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A study on prevalence of Methicillin and Vancomycin resistance among *Staphylococcus aureaus* isolates in an Iranian 1000-bed tertiary Care Hospital

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ABSTRACT

Methicillin resistant Staphylococcus aureus (MRSA) is one of the most important etiology agent of community and hospital acquired infections. In our country, the prevalence of MRSA is estimated to be 50%. The aim of present study was to determine the prevalence of MRSA and Vancomycin resistant Staphylococcus aureus isolates in an Iranian 1000-bed tertiary care hospital. During a period of September 2013 and June of 2014 a total of 220 non-duplicate S.aureus strains were isolated from clinical specimens. PCR and Cefoxitin disk diffusion methods were used for detection of MRSA. Vancomycin screen agar (BHI agar containing 6µ/ml Vancomycin) and E-test MIC method were used for detection of VISA VRSA. The susceptibility testing of isolates to other antibiotics was used by the disk diffusion method as recommended by CLSI. In our study 145 (65.90%) out of 220 isolates were isolated from urine and the others were isolated from other specimens such as wound and tracheal aspirates. By PCR method 105(47.72%) isolates were found to be MRSA. In our study the results of Cefoxitin disk diffusion method was in accordance to PCR. No VISA and VRSA isolates was determined by Vancomycin screenagar and E-test. The sensitivity and specificity of Cefxoitin disk diffusion method for detection of MRSA was 100%.It is concluded that the rate of MRSA and VRSA in our study was 47.72% and 0% respectively. In laboratories with limited sources for performance PCR, other methods such as Cefoxitin disk diffusion and Vancomycin screen agar are the best alternative methods for detection of MRSA, VISA and VRSA.

INTRODUCTION

Staphylococcus aureaus is a major causative agent of health problems both in hospitals and community. This organism is responsible for a verity of serious infections [1-2] *S.aureus* is an opportunistic pathogen and colonizes in nose, skin, mouth and other parts of healthy human body. [3-4]. Increasing resistance of *S.aureus* against antibiotics such as Methicillin is a major challenge. Methicillin was first introduced in 1959 for treatment of infection caused by *S.aureus* but shortly after introduction of Methicillin the first case of resistance to Methicillin was reported from UK hospitals[5-6] .In European countries approximately 20% of *S.aureus* isolates are reported as Methicillin resistant and in the USA the prevalence of MRSA is more than 50%[1]. According a systemic review and meta-analysis in Iran the mean prevalence of MRSA was 52.7% ±4.7 [7].Methicillin resistance in *S.aureus* is mediated by expression of *mecA* gene which results production of modified Penicillin–binding proteins (PBP2a).In addition recently other genes such as *femA* and auxiliary genes are known which can contribute to MRSA resistance.(8). MRSA isolates are resistant to beta-lactam and other routinely used antibiotics. A glycopeptoide antibiotic including Vancomycin is considered as a drug of choice for treatment infections caused by MRSA. However in 1996 the first Vancomycin –intermediate *S.aureus* (VISA) was reported from Japan and in 2002 the first Vncomycin resistant

S.aureus(VRSA) isolated in USA [1, 9-10]. The aim of this study was to determine prevalence of MRSA and Vncomycin resistance among clinical isolates of *S.aureus* in an Iranian 1000 tertiary care hospital.

MATERIALS AND METHODS

Strains

Identification of MRSA was carried of by PCR based method for detection of *mecA* gen as a gold standard method. Bacterial DNA was extracted by the rapid cell lysis method as described by Unal et al [11-12]. First micro tubes containing the bacteria in PBS were centrifuged at 10000 g for 3 minutes and then the supernatant was discarded. Sediment of bacteria STES and 20 ml solution 100 ml solution TE (pH = 7.6) was added to the micro tube multi-tap to scale well be solved in the solution.30 ml lysosomes were added to the mixture and were incubated at 37 ° C for one hour. Then, 30 ml proteinase (K) for 30 min at 56 ° C micro tubes was added. With regard to the total volume, half the volume of phenol and chloroform, add half the volume was centrifuged for 5 minutes at rpm 13000.After centrifugation, phase separation and the supernatant was transferred to another vial 0.1 volume of sodium acetate and 2.5 times the volume of cold absolute ethanol was added. Vials gently upside down several times, and then for 30 minutes in the freezer was - 20° C. Since then were centrifuged for 5 minutes at rpm 13000rpm. The supernatant was discarded and 500 ml 70% ethanol was added to the vial. After a few hit again for 5 minutes in a centrifuge at 13,000rpm around the supernatant was discarded and vials fixed until completely dry. In the last 100 ml was added to the solution with pH = 8 was for 18-16 hours at room temperature.

