Enterotoxin production, phage typing and serotyping of *Staphylococcus aureus* strains isolated from clinical materials and food

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SUMMARY

The production of enterotoxins A, B, C and F by strains of *Staphylococcus aureus* isolated from various clinical sources and from isolates implicated in food poisoning was investigated.

One hundred and ninety one of the 374 clinical strains (51.1 %) were found to be enterotoxigenic; of these, 81 (27.7 %) strains produced enterotoxin A, 57 (15.3 %) strains produced enterotoxin B, 23 (6.2 %) strains produced enterotoxin C, and 64 (17.1 %) strains produced enterotoxin F. These enterotoxigenic strains were most frequently lysed by phages of group III (21.5 %) or were not typable (22 %).

Eighteen of the 29 strains implicated in food poisoning were enterotoxigenic.

The correlation of antigens and bacteriophage patterns with enterotoxigenicity was determined: enterotoxin A being related to a₄ antigen, enterotoxin B to phages of 94/96 complex with c₁, o antigens, and enterotoxin F to phages of group I with 263₂, k₁k₂, m antigens.

INTRODUCTION

Certain strains of *Staphylococcus aureus* are known to produce different enterotoxins designated as A (Casman, 1960), B (Bergdoll, Surgalla & Dack, 1959), C (Bergdoll, Borja & Avena, 1965), D (Casman *et al.* 1967); E (Bergdoll *et al.* 1971), and F (Bergdoll *et al.* 1981). These enterotoxins play an important role in the pathogenesis of staphylococcal diseases, mainly in food poisoning outbreaks (Bergdoll, Huang & Schantz, 1974) and recently in an illness called toxic shock syndrome (Bergdoll *et al.* 1981).

The production of the enterotoxins by *S. aureus* strains of different origin has been reported from many countries (Girija, Gupta & Mithal, 1980; Petras & Maskova, 1980; Sourek, 1980; Mochmann *et al.* 1981; Reali, 1982). Limited local information regarding production of enterotoxins by *S. aureus*, especially of clinical origin, prompted us to seek the incidence and type of enterotoxins in some locally isolated human and food *S. aureus* strains. An attempt was also made to correlate phage groups and serotypes of the staphylococci with enterotoxigenicity.

MATERIALS AND METHODS

Strains. 403 local *S. aureus* strains were investigated: 374 were obtained from clinical materials isolated in the routine diagnostic bacteriology laboratories of Edouard Herriot and Neuro-cardiological hospitals in Lyon, 14 strains were isolated from employees of a restaurant implicated in food poisoning and 15 from different food materials (sausage, chicken, molluscs, minced meat and carrot).

Enterotoxin production. The cellophane over agar method (Hallander, 1965) with brain heart infusion agar (Jarvis & Lawrence, 1970) was used for the growth of the staphylococci strains. For the cellophane culture, sterile cellophane disks were placed on the agar in a 9 cm Petri dish. The surface of the cellophane was inoculated with an overnight culture (0.1 ml) of staphylococci (Minor & Marth, 1972) using a sterile applicator. Cultures were incubated at 37 °C for 24 h and then harvested, centrifuged and the supernatants were freeze dried (Sourek *et al.* 1979). The dried materials were redissolved at a 20-fold concentration in phosphate buffered saline (Sourek *et al.* 1979) containing 0.05 % sodium azide.

Enterotoxin detection. The microslide immunodiffusion method (Sourek *et al.* 1979) with agar gel dissolved in barbital buffer (Casman *et al.* 1967) was used. Reference enterotoxins (A, B, C, F) and antienterotoxin sera were kindly offered by M. S. Bergdoll (Food Research Institute, Wisconsin, Madison).

Phage typing. The international basic set of 23 typing phages (De Saxe & Rosendal, 1982) provided by Dr J. Fouace of Institut Pasteur Paris, was used. Cultures were typed at both routine test dilution (RTD) and 100 RTD.

Serotyping. The strains were serotyped according to Oeding, Haukenes-Grün system modified by Fleurette & Modjadedy (1976) using 18 factor sera.

RESULTS

One or more enterotoxins was produced by 191 (51.1 %) of 374 *S. aureus* strains isolated from human clinical materials (Table 1); of these, 158 (42.2 %) strains produced only a single enterotoxin, 32 (8.5 %) strains produced two enterotoxins (AB, AC, AF, CF) and one (0.3 %) strain only produced three enterotoxins (ABC). Production of enterotoxin A was detected in 13.1 % of strains, B in 14.7 %, C in 3.2 %, F in 11.2 %, AC in 2.4 %, AF in 5.6 %, and each of AB, CF, ABC in 0.3 %. When individual types of enterotoxin were considered staphylococcal enterotoxin A (SEA) was produced by 21.7 %, SEB by 15.3 %, SEC by 6.2 %, and SEF by 17.1 %.

