BACTERIAL PATHOGENICITY

Enterotoxin production by *Staphylococcus aureus* clones and detection of Brazilian epidemic MRSA clone (III::B:A) among isolates from food handlers

M. J. S. SOARES,* N. H. TOKUMARU-MIYAZAKI, A. L. S. NOLETO and A. M. S. FIGUEIREDO

Universidade Federal do Rio de Janeiro, Instituto de Microbiologia Prof. Paulo de Góes, Departamento de Microbiologia Médica, Laboratório de Biologia Molecular de Bactérias, CCS-BI I, Cidade Universitária, Ilha do Fundão, Rio de Janeiro, 21941.590 and *Universidade Federal do Piauí, Departamento de Parasitologia e Microbiologia, Campus Ministro Petrônio Portela, SG 16, 64049.550, Brazil

***Staphylococcus aureus* is a major bacterial pathogen involved in a wide range of diseases varying from infections to toxæmia. Staphylococcal food-poisoning syndrome is caused by ingestion of bacterial enterotoxins. These toxins are microbial superantigens and may also be virulence factors involved in staphylococcal infection. This study determined the enterotoxin types and pulsed-field gel patterns found among *S. aureus* isolates obtained from food handlers in community or hospital-located kitchens. Staphylococcal enterotoxin C was the most frequent enterotoxin produced. The data also suggested horizontal spread of *ent* genes among isolates belonging to the Brazilian epidemic MRSA clone III::B:A. A subclone of MRSA clone III::B:A was isolated from two hospital kitchen workers. This was the first report of this clone from a hospital in Teresina, Piauí State, although the presence of this MRSA clone has already been reported in six other Brazilian cities.**

Introduction

The importance of *Staphylococcus aureus* in diseases ranging from acute infections—which may be either localised or invasive—to acute toxæmia is well-known [1–3]. Staphylococcal food-poisoning syndrome is an intoxication caused by ingestion of staphylococcal enterotoxins (SEs). These toxins are characterised on the basis of their immunological reactivity and have been designated as SEA, SEB, SEC1, SEC2, SEC3, SED, SEE and SEH [4–11]. SEs are mitogenic for lymphocytes and are called microbial superantigens because of their ability to stimulate subsets of T cells by binding to MHC class II molecules (Ia) on the antigen-presenting cells [12, 13].

Genetic analysis has shown that the SEA gene (*entA*) is inserted within bacteriophage DNA that is present in at least two integration sites on the staphylococcal chromosome [14]. The SEB gene (*entB*) is associated with a large genetic element on the staphylococcal chromosome, but it is not known whether this is an integrated plasmid, phage or transposon [15]. The SEC

gene (*entC*) is also chromosomally located [16], but the SED gene (*entD*) is located on a plasmid that also carries penicillin and cadmium resistance genes [17]. Finally, the SEE gene (*entE*) is associated with a defective phage inserted in the chromosome [18]. Despite this detailed genetic characterisation, little is known of the evolution and spread of *ent* genes among *S. aureus* strains. However, Renaud *et al.* [19] studied 39 SEB strains and showed that these isolates derived from only three clones.

Acquisition of antibiotic resistance by *S. aureus* has been recognised as a major health problem. Numerous hospital infections, worldwide, are caused by methicillin-resistant *S. aureus* (MRSA) [20, 21] and a food-initiated hospital outbreak caused by MRSA has been described [22]. This paper investigated enterotoxin production by different *S. aureus* clones—including the Brazilian epidemic MRSA clone III::B:A [23]—isolated from food handlers in community and hospital-located kitchens.

Materials and methods

Study population

In total, 196 food handlers from four hospital kitchens and five community-located restaurants in Teresina

City, Piauí State, in the northeast of Brazil, were screened. There was no indication of staphylococcal infection in any of the food handlers and none had used antibiotics in the preceding 3 months.

Bacterial strains

Swabs from anterior nares were streaked on Trypticase Soy Agar (TSA; Difco) supplemented with sheep blood (Difco) 5% v/v. The plates were incubated at 37°C for 24 h. Fingernail specimens were streaked on Mannitol Salt Agar (MSA; Difco) and incubated at 37°C for 72 h. Five typical *Staphylococcus* colonies from both plates were selected. Identification as *S. aureus* was by routine tests [24]. Stock cultures were prepared in sterile glycerol 10% v/v and stored at -70°C. In addition, six multiresistant *S. aureus* hospital isolates belonging to the Brazilian epidemic clone III::B:A [23] were used for enterotoxin production and detection studies.