The bottles after it were refrigerator. PCR – Multiplex reaction for detection of *mecA* gene Primers for replication were provided form Cinagene company (Cinageneco. Tehran Iran)From this suspension, a 5µL volume was directly used as the template for the PCR amplification of the *mec A* gene fragments. The *mecA*-FTCCAGATTACAACTTCACCAGG and the *mecA*-R CCACTTCATATCTTGTAACG primers were used for the amplification of the 162bp fragment of the Methicillin-resistant gene(*mecA*)[11].The cycling conditions were as follows: 5 minutes at 94°C, followed by 32 cycles of denaturation at 94°C for 50seconds, annealing at 58°C for 50 seconds, extension at 72°Cfor 50 seconds and the final extension step at 72°C for 10 minutes. The PCR products were visualized on a Polyacrylamide gel (4ml Acrylamide- Bic acrylamide 50%, 2.5ml PCRbuffer 0.5XTBE, 180µl APS1%, 25 µl TEMED) dye under a UV transilluminator. Amplicons of 162bp were consistent with the *mecA* gene amplification.

Between September 2013 and June of 2014,two hundred twenty clinical isolates of *S.aureus* were collected. These strains were isolated from different clinical specimen such as blood, urine, sputum, tracheal aspirate ad others. Gram stain and biochemical test such as growth on Mannitol salt agar, Heat-stable nuclease (DNAse), Coagulase and Catalase tests and susceptibility to Novobiocin were used for identification of these isolates. All isolates were kept frozen at -70°C in trypticase soy broth containing 15% glycerol until for performance of susceptibility testing and MRSA detection.

Susceptibility testing of *S.aureus* to Vancomycin

Vancomycin screen agar plates containing6 µg/ml Vancomycin in brain heart infusion agar(CONDA Spain) was prepared in house. A direct suspension from fine isolated colonies of *S.aureus* equal to 0.5 Mc farland turbidity was prepared. Plates made up of brain heart infusion agar were spotted with a 10 micro liter inoculum of bacterial suspension and incubated for 24h .Growth of more than one colony signified as positive results. *S.aureu*s ATCC 25923 and *Enterococcus faecalis* ATCC 51299 were used as negative and positive control respectively. Positive screen-tests were reconfirmed by E-test MIC method.

Disk diffusion susceptibility testing was carried out using modified Kirby_Bauer agar method as recommended by CLSI. A direct suspicion of *S.aureus* isolate equivalent to 0.5 Mc far land standards was prepared .Muller-Hinton agar plates were overload with inoculum and were incubated at 35°C for24hours. Zone of inhibition for each tested antibiotic was measured and interpreted by guideline as recommended by CLSI. Antibiotics disks for susceptibility testing by disk diffusion method included :Penicillin(10U), Cefoxitin (30µg), Ciprofloxacin (5µg), Trimethoprim/sulfamethaxazole (1.25/23.75µg), Tetracycline(30 µg Erythromycin (15 µg) and Clindamycin (2 µg) .The quality control strains for detection of MRSA included methicillin resistant *S aureus*(ATCC 43300) and Methicillin sensitive *S.aureu*s (MSSA ATCC 25293). *Ecoli, Staphylococcus aureus* and *Pseudomonas aeruginosa* were used for quality control of antibiotic disks. All of these strains were provided from Iranian reference health laboratory

RESULTS

From 74572 specimens submitted to microbiology laboratory of milad hospital 220 *S.aureus* isolates were recovered from clinical specimens. Out of 220isolates 145 (65.90%)were isolated from urine and the others were isolated from

other specimens such as wound and tracheal aspirates. Out of 220 patients 56.4% were male and 43.6% female. Among 220*S.aureus* 105(47.72%) isolates were positive for the *mecA* gene by PCR method (Fig -1). In our study all isolates of *S.aureus* were susceptible to Vancomycin by Vancomycin screen agar and we did not observe any intermediate or resistant isolated of *S.aureus* to Vancomycin. For ensuring accuracy of Vancomycin screen agar nearly 10 % of isolates were rechecked by E-test MIC. The antibiotic resistance pattern of *S.aureus* isolates to other antibiotics is shown in table -1

