INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 4, No. 9, p. 169-176, 2014

RESEARCH PAPER OPEN ACCESS

Prevalence of toxic shock syndrome toxin I producing clinical isolates of *Staphylococcus aureus* strains isolated from hospitals in Tabriz, Iran

Morteza Teyhoo[1], Haedeh Mobaiyen[2*], Nour Amirmozafari[3], Seyyed Reza Moaddab[4], Shafegeh Mones rast[5]

[1]*Department of Microbiology, College of Basic Science, Lahijan Branch, Islamic Azad University, Lahijan, Iran*

[2]*Department of Microbiology, College of Medicine, Tabriz Branch, Islamic Azad University, Tabriz, Iran*

[3]*Department of Microbiology, Iran University of Medical Sciences, School of Medicine, Tehran, Iran*

[4]*Department of Microbiology, School of Para medicine, Tabriz University of Medical Sciences, Tabriz, Iran*

[5]*Shohada Hospital, Microbiology laboratory, Tabriz, Iran*

Key words: *Staphylococus aureus* , TSST-1 toxin, antibiotic resistance, PCR.

http://dx.doi.org/10.12692/ijb/4.9.169-1765 Article published on May 10, 2014

Abstract

Staphylococcus aureus is one of the most important etiological agents of hospital and community acquired infections, and drug-resistant strains, mostly of hospital origin, are rapidly on the rise in many parts of the world. The enterotoxins and toxic shock syndrome toxin (TSST-1) are among the most common pathogenic determinants elaborated by this bacterium. The incidence of TSST-1 elaborating strains is also very alarming. The aim of this investigation was to survey the prevalence of TSST-1 gene in the clinical isolates of *S. aureus* recovered from hospitalized patients in Shohada and Imam-Reza hospitals of Tabriz, Iran.During one year period, a total of 1,454 clinical specimens from Shohada and 11353 from Imam-Reza hospitals were subjected to bacterial culture. Strains of *Staphylococcus aureus* were recovered and identified by routine bacteriological methodologies. Their antibiotic susceptibility patterns were determined by agar disk diffusion method. Following genomic DNA extraction by boiling method, the presence of TSST-1 gene was analyzed by PCR. A total 100 *S. aureus* isolates were recovered. Antibiogram results indicated that all of the isolates were sensitive to linzolid; whereas, only 83% were resistant to methicillin. The prevalence rate of TSST-1 gene in the isolates was found to be 20% in shohada and 14% in Imam-Reza hospitals.The relatively high prevalence of TSST-1 gene in clinically recovered *S. aureus* strains in Tabriz region of Iran and their circulation in the community can have a potentially alarming influence in the general health of community as well as in hospitalized patients.

* **Corresponding Author:** Haedeh Mobaiyen ✉ drhmobaiyen@iaut.ac.ir

Introduction

Staphylococci are found everywhere and they are widely distributed in nature (Casey *et al.*, 2007). Enterotoxins and Toxic Shock Syndrome Toxin I (TSST-1) secreted from this bacterium are important virulence factors which along with Pyrogenic Toxin Super Antigens (PTSAgs) exert profound effects on their hosts. Their ability to develop resistance against antimicrobial agents is a major factor that has led to numerous health problems. These bacteria are responsible for more than %80 of infectious diseases with clinical symptoms (Saha *et al.*, 2008). Staphylococci are the most successful Pathogenic bacteria. If the host defense system is damaged due to injury or surgery and organisms penetrate into the tissue their latent invasion will be clear(Casey *et al.*, 2007- Kluytmans *et al.*, 1997- Saha *et al.*, 2008). Ability to develop resistance aginst antimicrobial agents is major factor that has led to numerous health problems in spite of the production of a broad spectrum antibiotics effective on Staphylococcus (in the past 40 years) (Fueyo *et al.*, 2005).