Enterotoxin production and detection

The cellophane-over-agar method described by Hallander [25], modified by Jarvis and Lawrence [26], was used for enterotoxin production. The optimum-sensitivity plate (OSP) method was used for enterotoxin detection [27]. Purified enterotoxins and specific antisera used in the immunodiffusion test were kindly provided by Dr M. S. Bergdoll (Food Research Institute, University of Wisconsin).

DNA preparation

All procedures for preparation of genomic or plasmid DNA were as described previously [28], except that *S. aureus* cell lysis was achieved following pre-incubation of the bacteria with 90 U of lysostaphin (Sigma) for 3 h at 37°C.

Pulsed-field gel electrophoresis

Preparation of bacterial DNA and digestion with *Sma*I were as described previously [29]. Pulsed-field gel electrophoresis (PFGE) was performed with a CHEF-DR III apparatus (BioRad) and a voltage of 6 V/cm, ramped with an initial forward time of 1 s and a final forward time of 30 s. Agarose 1% w/v gels were run for 23 h at 11.3°C in 50 mM Tris buffer containing 50 mM boric acid and 0.2 mM EDTA, pH 8.0 (0.5 × TBE). Gel staining and photography were as described previously [28]. Strains that shared the same PFGE pattern were assigned a common capital letter. Strains that differed in one-to-three bands were assigned a common capital letter and a subtype number. Strains with more than three band differences were assigned a different capital letter and considered to be another clone [30, 31].

Antibiotic susceptibility

The Kirby-Bauer test was performed as recommended by the National Committee for Clinical Laboratory Standards [32]. The disks (Cecon Ltd., Brazil) used contained the following antibiotics: penicillin G (PN, 10 U); oxacillin (OX, 1 µg); ampicillin (AP, 10 µg); gentamicin (GN, 10 µg); amikacin (AM, 30 µg); tetracycline (TC, 30 µg); cephalothin (CF, 30 µg); erythromycin (EI, 15 µg); clindamycin (CL, 2 µg); imipenem (IM, 10 µg); trimethoprim-sulphamethoxazole (SFT, 25 µg); and vancomycin (VC, 30 µg).

Screening test for methicillin resistance

This test was performed as described previously [33], except that methicillin 25 mg/L agar plates were prepared with Mueller-Hinton Agar (MHA, Difco) instead of TSA. After an incubation period of 24 h at 37°C, any growth was taken as an indication of resistance and examined further with a *mecA*-specific DNA probe (see below).

Preparation of the *mecA* DNA probe

The fluorescein-labelled *mecA* DNA probe used in this study was the 1.196-kb *Pst*I-*Xba*I fragment (MF13) from the *mecA* gene of the Australian type strain ANS46, cloned in pTZ219 [34]. The probe was recovered following electrophoresis of restriction endonuclease-digested plasmid DNA on an agarose 0.8% w/v gel with a Sephaglas Bandprep Kit (Pharmacia) and labelled with fluorescein by the ECL Random Prime Labelling System (Amersham International) as recommended by the manufacturer.

Dot-blot hybridisation

Samples (3 µl) of genomic DNA preparations were blotted on to PhotoGene Nylon Membranes (Gibco BRL) after denaturation of the DNA at 100°C for 5 min. The DNA was fixed by baking at 80°C for 2 h. Hybridisation and wash conditions were according to the ECL protocol. Positive hybridisation results were detected following incubation with anti-fluorescein-HRP conjugate as recommended for the ECL system. The signals generated were recorded on X-ray film (X OMAT XK 1; Kodak). *S. aureus* strain Col was used as a positive control for *mecA* and was obtained from Dr A. Tomasz, Rockefeller University, New York.

Results

S. aureus colonisation

S. aureus was isolated from 91 (46.4%) of the 196 food handlers studied. Among the 91 carriers, isolation of *S. aureus* from the nares was more common than isolation from hands (Table 1). It was also observed that the frequency of nasal *S. aureus* carriage was higher amongst hospital workers than in workers from

Table 1. Carriage of *S. aureus* by 196 food handlers working in community- or hospital-located kitchens

Kitchen type	Number of <i>S. aureus</i> carriers/number of workers examined (%)	Number of colonisation sites (percentage of <i>S. aureus</i> carriers)		
		Nares	Hands	Nares and hands
Community	42/97 (43.3)	21 (50.0)	11 (26.2)	10 (23.8)
Hospital	49/99 (49.5)	39 (79.6)	4 (8.2)	6 (12.2)
Total	91/196 (46.4)	60 (65.9)	15 (16.5)	16 (17.6)

community-located kitchens. In contrast, hand colonisation with *S. aureus* was commoner among food handlers from community-located kitchens than among hospital kitchen workers (Table 1).