Table-1 Antibiotic resistance profile of *S.aureus* isolates

Antibiotic	Content µg	Sensitive		Intermediate		Resistant	
		Number	Percent	Number	Percent	Number	Percent
Penicillin	10	1	0.5%	0	0%	99	99.5%
Cefoxitin	30	101	43.3%	14	10%	105	47.72%
Ciprofloxacin	5	145	66%	13	6%	62	18%
Co-trimoxazole	25	198	90%	0	0%	22	10%
Tetracycline	30	114	52%	1	0.5%	105	47.5%
Erythromycin	15	44	20%	92	42%	84	38%
Clindamycin	2	129	59%	8	4%	83	37%

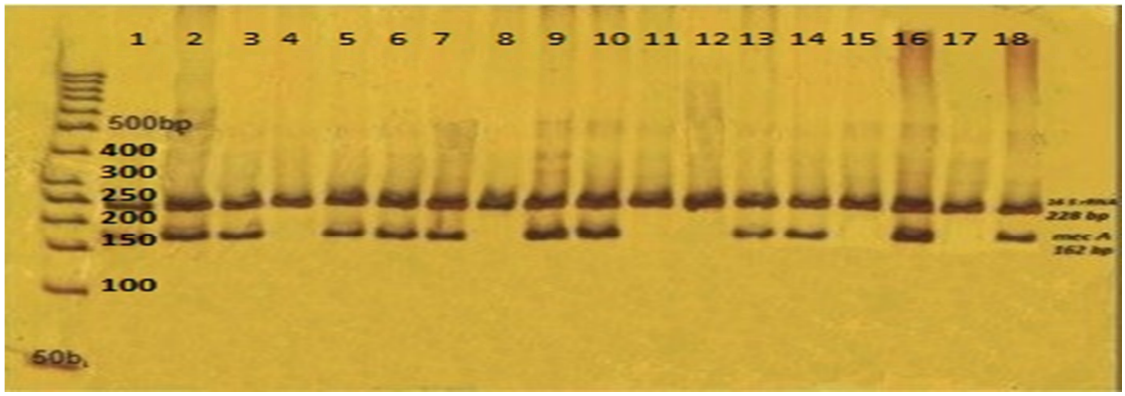

Figure (1): Agarose gel electrophoresis of *mecA* gene by PCR assay

The first Lane from left is ladder.
The lanes 1,2,3,5,6,7,9,10,13,14,and 16 are positive for mecA gene
The lanes 4, 8, 11, 12 and 15 are negative for *mecA* gene
The lane 17 is negative and 18 is positive control.
Fig-2 Prevalence of MRSA in different provinces in Iran