Staphylococcus aureus is part of the normal nasal flora. About 20 percent of the world population carries *Staphylococcus aureus* for long durations (Peck *et al.*, 2009). A group of serious medical problems such as skin and soft tissue infections (SSTIs), surgical sites infections, endocarditis and hospital-acquired bacterial infections can be caused. Infections are often attributed to the use of medical devices such as catheters and prosthetics or using medications that suppress the immune system (Casey *et al.*, 2007- Fueyo *et al.*, 2005- Kluytmans *et al.*, 1997- Saha *et al.*, 2008). Staphylococcus extreme flexibility always leads to difficulty in identifying of Staphylococcus aureus .The importance of this flexibility (in medicine and veterinary medicine) has not identified until identification of resistant strains against penicillin (at first in penicillin and then in other antibiotics). Different treatment and epidemiologic problems were caused by these resistant strains against drugs that uncovered necessity of genetic studies on Staphylococci (Omoe *et al.*, 2005). Staphylococcal severe diseases commonly are hospital infections and mostly originated by coagulase negative staphylococci. This bacterium transfers to host from the wound resulted from catheters, shunts, needle and other medical devices. A functioning phagocytosis system is essential for the host's natural defenses and defects in humeral immune response may contribute to disease susceptibility. It is obvious that in the *Staphylococcus aureus* similar to *Enterobacteriaceae* most common antibiotic resistance was associated to plasmids and Staphylococcus genetic is fundamentally similar to genetics of *Escherichia coli*. Staphylococci resistance to antibiotics has increased physicians concern because 60%-90% of *Staphylococcus aureus* strains are resistant against to penicillin (Fueyo *et al.*, 2005).

Staphylococcus enterotoxins and toxic shock syndrome toxin (TSST-1) are major virulence factors of this bacterium. They constitute parts of PTSAgs which exert significant effects on their hosts. Toxic shock syndrome-1 toxin, a protein with a molecular weight of approximately 24,000 Daltons and isoelectric point of 6.8-7.2 has been considered as the most important cause of TSS disease. This protein (TSST-1) is seen in 90%-100% of *S. aureus* strains isolated from women with menstrual TSS (El-Ghodban *et al.*, 2006- Fueyo *et al.*, 2005). Toxic shock syndrome (TSS) is a systemic disease caused by *Staphylococcus aureus* which *was* initially reported by Todd *et al* (Todd *et al.*, 1984). This protein is synthesized almost by all strains of *Staphylococcus aureus* strains associated with menstrual period and several of non-menstrual associated strains. It may also be synthesized by *Staphylococcus aureus* strains isolated from healthy people. This disease is associated with symptoms such as fever, vomiting, diarrhea, muscle pain, and skin rashes (scarlet like), and in severe cases hypotension, lymphadenopathy, liver failure and renal failure were seen (El-Ghodban *et al.*, 2006- Todd *et al.*, 1984). Considering the importance of this toxin, this investigation was undertaken to evaluate the prevalence of TSST-1 production in *Staphylococcus aureus* strains isolated from hospitalized patients in Shohada and Imam Reza hospitals in Tabriz, Iran.

Materials and methods

Biological materials

A total of 1,454 and 11,353 clinical samples such as urine, blood, wounds and various secretions were collected from patients hospitalized in Tabriz Shohada and Imam Reza hospitals respectively, during 2011-2012. The specimens were processed for bacterial culture. *Staphylococcus aureus* strains were isolated by standard bacteriological procedures. Antibiotic susceptibility of the strains was evaluated by agar disk diffusion method according to CLSI guidelines (Chapaval *et al.*, 2008). Antibiotics used in this study were methicillin (5µg), co-trimoxazole (25µg), linezolid (30µg), tetracycline (30µg), gentamicin (10µg) and ciprofloxacin (5µg) (Mast Co., UK).

DNA extraction

Genomic DNA extraction was carried out using a commercial kit (Cinnagen Co.) as well as by boiling method (Sindhu *et al.*, 2010). Isolates were cultured in Luria Bertoni broth for 24 hr at 37 º C. About 1 ml of the bacterial culture was centrifuged at 4500 rpm for 10 min and the supernatant was discarded. One hundred microliters of Gram positive Pre-lysis buffer and 20 microliters of Lyzosyme were added to the cell pellet and following vortexing, they were incubated for 30 min at 37°C. The incubation temperature was increased to 55 ° C and following addition of 10 microliters of RNase it was placed at this temperature for 30 min. According to the kit instruction, after addition of the elution buffer, purified DNA preparations were eluted. For purity confirmation, the DNA extracts were electrophoresed on 1% agarose gel.

