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Detection the *Staphylococcus aureus* Producing Enterotoxin Isolated from Skin Infections in Hospitalized Patients

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Abstract: *Staphylococcus aureus* is a major human pathogen that produces a wide array of toxins, thus causing various type of disease symptoms. Staphylococcal enterotoxins (SES), a family of 9 major serological types of heat-stable enterotoxins, are a main cause of gastroenteritis and skin infection. In this study to determine the extent of enterotoxin-producing *S. aureus* in skin infections of hospitalized patients, their samples were screened and the results showed that 42% of totally 200 patients studied in this research carried *S. aureus* and 45% of these *S. aureus* produced Staphylococcal enterotoxins. Twenty percent produced enterotoxin A, 25% produced enterotoxin B and 4.7% produced both enterotoxin A and B. The results demonstrated a high level of enterotoxigenic and multi drug resistance *S. aureus* in skin infections of hospitalized patients.

Key words: *Staphylococcus aureus*, enterotoxin, antibiotics, skin infections

INTRODUCTION

Staphylococcus aureus strains have long been recognized as an important pathogen in human disease. They populate the skin, mucous, membranes, anterior nares, eyes and gastrointestinal tract of asymptomatic individuals (Bradley *et al.*, 2005; Marples *et al.*, 1990; Heczko *et al.*, 1990). They can exist in these domains as residents or as temporary members of normal flora without causing disease (Alttoparlak *et al.*, 2004). The mean carrier rate in general population were found as 37.2%, in health care worker as 26.6% and in patient pf admission as 35.7% (Kluitmans *et al.*, 1997). However, some strains of *S. aureus* also cause infections in humans including skin infections and food poisoning (Bergdoll *et al.*, 1979; Noble and White, 1987). *S. aureus* also account for about 47% of all nosocomial infections (Wieneke *et al.*, 1993). *S. aureus* generates a wide range of toxins and enzymes that facilitate it in overcoming the host defenses. Some strains produce one or more of nine serologically related enterotoxins (type A to R) (Bradley *et al.*, 2005; Balaban *et al.*, 2000). The human anterior nares and finger tips are important sources of enterotoxigenic and non enterotoxigenic staphylococci (Balaban *et al.*, 1990; Chow *et al.*, 1989). Nasal carrier of antibiotic-resistant *S. aureus* among hospital workers and hospitalized patients has been widely studied, but data on the enterotoxigenic *S. aureus* is less extensive

(Kluitmans *et al.*, 1997). This study was designed to determine whether there was a high frequency of enterotoxin (SEA-SEB) producing strain *S. aureus* in hospitalized patients and try to establish whether the most of these strains were multidrug resistant. The results revealed a significant relation between enterotoxigenic *S. aureus* isolated from hospitalized patients and hospital workers carrying *S. aureus*.

MATERIALS AND METHODS

Sample collection: Two hundred patients hospitalized with skin infections in Tehran during Feb. 2005 until Sep 2005 were selected. Sterile swabs moistened in sterile normal saline and secretion of lesions was collected. Swabs were rotated five to six times and used to inoculate Mannitol Salt Agar (MSA) and blood agar plates (Merck, Oxoid, High media) in duplicate and incubated at 37°C. Plates were examined after 24 and 48 h incubation. Isolates identified as *S. aureus* by growth characteristics on blood agar and MSA, gram stain reaction, catalase test, tube coagulase test and DNase test. Pureculture of isolates were stored in skimmed milk broth at -75°C. Working cultures were maintained on brain heart infusion agar slopes at 4°C.

Detection of enterotoxins: Staphylococcal enterotoxins were obtained by Sac-culture as published previously

(Robbins *et al.*, 1974). Detection of enterotoxins were evaluated with ochtorlony test (Braga *et al.*, 2005). Reference antitoxins were provided from Sigma Company.

Antibiotic susceptibility testing: The antibiotic susceptibility was determined by agar disc diffusion method as recommended by the National Committee for Clinical Laboratory Standards (standard M-2 A5/1995) with the following discs (Table 2). The susceptibility tests were performed on Mueller-Hinton agar with *S. aureus* strain ATCC 25923 as a control. The Mast diagnostic instruction sheet was followed for the zone size interpretation.

RESULTS

Detection the *S. aureus* isolated from skin infections: To assess the *S. aureus* in the skin infection, 200 patients with skin diseases were estimated and the protocol in Table 1 was performed the results showed that 84 *S. aureus* were isolated from skin infections in hospitalized patients and 45% of the *S. aureus* produced staphylococcal enterotoxins, that 20% of them produced SEA, 25% of them produced SEB, 4.7% of them produced SEA+SEB. The hospital studied in this research is one of the reference center in Tehran.