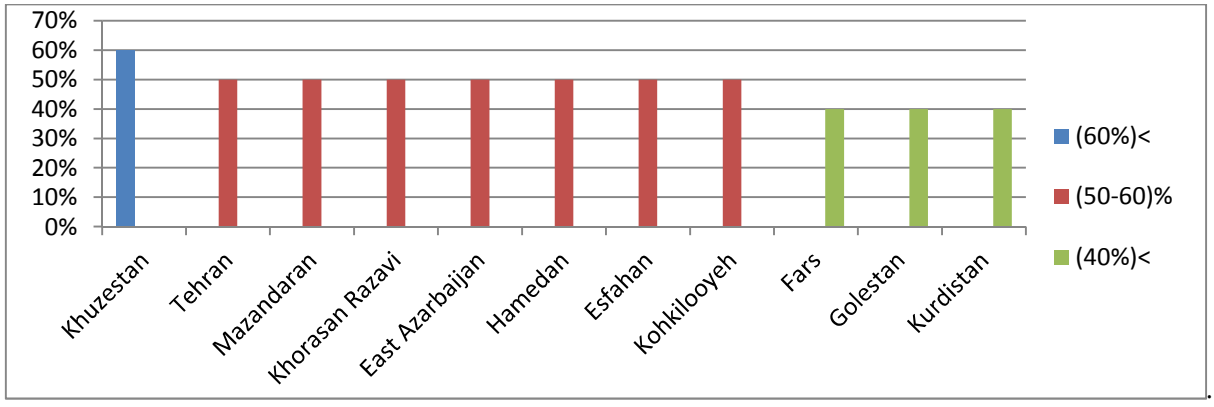

Table-2 Prevalence of MRSA in different countries

Argentina	42.7%
Brazil	33.7%
Chile	45.3%
Colombia	8.6%
Mexico	11.4%
Canada	5.7%
United States	34.2%
Austria	9.4%
Belgium	25.6%
England	27.5%
France	21.4%
Germany	4.9%
Greece	34.4%
Italy	0.5%
Netherlands	2%
Poland	25.8%
Portugal	54.4%
Spain	19.3%
Switzerland	1.8%
Turkey	37.7%
Australia	23.6%
Hong Kong	73.8%
Japan	71.6%
Singapore	62.3%
South Africa	42.9
Taiwan	61.9%
Present study	47.72%

DISCUSSION

The susceptibility testing performance for clinical isolates of *S.aureus* is an important issue for optimal treatment of infections caused by this organism. Methicillin and multidrug resistant *S.aureus* strains are important pathogens responsible for nosocomial and community acquired infections. MRSA and MDR *S.aureus* have few therapeutic options. Unfortunately many of these antibiotics have high cost and in some cases there are not available in developing countries. [13].Microbiology laboratories have a vital role in detection of MRSA and finally therapeutic guideline. Results of our study revealed that the prevalence of MRSA in our hospital is 47.72%. Other studies in different parts of our country have also indicated such a high prevalence. A systemic review and meta-analysis study which carried out in 2012 by Askari E and et al the prevalence of MRSA ranging was from 20.48% to 90% (7) .Variation in prevalence of MRSA may be due to several factors such as efficacy of infection control programs anabatic usage and laboratory methods for detection of MRSA. Fig-2 shows prevalence of MRSA in various provinces of Iran.

Vancomycin is the last choice for treatment of infections caused by MRSA isolates and the emergence of VRSA is an urgent warning for public health [14]. In our study all MRSA were sensitive to Vancomycin. Our finding is in accordance with other studies [15-16]. There is a few reports of Vancomycin resistant *Staphylocoocus aureus* (VRSA)from our country ,however there is not any evidence of confirmation by reference laboratories[17]. Literature review shows there is only one VRSA isolate which has been confirmed by international institutes [18]

Studies in other regional countries have been revealed that, the prevalence of MRSA in Iran in comparison with other neighbor countries is high with exception of Iraq. Studies also have been shown a higher prevalence of MRSA in Asian countries compared to Iran. prevalence of MRSA in other countries is also variable .Prevalence of MRSA in Latin American countries such as Argentine and Mexico is the same as our country and .mean prevalence of MRSA in our country is lower than USA and higher than Australia .In European counties prevalence of MRSA is variable and in total the mean prevalence of MRSA in these countries in lower than Iran. But in some countries such as Portugal similar rate of MRSA to our country has been reported [7, 19].The prevalence of MRSA in different countries has been showed in table-.2

CONCLUSION

It is concluded there is a high rate of MRSA in Iran. The Vancomycin seems to be a drug of choice for treatment of infections caused by MRSA .It is obvious that the most effective way for prevention of MRSA isolates needs a reliable laboratory method for detection of MRSA and establishment of drug resistance surveillance in country.

