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Frequency of *mecA, pvl, arcA, speG* genes in *Staphylococcus aureus* isolated from Burn Wounds in Tabriz, Iran

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Abstract

Background: Burn wounds provide a proper position for bacterial growth and are more persistent richer sources of infection than other wounds. Staphylococcus aureus (S. aureus) is one of the most commonly isolated pathogens in burn wound infections. Methods: One hundred S. aureus isolates were collected from burn wound infections. Identification of S. aureus was performed by phenotypic and molecular methods. The Kirby-Bauer and agar dilution methods were performed for determination of antibiotic susceptibility patterns. Polymerase chain reaction (PCR) was carried out for detection of mecA, pvl, nuc, arcA and speG genes. Results: Methicillin resistant S. aureus (MRSA) was detected in 21 % of isolates by mecA gene PCR and phenotypic methods. All isolates were negative for arcA and speG genes. All of isolates were susceptible to linezolid, vancomycin and mupirocin. The resistance rate to erythromycin and clindamycin were high. Conclusion: Our findings showed that S. aureus isolates have high-level resistance to antimicrobial agents and that appropriate antibiotic therapy, based on the antibiotic susceptibility pattern, is essential to ensure a good result. Thus, strict consideration for S. aureus infection and proper usage of antibiotic policy are recommended in decreasing the incidence and occurrence of multidrug resistant S. aureus infections in burn wound infections in this hospital.

Keywords: Antibiotic Susceptibility Patterns, Burn Wound Infection, Minimum Inhibitory Concentration, *Staphylococcus aureus.*

Introduction

Burn wound is a main problem in many parts of the worldwide. It has been predictable that 75% of all deaths following thermal wounds are associated with infection (Srinivasan et al.,2009). Thermal wound destroys the skin barrier that normally prevents invasion by microorganisms such as *Staphylococcus aureus* (*S. aureus)*, making the burn wound the most frequent origin of sepsis in these patients (Saha et al.,2011). *S. aureus* has long been recognized as an important human pathogen that causes various infections, often with high morbidity and mortality. In addition to, dependent on its intrinsic virulence or the ability of the host to contain its opportunistic behavior, S aureus can cause a dangerous diseases in human (Pires et al.,2018;, Islam et al.,2012).

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USA 300 clone was found to be responsible for outbreaks of methicillin resistant *S. aureus* (MRSA) in the United States and other parts of the world. To date, isolation of USA300 community acquired-MRSA has been gradually reported in diverse from different countries (Azimian et al.,2014). It has been related to skin and soft-tissue infections, community-acquired pneumonia, catheter-related bloodstream infections, and other systemic infections. USA300 typically carries Panton-Valentine leucocidin (PVL)-encoding genes, SCCmec element type IV, and arginine catabolic mobile element (ACME) (Planet et al.,2013).

S.aureus readily acquires resistance against all classes of antibiotics by one of two different mechanisms: mutation of an existing bacterial gene or horizontal transfer of a resistance gene from another bacterium. Staphylococcal resistance to β-lactam antibiotics is mediated by either of two mechanisms: (i) production of -lactamase and (ii) production of an altered target penicillin-binding protein (PBP2a), which is encoded by the mecA gene (Brumfitt & Hamilton-Miller,1989; Pinho et al.,2001). *Pvl* is a leukocytolytic toxin produced by less than 5% of S. aureus strains worldwide and has generated considerable interest in recent years, both in medical and media circles (Prevost et al.,1995). The presence of this toxin is associated with skin and soft tissue infections such as recurrent boils, furuncles, abscesses, necrotizing pneumonia (with a high mortality), bone and joint infections (Clark,2008).

The ACME locus from USA300 strains is composed of at least 33 putative genes and 2 operons, referred to as arc and opp. The arc operon encodes genes that are thought to be involved in arginine catabolism and has recently been shown to be important for survival of USA300 in acidic environments (Thurlow et al.,2013). The ACME speG gene, which encodes a spermidine acetyltransferase (SpeG), confers the ability to survive levels of the polyamines spermidine and spermine that are lethal for other strains of S. aureus (Planet et al.,2013, Seiler & Atanassov,1994).