Phage typing of the 374 strains (Table 2) indicated that the majority of them belonged to group III phages (16.3 %) or were not typable (26.2 %) with the basic set at 100 RTD; however all other groups were represented. This predominancy was also observed in the enterotoxigenic strains. The distribution of the enterotoxin type within the phage groups was as follows: the majority of SEA strains were lysed by group III phages or were not typable, SEB strains were mostly lysed by phages of the 94/96 complex or by group II phages, and SEF strains were mostly lysed by group I phages or were not typable. SEB strains were the only enterotoxigenic isolates lysed by phages of the 94/96 complex (Table 2).

The relation between production of enterotoxins and the serotype of the strains is given in Fig. 1. Most of the antigens (a_4 , a_5 , b_1 , c_1 , h_2 , k_1k_2 , l , m , o , 263₁, 263₂)

Table 1. *Staphylococcus aureus* strains of clinical origin and enterotoxin (A, B, C and F) production

Site of isolation	Strains		Enterotoxin type								
	No.	Enterotoxigenic	A	B	C	F	AB	AC	AF	CF	ABC
Skin	127	62	16	16	4	15	1	5	5	—	—
Respiratory tract	90	53	13	18	3	9	—	1	7	1	1
Cerebrospinal fluid	14	7	1	5	—	1	—	—	—	—	—
Peritoneal fluid	10	4	1	1	—	1	—	—	1	—	—
Urine	5	2	1	—	—	1	—	—	—	—	—
Blood	95	46	15	12	3	8	—	—	8	—	—
Vagina	33	17	2	3	2	7	—	3	—	—	—
Total											
No.	374	191	49	55	12	42	1	9	21	1	1
%	100	51.1	13.1	14.7	3.2	11.2	0.3	2.4	5.6	0.3	0.3

Table 2. *Phage typing and enterotoxin A, B, C and F production of Staphylococcus aureus strains isolated from clinical specimens*

Enterotoxin type	Number of strains lysed by phage group										Total
	I	II	III	I/II	I/III	II/III	Miscellaneous, 81, 94, 95, 96	94/96	Mixed	Not typable, 100 RTD	
A	3	2	19	—	2	—	—	—	4	19	49
B	—	11	7	—	—	1	5	21	5	5	55
C	—	—	3	—	—	—	2	—	4	3	12
F	22	—	3	—	3	—	1	—	5	8	42
Comb. A-C and F	12	—	9	1	—	—	1	—	3	7	33
Total enterotoxin-positive	37	13	41	1	5	1	9	21	21	42	191
No.	19.4	6.8	21.5	0.5	2.6	0.5	4.7	11	11	22	100
%											
Total enterotoxin-negative	14	34	20	0	9	1	17	17	15	56	183
No.	7.7	18.6	10.9	0	4.9	0.5	9.3	9.3	8.2	30.6	100
%											
Total	51	47	61	1	14	2	26	38	36	98	374
%	13.6	12.6	16.3	0.3	3.7	0.5	7	10.2	9.6	26.2	100

Fig. 1. Occurrence of agglutinogens among the enterotoxigenic strains isolated from clinical materials.

Table 3. *Staphylococcus aureus* strains implicated in food poisoning outbreaks and enterotoxin, A, B, C, F production

Origin	Strains		Enterotoxin type							
	No.	Enterotoxigenic	A	B	C	F	AB	AC	AF	ACF
Employees of the restaurant	14	7	1	1	2	1	—	—	2	—
Food	15	11	2	1	1	—	1	1	3	2
Total										
No.	29	18	3	2	3	1	1	1	5	2
%	100	62	10.4	6.9	10.4	3.4	3.4	3.4	17.2	6.9

were found with the SEA and SEB strains while the antigens a₅, b₁, c₁, h₂, l, m, o with SEC strains and the antigens a₅, c₁, h₂, k₁k₂, l, m, o, 263₁, 263₂, with SEF strains. The most frequent antigens found with SEA were the antigens h₂ (71.4 %) and o (34.7 %), with SEB were c₁ (67.3 %) and o (69.1 %), with SEC were h₂ (83.3 %) and o (75 %), with SEF were h₂ (78.6 %) and 263₂ (78.8 %). The SEC and SEF producer strains were found to be excluded from a₄ antigen whilst this antigen was found at a frequency of 22.5 % with SEA strains. The antigens k₁k₂ (55 %) and m (68 %) were observed with SEF strains.