Enterotoxin production

Of 91 carriers, 26 (28.6%) were colonised by enterotoxin-producing strains. Sixteen (61.5%) of these 26 workers harboured enterotoxigenic strains of *S. aureus* in their nose only, seven (27%) on their hands only, and three (11.5%) at both sites. The strains produced SEA, SEB, SEC, SED or SEE, or combinations of these toxins. The percentage of carriers from whom enterotoxin-producing strains were isolated was 32.6% for hospital workers and 23.8% for food

handlers from community-located kitchens. Overall, SEC was the toxin produced most frequently (Table 2). Isolates that produced one enterotoxin type alone were most common; however, seven (26.9%) of the 26 carriers yielded strains that produced a combination of different enterotoxin types (Table 2).

Antibiotic susceptibility and detection of the *mecA* gene

The susceptibility profiles of 130 *S. aureus* isolates from the food handlers were analysed (Table 3). Although 81% and 94% of the strains studied, isolated from community- and hospital-located kitchen carriers, respectively, were resistant to penicillin and ampicillin, most of the strains were susceptible to the remaining

Table 2. Production of staphylococcal enterotoxins by *S. aureus* isolates obtained from food handlers

Kitchen	Food handler no.	Number of enterotoxigenic strains/number of strains tested	Enterotoxins produced by isolates from	
			Nares	Hands
Community	I			
	1	4/4	B	Nd
	2	2/7	Nd	ABE
	3	1/7	Nd	AB
	4	7/10	B*	B
	II	5	9/9	C
	6	2/3	ABE†; C‡	A; CD
	7	1/4	Nd	A
	III	8	6/6	B§
	IV	9	4/5	C
	V	10	3/3	C; AB
Hospital	I			
	11	2/3	C	Nd
	12	5/5	C	Nd
	13	1/1	A	Nd
	14	1/1	A	Nd
	15	2/4	Nd	A
	16	1/1	Nd	AE
	17	5/5	B	Nd
	II	18	2/2	C; AC
	19	1/1	C	Nd
	20	1/2	C	Nd
	21	1/3	A	Nd
	22	1/1	Nd	C
	23	4/4	C	Nd
	III	24	1/5	C
	IV	25	5/5	C
	26	3/3	C	Nd

Nd, no enterotoxin-producing strain of *S. aureus* detected.

*Five SEB-producing colonies were obtained from nares and two from hands.

†Strain carrying the *mecA* gene.

‡Five SEC-producing colonies were isolated from nares and three from hands.

§Five SEB-producing colonies were isolated from nares and one from hands.

||Two colonies producing SEA and SEB were isolated from nares.

Table 3. Antibiotic susceptibility patterns of 130 *S. aureus* isolates from food handlers

Kitchen type	Number of strains tested*	Number of strains resistant to											
		PN	CF	AP	AM	IMP	TC	ER	CL	VC	OX	GM	SFT
Community	63	51	1	51	1	1	13	5	1	0	1 [†]	1	1
Hospital	67	63	2	63	2	2	17	22	2	0	2 [†]	2	2

PN, penicillin; CF, cephalothin; AP, ampicillin; AM, amikacin; IMP, imipenem; TC, tetracycline; ER, erythromycin; CL, clindamycin; VC, vancomycin; OX, oxacillin; GM, gentamicin; SFT, sulphamethoxazole-trimethoprim.

*In total, 130 strains of *S. aureus* were obtained from 91 food handlers.

[†]Strains identified as MRSA by an agar screen and confirmed with a probe for *mecA*.

antibiotics tested. However, increased resistance to erythromycin (21%) and tetracycline (23%) was observed, mainly among hospital-worker isolates. When disk diffusion tests were used, all of the strains studied were oxacillin-susceptible. However, screening on methicillin 25 mg/L agar detected MRSA isolates from the nares of three carriers. The methicillin-resistant strains were multiresistant and showed susceptibility only to vancomycin. The presence of the *mecA* gene in these strains was confirmed with the specific *mecA* probe (Fig. 1).

