Evolution of slime production by coagulase-negative staphylococci and enterotoxigenic characteristics of *Staphylococcus aureus* strains isolated from various human clinical specimens

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The present study was designed to determine the slime production of coagulase-negative staphylococci (CoNS) and the enterotoxigenic properties of *Staphylococcus aureus* strains, and to evaluate the clinical importance of slime-producing CoNS and enterotoxigenic *S. aureus* strains isolated from various human clinical specimens. For this purpose, a total of 120 *Staphylococcus* strains were isolated and identified, and further characterized for their slime production and enterotoxigenicity. Of the clinical isolates, 55 (45.8%) were found to be *S. aureus*, and the others (54.2%) were identified as CoNS. Of the CoNS, 20 (16.7%) were further identified as *Staphylococcus hominis*, 18 (15%) as *Staphylococcus epidermidis*, six (5%) as *Staphylococcus xylosus*, six (5%) as *Staphylococcus warneri*, five (4.2%) as *Staphylococcus sciuri*, four (3.3%) as *Staphylococcus haemolyticus*, and two each (1.7%) as *Staphylococcus simulans*, *Staphylococcus capitis* and *Staphylococcus saprophyticus*, respectively. Thirty-nine (60%) of 65 CoNS were found to be slime producers. Slime production was observed in all CoNS, except *S. capitis*, mostly from blood (38.5%), tracheal aspiration (20.5%) and urine (12.8%) specimens. In addition, of the 55 *S. aureus* isolates, 46 (83.6%) were found to be enterotoxigenic, and of these *S. aureus* strains, 39 (84.7%) were positive for staphylococcal enterotoxin (SE)A. The results of this study showed that the slime-producing CoNS were mostly found in clinical specimens of blood, tracheal aspirate and urine. SEA was the predominant enterotoxin type detected in *S. aureus* strains from human clinical specimens.

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INTRODUCTION

Coagulase-negative staphylococci (CoNS), inhabitants of skin and mucous membranes, are known to be opportunistic pathogens, causing bacterial keratitis and infections in immunocompromised hosts or in patients implanted with medical devices such as intravascular and peritoneal dialysis catheters, prosthetic heart valves and orthopaedic prostheses (Kogan *et al.*, 2006). CoNS are characterized by an ability to colonize the surfaces of biomaterials by adhering in biofilm-structured communities of cells encased in a self-produced polymeric matrix, an amorphous slimy material that is loosely bound to staphylococcal cells. Slime is believed to make the

micro-organisms more resistant to administered antibiotics and to host-defence mechanisms (Götz, 2002).

Staphylococcus aureus strains are widespread in nature. They inhabit the skin, mucous membranes, anterior nares, eyes and gastrointestinal tract of asymptomatic individuals, where they can exist as resident or transient members of the normal flora without causing disease. However, some strains are responsible for many nosocomial infections, and in addition are the main causative agent of food intoxication by virtue of their variety of enterotoxins (Stephan *et al.*, 2001).

S. aureus produces a wide variety of toxic proteins, such as toxic shock syndrome toxin 1, exfoliative toxins, and staphylococcal enterotoxins (SEs). In addition to the classical five major antigenic types of SE (SEA, SEB, SEC, SED and SEE), four additional SEs (SEG, SEH, SEI and

Abbreviations: CoNS, coagulase-negative staphylococci; SE, staphylococcal enterotoxin.

SEJ) have been reported, and their corresponding genes have been described (Rosec & Gigaud, 2002). In recent times, data resulting from partial or complete genome sequence analyses have led to a rapid expansion of the 'se alphabet' and the description of further 'new' genes (*sek*, *sel*, *sem*, *sen*, *seo*, *sep*, *seq*, *ser* and *seu*) (Letertre *et al.*, 2003).

Recently, many studies have mainly been focused on the slime production of CoNS, but the results of these studies mostly do not reflect the importance of slime-producing CoNS from different clinical sources, including blood, urine and wound samples, and surgical instruments. On the other hand, there is little information on the enterotoxigenic properties of *S. aureus* strains isolated from various human clinical specimens in Turkey or in other countries.

