isolates Rep-types which were grouped into seven main clusters (H1–H7) Figure 2. Complex patterns of fingerprints have been obtained for all the examined strains. Generally, the electrophoretic analysis of the PCR reaction products has revealed that the number of bands in particular electrophoretic paths ranged from six to15. The sizes of the PCR products ranged from 100 bp to about 2500bp. Isolates of first groupmakesupto 28.4% of total isolates that their similarity is 16%, as shown in Table2. The characterization of 8patterns and genetic diversity of each pattern that contain more than one isolates on each has been shown in Table 3.

Table 1. Antibiotic resistanceof 88 *S.aureus* strains

Staphylococcus	**Gentamicin(%)**	**Ciprofloxacin**(%)	**Vancomycin**(%)	**Erythromycin(%)**
Resistance	64(72.7)	47(53.4)	34(38.6)	79(89.8)
Sensitive	24(27.3)	41(47)	54(61.4)	9(10.2)

Table 2. Clustering and similarity of *S. aureus* isolates and Number of strains in each cluster

Clusters	**Dendrogram similarity in clusters**	**Number of strains in each cluster(%)**	**Subclusters**	**Dendrogram similarity in Subclusters**	**Number of strians in each Subcluster(%)**
H1	16%	25(28.4)	H1'	18%	21(24)
			H1"	24%	4(4.55)
H2	20%	37(42)	H2'	38%	11(12.5)
			H2"	25%	26(29.5)
H3	27%	6(6.8)	H3'	42%	3(3.41)
			H3"	51%	3(3.41)
H4	18%	13(14.8)	H4'	21%	4(4.5)
			H4"	23%	9(10.2)
H5	0.00%	1(1.14)			1(1.14)
H6	25%	3(3.41)			3(3.41)
H7	25%	3(3.41)			3(3.41)

Table 3. Genetic diversity and characterization of 8 patterns of 88 *S.aureus*clinical isolates

Pattern	Clusters (no)	Hospital(%)		Specimens(%)				Hospital ward(%)			Antibiotic(%)			
		T	B	U	B	N/S	E	ICU	P	I	VA	E	CIP	GM
N1	H1 (2)	2(10.5)		2(10.5)						2(10.5)	2(10.5)	2(10.5)	2(10.5)	2(10.5)
N7	H1 (2)		2(10.5)		2(10.5)				2(10.5)		1(5.26)	2(10.5)	1(5.26)	1(5.26)
N12	H1 (3)	1(5.26)	2(10.5)		3(16)				2(10.5)	1(5.26)	0.00	1(5.26)	1(5.26)	1(5.26)
N30	H2 (2)		2(10.5)		1(5.26)		1(5.26)		2(10.5)		2(10.5)	2(10.5)	0.00	2(10.5)
N35	H2 (2)		2(10.5)		2(10.5)				1(5.26)	1(5.26)	1(5.26)	2(10.5)	2(10.5)	2(10.5)
N50	H2 (4)		4(21)				4(21)	4(21)			3(16)	4(21)	1(5.26)	3(16)
N71	H4 (2)	2(10.5)				2(10.5)		1(5.26)		1(5.26)	2(10.5)	2(10.5)	1(5.26)	0.00
N74	H6 (2)	2(10.5)		1(5.26)	1(5.26)			1(5.26)		1(5.26)	0.00	2(10.5)	1(5.26)	0.00
Total	7 (19)	36.84	63.61	15.79	36.84	21.05	26.32	31.58	36.84	31.58	57.89	89.47	47.37	57.89

Abbreviation: **T**:Toohid, **B**:Beasat, **U**:Urine, **B**:Blood, **N/S**:Nasopharynx swab, **E**: Environment, **ICU**: Intensive Care Unit, **P**:Pediatric, **I**:Internal, **VA**: vancomycin, **E**: erythromycin , **CIP**: ciprofloxacin , **GM**: gentamicin