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REFERENCES

[1] Appelbaum PC1. MRSA--the tip of the iceberg. ClinMicrobiol Infect. **2006** Apr; 12Suppl 2:3-10.
[2] Arora S, Devi P, Arora U, Devi B.Prevalence of Methicillin-resistant *Staphylococcus* aureus (MRSA) in a Tertiary Care Hospital in Northern India. Lab Physicians.**2010**; 2(2):78-81.
[3] Lozano C, Gómez-Sanz E, Benito D, Aspiroz C, Zarazaga M, Torres C.*Staphylococcusaureus* nasal carriage, virulence traits, antibiotic resistance mechanisms, and genetic lineages in healthy humans in Spain, with detection of CC398 and CC97 strains.Int J Med Microbiol. **2011**; 301(6): 500-5.
[4] Verhoeven PO, Gagnaire J, Botelho-Nevers E, Grattard F, Carricajo A, Lucht F and et al . Detection and clinical relevance of *Staphylococcus aureus* nasal carriage: an update. Expert Rev Anti Infect Ther. **2014**, 12 (1):75-89
[5]Askarian M1, Zeinalzadeh A, Japoni A, Alborzi A, Memish ZA.Prevalence of nasal carriage of methicillin-resistant Staphylococcus aureus and its antibiotic susceptibility pattern in healthcare workers at Namazi Hospital, Shiraz, Iranian J Infect Dis. **2009** Sep;13(5):e241-7. .
[6]Enright MC, Robinson DA, Randle G, Feil EJ, Grundmann H, Spratt BG. The evolutionary history of methicillin-resistant *Staphylococcus aureus* (MRSA) ProcNatlAcadSci U S A. **2002** May 28; 99(11):7687-92.
[7] Askari E, Soleymani F, Arianpoor A, Tabatabai SM, Amini A, Naderinasab M. Epidemiology of mecA-Methicillin Resistant *Staphylococcus aureus* (MRSA) in Iran: A Systematic Review and Meta-analysis. Iran J Basic Med Sci. **2012**; 15(5):1010-9.
[8] Boşgelmez-Tinaz G, Ulusoy S, Aridoğan B, Coşkun-Ari F. Evaluation of different methods to detect oxacillin resistance in *Staphylococcus aureus* and their clinical laboratory utility.Eur J ClinMicrobiol Infect Dis. **2006**; 25(6):410-2.
[9] Zorgani AA1, Elahmer O, Abaid A, Elaref A, Elamri S, Aghila E, Tubbal A.Vancomycin susceptibility trends of methicillin-resistant *Staphylococcus aureus* isolated from burn wounds: a time for action. J Infect Dev Ctries. **2015** ; 9(11):1284-8.
[10]Chambers HF. The changing of epidemiology of *Staphylococcus aureus*.Em. Infect Dis. **2001**: 792): 178-182.
[11] Unal S, Hoskins J, Flokowitsch JE, Wu CY, Preston DA, Skatrud PL. Detection of Methicillin-resistant staphylococci by using the polymerase chain reaction. J ClinMicrobiol **1992**; 30:1685-91.
[12] Perez-Roth E, Claverie-Martin F, Villar J, Mendez-Alvarez S. MultiplexPCR for the simultaneous identification of *Staphylococcus aureus* and the detection of methicillin and mupirocin resistance. J ClinMicrobiol **2001**; 39:4037-41
[13]SathishJV,Janakiram K, Vijya D. Inducible clindamycin resistance in *Staphylococcus aureus*: reason for treatment failure. JIMD. **2015**:2(2): 97-103
[14] Rahimi F, Bouzari M, Katouli M, Pourshafie MR. Antibiotic resistance pattern of methicillin resistant and methicillin sensitive *Staphylococcus aureus* isolates in Tehran, Iran .JundishapourJ.Microbiol. : **2013**: 6(2) 144-49.
[15] FarhadianA BehzadianNejad Q, Najarpeerayeh SH, Rahbar M andVaziri F.Determination of Vancomycin and Methicillin Resistance in Clinical Isolates of *Staphylococcus aureus* in Iranian Hospitals.British Microbiology Research Journal. **2014**; 4(4): 454-461
[16] Aghazadeh,, M,Rahba M, KabehMonnavar M ,SavaheliMoghadamM.Sensitivity pattern of methicillin resistant and methicillinsensitive*Staphylococcus aureus* isolates, against lseveral antibiotics including tigecycline in Iran: A hospital based study.Pak J Med Sci April - June **2009** (Part-II) Vol. 25 No. 3 443-446
[17] Moravvej Z1, Estaji F, Askari E, Solhjou K, NaderiNasab M, Saadat S.Update on the global number of vancomycin-resistant *Staphylococcus aureus* (VRSA) strains.Int J Antimicrob Agents. **2013**; 42(4):370-1.
[18] Azimian A, Havaei SA, Fazeli H, Naderi M, Ghazvini K, Samiee SM, Soleimani M, PeerayehSN.Genetic characterization of a vancomycin-resistant *Staphylococcus aureus* isolate from the respiratory tract of a patient in a university hospital in northeastern Iran.J ClinMicrobiol.**2012** ;50(11):3581-5.
[19] Diekema DJ1, Pfaller MA, Schmitz FJ, Smayevsky J, Bell J, Jones RN, Beach M; SENTRY ParticipantsGroup. Survey of infections due to Staphylococcus species: frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, Latin America, Europe, and the Western Pacific region for the SENTRY Antimicrobial Surveillance Program, 1997-1999.Clin Infect Dis. **2001** May 15; 32 Suppl 2:S114-32.