The purpose of the present study was to detect the slime production of CoNS and the enterotoxigenic properties of *S. aureus* strains isolated from various human clinical specimens and to evaluate the importance of the distribution of these pathogens in human clinical specimens.

METHODS

Source of micro-organisms. The staphylococci tested in this study were isolated from various clinical specimens of patients in the hospital of the University of Yuzuncu Yıl, Van, Turkey, over a period of 6 months. Basic identification of staphylococci was determined by colony morphology, Gram-staining, and catalase and tube coagulase tests (Koneman *et al.*, 1997). *Staphylococcus* isolates were further identified by the Sceptor bacterial identification system (Becton Dickinson).

Reference strains. The following *S. aureus* reference strains were kindly provided by Dr B. Holmes, National Collection of Type Cultures (NCTC): SEA 10652 FDA 196E, SEB 10654 FDA 243, SEC 10655 137 and SED 10656 494. Other reference strains (*Staphylococcus epidermidis* ATCC 35983, *Staphylococcus hominis* ATCC 35982) were obtained from our culture collection.

Slime-production test. The ability of CoNS to produce slime was tested as described by Christensen *et al.* (1982). For the test, four colonies from freshly prepared 5% sheep blood agar plates were transferred to 10 ml tryptic soy broth (Difco) in 15 ml plastic conical tubes. After incubation for 18–24 h at 37 °C under aerobic conditions, the broth was poured gently to examine for the presence of a film on the inside walls of the tubes. The absence of a film or a ring at the liquid–air interface was interpreted as a negative result. Results were evaluated as negative, weak positive (+1), moderately positive (+2) and strongly positive (+3). *S. epidermidis* ATCC 35983 (tube adherence test positive) and *S. hominis* ATCC 35982 (tube adherence test negative) were used as control organisms.

Detection of enterotoxins. The enterotoxin types of *S. aureus* strains were determined using the SET-RPLA kit (an SEA, -B, -C and -D detection kit employing reversed passive latex agglutination; Oxoid) following the manufacturer's recommendations. *S. aureus* strains (SEA 10652 FDA 196E, SEB 10654 FDA 243, SEC 10655 137 and SED 10656 494) were used as positive controls; *S. epidermidis* 33 served as a negative control strain.

RESULTS AND DISCUSSION

In this study, slime-factor production by CoNS and the enterotoxigenic properties of *S. aureus* strains isolated from various human clinical specimens were determined.

Bacterial isolates

Out of a total of 120 staphylococci isolated from 280 human clinical specimens of various types, 65 (54.2%) were identified as CoNS and 55 (45.7%) as *S. aureus*. The largest number of staphylococci (44.1%) were isolated from human blood specimens (Table 1).

Slime production by CoNS

Thirty-nine (60%) of CoNS were found to be positive for slime production. Eleven of 39 isolates were found to be weakly positive (+1), 24 moderately positive (+2), and four strongly positive (+3) (Table 2). The distribution of the 39 slime-producing CoNS strains by clinical specimen type is presented in Table 3. Slime production was mostly found in *S. hominis* (28.2%) and *S. epidermidis* (28.2%). *Staphylococcus capitis* did not produce slime factor. Blood (38.5%), tracheal aspiration (20.5%) and urine (12.8%) samples were the clinical specimens from which slime-producing CoNS were most frequently isolated.

Slime production by CoNS isolated from nosocomial bloodstream infections has been observed in 13 of 14 clinical isolates, but in only three of 13 contaminants, and four of 27 skin isolates (Ishak *et al.*, 1985). Kotilainen (1990) reported that the analysis of 64 strains from adult septicaemias disclosed 34 (53%) adherent slime producers, while only 142 (29%) of 489 single blood culture isolates were adherent slime producers. One study showed that 496 isolates (31%) from urine samples, 61 (26%) from catheter tips, and 24 (17%) from cerebrospinal fluid, were slime producers. In another study (Ammendolia *et al.*, 1999), it has been stated that 76.7% of *S. epidermidis* produce slime, while the incidence of slime formation is

Table 1. Distribution of CoNS and *S. aureus* by specimen origin

n, Number of tested specimens.