PCR reaction

Specific primers (Table 1) and PCR thermal cycling reaction were used for tst-1 gene amplification as described by Deurenberg *et al* (Deurenberg *et al.*, 2005). The PCR products were subjected to electrophoresis in agarose gel using 100 mv voltage.

Results

In the present study, 11,353 clinical specimens of patients hospitalized in Imam Reza hospital in Tabriz were studied. Of those, 3,241 cases (28.5%) were specimens obtained from female patients and 8,112 cases (71.4%) were from male. Following bacteriological culturing, 2,276 samples (20.04%) were positive for bacterial growth and 9,077 (79.90%) were negative. A total of 136 isolates were identified as Staphylococcus and *84* strains (%3.69) were confirmed as *Staphylococcus aureus*. In the same period at Tabriz Shohada hospital, 1,454 clinical specimens were processed. Of those, 483 cases (33.2%) were from females and 971 cases (66.8%) were male samples. A total of 556 samples (38.23%) were positive from bacterial growth and 898 (61.76%) were negative. Among 132 Staphylococcal isolates, 100 strains (6.87%) were confirmed as *Staphylococcus aureus* (Table 3).

Table 1. Sequences of primers used in the detection of TSST-1 gene in strains of *Staphylococcus aureus* isolated from Shohada and Imam Reza hospitals in Tabriz - Iran (Deurenberg *et al.*, 2005).

Primer		Sequence
TSST	*Forward primer*	5'CATGAATAGAATAAAAGTTGCAATA3'
	Reverse primer	5'CCCCTTTAACGCTAATACGACGATCAA3'

Table 2. Frequency of antibiotic-resistant strains of the *Staphylococcus aureus* isolates.

Antibiotic	Shohada hospital		Imam Reza hospital	
	No.	Percentage	No.	Percentage
Co-trimoxazole	11	11	10	12
Tetracycline	39	39	40	48
Ciprofloxacin	15	15	14	17
Gentamicin	21	21	22	27
Methicillin	83	83	73	87
Linezolid	0	0	0	0

With disk diffusion method, antibiotic susceptibility patterns of the *S. aureus* isolates were determined and the results are depicted in Table 2.

Of the 100 *S. aureus* isolates from Shohada hospital, 20 (20%) were carriers of TSST-1 gene. Whereas, 12 isolates (14.3%) among those recovered from Imam Reza hospital were TSST-1 positive (Figure1). The positive bacteria were mainly isolated from wounds and abscess (Tables 5 and 6).

Table 3. Microorganisms isolated from clinical specimens obtained from patients in Tabriz Imam Reza and Shohada hospitals.

Microorganism	Shohada hospital		Imam Reza hospital	
	No.	%	No.	%
Escherichia coli	36	2.4	855	7.5
Klebsiella spp.	15	1.0	5	0.04
Enterobacter spp.	-	-	158	1.4
Serratia marcescene	-	-	5	0.04
Pseudomonas aeruginosa	46	3.1	149	1.3
Acinetobacter spp.	-	-	29	0.25
Other Gram Negative Bacilli	205	14.1	484	4.3
Staphylococcus aureus	100	6.9	84	0.73
Staphylococcus epidermidis	-	-	93	0.81
Other Coagulase Negative *Staphylococcus*	93	6.3	18	0.15
Streptococcus spp.	12	0.82	0.60	0.52
Enterococcus spp.	-	-	97	0.85
Various fungi	27	1.8	137	1.2

All PCR products of tst gene had the expected size and no nonspecific bands were seen. Two of the amplified PCR products were sent for sequence determination to Bioneer Inc. The sequence results showed gene similarities of these strains with tst gene (483bp). These strains were considered as positive control.

Table 4. Characteristics of tst gene positive Staphylococcus *aureus* strains isolated from hospitalized patients in Shohada hospital.