PFGE patterns

Fourteen major PFGE patterns were observed following analysis of 24 enterotoxigenic *S. aureus* isolates (Table 4). All the SEA-producing strains tested had the same PFGE pattern, except for two multiresistant SEA strains. Four SEB-producing strains yielded three different PFGE patterns. Finally, 13 SEC-producing

strains yielded eight different PFGE patterns. Two of the three MRSA isolates had a PFGE pattern similar to that of the Brazilian epidemic multiresistant clone III::B:A (Fig. 2a) [23], differing by only one PFGE band (Fig. 2b). In addition to these two MRSA strains, six hospital isolates belonging to clone III::B:A were tested for enterotoxin production. Most of these strains produced different combinations of enterotoxins, although they appeared to be closely related by PFGE typing (Table 5).

Discussion

It has been reported previously that 10–50% of the human population are healthy carriers of *S. aureus* [35, 36] and that about 30% of these strains are enterotoxigenic *S. aureus* [9]. However, lower (6.4–16.7%) and higher (47–76%) percentages of enterotoxin-producing *S. aureus* isolates have also been described [9, 37–42]. The study presented in this paper found that 91 (46.4%) of 196 kitchen workers harboured *S. aureus* in their anterior nares, on their hands or at both sites. The total incidence of *S. aureus* was similar between workers from community- and hospital-located kitchens (43.3% and 49.5%, respectively). However, as observed previously [35, 43], the frequency of nose isolates was significantly higher ($p < 0.05$; χ^2) among hospital workers. The lower recovery of *S. aureus* from the hands of hospital kitchen workers might be related to implementation of hospital control measures to stop transmission of epidemic bacteria. The overall incidence of enterotoxigenic *S. aureus* was 32.6% and 23.8% for hospital- and community-located kitchen isolates, respectively. However, this difference was not significant.

The predominance of specific enterotoxin types among *S. aureus* isolates from human carriers is variable. Adesiyun *et al.* [44] found that SEA was produced by 32.7% of all enterotoxigenic strains, while SEC and SED were produced by 6.8% and 6.3%, respectively, of the strains examined. In contrast, Reali [42] found that *S. aureus* strains producing SEB were the most common isolates. The present study found that 50% of the *S. aureus* isolates produced SEC, 23.1% SEA and 15.4% SEB. A predominance of SEC-producing *S.*

Fig. 1. Dot-blot hybridisation with a *mecA*-specific probe. Dilutions of DNA preparations of *S. aureus* strains 21r, 12r and 5r (strains that grew on methicillin 25 mg/L screening agar) were hybridised with the *mecA* probe as described in *Materials and methods*. *S. aureus* strain Col was a known *mecA*-positive control and strain 27 (susceptible on screening agar) was used as a *mecA*-negative control.

Table 4. Genotypic and phenotypic characterisation of enterotoxigenic and methicillin-resistant isolates of *S. aureus*

Food handler no.	Enterotoxin types or combinations	PFGE pattern	Kitchens*	Disk diffusion test results											
				PN	CF	AP	AM	IMP	TC	ER	CL	VC	OX	GM	SFT
MSSA 1	B	A	CK I	R	S	R	S	S	S	S	S	S	S	S	S
4	B	A1	CK I	R	S	R	S	S	S	S	S	S	S	S	S
17	B	B	HK I	R	S	R	S	S	R	R	S	S	S	S	S
8	B	C	CK III	R	S	R	S	S	S	S	S	S	S	S	S
6	A	D	CK II	R	S	R	S	S	S	S	S	S	S	S	S
7	A	D1	CK II	R	S	R	S	S	R	R	S	S	S	S	S
13	A	D2	HK I	R	S	R	S	S	R	R	S	S	S	S	S
14	A	D2	HK I	R	S	R	S	S	I	R	S	S	S	S	S
15	A	D2	HK I	R	S	R	S	S	R	R	S	S	S	S	S
25	C	E	HK IV	R	S	R	S	S	S	S	S	S	S	S	S
26	C	F	HK IV	S	S	S	S	S	S	S	S	S	S	S	S
10	C	F	CK V	R	S	R	S	S	S	S	S	S	S	S	S
5	C	G	CK II	R	S	R	S	S	S	S	S	S	S	S	S
18	C	H	HK II	R	S	R	S	S	S	R	S	S	S	S	S
19	C	G	HK II	R	S	R	S	S	R	S	S	S	S	S	S
20	C	I	HK II	R	S	R	S	S	S	S	S	S	S	S	S
11	C	J	HK II	R	S	R	S	S	S	S	S	S	S	S	S
12	C	J1	HK I	R	S	R	S	S	S	S	S	S	S	S	S
22	C	I	HK I	R	S	R	S	S	S	S	S	S	S	S	S
23	C	K	HK II	R	S	R	S	S	R	S	S	S	S	S	S
24	C	K1	HK III	R	S	R	S	S	S	S	S	S	S	S	S
9	C	L	CK IV	R	S	R	S	S	S	S	S	S	S	S	S
MRSA 5r	ABE	M	CK II	R	R	R	R	R	R	R	R	S	R	R	R
12r	Nd	N	HK I	R	R	R	R	R	R	R	R	S	R	R	R
21r	A	N	HK II	R	R	R	R	R	R	R	R	S	R	R	R