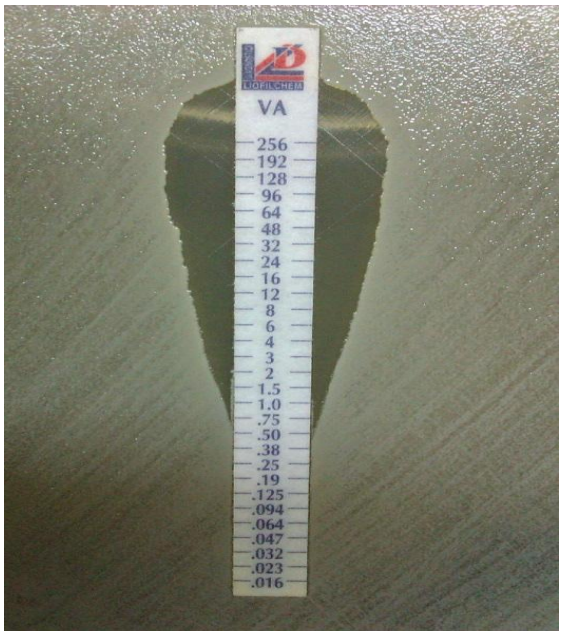

Figure 1. MIC of vancomycin using E-test strips

Discussion

Staphylococcus aureus is one of the most cause of nosocomial and community-acquired infections worldwide(18). This is the first study in this region to detect Molecular epidemiology of the *Staphylococcus aureus* by Rep-PCR method in Sanandaj hospitals. There are several methods for determination of transmission of bacterial trace in community and hospital. Rep_PCR is a partial, suitable method, which can be used for screening, especially in laboratories that lack more specialized equipment such as those used for PFGE or DNA sequences. This technique provide a simple and rapid discriminatory means of molecular epidemiology typing *Staphylococcus aureus* involved in nosocomial infections(25). Rep-PCR typing method showed 77 patterns for 88 clinical isolates. Also bacterial pattern were further divided into seven main clusters (H1–H7) which H2 was the largest cluster. Sixty nine (78.4%) of isolates has single profile whereas 19 (21.5%) have shared pattern and indicate the same origin of dissemination. According to the study that we carried out the greatest resistance was observed to erythromycin. These results are in agreement with the study of Safdari *et al*, but higher than rate reported(26). Blood and environmental isolates taken from the Pediatric ward had the same genetic pattern, PatternN30. Blood samples of patients of the Pediateric and Internal wards had the same genetic pattern, PatternN35. Nasopharynx swabs samples taken from the ICU, and internal staffs as well as blood and urine samples of patients in these wards showed the same genetic in pattern, N74 and similar pattern, N71 and there was a correlation between ICU and Internal staffs. The aim of this study was the showing of genetic relationship in *S. aureus* isolates and its transmission in hospital. Most of isolates have unique pattern and low similarity between the most isolates. The patterns indicate that rate of nosocomial infections are very low and that the sources of infections are variable in Sanandaj hospitals. The results of shared patterns, especially among ICU, Pediatric and all Internal wards indicate the exist of nosocomial infections in these wards. Staphylococcal bacteremia is one of the most causes of mortality and morbidity, especially in pediatric patients and ICU. ICU is one of the important hospital wards critical in the treatment of many serious diseases, which needs particular cares (14, 16).There was not any data in regard to prevalence of *S.aureus*in Iran by Rep-PCR.

In conclusion, our results showed more diversity in Rep-PCR patterns in *S. aureus* isolates. Therefore patterns indicates the low rate of hospital accrued infections in Sanandaj hospitals and the results of the shared patterns especially among ICU, Pediatric and Internal wards indicate the exist of nosocomial infections in these wards. Therefore control of hospital infection, although costly, difficult and time consuming, but is necessary and affordable. Resistance to vancomycin disk diffusion test was performed as

reported in this study that may be due heterogeneous vancomycin resistance Staphylococcus aureus (hVRSA) or genes van A, van B resistances, therefore MIC of vancomycin using E-test strips were placed by all who are sensitive so that the Disc Diffusionsare not reliable at all.

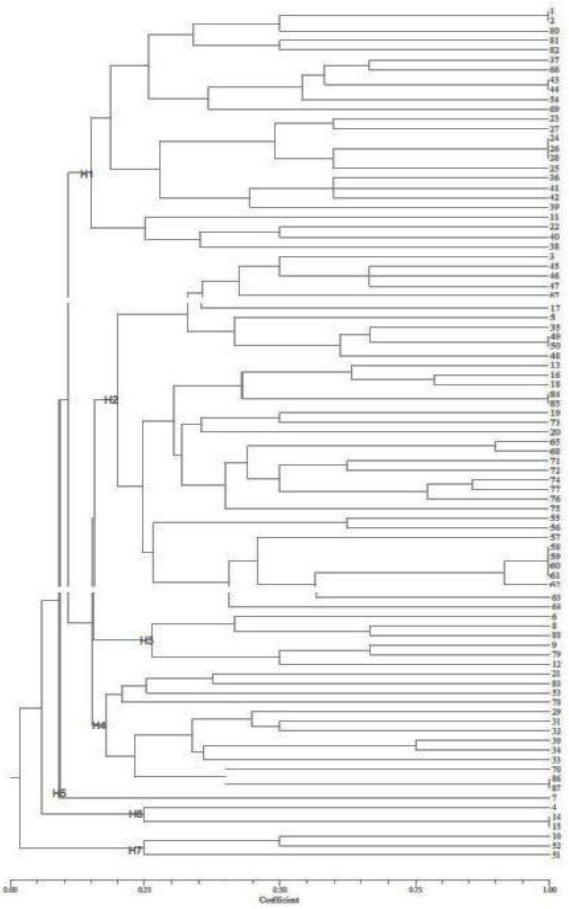

Figure 2. Dendrograms of genomic similarity of 88 *S.aureus* strains in Sanandajhospitalsthe strains were determined on the basis of the Jaccard similarity coefficient in the SAHN program of the NTSYS-pc software.

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