The aim of this study was to determine the prevalence of MRSA and antibiotic susceptibility patterns, arcA, speG and pvl genes of S. aureus isolates obtained from burn wound infections.

Materials and Methods

Bacterial isolates

One hundred S. aureus isolates were collected by using sterile applicator stick with cotton swabs from wound pus swabs specimens. Specimens were collected aseptically from all inpatients and outpatients during the study period attending Sina hospital burn unit, Tabriz, Iran. All specimens were transported to microbiology department laboratory for cultures and identifications. The Ethic Commission of Tabriz University of Medical Sciences approved this study (IR.TBZMED.REC.1396.139).

First, a Gram stain smear was processed for the detection of S. aureus in specimens. For the isolation of S. aureus, specimens were plated onto sheep blood (5 %) and mannitol salt agar plates. The plates were incubated at 37 °C under 10 % CO2 and examined at 24 and 48 h. These isolates were identified by Gram staining, catalase, coagulase, DNase activities and presence of nuc gene.

Antimicrobial susceptibility testing

Kirby-Bauer method of antibacterial susceptibility test was performed on Mueller–Hinton agar as per CLSI guidelines (CLSI,2009). The disks used for antibacterial susceptibility test included erythromycin (15 µg), vancomycin (30 µg), clindamycin (2 µg), gentamicin (10 µg), cefoxitin (30 µg), linezolid (30 µg), co-trimoxazole (25 µg) and mupirocin (20 µg). All disks were provided from Mast Ltd., England. The agar dilution assay was used to determine the erythromycin, vancomycin, clindamycin, gentamicin, cefoxitin, linezolid, co-trimoxazole and mupirocin. Minimum inhibitory concentration (MIC) according to the CLSI guideline (CLSI,2009). S.aureus ATCC25923 was used as control strain.

Detection of MRSA

Resistance to methicillin was determined by cefoxitin (30 µg) disk diffusion method and genotypically confirmed by detection of mecA gene using PCR method. For PCR reaction, specific primers of 310-bp fragments for mec A (forward: 5-GTAGAAATGACTGAACGTCCGATAA-3 and reverse: 5- CCAATTCCACATTGTTTCGGTCTAA-3) were used (Sadeghi & Mansouri,2014).

Detection of pvl, arcA and speG genes

A loopful of S. aureus culture was suspended in 300 ml of TE buffer (10 mM Tris-HCL, 1 mM EDTA, pH 8.0) and placed at 80 °C for 20 min to kill the bacteria. DNA was extracted by CTAB method (14). PCR was carried out as previously described (Azimian et al.,2014; Lin et al.,2013; Stegger et al.,2012) , specific primers of pvl, arcA and speG genes were used. DNA amplifications was

performed in 20 µl volumes that contained 10 to 100 ng of DNA, 0.5 µM of each primer, in the presence of 2 mM MgCl2, 100 µM of each dNTP, 50 µm KCl, 20 mM Tris-HCL, pH 8.4, and 2.5 U recombinant DNA polymerase. Gel electrophoresis was performed for 45 min on a 1.2 % agarose gel at 80 V and after staining with 0.5 µg/ml ethidium bromide visualized under UV light.

Results

Sixty (60%) male and forty (40%) female with a mean age of 38/8 years were studied during a determined period. We evaluated 100 *S. aureus* isolates, which were obtained from burn ward. All of the isolates that were positive for the nuc gene by PCR, with a 267 bp on agarose gel electrophoresis, were confirmed as S. aureus and were included in this study. Table 1 shows antibiotic susceptibility of *S. aureus* isolates obtained from burn wards. The highest rate of resistance was in erythromycin, clindamycin and cefoxitin with 39%, 35% and 24%, respectively. All isolates were susceptible to linezolid, vancomycin and mupirocin. In this study, 24 (24%) isolates were MRSA according to cefoxitin disk and oxacillin MIC.