Origin	<i>n</i>	CoNS (%)	<i>S. aureus</i> (%)	Total (%)
Blood	88	33 (27.5)	20 (16.7)	53 (44.1)
Tracheal aspiration	47	11 (9.2)	10 (8.3)	21 (17.5)
Urine	43	5 (4.2)	9 (7.5)	14 (11.7)
Wound/vaginal swab	28	6 (5)	5 (4.2)	11 (9.2)
Catheter	15	2 (1.7)	3 (2.5)	5 (4.2)
Needle	20	2 (1.7)	3 (2.5)	5 (4.2)
Cerebrospinal fluid	21	2 (1.7)	1 (0.8)	3 (2.5)
Mixed	18	4 (3.3)	4 (3.3)	8 (6.6)
Total	280	65 (54.2)	55 (45.8)	120 (100)

Table 2. Slime-factor results and titres of CoNS strains

Titres: +1, weak positive; +2, moderately positive; +3, strongly positive.

CoNS	Frequency	No. slime positive (%)	+1	+2	+3
<i>S. hominis</i>	20	11 (55)	—	11	—
<i>S. epidermidis</i>	18	11 (61.1)	6	4	1
<i>S. xylosum</i>	6	4 (66.7)	2	1	1
<i>Staphylococcus warneri</i>	6	3 (50)	1	2	—
<i>S. sciuri</i>	5	3 (60)	1	2	—
<i>S. haemolyticus</i>	4	3 (75)	—	2	1
<i>S. simulans</i>	2	2 (100)	—	1	1
<i>Staphylococcus saprophyticus</i>	2	2 (100)	1	1	—
<i>S. capitis</i>	2	0 (0)	—	—	—
Total	65	39 (60)	11	24	4

47.8% in other CoNS, e.g. *Staphylococcus haemolyticus*, *Staphylococcus xylosum* and *Staphylococcus simulans*. In contrast, another report (Mehrotra *et al.*, 2000) has shown that 58 (17.9%) of CoNS strains are positive for slime production.

Stepanovic *et al.* (2001) reported that out of 107 (88.4%) slime-producing *Staphylococcus sciuri* strains, 26 (24.3%) were strongly positive and 31 (28.9%) were moderately positive, whereas 50 (46.7%) were weak slime producers. In the present study, two of five *S. sciuri* strains were found to be moderately positive, and one was weakly positive.

In agreement with our results, Arciola *et al.* (2002) also reported that 65 (57.5%) of the 113 clinical CoNS isolates were slime-producer strains. Similar studies (Yılmaz *et al.*, 2004; Kogan *et al.*, 2006; Foka *et al.*, 2006) have also shown that the proportion of slime-producing CoNS strains from various human clinical specimens can vary from 20 to 89%. On the other hand, Altöparlak *et al.* (2004) stated that slime production was mostly detected in CoNS from a patient group (27.9%), compared with the CoNS from a control healthy group (8.7%).

When our results are compared to those of others, some differences are observed. These variations can be explained by the variation in strain numbers as well as by the assumptions that the *Staphylococcus* species tested in some studies were isolated under different environmental conditions and that the nature of the strains was different. On the other hand, it should also be considered that slime production may be affected by various factors, such as medium composition, the presence of carbohydrate, iron and CO₂, and oxidation (Götz, 2002).

Results of enterotoxigenicity of *S. aureus*

In the present study, 46 (83.6%) of 55 *S. aureus* strains were found to be enterotoxigenic. Most (84.7%) of the enterotoxigenic *S. aureus* strains were positive for SEA (Table 4).

al Bustan *et al.* (1996) stated that 115 (86.6%) of 133 *S. aureus* isolates from nasal swabs of restaurant workers in Kuwait City produced enterotoxins. They also reported that 33 (28.6%) of enterotoxin-producing strains were positive for SEA, 33 (28.6%) for SEB, 19 (16.4%) for SEC,

Table 3. Distribution of 39 slime-producing CoNS strains among human clinical specimens