Strain No.	Gender	Age	Type of sample	Antibiotic resistance
1	male	47	abscess	Methicillin, Co-trimoxazole
2	male	38	Femur secretion	Methicillin
3	male	28	Femur secretion	Methicillin
4	male	53	nasal excretion	Methicillin, Tetracycline, Co-trimoxazole, Ciprofloxacin, Gentamicin
5	male	62	wound	Methicillin, Tetracycline
6	female	30	wound	Methicillin
7	male	55	trachea	Methicillin
8	male	35	wound	Methicillin
9	male	42	blood	Methicillin, Tetracycline
10	male	41	abscess	Methicillin
11	female	38	CSF	Methicillin
12	male	24	blood	Methicillin, Tetracycline, Co-trimoxazole
13	female	40	wound	Methicillin, Tetracycline
14	male	34	wound	Tetracycline
15	female	29	Abscess	Methicillin, Tetracycline, Co-trimoxazole
16	female	19	wound	Methicillin, Tetracycline
17	male	57	femur	Methicillin, Tetracycline
18	male	35	wound	Methicillin
19	female	28	wound	Methicillin
20	male	39	abscess	Tetracycline

Discussion

Staphylococcus aureus is an important pathogen which has been responsible for many different infections in community as well as in hospital environments (Nnis, 2002). Infections caused by *Staphylococcus aureus* are frequently reported in patients hospitalized with severe complications despite antibiotic treatment (Peacock *et al.*, 2001), (Vandenbergh *et al.*, 1999). In this survey, 84 of the 2,276 culture positive specimens obtained from patients hospitalized in Imam Reza hospital and 100 of the 556 cultivable samples in Shohada hospital were identified as *Staphylococcus aureus*. This bacterium was the second most encountered infectious agent in the studied population. They were mostly recovered from blood, wound, urine and abscess. This is consistent with Varshney *et al* (Varshney *et al.*, 2009), results reported in 2009 which over 50% of the blood, wound and abscess specimens obtained from patients hospitalized in New Jersey were infected with *S. aureus*. Shokoohi *et al* (Shokoohi *et al.*, 2009) isolated 180 *S. aureus* strains from a total of 511 clinical samples collected from patients hospitalized at Loghman hospital in Tehran, Iran during 1998-2004. The *S. aureus* isolates were mostly recovered from blood and urine samples. Increase in isolation rate of this bacterium demonstrates the role and importance of this microorganism in public health. Several studies looked at the presence of *S. aureus* in the nose of healthy people. Nasal cavity constitutes the main source of Staphylococcal nosocomial infections.

Table 5. Characteristics tst gene positive *Staphylococcus aureus* strains isolated from hospitalized patients in Imam Reza hospital.

Strain No.	Gender	Age	Type of sample	Antibiotic resistance
1	male	80	wound	Methicillin, Gentamicin
2	female	24	abscess	Methicillin
3	male	64	trachea	Methicillin, Gentamicin, Ciprofloxacin
4	male	70	wound	Methicillin, Tetracycline
5	male	47	pharynx	Methicillin
6	male	62	femur	Methicillin, Gentamicin
7	male	70	blood	Methicillin
8	female	25	nasal excretion	Methicillin, Tetracycline
9	male	22	abscess	Methicillin, Co-trimoxazole, Tetracycline, Gentamicin
10	male	59	wound	Methicillin, Tetracycline
11	male	37	CSF	Methicillin
12	female	37	blood	Methicillin, Tetracycline

Johnson *et al* demonstrated that PCR is a convenient, sensitive, and rapid method for detection of tst gene (Johnson *et al.*, 1991). Parsonnet *et al* (2008) studied 159 S. aureus strains isolated from women. Of these, 14(%9) strains were tst positive and 2 of the toxigenic strains were methicillin resistant (Parsonnet *et al.*, 2008).