PN, penicillin; CF, cephalothin; AP, ampicillin; AM, amikacin; IMP, imipenem; TC, tetracycline; ER, erythromycin; CL, clindamycin; VC, vancomycin; OX, oxacillin; GM, gentamicin; SFT, sulphamethoxazole + trimethoprim; R, resistant; S, sensitive; Nd, no enterotoxin production detected.

*CKI-V, community-located kitchens; HKI-IV, hospital-located kitchens.

aureus strains among food handlers has been observed previously [37, 40, 41, 45]. In Brazil, few food-borne intoxication outbreaks caused by enterotoxigenic *S. aureus* have been reported. In these studies the most frequent enterotoxin types produced were SEA, or SEA and SEB [46–48]. However, Bergdoll *et al.* [49, 50] found that SEC was involved in four different food-poisoning outbreaks in Curitiba City, Brazil. The present study observed production of two or more enterotoxin types by isolates from three workers. Co-production of different toxin types might be important in increasing the toxigenic effect of these superantigens. Some studies have suggested that the production of enterotoxins by *S. aureus* isolates may also be important in the pathogenesis of infections other than staphylococcal food poisoning [38, 51, 52]. However, the possible involvement of these toxins in other staphylococcal diseases is not well documented.

Of the 130 *S. aureus* strains analysed, 81–94% were resistant to penicillin G and ampicillin. Increased resistance (33%) to erythromycin was found in hospital isolates compared with community isolates (8%). This increase might be related to the frequent use of this drug in hospitals. About 3% of the strains carried the *mecA* gene, and two of three MRSA isolates had a PFGE pattern very similar to that of the Brazilian epidemic MRSA clone, designated previously as clone III::B:A [23] and assigned PFGE

pattern N in this study (Table 4). This clone has caused hospital outbreaks in six different Brazilian cities [23, 53], ranging from Manaus in the north to Porto Alegre in the south (5300 km from Manaus), and the results show that a subclone of this epidemic MRSA clone is also colonising a hospital in Teresina.

A food-related hospital outbreak of infection caused by MRSA has been described previously [22] and, combined with the results from the present study, these data indicate that kitchen workers may contribute to hospital dissemination of epidemic multi-resistant *S. aureus* clones. Hospital kitchen workers carrying MRSA seem not to be rare and should be given special attention during control measures to stop MRSA transmission during outbreaks. A further MRSA clone was isolated from a carrier in a community-located kitchen. Clone III::B:A and other MRSA clones have also been observed among *S. aureus* isolates from healthy carriers in Rio de Janeiro [54]. The wide dissemination of these multiresistant *S. aureus* clones in the community would be a serious health problem and health counsellors should not underestimate this risk.

PFGE analysis of the enterotoxigenic strains revealed clonal diversity, with the exception of the five SEA-producing strains that belonged to the same clone. Similar diversity was reported previously among SEC-producing *S. aureus* isolates from human and food

Fig. 2. Examples of *Sma*I PFGE patterns. **A**, strains representative of Brazilian Epidemic Clone III::B:A. **B**, strains isolated from hospital food handlers, showing that strains 12r and 21r had essentially the same *Sma*I PFGE pattern as the representatives of clonal type III::B:A, while strain 5r belonged to a different *Sma*I PFGE type.

sources [55]. In contrast, Renaud *et al.* [19] found that 39 SEB-producing strains probably originated from only three clones.

Eight isolates belonging to MRSA clone III::B:A varied from non-enterotoxin producers to producers of different combinations of enterotoxin types (Table 5). Thus, acquisition of *ent* genes by this clone seems to have occurred after the acquisition of *mecA* and the Tn554 transposon. The diversity of enterotoxin types produced by this clone suggests that the acquisition of *ent* genes might have occurred by horizontal transmission. Marr *et al.* [55] studied 15 SEC-producing strains and confirmed the wide spread transmission of this gene among *S. aureus* isolates.

In conclusion, these results suggest that the recent acquisition of *ent* genes by isolates belonging to MRSA clone III::B:A occurred by horizontal transfer and that this phenomenon might be a common mechanism for spread of *ent* genes among strains of *S. aureus*. Attention should be given to the dissemination of MRSA clone III::B:A and to the presence of MRSA clones colonising the nares of carriers in hospital- or community-located kitchens.

