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Detection and Antimicrobial Resistance Properties of *Staphylococcus aureus* Strains Isolated from the Human Clinical Infections

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ABSTRACT

The present investigation was carried out to study the prevalence of *Staphylococcus aureus* and its antimicrobial resistance pattern isolated from various types of human clinical infections. One hundred and fifty clinical specimens were collected from the educational hospitals of Iran. Samples were cultured and those that were *S. aureus*-positive were confirmed using the PCR. Antimicrobial resistance pattern was analyzed using the disk diffusion method. Of 150 samples studied, 50 samples (33.33%) were positive for *S. aureus*. The results of the culture method were confirmed using the PCR amplification of *16S rRNA* gene of the *S. aureus*. Burn infections had the highest prevalence of *S. aureus* (40%). *S. aureus* strains of our study harbored the highest levels of resistance against penicillin (94%), tetracycline (92%), oxacillin (90%) and azithromycin (80%). Keep the skin and especially wound and burn infections clean away from the polluted environment of hospitals and regular prescription of imipenem, methicillin and vancomycin may be good instructions to reduce the risk of *S. aureus* infections in the cases of human clinical infections.

Key words: *Staphylococcus aureus*, Detection, Antibiotic resistance properties, clinical infections, Iran.

INTRODUCTION

Staphylococcus aureus (*S. aureus*) has long been deliberated as a main pathogen of hospital infections all-around the world. *It* is a bacterium that frequently colonizes the human skin. The bacterium can exist in this form without harming its host or causing symptoms. However, if there is a break in someone's skin from a wound, burn or surgery, or if there is a suppression of a person's immune system, then colonizing *S. aureus* can cause an infection. It is documented that the *S. aureus* is one of the most routine causes of skin and soft-tissue infections and especially superficial wounds, post-surgical wounds and burn infections[1-5]. *S. aureus* may also infect others as it can be passed from both infected and colonized people to other people through skin contact or through sharing contaminated objects, such as towels or razors. Therefore, consideration of the *S. aureus* as a threating pathogens for human health especially in the cases of skin infections has critical health importance[6].

The ability of *S. aureus* to resistance against wide range of antibiotics causing severe problems in treatment of hospital infections. *S. aureus* has developed resistance to multiple classes of antibiotics, especially beta-lactams. It has been documented that majority of *S. aureus* strains were resistant to sulfamethoxazole-trimethoprim, erythromycin, oxacillin, ampicillin, penicillin,

tetracycline, chloramphenicol, cotrimoxazole, gentamicin, cefexim and clindamycin[7, 8]. In recent years prevalence of resistance against methicillin and vancomycine has been increased throughout the world [9-13].

MATERIALS AND METHODS

Ethical consideration

The present study was accepted by the ethical committees of the educational Hospitals. Written informed consent was obtained from all of the study patients or their parents.

Samples collection

Overall 150 clinical samples from various types of infections including superficial wound (n=50), post-surgical wounds (n=50) and burn infections (n=50) were collected from hospitalized patients of major educational hospitals of Tehran, Iran. All samples were immediately transferred to the laboratory at 4°C in a cooler with ice packs.

Staphylococcus aureus identification

All samples were directly cultured into 7% sheep blood agar (Merck, Darmstadt, Germany) and incubated aerobically at 37°C for 48 h. After incubation, suspicious colonies were examined by the use of morphologies compatible with *Staphylococcus* spp. (microscopical morphology, catalase and coagulase production). Studied colonies were cultured on Tryptic Soy Broth (TSB) (Merck, Darmstadt, Germany) and Tryptic Soy Agar (TSA) (Merck, Darmstadt, Germany). After growth, staphylococci were identified on the basis of colony characteristics, Gram staining, pigment production, hemolytic and the following biochemical reactions: catalyses activity, coagulated test (rabbit plasma), Oxidase test, glucose O/F test, resistance to bacitracin (0.04 U), mannitol fermentation on Mannitol Salt Agar (MSA) (Merck, Darmstadt, Germany), urease activity, nitrate reduction, novobiocin resistance, phosphatase, deoxyribonuclease (DNase) test and carbohydrate (xylose, sucrose, trehalose and maltose, fructose, lactose, mannose) fermentation test[14].