Vink *et al* (2005) studied 103 *S. aureus* isolates from the community, hospitals and granulomatous patients. Twelve strains of the 51 community isolated strains (%24), 5 strains of the 36 hospital associated strains (%14), and 4 strains of the 16 granulomatous recovered isolates were tst positive (%25) (Deurenberg *et al.*, 2005). Rahman *et al* (2007) examined 73 samples of *S. aureus* from surgical wound and ear infections (Islam *et al.*, 2007). Only one strain was tst positive and resistance to penicillin G and sulfamethoxazole was reported to be 83.3% and 70.0%, respectively. Prevalence of MRSA was 23.3%. Ran Peck *et al* (2008) studied 70 clinical samples during 5 months and took 95 nose excretion

samples from hospitalizes patients in Korea. They explored for different enterotoxin genes (sea-sek) as well as for tst by PCR. Among the 70 clinical isolates; 50 (71.4%) had at least one enterotoxin gene and 44.3% of those harbored the tst gene (Tsen *et al.*, 1998).

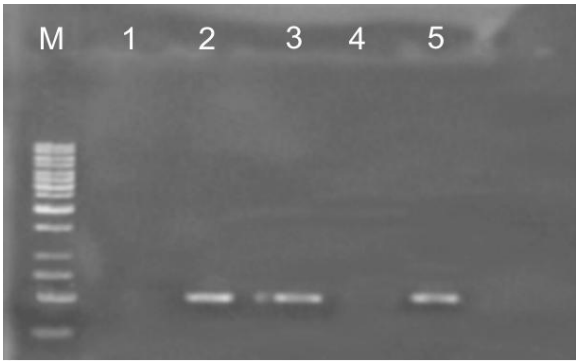

Fig. 1. PCR product of tst gene as shown in stained 1% agarose gel M: Size maker, Lane 1: negative control (deionized water), Lane 2: *Staphylococcus aureus* isolate harboring tst gene from Imam Reza hospital, Lane 3: *Staphylococcus aureus* isolate harboring tst gene from Shohada hosptial, Lane 4: *Staphylococcus aureus* lacking tst gene, Lane 5: the positive control.

In this study, tst gene was detected in 20 strains (20%) of the samples from Shohada hospital and 12 strains (14.3%) of Imam Reza hospital samples. This finding is consistant with those of Mehrotra *et al* who reported 24% tst carriage rate in the 154 *S. aureus* strains isolated from patients in Canada and the Netherlands (Mehrotra *et al.*, 2000). However, El-Ghodban *et al* in Libya reported lower frequency of tst gene isolation, only 7.5% in 40 clinical isolates (El-Ghodban *et al.*, 2006). Tsen *et al* in Taiwan detected 4.8% tst gene carriage in a total of 62 clinical isolates and no toxigenic strain was seen among the food associated isolates (Tsen *et al.*, 1998).

Antibiotic susceptibility tests revealed that 83% of our isolates were resistant to methicillin. All strains were sensitive to linezolid. Additionally, 39% were resistant to tetracycline, 15% to ciprofloxacin, 21% to gentamicin and 11% of the strains were resistant to co-trimoxazole. Seventy five percent of the tst positive strains were resistant to methicillin, 50% to tetracycline, and 5% was the resistant rate to co-trimoxazole. One isolate (5%) was resistant to three antibiotics; methicillin, co-trimoxazole, and tetracycline. Two isolates were resistant to methicillin and tetracycline. These results are consistent to those of Tokajian *et al* (2011) who examined enterotoxin, tst and antibiotic resistance in *S. aureus* hospital isolates (Sima *et al.*, 2011). Seventy two percents of their strains were resistant to methicillin and 5% harbored tst gene. The highest resistance rate after methicillin was reported for tetracycline (42%), oxacillin (32%), azithromycin (25%), erythromycin (24%), co-trimoxazole (14%) and ciprofloxacin (5%).

The prevalence of *S. aureus* in Iran is quite remarkable. Particularly, isolates with tst gene are concerning issue. Circulation of these strains among high risk population like children, hospitalized patients and the immune-deficient's can become a major health predicament. Considering the high rate of Staphylococcus colonization in healthy individuals which can reach up to 50-60% in nasopharynx and 5-30% in the skin and hair, the consequences can be alarming (Deurenberg *et al.*, 2005- Normanno *et al.*, 2007). Continuous monitoring and control of these strains require an effective monitoring system (Fueyo *et al.*, 2005). Studies are necessary particularly in epidemiology and identification of dominant strains with genotypic and phenotypic typing techniques.