This work was partially supported by grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação Coordenação de Pessoal de Nível Superior (CAPES) and Financiadora de Estudos e Projetos (FINEP).

Table 5. Production of enterotoxins by isolates belonging to a single MRSA clone

<i>S. aureus</i> isolate	Genotype*	Description	Enterotoxin types†
BMB4792	III::B:A	Hospital isolate from invasive infection	SEC
BMB5292	III::B:A	Hospital isolate from invasive infection	Nd
BMB5592	III::B:A	Hospital isolate from invasive infection	SEA, SEB, SEC
BMB6492	III::B:A	Hospital isolate from invasive infection	SEA
BMB6792	III::B:A	Hospital isolate from invasive infection	SEA, SEC
BMB7192	III::B:A	Hospital isolate from invasive infection	SEA, SEB, SEC
12r	PFGE (N)‡	Hospital isolate from food handler	Nd
21r	PFGE (N)‡	Hospital isolate from food handler	SEA

Nd, no production of enterotoxin detected.

*Characterisation of BMB isolates listed has been described previously [23].

†Determined by the optimum sensitivity plate (OSP) method [26].

‡Strain with a *Sma*I PFGE pattern essentially identical to strains belonging to clone III::B:A (see Fig. 2).

References

- Baird-Parker AC. The staphylococci: an introduction. *Soc Appl Bacteriol Symp Ser* 1990; **19**: 1S–8S.
- Musher DM, McKenzie SO. Infection due to *Staphylococcus aureus*. *Medicine* 1977; **56**: 383–409.
- Cohen ML. *Staphylococcus aureus*: biology, mechanisms of virulence, epidemiology. *J Pediatr* 1986; **108**: 796–799.
- Casman EP. Further serological studies of staphylococcal enterotoxin. *J Bacteriol* 1960; **79**: 849–856.
- Bergdoll MS, Sugiyama H, Dack GM. Staphylococcal enterotoxin. I. Purification. *Arch Biochem* 1959; **85**: 62–69.
- Borja CR, Bergdoll MS. Purification and partial characterization of enterotoxin C produced by *Staphylococcus aureus* strain 137. *Biochemistry* 1967; **6**: 1467–1473.
- Avena RM, Bergdoll MS. Purification and some physicochemical properties of enterotoxin C, *Staphylococcus aureus* strain 361. *Biochemistry* 1967; **6**: 1474–1480.
- Reiser RF, Robbins RN, Noletto AL, Khoe GP, Bergdoll MS. Identification, purification and some physicochemical properties of staphylococcal enterotoxin C3. *Infect Immun* 1984; **45**: 625–630.
- Casman EP, Bennett RW, Dorsey AE, Issa JA. Identification of a fourth staphylococcal enterotoxin, enterotoxin D. *J Bacteriol* 1967; **94**: 1875–1882.
- Bergdoll MS, Borja CR, Robbins RN, Weiss KF. Identification of enterotoxin E. *Infect Immun* 1971; **4**: 593–595.
- Su Y-C, Wong ACL. Identification and purification of a new staphylococcal enterotoxin, H. *Appl Environ Microbiol* 1995; **61**: 1438–1443.
- Janeway CA, Yagi J, Conrad PJ *et al.* T-cell responses to M1s and bacterial proteins that mimic its behaviour. *Immunol Rev* 1989; **107**: 61–88.
- Fraser JD. High-affinity binding of staphylococcal enterotoxins A and B to HLA-DR. *Nature* 1989; **339**: 221–223.
- Betley MJ, Mekalanos JJ. Staphylococcal enterotoxin A is encoded by phage. *Science* 1985; **229**: 185–187.
- Johns MB, Khan SA. Staphylococcal enterotoxin B gene is associated with a discrete genetic element. *J Bacteriol* 1988; **170**: 4033–4039.