CoNS	Specimen type:								
	No. positive strains (%)	Blood	Tracheal aspiration	Urine	Wound/vaginal swab	Catheter	Needle	Cerebro-spinal fluid	Mixed
<i>S. hominis</i>	11 (28.2)	4	2	2	1	0	1	0	1
<i>S. epidermidis</i>	11 (28.2)	6	0	2	1	0	0	2	0
<i>S. xylosum</i>	4 (10.2)	3	1	0	0	0	0	0	0
<i>S. warneri</i>	3 (7.7)	0	3	0	0	0	0	0	0
<i>S. sciuri</i>	3 (7.7)	0	0	0	0	1	0	0	2
<i>S. haemolyticus</i>	3 (7.7)	0	0	1	1	0	0	0	1
<i>S. simulans</i>	2 (5.1)	1	1	0	0	0	0	0	0
<i>S. saprophyticus</i>	2 (5.1)	1	1	0	0	0	0	0	0
<i>S. capitis</i>	0 (0)	0	0	0	0	0	0	0	0
Total	39 (100)	15 (38.5)	8 (20.5)	5 (12.8)	3 (7.7)	1 (2.6)	1 (2.6)	2 (5.1)	4 (10.2)

Table 4. Distribution of enterotoxigenic properties of *S. aureus* strains by specimen origin

Sample	No. <i>S. aureus</i> isolates	Enterotoxin type				Total (%)
		A	B	C	D	
Blood	20	12	2	—	2	16 (34.8)
Tracheal aspiration	10	9	1	—	—	10 (21.7)
Urine	9	8	—	—	—	8 (17.4)
Wound/vaginal swab	5	2	—	1	—	3 (6.5)
Catheter	3	2	—	1	—	3 (6.5)
Needle	3	3	—	—	—	3 (6.5)
Cerebrospinal fluid	1	1	—	—	—	1 (2.2)
Mixed	4	2	—	—	—	2 (4.3)
Total	55	39	3	2	2	46 (83.6)

and four (3.5 %) for SED, whereas 10 isolates produced both SEA and SEB or SEC. In another study (Becker *et al.*, 1998), of 50 clinical isolates, six (12 %) were positive for SEA, two (4 %) for SEB and SED, and one (2 %) for SED. Similar results have also been published by Larsen *et al.* (2000). Fueyo *et al.* (2001) reported that 32 (29 %) of *S. aureus* strains from human clinical isolates were enterotoxigenic, and of these strains, 14 were positive for SEA, two for SEB, 10 for SEC, two for SED, one for SEA plus SEC, and four for SEC plus SED.

Mehrotra *et al.* (2000) showed that 37 (34.6 %) of 107 healthy individuals were carriers of enterotoxin-producing *S. aureus* strains, and determined by PCR that 21 (19.6 %) of these strains carried *sea*, six (5.6 %) *seb*, eight (7.5 %) *sec*, and two (1.9 %) *sed*.

Hazariwala *et al.* (2002) found that over 51 % of 41 human *S. aureus* isolates were positive for SE genes by PCR. They also showed by latex agglutination test that 12.2 % of these strains were SEA, 2.4 % SEB, 22 % SEC and 24.4 % SED. Becker *et al.* (2003) reported that 17 (40 %) human *S. aureus* strains were positive by PCR for *sea*, 10 (2.3 %) for *seb*, five (1.2 %) for *sec* and three (0.7 %) for *seh* genes. Similar results were reported by Letertre *et al.* (2003).

Boerema *et al.* (2006) stated that 11 (42.3 %) of *S. aureus* were positive for 'old' *se* genes (A–E), whereas 21 (80.7 %) were positive for all *se* genes (A–O). A PCR-based study from Jordan (Naffa *et al.*, 2006) reported that the prevalences of the classic enterotoxin *sea*, *sec*, and *sea* plus *sec* genes in *S. aureus* isolates from clinical cases were 15, 4 and 4 %, respectively.