Antimicrobial susceptibility test

Pattern of antimicrobial resistance was studied using the simple disk diffusion technique. The Mueller–Hinton agar (Merck, Germany) medium was used for this purpose. Antibiotic resistance of *S. aureus* strains against 15 commonly used antibiotics in the cases of UTIs was determined using the instruction of Clinical and Laboratory Standards Institute guidelines[15]. Susceptibility of *S. aureus* isolates were tested against ampicillin (10 u/disk), gentamycin (10 µg/disk), amikacin (30 u/disk), imipenem (30 u/disk), methicillin (30 µg/disk), tetracycline (30 µg/disk), vancomycine (5 µg/disk), norfloxacin (30 µg/disk), cotrimoxazole (30 µg/disk), clindamycin (2 µg/disk), *trimethoprim-sulfamethoxazole* (25 *ìg/disk*), penicillin (10 u/disk), oxacillin (1µg/disk), erythromycin (15µg/disk), azithromycin (15 µg/disk) and cefexime (5 *ìg/disk*) antibiotic agents (Oxoid, UK). The plates containing the discs were allowed to stand for at least 30 min before incubated at 35°C for 24 h. The diameter of the zone of inhibition produced by each antibiotic disc was measured and interpreted using the CLSI zone diameter interpretative standards (CLSI 2012)[15]. *S. aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 were used as quality control organism in antimicrobial susceptibility determination.

DNA extraction and PCR confirmation

Total genomic DNA was extracted from the bacterial colonies. A single colony was inoculated on 5ml of brain heart infusion broth and incubated over night at 37ºC. Then 1.5 ml of a saturated culture was harvested with centrifugation for 5 min. at 14,000 rpm. The cell pellet was resuspended and lysed in 200µl of lysis buffer (40 mM Tris-acetate pH 7.8, 20 mM sodium-acetate, 1 mM EDTA, 1% SDS) by vigorous pipetting. To remove most proteins and cell debris, 66 µl of 5M NaCl solution was added and mixed well, and then the viscous mixture was centrifuged at 12,000 rpm for 10min. at 4ºC. After transferring the clear supernatant into a new eppendorf tube, an equal volume of chloroform was added, and the tube was gently inverted at least 50 times when a milky solution was completely formed. Following centrifugation at 14,000 rpm for 5min., the supernatant is then removed to another eppendorf tube and double volume of 100% ethanol was added. The tubes were inverted 5 to 6 times gently, then centrifuged at 10,000rpm for 5minutes. The supernatant was discarded and 1ml of ethanol (70%) was added to the pellet, and tubes

centrifuged at 10,000 rpm for 5 minutes. Finally the supernatant discarded and the pellet was dried for 10 min at room temperature, the pellet was resuspended by 100µl H2O. The stock was kept at -20ºC until use. The DNA concentration has been determined by measuring absorbance of the sample at 260 nm using spectrophotometer[16]. Presence of *S. aureus* in each DNA samples was confirmed using the Daniel *et al*. (1994)[17] method. The PCR reaction mix consist of 1 X PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl and 0.001% (w/v) gelatin) with 4 mM MgCl2, 250 mM of each nucleotide (deoxynucleoside triphosphate), 0.5 mM of each primer (F: 5'-GGAATTCAAAGGAATTGACGGGGGC -3' and R: 5'- CGGGATCCCAGGCCCGGGAACGTATTCAC -3') (479 bp size of product for *16S rRNA* gene of the *S. aureus*), 4 ng of the molecular beacon and 4 U of Jumpstart Taq DNA polymerase (Fermentas, Germany).

Statistical analysis

Statistical analysis was performed using SPSS/16.0 software for significant relationships. The incidences of serogroups, virulence factors and antibiotics resistance properties of *S. aureus* isolated from various types of infectious samples were statistically analyzed. Statistical significance was regarded at a P value < 0.05.

RESULTS

Total distribution of *S. aureus* in various types of human infections samples of Iranian educational hospitals is shown in table 1. Of 150 samples studied, 50 samples (33.33%) were positive for *S. aureus*. The results of the culture method were confirmed using the PCR amplification of *16S rRNA* gene of the *S. aureus* (Figure 1). Burn infections had the highest prevalence of *S. aureus* (40%). Significant statistical analysis was found for the prevalence of *S. aureus* between superficial and burn infections (P < 0.05). Pattern of antibiotic resistance among the *S. aureus* strains of various types of clinical infections is shown in table 2. *S. aureus* strains of our study harbored the highest levels of resistance against penicillin (94%), tetracycline (92%), oxacillin (90%) and azithromycin (80%). Bacterial strains of our investigation harbored the lowest levels of resistance against imipenem (4%), methicillin (8%) and vamcomycine (10%). Significant statistical analysis was found for the prevalence of resistance between penicillin and vancomycine (P < 0.05), tetracycline and imipenem (P < 0.05), tetracycline and vancomycine (P < 0.05) and oxacillin and methicillin (P < 0.05).