The distribution of the enterotoxins (A, B, C, and F) among the 29 strains collected from food poisoning cases is shown in Table 3. Eighteen of the 29 investigated strains produced enterotoxin (62 %); of these, nine (31.1 %) produced

Table 4. *Relation between bacteriophage type and enterotoxin production of Staphylococcus aureus strains implicated in food poisoning outbreaks*

Enterotoxin type	Number of strains lysed by phage group							Total
	I	II	III	II/III	Miscellaneous, 81, 94, 95, 96	Mixed	94/96	Not typable, 100 RTD
A	—	—	1	1	—	1	—	—
B	—	—	—	—	—	—	2	—
C	—	—	—	—	2	—	—	1
F	1	—	—	—	—	—	—	—
Comb. A-C and F	2	—	2	—	—	2	—	3
Total enterotoxin positive No.	3	0	3	1	2	3	2	4
Total enterotoxin negative No.	0	4	2	0	0	0	1	4
Total No.	3	4	5	1	2	3	3	8

single enterotoxin, seven (24 %) produced two enterotoxins (AB, AC, AF), and two (6.9 %) strains produced three enterotoxins (ACF). Considering individual types of enterotoxins, SEA was produced by 41.3 %, SEB by 10.3 %, SEC by 20.7 %, and SEF by 27.5 %. Eleven (73.3 %) of the 15 food strains were enterotoxigenic, while seven (50 %) of the 14 restaurant-employees strains produced enterotoxins. No production of SEF alone was observed amongst the food strains tested. Phage typing (Table 4) and serotyping patterns of the strains implicated in food poisoning were almost similar to those of the other isolates.

DISCUSSION

Between 25 and 50 % of the human population are carriers of *S. aureus* (Williams, 1963) and more than 50 % of such isolates are enterotoxigenic (Bergdoll, 1979). The incidence of enterotoxigenic staphylococci isolated from clinical materials and from food products connected with food poisoning outbreaks was found to be 53.5 % (Petras & Maskova, 1980) and 96.2 % (Casman *et al.* 1967) respectively. In the present study, 191 (51.1 %) of the 374 strains isolated from clinical materials produced enterotoxins, whilst 18 (62 %) of the 29 food poisoning strains were also enterotoxigenic. Similar results have been reported by other investigators (Piotrowska & Jozefczyk, 1976).

The enterotoxigenic strains isolated in this study most often produced enterotoxin A (21.6 % clinical and 41.1 % food poisoning strains). The same findings were reported by Wieneke (1974), Petras & Maskova (1980), De Buyser & Janin (1981) and by Melconian, Brun & Fleurette (1982), whereas others have reported a high frequency of SEB production (Girija, Gupta & Mithal, 1980; Reali, 1982). This difference could be related to the origin of the strains studied since the latter authors isolates possessed the potentiality of producing enterocolitis (Girija, Gupta & Mithal, 1980) or were not connected with food poisoning outbreaks (Reali, 1982).

The production of enterotoxin F by strains isolated from clinical materials (17.1 %) corresponded with the results reported by Bergdoll *et al.* (1981). These findings are in contrast to the high percentage of F produced strains (42 %) reported by De Nooij, Van Leeuwen & Notermans (1982). Enterotoxin F was produced by eight strains implicated in food poisoning outbreaks, (Table 3); all but one of these strains produced enterotoxin A. Similar results were reported by Bergdoll *et al.* (1982) when testing six strains isolated from food connected in food poisoning. No particular association between enterotoxigenicity and bacteriophage group has been reported, but staphylococci implicated in food poisoning are most likely to be lysed by phages of group III (Simkovicova & Gilbert, 1971). Non-typable strains formed the largest group amongst our clinical and food poisoning strains (Tables 2 and 4) which supports the findings obtained by Payne & Wood (1974) while testing strains isolated from foods. Asheshov, Coe & Porthouse (1976) reported the relation between the SEB strains and the phages of 94/96 complex. In this study all our enterotoxigenic strains showed resistance to attack by these phages except the SEB ones. These latter strains most frequently possessed the antigens c_1 and o . An earlier study by Fleurette & Brun (1981) showed that 90 % of the 94/96 strains possessed the antigens c_1 and o .

Strains producing enterotoxin F were related mostly with the group I phages

and with the h_2 and 263₂ antigens. This supports the relation found by Altemeier *et al.* (1982) between the phage types 29, 52 (group I), exotoxin type C and SEF production.

Our findings showed no significant difference in antigenic pattern of the enterotoxigenic strains from that of the previous report (Flandrois, Fleurette & Behr, 1978) except for SEB strains associated with C, antigen.

The investigation indicates that more than 50 % of our locally isolated *S. aureus* strains are enterotoxigenic and confirms that the circulation of the enterotoxigenic strains in human is far from being negligible, and constitutes a risk factor in some cases. Hence the diagnosis of a clinical syndrome could be misinterpreted or affected by an associated isolate producing one or more enterotoxins. It is thus in certain benign forms of toxic shock syndrome.

This discrepancy calls for more work on the correlation between epidemiology and enterotoxigenicity of *S. aureus* strains.

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