Multi drug resistant (MDR) isolates were observed in 26 (26%) isolates. MDR was defined as acquired nonsusceptibility to at least one agent in three or more antimicrobial categories.

According to MIC test, figure 1 shows the distribution of antibiotic MIC in *S. aureus* isolates. The high rate of MIC was in erythromycin and clindamycin with 30% and 28%, respectively.

MecA and *pvl* genes were detected in 21 (21 %) and three (3 %) isolates (Table 2). When these isolates were subjected to PCR to screen for the presence of the speG and arcA genes, all isolates were negative. The components of PCR reactions were cross-checked for the confidence of action and repeated 3 times.

Table 1- Antibiotic resistance of S. aureus isolates obtained from burn ward

Antibiotic	Disk diffusion	MIC
Erythromycin	39%	30%
Clindamycin	35%	28%
Gentamicin	22%	18%
Cefoxitin	24%	24%
Co-trimoxazole	19%	17%
Vancomycin	-	0
Linezolid	0	0
Mupirocin	0	0

Table 2- Detection of meticillin resistance among S. aureus isolates by presence of the mecA gene, and MIC of oxacillin.

Breakpoint of oxacillin (µg/ml)	**PCR detection**	
	mecA negative (n= 79)	*mecA* positive (n= 21)
≤0.25	23	0
1	27	0
2	26	0
4	3	0
8	0	10
16	0	7
32	0	4
64	0	0
128	0	0
≥256	0	0

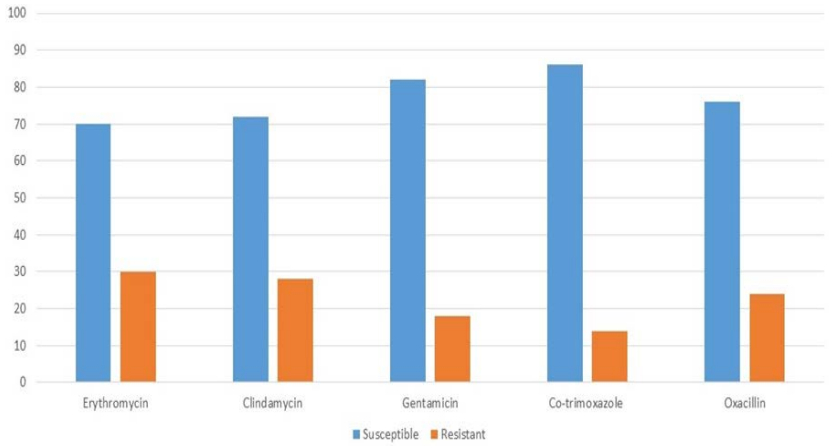

Fig. 1: Distribution of antibiotics MIC (minimum inhibitory concentration) in S. aureus isolates.

Discussion

Despite advances in the use of topical and parenteral antimicrobial therapy, bacterial infection specially *S. aureus* infection remains a major problem in the management of burn (Papini et al.,1995).

S. aureus can colonized in nasal and skin sites, especially anus and armpits of human. Burn patients have lost their primary barrier and exposed to microorganism invasion continually and chronically in the pathogens (Alebachew et al.,2012). The principal normal flora of burn wounds in hospitalization including Gram-positive bacteria such as staphylococci and Gram-negative bacteria like Pseudomonas aeruginosa (Warner et al.,2009).
S. aureus was frequently isolated pathogen in both community and hospital practices. The antimicrobial susceptibility pattern of S. aureus change, especially in developing countries making antimicrobial agents increasingly less effective. Antibiotic sensitivity patterns attended as a useful guideline for selecting suitable drugs (Bukhari et al.,2004).