Conclusions

Infections caused by *Staphylococcus aureus* had high prevalence in the studied hospitals. Some strains were simultaneously resistant to several antibiotics and a high percentage of them produced TSST-1. This can have major health ramifications. It is recommended to use PCR reaction for search of tst gene in *Staphylococcus aureus* isolated from various sources.

Acknowledgment

The authors thank the authorities of Tabriz Medical faculty of Islamic Azad University and Tabriz University of Medical Sciences and Research Centre of Tuberculosis and Lung Diseases Research facilities for their assistance.

References

Casey AL, Lambert PA, Elliott TSJ. 2007. Staphylococci. International journal of antimicrobial agents **29,** S23-S32. http://dx.doi.org/10.1016/S0924-8579(07)72175-1

Chapaval L, Moon D, Gomes J, Duarte F, Tsai S. 2008. An Alternative Method for Staphylococcus Aureus DNA Isolation. Arquivo Brasileiro de Medicina Veterinária e Zootecnia **60,** 299-306. http://dx.doi.org/10.1590/S010209352008000200004

Deurenberg RH, Nieuwenhuis RF, Driessen C, London N, Stassen FR, Tiel FH, Stobberingh EE, Vink C. 2005. The Prevalence of the Staphylococcus Aureus Tst Gene among Community, and Hospital, Acquired Strains and Isolates from Wegener's Granulomatosis Patients. FEMS microbiology letters **245,** 185-189.

El-Ghodban A, Ghenghesh KS, Márialigeti K, Esahli H, Tawil A. 2006. Pcr Detection of Toxic Shock Syndrome Toxin of Staphylococcus Aureus from Tripoli, Libya. Journal of medical microbiology **55,** 179-182. http://dx.doi.org/10.1099/jmm.0.46162-0

Fueyo J, Mendoza MC, Rodicio MR, Muniz J, Alvarez M, Martín MC. 2005. Cytotoxin and Pyrogenic Toxin Superantigen Gene Profiles of Staphylococcus Aureus Associated with Subclinical Mastitis in Dairy Cows and Relationships with Macrorestriction Genomic Profiles. Journal of clinical microbiology **43,** 1278-1284. http://dx.doi.org/10.1128/JCM.43.3.1278-1284.2005

Fueyo JM, Mendoza MC, Martín MC. 2005. Enterotoxins and Toxic Shock Syndrome Toxin in< I> Staphylococcus Aureus</I> Recovered from Human Nasal Carriers and Manually Handled Foods: Epidemiological and Genetic Findings. Microbes and infection **7,** 187-194. http://dx.doi.org/10.1016/j.micinf.2004.10.009

Islam M, Uddin M, Nasrin M, Nazir K, Rahman M, Alam M. 2007. Prevalence of Enterotoxigenic and Toxic Shock Syndrome Toxin-1 Producing Coagulase Positive Staphylococcus Aureus in Human and Their Characterization. Bangladesh Journal of Veterinary Medicine **5,** 115-119. http://dx.doi.org/10.3329/bjvm.v5i1.1328

Johnson W, Tyler S, Ewan E, Ashton F, Pollard D, Rozee K. 1991. Detection of Genes for Enterotoxins, Exfoliative Toxins, and Toxic Shock Syndrome Toxin 1 in Staphylococcus Aureus by the Polymerase Chain Reaction. Journal of clinical microbiology **29,** 426-430.

Kluytmans J, van Belkum A, Verbrugh H. 1997. Nasal Carriage of Staphylococcus Aureus: Epidemiology, Underlying Mechanisms, and Associated Risks. Clinical microbiology reviews **10,** 505-520.

Mehrotra M, Wang G, Johnson WM. 2000. Multiplex Pcr for Detection of Genes Forstaphylococcus Aureus Enterotoxins, Exfoliative Toxins, Toxic Shock Syndrome Toxin 1, and Methicillin Resistance. Journal of clinical microbiology **38,** 1032-1035.

NNIS. 2002. National Nosocomial Infections Surveillance (Nnis) System Report, Data Summary from January 1992 to June 2002, Issued August 2002. American Journal of Infection Control **30,** 458-475.