The conflicting results of our study and those of other investigations concerning the occurrence of enterotoxigenic *S. aureus* isolates from clinical specimens may be due to geographical and methodical variations. Recently, new enterotoxin types (SEG–SEU) have been described (Letertre *et al.*, 2003), but in the current study we could not screen for these enterotoxin types, since the RPLA test

kit did not include antisera to detect the new enterotoxins. In addition, *S. aureus* may be able to produce unidentified enterotoxin types that cannot be determined at present (Rosec & Gigaud, 2002; Boerema *et al.*, 2006). It should also be emphasized that the RPLA method used in this study detects the enterotoxins SEA, SEB, SEC and SED only. Nevertheless, the present study showed a relatively high percentage of enterotoxin-forming *S. aureus* strains from human clinical specimens that seems to be in agreement with the findings of other publications (al Bustan *et al.*, 1996; Fueyo *et al.*, 2001; Hazariwala *et al.*, 2002). In another study we have found that 25.5 % of *S. aureus* strains isolated from subclinical bovine mastitis are enterotoxigenic and that SEA is the predominant enterotoxin type (23.6 %) detected in these strains (B. Boynukara and others, unpublished data).

In conclusion, SEA is the most frequent enterotoxin type among *S. aureus* strains from clinical human isolates (Mehrotra *et al.*, 2000; Fueyo *et al.*, 2001; Naffa *et al.*, 2006), and this was confirmed in our study; however, some reports (Hazariwala *et al.*, 2002) have stated that SEC is the predominant enterotoxin type detected in human *S. aureus* strains.

The results of this study also showed that the incidence of slime formation in CoNS was high in human clinical specimens, and slime production was observed predominantly in CoNS isolated from blood, tracheal aspiration and urine samples. Further studies are necessary for the detection of the new enterotoxin types of *S. aureus* (SEG–SEU) and the corresponding enterotoxin-encoding genes (*seg–seu*).

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REFERENCES

- al Bustan, M. A., Udo, E. E. & Chugh, T. D. (1996). Nasal carriage of enterotoxin-producing *Staphylococcus aureus* among restaurant workers in Kuwait City. *Epidemiol Infect* **116**, 319–322.
- Altöparlak, U., Kadanali, A. & Çelebi, S. (2004). Slime factor positivity in coagulase negative staphylococci isolated from nasal samples of haemodialysis patients. *Int J Clin Pract* **58**, 1112–1114.
- Ammendolia, M. G., Di Rosa, R., Montanaro, L., Arciola, C. R. & Baldassarri, L. (1999). Slime production and expression of slime-associated antigen by staphylococcal clinical isolates. *J Clin Microbiol* **37**, 3235–3238.
- Arciola, C. R., Campoccia, D., Gamberini, S., Cervellati, M., Donati, E. & Montanaro, L. (2002). Detection of slime production by means of an optimized Congo red agar plate test based on a colourimetric scale in *Staphylococcus epidermidis* isolates genotyped for *ica* locus. *Biomaterials* **23**, 4233–4239.
- Becker, K., Roth, R. & Peters, G. (1998). Rapid and specific detection of toxigenic *Staphylococcus aureus*: use of two multiplex PCR enzyme

immunoassays for amplification and hybridization of staphylococcal enterotoxin genes, exfoliative toxin genes, and toxic shock syndrome toxin 1 gene. *J Clin Microbiol* **36**, 2548–2553.

Becker, K., Friedrich, A. W., Lubritz, G., Weilert, M., Peters, G. & von Eiff, C. (2003). Prevalence of genes encoding pyrogenic toxin superantigens and exfoliative toxins among strains of *Staphylococcus aureus* isolated from blood and nasal specimens. *J Clin Microbiol* **41**, 1434–1439.

Boerema, J. A., Clemens, R. & Brightwell, G. (2006). Evaluation of molecular methods to determine enterotoxigenic status and molecular genotype of bovine, ovine, human and food isolates of *Staphylococcus aureus*. *Int J Food Microbiol* **107**, 192–201.

Christensen, G. D., Simpson, W. A., Bisno, A. L. & Beachey, E. H. (1982). Adherence of slime-producing strains of *Staphylococcus epidermidis* to smooth surfaces. *Infect Immun* **37**, 318–326.