- Betley MJ, Borst DW, Regassa LB. Staphylococcal enterotoxins, toxic shock syndrome toxin and streptococcal pyrogenic exotoxins: a comparative study of their molecular biology. *Chem Immunol* 1992; **55**: 1–35.
- Bayles KW, Iandolo JJ. Genetic and molecular analysis of the gene encoding staphylococcal enterotoxin D. *J Bacteriol* 1989; **171**: 4799–4806.
- Couch JL, Soltis MT, Betley MJ. Cloning and nucleotide sequence of the type E staphylococcal enterotoxin gene. *J Bacteriol* 1988; **170**: 2954–2960.
- Renaud F, Bornstein N, Meugnier H, Forey F, Bes M, Fleurette J. Clonal study of enterotoxin-B producing strains of *Staphylococcus aureus*. *Epidemiol Infect* 1994; **112**: 501–516.
- Maple PAC, Hamilton-Miller JMT, Brumfitt W. World-wide antibiotic resistance in methicillin-resistant *Staphylococcus aureus*. *Lancet* 1989; **1**: 537–540.
- Voss A, Milatovic D, Wallrauch-Schwarz C, Rosdahl VT, Braveny I. Methicillin-resistant *Staphylococcus aureus* in Europe. *Eur J Clin Microbiol Infect Dis* 1994; **13**: 50–55.
- Klutymans J, van Leeuwen W, Goessens W *et al.* Food-initiated outbreak of methicillin-resistant *Staphylococcus aureus* analyzed by pheno- and genotyping. *J Clin Microbiol* 1995; **33**: 1121–1128.
- Teixeria LA, Resenade CA, Ormonde LR *et al.* Geographic spread of epidemic multiresistant *Staphylococcus aureus* clone in Brazil. *J Clin Microbiol* 1995; **33**: 2400–2404.
- International Commission on Microbiological Specifications for Foods (ICMSF). Microorganisms in foods 1: their significance and methods of enumeration, 2nd edn. Toronto, University of Toronto Press, 1976; 218, 256.
- Hallander HO. Production of large quantities of enterotoxin B and other staphylococcal toxin on solid media. *Acta Path Microbiol Scand* 1965; **63**: 299–305.
- Jarvis AW, Lawrence RC. Production of high titers of enterotoxins for the routine testing of staphylococci. *Appl Microbiol* 1970; **19**: 698–699.
- Robbins R, Gould S, Bergdoll MS. Detecting the enterogenicity of *Staphylococcus aureus* strains. *Appl Microbiol* 1974; **28**: 946–950.
- Sambrook J, Fritsch EF, Maniatis T. Molecular cloning. A laboratory manual, 2nd edn. Cold Spring Harbor, New York, Cold Spring Harbor Laboratory Press, 1989.
- de Lencastre H, Couto I, Santos I, Melo-Cristino J, Torres-Pereira A, Tomasz A. Methicillin-resistant *Staphylococcus aureus* in a Portuguese hospital: characterization of clone types by a combination of DNA typing methods. *Eur J Clin Microbiol Infect Dis* 1994; **13**: 64–73.
- Struelens MJ, Deplano A, Godard C, Maes N, Serruys E. Epidemiologic typing and delineation of genetic relatedness of methicillin-resistant *Staphylococcus aureus* by macrorestriction analysis of genomic DNA by using pulsed-field gel electrophoresis. *J Clin Microbiol* 1992; **30**: 2599–2605.
- Tenover FC, Arbeit RD, Goering RV *et al.* Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* 1995; **33**: 2233–2239.
- National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial disk susceptibility tests.