Normanno G, La Salandra G, Dambrosio A, Quaglia N, Corrente M, Parisi A, Santagada G, Firinu A, Crisetti E, Celano G. 2007. Occurrence, Characterization and Antimicrobial Resistance of Enterotoxigenic *Staphylococcus Aureus* Isolated from Meat and Dairy Products. International journal of food microbiology **115,** 290-296. http://dx.doi.org/10.1016/j.ijfoodmicro.2006.10.049

Omoe K, Hu DL, Takahashi- Omoe H, Nakane A, Shinagawa K. 2005. Comprehensive Analysis of Classical and Newly Described Staphylococcal Superantigenic Toxin Genes in Staphylococcus Aureus Isolates. FEMS microbiology letters **246,** 191-198.

Parsonnet J, Goering RV, Hansmann MA, Jones MB, Ohtagaki K, Davis CC, Totsuka K. 2008. Prevalence of Toxic Shock Syndrome Toxin 1 (Tsst-1)-Producing Strains of Staphylococcus Aureus and Antibody to Tsst-1 among Healthy Japanese Women. Journal of clinical microbiology **46,** 2731-2738.
http://dx.doi.org/10.1128/JCM.00228-08

Peacock SJ, de Silva I, Lowy FD. 2001. What Determines Nasal Carriage of Staphylococcus Aureus? Trends Microbiol. **9,** 605-610.
http://dx.doi.org/10.1016/S0966-842X(01)02254-5

Peck KR, Baek JY, Song J-H, Ko KS. 2009. Comparison of Genotypes and Enterotoxin Genes between Staphylococcus Aureus Isolates from Blood and Nasal Colonizers in a Korean Hospital. Journal of Korean medical science **24,** 585-591.
http://dx.doi.org/10.3346/jkms.2009.24.4.585

Saha B, Singh AK, Ghosh A, Bal M. 2008. Identification and Characterization of a Vancomycin-Resistant Staphylococcus Aureus Isolated from Kolkata (South Asia). Journal of medical microbiology **57,** 72-79.
http://dx.doi.org/10.1099/jmm.0.47144-0

Shokoohi SH, Aminzadeh Z, Shrafi k, Ashrafi M. 2009. Evaluation of Antibiotic Resistance of Resistant Staphylococcus Aureus to Acquired Metisilin. Journal Microbiology **1,** 59.

Sima T, Dominik H, Rana A, Fuad H, George A. 2011. Toxins and Antibiotic Resistance in Staphylococcus Aureus Isolated from a Major Hospital in Lebanon. ISRN microbiology **2011**.

Sindhu N, Sharma A, Jain V. 2010. Coagulase Gene Based Molecular Detection of Staphylococcus Aureus Directly from Mastitic Milk Samples of Murrah Buffalo. Buffalo Bulletin **29,** 52-59.

Todd JK, Franco-Buff A, Lawellin DW, Vasil ML. 1984. Phenotypic Distinctiveness of Staphylococcus Aureus Strains Associated with Toxic Shock Syndrome. Infection and immunity **45,** 339-344.

Tsen H, Yu G, Wang K, Wang S, Chang M, Lin L. 1998. Comparison of the Enterotoxigenic Types, Toxic Shock Syndrome Toxin I (Tsst-1) Strains and Antibiotic Susceptibilities for Enterotoxigenic *Staphylococcus Aureus* Strains Isolated from Food and Clinical Samples. Food microbiology **15,** 33-41.

VandenBergh MF, Yzerman EP, van Belkum A, Boelens HA, Sijmons M, Verbrugh HA. 1999. Follow-up of Staphylococcus Aureus Nasal Carriage after 8 Years: Redefining the Persistent Carrier State. Journal of clinical microbiology **37,** 3133-3140.

Varshney AK, Mediavilla JR, Robiou N, Guh A, Wang X, Gialanella P, Levi MH, Kreiswirth BN, Fries BC. 2009. Diverse Enterotoxin Gene Profiles among Clonal Complexes of Staphylococcus Aureus Isolates from the Bronx, New York. Applied and Environmental Microbiology **75,** 6839-6849. doi: 6810.1128/AEM.00272-00209. Epub 02009 Sep 00211.
http://dx.doi.org/10.1128/AEM.00272-09