In our study, high-level resistance was observed to erythromycin (39%) and clindamycin (35%) that similar to the other studies carried out by Akhi et al and Alebachew et al (Warner et al.,2009; Akhi et al.,2015).

In addition to, all of S. aureus isolates are susceptible to vancomycin, mupirocin and linezolid. This result is in agreement with Uchenna et al and Gebreselassie et al studies (Ozumba,2005; Gebreselassie,2002).

Increasing antimicrobial resistance among S. aureus isolates is a matter of concern, with limited treatment options available for multidrug-resistant strains (Bhat & Vasaikar,2010).

In this study, 24 % of S. aureus isolates were methicillin resistant that was determined by using the 30 µg cefoxitin disk. This is in agreement with other results reported by Zubair et al and El-Tahawy from other wounds (25, 26). As well as, different studies reported frequency of MRSA were 24 % to 58 % (24, 27). This could be due to prolonged antibiotic therapy and use of broad-spectrum antibiotics that may increase incidence of antibiotic resistant organism (Akhi et al.,2015).

In the present study, 26% of the S. aureus isolates were MDR. This could be due to continuous usage of broad-spectrum antibiotics and non-adherence to a hospital antibiotic policy. Additionally, selective pressure in the hospital wards could also be taken as the most probable factor for the increased resistance in isolates from the patients. Because MDR is resistant to several antibiotics, infections with these bacteria could be treated with extended spectrum antibiotics for longer duration. Consequently, duration of hospital stay for infections with MDR can be longer and their management can be more costly (Alebachew et al.,2012; Akhi et al.,2015; Bhat & Vasaikar,2010).

In the present research, from all MRSA, three isolates were methicillin-resistant by cefoxitin disk diffusion and oxacillin agar dilution methods, but did not show the amplification of the mecA gene. Similar to other research carried out by Perez et al. and Sadeghi et al. difference between the phenotypic and genotypic tests was reported (Sadeghi & Mansouri,2014; Perez et al.,2008). This discrimination between the phenotypic and genotypic evaluates may be due to culture settings (as temperature, configuration of culture medium, size of inoculums, and time of incubation). In addition, an association of resistance mechanisms and genetic background (translated by interfering of other genes in the control of resistance appearance to oxacillin) prevent the standardization of approaches for detecting

MRSA. In addition to, the PCR assay is unable to detect methicillin resistant mechanism mediated by other than the mecA gene, and the cefoxitin disk may fail in showing low-level or heterogeneous resistance strains (Palazzo et al.,2007).

In recent years, the prevalence of pvl in S. aureus strains is increasing due to global distribution of pvl-producing MRSA strains. In our investigation, 3 % of isolates were pvl -positive. Our results are consistent with the results of Kilic et al. and Sadeghi et al. that the prevalence of pvl-positive S. aureus was reported to be 1.3% 3.08%, respectively (Sadeghi & Mansouri,2014; Kilic et al.,2008). In contrast, in the study of Havaei et al., 24.16% of S. aureus isolates were reported to be pvl-positive (Havaei et al.,2010).

Conclusion

This study result may pave a way for providing useful guidelines in choosing to empirical antimicrobial therapy especially in areas where culture facility is not available against S. aureus isolate from burn patients. Regular surveillance of burn wound organisms and their antimicrobial resistance patterns will help in formulating empirical antibiotic therapy and reducing mortality from septic events. Our results revealed that the prevalence of MRSA isolates in this region is slightly high. On the other hand, with the existence of PVL in the isolates, the combination of the mecA gene and the pvl gene would create a super adaptable S. aureus strain that is multidrug-resistant and capable of rapid spreading. At the results, screening of S. aureus for mecA and pvl genes can help to prevent of rapid spreading and sever infections.

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Conflict of interest

The authors have reported no conflict of interest

References

Akhi, M.T., Ghotaslou, R., Asgharzadeh, M., Varshochi, M., Pirzadeh, T., Memar, M.Y., et al. (2015). Bacterial etiology and antibiotic susceptibility pattern of diabetic foot infections in Tabriz, Iran. GMS hygiene and infection control.10.