- M2-A5, 5th edn., V. 13 N°. 24. Villanova, PA, National Committee for Clinical Laboratory Standards. 1993.
33. de Lencastre HSE, Figueiredo AM, Urban C, Rahal J, Tomasz A. Multiple mechanisms of methicillin resistance and improved methods for detection in clinical isolates of *Staphylococcus aureus*. *Antimicrob Agents Chemother* 1991; **35**: 632-639.
34. Matthews PR, Reed KC, Stewart PR. The cloning of chromosomal DNA associated with methicillin and other resistances in *Staphylococcus aureus*. *J Gen Microbiol* 1987; **133**: 1919-1929.
35. Williams REO. Healthy carriage of *Staphylococcus aureus*: its prevalence and importance. *Bacteriol Rev* 1963; **27**: 56-71.
36. Noble WC, Valkenburg HA, Wolters CHL. Carriage of *Staphylococcus aureus* in random samples of a normal population. *J Hyg* 1967; **65**: 567-573.
37. Castro MMMV, Iaria ST. *Staphylococcus aureus* enterotoxigênico no vestibulo nasal de manipuladores de alimentos em cozinhas de hospitais do município de João Pessoa PB. [Enterotoxigenic *Staphylococcus aureus* in the nasal vestibule of food handlers in hospital kitchens of the municipality of João Pessoa, Paraíba.] *Rev Saude Publica* 1984; **18**: 235-245.
38. Humphreys H, Keane CT, Hone R *et al.* Enterotoxin production by *Staphylococcus aureus* isolates from cases of septicaemia and from healthy carriers. *J Med Microbiol* 1989; **28**: 163-172.
39. Noleto ALS, da Costa Cesar E, Bergdoll MS. Antibodies to staphylococcal enterotoxins and toxic shock syndrome toxin 1 in sera of patients and healthy people in Rio de Janeiro, Brazil. *J Clin Microbiol* 1986; **24**: 809-811.
40. Iaria ST, Furlanetto SMP, Cerqueira-Campos ML. Pesquisa de *Staphylococcus aureus* enterotoxigênico nas fossas nasais de manipuladores de alimentos em hospitais [Research of enterotoxigenic *Staphylococcus aureus* in the nasal fossa of hospital food handlers in Sao Paulo, 1976.] *Rev Saude Publica* 1980; **14**: 93-100.
41. Melconian AK, Brun Y, Fleurette J. Enterotoxin production, phage typing and serotyping of *Staphylococcus aureus* strains isolated from clinical materials and food. *J Hyg* 1983; **91**: 235-242.
42. Reali D. Enterotoxin A and B production in strains of *Staphylococcus aureus* isolated from human beings and foods. *J Hyg* 1982; **88**: 103-106.
43. Ballou WR, Cross AS, Williams DY, Keiser J, Zierdt CH. Colonization of newly arrived house staff by virulent staphylococcal phage types endemic to a hospital environment. *J Clin Microbiol* 1986; **23**: 1030-1033.
44. Adesiyun AA, Raji I, Yobe V. Enterotoxigenicity of *Staphylococcus aureus* from anterior nares of dining hall workers. *J Food Protect* 1986; **49**: 955-957.
45. Adesiyun AA, Prabhakar P, Ali C, Lewis M. Characteristics of *Staphylococcus aureus* isolated from clinical and non-clinical human sources in Trinidad: susceptibility to bacteriophages and antimicrobial agents, and toxigenicity. *Zentralbl Bakteriell* 1995; **282**: 519-532.
46. Carmo LS, Bergdoll MS. Staphylococcal food poisoning in Belo Horizonte (Brazil). *Rev Microbiol* 1990; **21**: 320-323.
47. Noleto ALS, Tibana A. Outbreak of staphylococcal enterotoxin B food poisoning. *Rev Microbiol* 1987; **18**: 144-145.
48. Pereira ML, do Carmo LS, dos Santos EJ, Bergdoll MS. Staphylococcal food poisoning from cream-filled cake in a metropolitan area of south-eastern Brazil. *Rev Saude Publica* 1994; **28**: 406-409.
49. Bergdoll MS, Carmo LS, Sikorski Moscalewski W, Oliveira Branco Filho M. Staphylococcal food poisoning in Brazil. Proceedings of the 3rd World Congress on Foodborne Infections and Intoxications, Berlin. 1992. V1: 320-323.
50. Pereira ML, Carmo LS, Lara MA, Dias RS, Bergdoll MS. Enterotoxigenic staphylococci from food handlers working in an industrial kitchen in Belo Horizonte, MG (Brazil). *Rev Microbiol* 1994; **25**: 161-165.
51. Crass BA, Bergdoll MS. Involvement of staphylococcal enterotoxins in nonmenstrual toxic shock syndrome. *J Clin Microbiol* 1986; **23**: 1138-1139.
52. Lehn N, Schaller E, Wagner H, Kronke M. Frequency of toxic shock syndrome toxin- and enterotoxin-producing clinical isolates of *Staphylococcus aureus*. *Eur J Clin Microbiol Infect Dis* 1995; **14**: 43-46.
53. Santos Filho L, Sader HS, Bortolotto VI, Gontijo Filho PP, Pignatari AC. Analysis of the clonal diversity of *Staphylococcus aureus* methicillin-resistant strains isolated at João Pessoa, State of Paraíba, Brazil. *Mem Inst Oswaldo Cruz* 1996; **91**: 101-105.
54. Falcão MHL, Borges-Neto AA, Fracalanza SEL, Seldin L, Figueiredo AMS. Associations of methicillin-resistant and susceptible strains of *Staphylococcus aureus* isolated from the same colonization site from healthy humans in Rio de Janeiro. *Program and Abstracts of The 94th General Meeting of the American Society for Microbiology*, 1994: 563.
55. Marr JC, Lyon JD, Roberson JR, Lupher M, Davis WC, Bohach GA. Characterization of novel type C staphylococcal enterotoxins: biological and evolutionary implications. *Infect Immun* 1993; **61**: 4254-4262.