DISCUSSION

The present study showed that the burn, post-surgical wound and superficial wound infections were infected with resistant strains of *S. aureus*. Our results showed that the prevalence of *S. aureus* in the surficial, post-surgical and burn infections of hospitalized patients of Iranian hospitals were 26%, 34% and 40%, respectively. The burn wound is considered as one of the major health problems in the world[18]. In the present study, *S. aureus* was the most common isolate which is similar to other findings[19, 20]. One possible explanation for the high prevalence of *S. aureus* in the clinical samples of patients of our study is the fact that the hospital environment is so contaminated and antimicrobial agents are prescribed in an irregular and impermissible manner. In a study which was conducted on Addis Ababa, Ethiopia on the burn wound infections[21], bacterial infection was observed in 95 out of 114 patients (83.3%) of which, 66 (69.5%) had *S. aureus* infection. Alebachew *et al*. (2012)[21] reported that most of the *S. aureus* strains of wound infections were sensitive to vancomycin, clindamycin, kanamycin and erythromycin, but highly resistant to penicillin. They showed that all isolates were multi drug resistant, and one isolate was resistant to all the tested drugs. In an investigation which was conducted on Ahvaz[22], results showed that 27.8% of wound and blood specimens were infected by Staphylococci and among these 60% were identified as methicillin resistant. Ekrami *et al*. (2010)[22] showed that the highest resistance percentage belonged to ciprofloxacin (81.2%) and then amikacin (81%), carbenicillin (64.6%) and gentamicin (64.3%). Momtaz and Hafezi (2014)[23] reported that of 132 clinical samples, 66 were positive for *S. aureus*. Superficial and surgical wounds had the highest incidence of *S. aureus* (66.12%), while blood samples had the lowest incidence (15.38%). They showed that the *S. aureus* isolates harbored the

highest levels of antibiotic resistance against azithromycin (62.12%), tetracycline (57.57%) and erythromycin (54.54%) which was similar to our results.

Infection is the most important problem in the treatment of burn patients. The bacteriology of burn wounds is often poly-microbial in nature, and the presence of multidrug-resistant organisms is often associated with more severe clinical manifestations and poor response to antimicrobial therapy. Antibiotic susceptibility patterns served as a useful guideline for choosing an appropriate antibiotic. The results of our investigation showed that the *S. aureus* strains of our study harbored the highest levels of resistance against penicillin (94%), tetracycline (92%), oxacillin (90%) and azithromycin (80%). In the other hand, the *S. aureus* strains of our study were susceptible to imipenem, methicillin and vamcomycine antibiotics. Similar results have been reported previously[21-23]. Methicillin is one of the best choices for *S. aureus* clinical infections. The rate of resistance against this antibiotic was 8% in our study. Important role of methicillin resistant *S. aureus* (MRSA) as a causative agent of human clinical infections has been reported previously[21-25]. High prevalence of antibiotic resistance in our study is maybe due to the fact that the prescription of methicillin is very high in Iranian health center and hospitals. In addition, this finding showed that the Iranian hospital's environments are so infected. Also, our results showed that antibiotics were used in a highly irregular manner in Iranian hospitals.

Table 1: Distribution of *Staphylococcus aureus* in various types of human infections

Type of samples	No. samples collected	Positive strains (%)	PCR confirmation (%)
Superficial wound	50	13 (26)	13 (26)
Post-surgical wounds	50	17 (34)	17 (34)
Burn infections	50	20 (40)	20 (40)
Total	150	50 (33.33)	50 (33.33)

Table 2: Susceptibility of *Staphylococcus aureus* strains of various types of clinical infections against commonly used antibiotics