Alebachew, T., Yismaw, G., Derabe, A., Sisay, Z. (2012).Staphylococcus aureus burn wound infection among patients attending Yekatit 12 hospital burn unit, Addis Ababa, Ethiopia. Ethiopian journal of health sciences. 22(3).

Azimian, A., Havaei, S.A., Khosrojerdi, M., Naderi, M., Samiee, S.(2014). Isolation of PVL/ACME-Positive, Community Acquired, Methicillin-Resistant Staphylococcus aureus (USA300) from Iran. Journal of Medical Microbiology and Infectious Diseases.2(3):100-4.

Bhat, V., Vasaikar, S. (2010). Bacteriological profile and antibiogram of aerobic burn wound isolates in Mthatha, Eastern Cape, South Africa. Southern African Journal of Epidemiology and Infection. 25(4):16-9.

Brumfitt, W., Hamilton-Miller, J. (1989). Methicillin-resistant Staphylococcus aureus. New England Journal of Medicine.320(18):1188-96.

Bukhari, M., Iqbal, N, Naeem, S., Qureshi, G., Naveed, I., Iqbal, A., et al. (2004). A laboratory study of susceptibility of methicillin resistant Staphylococcus aureus (MRSA). Pakistan Journal of Medical Sciences. 20(3):229-33.

Clark, J. (2008).A brief review of Panton-Valentine leukocidin producing staphylococcal infections in the intensive therapy unit. Current Anaesthesia & Critical Care.19(5-6):330-2.

CLSI, C. (2009). Performance of standards for antimicrobial disk susceptibility tests; approved standards. Clinical and Laboratory Standard Institute Wayne, PA. USA.

El-Tahawy, A.T. (2000). Bacteriology of diabetic foot. Saudi Medical Journal. 21(4):344-7.

Gebreselassie, S. (2002). Patterns of isolation of common gram positive bacterial pathogens and their susceptibilities to antimicrobial agents in Jimma Hospital. Ethiopian medical journal. 40(2):115-27.

Havaei, S., Moghadam, S.O., Pourmand, M.R., Faghri, J. (2010). Prevalence of genes encoding bi-component leukocidins among clinical isolates of methicillin resistant Staphylococcus aureus. Iranian journal of public health. 39(1):8.

Islam, M., Yusuf, M.A., Chowdhury, M., Hossain, M.A. (2012). ESBL producing gram negative aerobic bacteria isolated from burn wound infection with their antibiogram in dhaka. Journal of Science Foundation.10(2):1728-7855.

Kilic, A., Guclu, A.U., Senses, Z., Bedir, O., Aydogan, H., Basustaoglu, A.C. (2008). Staphylococcal cassette chromosome mec (SCCmec) characterization and panton-valentine leukocidin gene occurrence for methicillin-resistant Staphylococcus aureus in Turkey, from 2003 to 2006. Antonie Van Leeuwenhoek.94(4):607.

Lin, Y-T., Tsai, J-C., Chen, H-J., Hung, W-C., Hsueh, P-R., Teng, L-J. (2013). A novel staphylococcal cassette chromosomal element, SCCfusC, carrying fusC and speG in fusidic acid-resistant methicillin-resistant Staphylococcus aureus. Antimicrobial agents and chemotherapy. AAC. 01772-13.

Naqvi, Z.A., Hashmi, K., Kharal, S.A. (2007). Methicillin resistant Staphylococcus aureus (MRSA) in burn patients. Pakistan Journal of Pharmacology.24(2):7-11.

Ozumba, U.C. (2005). Antimicrobial resistance problems in a university hospital. Journal of the National Medical Association.97(12):1714.