Susceptibility of *S. aureus* isolated from skin infections to antibiotics: To assess the susceptibility of the isolated *S. aureus* in the skin infection 200 patients were estimated and the protocol in Table 2 was performed the results for susceptibility of the isolate to 12 different antibiotics are shown in Table 2. While 1% of strains are resistant to

Table 1: Staphylococcal Enterotoxin (SE) from skin infections in hospitalized patients

Type of enterotoxin	No. of <i>S. aureus</i> isolated (n= 84) (%)
SEA	17 (20)
SEB	21 (25)
SEA+SEB	4 (4.7)

Table 2: Susceptibility of the *Staphylococcus aureus* isolates from skin infections in hospitalized patients

Antibiotic (µg/disc)	Symbol	<i>S. aureus</i> (%)		
		R	S	I
Vancomycin (10)	Va	1	99	0
Carbenicillin (100)	Cb	48	52	0
doxycycline (30)	D	50	45	5
Erythromycin (15)	E	50	48	2
Tetracycline (30)	T	50	45	5
Amoxycillin (10)	Am	72	21	7
Ampicillin (10)	A	65	30	5
Ciprofloxacin (1)	Cf	4	56	1
Trimethoprim (10)	Tr	44	55	1
Clindamycin (10)	Cl	43	56	1
Penicillin G (10IU)	P	77	18	5
Gentamycine (10)	G	16	84	0

vancomycin. There were some variations in antibiotic sensitivity patterns, but there was no significant difference between extent of toxin production and antibiogram patterns, which was an important observation.

DISCUSSION

An intention of this study was to determine about the population enterotoxigen *S. aureus* from skin infection on the hospitalized patients. *S. aureus* produces a set of exotoxins including SEs and toxic shock syndrome toxine-1(TSST-1) which cause skin infections, food poisoning and a life threatening toxic shock syndrome (TSS), respectively (Edwards *et al.*, 1996; Tomi *et al.*, 2005). The results showed that 42% causes of skin infection was *S. aureus*, which is higher than reported by previous studies (Umolu *et al.*, 2002). This frequency was high compared to carrier in the hospital workers and general population which is 25 and 38%, respectively (Heczko *et al.*, 1990; Balaban *et al.*, 1990; Paul Moeatl, 1982; Shanson *et al.*, 1980). These toxins are intermediate Molecular Weight proteins (22-30 KD) that also act as superantigens due to their ability to bind to MHC-II molecules on APCs and stimulate all T-cells bearing particular Vβ on their T-cell receptors (White *et al.*, 1989; Herman *et al.*, 1991). Although these are two separate functions localized on separate domains between these activities and in most cases a loss of superantigen activity (because of genetic mutation) results in loss of enterotoxigenic activity as well (Harris *et al.*, 1993). However 45% of the *S. aureus* strains produced enterotoxins. This figure was more compatible than that reported by Naidu (Naidu *et al.*, 1989), but higher than that reported by Edward (Edwards *et al.*, 1996) and lower than that reported by Tomi *et al.* (2005). Isolates from skin infection exhibited high degree of enterotoxigenicity compared to nasal carrier of enterotoxin-producing *S. aureus* (Al bustan *et al.*, 1996) also, the relationship between *S. aureus* nasal carrier and staphylococcal skin infection was significance (Toshkova *et al.*, 2001; Tulloch, 1954). It seems that there is a relation between productivity of enterotoxin by *S. aureus* and the intensity of skin infections. In order to establish the fact it requires more studies, because enterotoxigenic *S. aureus* was present in more than 50% of patients with Psoriasis and Atopic dermatitis significantly correlate to enterotoxin production of isolated *S. aureus* strains (Tomi *et al.*, 2005). The result of antibiogram showed that more of enterotoxigen *S. aureus* that cause skin infection in comparison with Umolu study are more resistance while related to Vancomycin showed only 1% resistance (Table 2)

(Umolu *et al.*, 2002). This probably depends to the fact that isolates from skin infection in our study originate from hospital strains and/or infection from surgical procedure that Weinstein also established that (Toshkova *et al.*, 2001; Weinstein, 1959). It seems that the nosocomial strains are more resistant than general or food poisoning strains. A possible explanation for this result is that resistance mutant strains may be produced due to continuous encounter of nosocomial strains with different antibiotic used in hospital (Essawi *et al.*, 1998). The present findings strongly confirmed this explanation and suggest that only choice drug for treatment is Vancomycine. However it is recommended that treatment against these strains could be prescribed as multidrug (synergism). Several methods have proposed and are in use for production and analysis of the enterotoxins. In the analysis of a strain for enterotoxigenicity only a small amount of culture supernatant is needed thus methods have been provided that give a few milliliters of relatively concentrated enterotoxin. These methods including the Sac-culture, the semi- solid agar plate and shake-flask (Robbins *et al.*, 1974). The results obtained from our previous study concerning a comparison of the above method production of enterotoxins supported that the sac-culture method was superior (Imanifooladi *et al.*, 2004).

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