Antimicrobial agents	Types of infections (%)			
	Superficial wound (13)	Post-surgical wounds (17)	Burn infections (20)	Total (50)
Ampicillin	2 (15.38)	4 (23.52)	6 (20)	12 (24)
Gentamycin	2 (15.38)	3 (17.64)	5 (25)	10 (20)
Imipenem	-	-	2 (10)	2 (4)
Tetracycline	7 (53.84)	9 (52.94)	30 (15)	46 (92)
Vancomycine	1 (7.69)	1 (5.88)	3 (15)	5 (10)
Methicillin	1 (7.69)	1 (5.88)	2 (10)	4 (8)
Norfloxacin	3 (23.07)	5 (29.41)	7 (35)	15 (30)
Cotrimoxazole	3 (23.07)	4 (23.52)	7 (35)	14 (28)
Clindamycin	2 (15.38)	2 (11.76)	5 (25)	9 (18)
Trimethoprim-sulfamethoxazole	3 (23.07)	5 (29.41)	10 (50)	18 (36)
Penicillin	5 (38.46)	13 (76.47)	19 (95)	47 (94)
Oxacillin	3 (23.07)	15 (88.23)	17 (85)	45 (90)
Erythromycin	2 (15.38)	10 (58.82)	12 (60)	23 (46)
Azithromycin	2 (15.38)	8 (47.05)	9 (45)	40 (80)
Cefexime	1 (7.69)	2 (11.76)	5 (25)	35 (70)

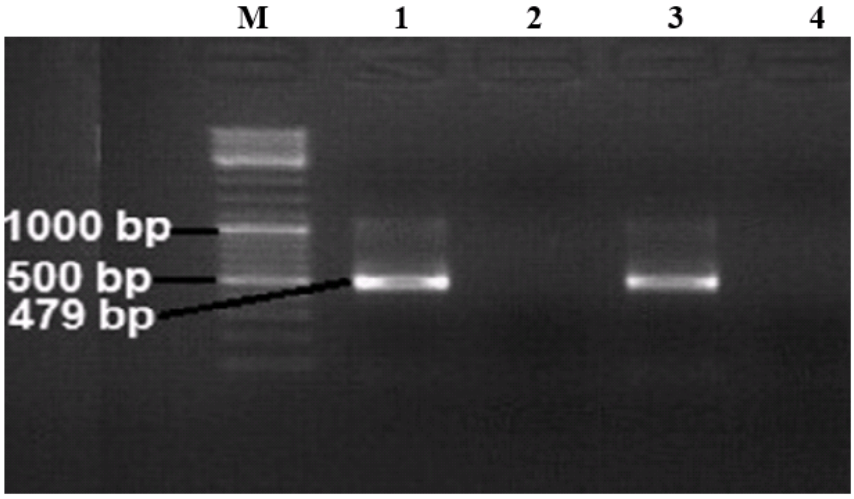

Fig. 1: Results of the gel electrophoresis for identification of *16S rRNA* gene of the *S. aureus* strains. M: 100 bp DNA ladder (Fermentas, Germany), Line 1: Positive samples for *16S rRNA* gene of the *S. aureus*, Line 2: Negative sample, Line 3: Positive control and Line 4: Negative control

High prevalence of MRSA in various types of clinical infections were also reported by Alghaithy *et al*., (2000) (61% in Saudi-Arabia)[26], M³ynarczyk *et al*., (2001) (40% in Warszawie)[27] and Rijal *et al*., (2008) (56.1% in Pokhara) (28). Virdis *et al*., (2010)[29] showed that the prevalence of resistance of *S. aureus* against kanamycin, oxytetracycline and ampicillin were 28%, 16% and 12%, respectively. Deng *et al*., (2013)[30] reported that the high prevalence of resistance of the *S. aureus* against most commonly used antibiotics including naficillin, oxacillin, vancomycin and cefathiamidine. Nishijima and Kurokawa (2002)[31] showed that the prevalence of *S. aureus* resistance against penicillin, cephalosporins and clindamycin were 20 to 30%. They showed that the prevalence of resistance against gentamycin, erythromycin and methicillin were 55.2%, 39.6% and 21%, respectively. Kumar *et al*., (2011)[32] revealed that the *S. aureus* isolates of clinical infections were highly resistant to different antibiotics, i.e. 33.6% were resistant to oxytetracycline, 36.4% to streptomycin, 29.9% to gentamycin and 26.2% each to chloramphenicol, pristinomycin and ciprofloxacin which was similar to our results.

CONCLUSIONS

The results of the present investigation showed that the *S. aureus* is one of the most important cause of infections in superficial, post-surgical and burn wounds. Therefore, its accurate diagnosis of in hospitals, patients and health care units is an important need. Also the dissemination of MRSA strains with high resistance to different antibiotics in Iranian hospitals is a warning for patient's public health. Accurate and continuous surveillance of antibiotic resistance patterns among *S. aureus* strains should be considered in pediatrics. We recommended prescription of imipenem, methicillin and vancomycin in a highly regular pattern for treatment of cases of superficial, post-surgical and burn wound infections.

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