Palazzo, I.C.V., Rehder, A., Darini, A.L.C. (2007). Quantitative disk diffusion as a convenient method for determining minimum inhibitory concentrations of oxacillin for staphylococci strains. Journal of microbiological methods.71(3):186-90.

Papini, R., Wilson, A., Steer, J., McGrouther, D., Parkhouse, N. (1995). Wound management in burn centres in the United Kingdom. BJS. 82(4):505-9.

Perez, L.R.R., Dias, C., d'Azevedo, P.A. (2008). Agar dilution and agar screen with cefoxitin and oxacillin: what is known and what is unknown in detection of meticillin-resistant Staphylococcus aureus. Journal of medical microbiology.57(8):954-6.

Pinho, M.G., de Lencastre, H., Tomasz, A. (2001). An acquired and a native penicillin-binding protein cooperate in building the cell wall of drug-resistant staphylococci. Proceedings of the National Academy of Sciences.98(19):10886-91.

Pires, B.M.F.B., de Oliveira, F.P., de Oliveira, B.G.R.B., Fuly, Pd.S.C., Ferreira-Carvalho, B.T., de Paula, G.R., et al. (20108). Monitoring and molecular characterization of Staphylococcus aureus isolated from chronic wounds. Advances in Skin & Wound Care.

Planet, P.J., LaRussa, S.J., Dana, A., Smith, H., Xu, A., Ryan, C., et al. (2013). Emergence of the epidemic methicillin-resistant Staphylococcus aureus strain USA300 coincides with horizontal transfer of the arginine catabolic mobile element and speG-mediated adaptations for survival on skin. MBio.4(6):e00889-13.

Prevost, G., Couppie, P., Prevost, P., Gayet, S., Petiau, P., Cribier, B., et al. (1995). Epidemiological data on Staphylococcus aureus strains producing synergohymenotropic toxins. Journal of medical microbiology.42(4):237-45.

Sadeghi, J., Mansouri, S. (2014). Molecular characterization and antibiotic resistance of clinical isolates of methicillin-resistant Staphylococcus aureus obtained from Southeast of Iran (Kerman). Apmis.122(5):405-11.

Saha, S., Muazzam, N., Begum, S., Chowdhury, A., Islam, M., Parveen, R. (2011). Study on time-related changes in aerobic bacterial pattern of burn wound infection. Faridpur Medical College Journal.6(1):41-5.

Seiler, N., Atanassov, C. (1994). The natural polyamines and the immune system. Progress in Drug Research/Fortschritte der Arzneimittelforschung/Progrès des recherches pharmaceutiques: Springer. p. 87-141.

Srinivasan, S., Vartak, A.M., Patil, A., Saldanha, J. (2009). Bacteriology of the burn wound at the Bai Jerbai Wadia hospital for children, Mumbai, India—A 13-year study, Part I-Bacteriological profile. Indian journal of plastic surgery: official publication of the Association of Plastic Surgeons of India.42(2):213.

Stegger, á., Andersen, P., Kearns, A., Pichon, B., Holmes, M., Edwards, G., et al. (2012). Rapid detection, differentiation and typing of methicillin-resistant Staphylococcus aureus harbouring either mecA or the new mecA homologue mecALGA251. Clinical Microbiology and Infection.18(4):395-400.

Thurlow, L.R., Joshi, G.S., Clark, J.R., Spontak, J.S., Neely, C.J., Maile, R., et al. (2013). Functional modularity of the arginine catabolic mobile element contributes to the success of USA300 methicillin-resistant Staphylococcus aureus. Cell host & microbe.13(1):100-7.

Warner, P., Neely, A., Bailey, J.K., Yakuboff, K.P., Kagan, R.J. (2009). Methicillin-resistant staphylococcus aureus furunculitis in the outpatient burn setting. Journal of burn care & research.30(4):657-60.

Zubair, M., Malik, A., Ahmad, J. (2011). Clinico-microbiological study and antimicrobial drug resistance profile of diabetic foot infections in North India. The Foot.21(1):6-14.