Foka, A., Chini, V., Petinaki, E., Kolonitsiou, F., Anastassiou, E. D., Dimitracopoulos, G. & Spiliopoulou, I. (2006). Clonality of slime-producing methicillin-resistant coagulase-negative staphylococci disseminated in the neonatal intensive care unit of a university hospital. *Clin Microbiol Infect* **12**, 1230–1233.

Fueyo, J. M., Martin, M. C., Gonzalez-Hevia, M. A. & Mendoza, M. C. (2001). Enterotoxin production and DNA fingerprinting in *Staphylococcus aureus* isolated from human and food samples. Relations between genetic types and enterotoxins. *Int J Food Microbiol* **67**, 139–145.

Götz, F. (2002). *Staphylococcus* and biofilms. *Mol Microbiol* **43**, 1367–1378.

Hazariwala, A., Sanders, Q., Hudson, C. R., Hofacre, C., Thayer, S. G. & Maurer, J. J. (2002). Distribution of staphylococcal enterotoxin genes among *Staphylococcus aureus* isolates from poultry and humans with invasive staphylococcal disease. *Avian Dis* **46**, 132–136.

Ishak, M. A., Gröschel, D. H. M., Mandell, G. L. & Wenzel, R. P. (1985). Association of slime with pathogenicity of coagulase-negative staphylococci causing nosocomial septicemia. *J Clin Microbiol* **22**, 1025–1029.

Kogan, G., Sadovskaya, I., Chaignon, P., Chokr, A. & Jabbouri, S. (2006). Clinical strains of *Staphylococcus* that do not contain polysaccharide intercellular adhesin. *FEMS Microbiol Lett* **255**, 11–16.

Koneman, E. W., Allen, S. D., Janda, W. M., Schreckenberger, P. C. & Winn, W. C. (1997). The Gram-positive cocci, part I: staphylococci. In *Color Atlas and Text Book of Diagnostic Microbiology*, 5th edn, pp. 511–576. Philadelphia, PA: Lippincott.

Kotilainen, P. (1990). Association of coagulase-negative staphylococcal slime production and adherence with the development and outcome of adult septicemias. *J Clin Microbiol* **28**, 2779–2785.

Larsen, H. D., Huda, A., Eriksen, N. H. R. & Jensen, N. E. (2000). Differences between Danish bovine and human *Staphylococcus aureus* isolates in possession of superantigens. *Vet Microbiol* **76**, 153–162.

Letertre, C., Perelle, S., Dilasser, F. & Fach, P. (2003). Detection and genotyping by real-time PCR of the staphylococcal enterotoxin genes *sea* to *sej*. *Mol Cell Probes* **17**, 139–147.

Mehrotra, M., Wang, G. & Johnson, W. M. (2000). Multiplex PCR for detection of genes for *Staphylococcus aureus* enterotoxins, exfoliative toxins, toxic shock syndrome toxin 1, and methicillin resistance. *J Clin Microbiol* **38**, 1032–1035.

Naffa, R. G., Bdour, S. M., Migdad, H. M. & Shehabi, A. A. (2006). Enterotoxigenicity and genetic variation among clinical *Staphylococcus aureus* isolates in Jordan. *J Med Microbiol* **55**, 183–187.

Rosec, J. P. & Gigaud, O. (2002). Staphylococcal enterotoxin genes of classical and new types detected by PCR in France. *Int J Food Microbiol* **77**, 61–70.

Stepanovic, S., Vukovic, D., Trajkovic, V., Samardzic, T., Cupic, M. & Svabic-Vlahovic, M. (2001). Possible virulence factors of *Staphylococcus sciuri*. *FEMS Microbiol Lett* **199**, 47–53.

Stephan, R., Senczek, D. & Dorigon, V. (2001). Enterotoxin production and other characteristics of *Staphylococcus aureus* strains isolated from human nasal carriers. *Arch Lebensmittelhyg* **52**, 7–9.

Yilmaz, M., Aral, M. & Erkman, N. (2004). Determination of slime production by coagulase-negative staphylococci: comparison of two methods. *Turk J Infect* **18**, 53–58.