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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 a_4 antigen, enterotoxin B to phages of 94/96 complex with c_1, o antigens, and enterotoxin F to phages of group I with 263_2, k_1k_2, 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, 263_1, 263_2)

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

Site of isolation	Strains: No.	Strains: Enterotoxigenic	Enterotoxin type: 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*

	Number of strains lysed by phage group: I	II	III	I/II	I/III	II/III	Miscellaneous, 81, 94, 95, 96	94/96	Mixed	Not typable, 100 RTD	Total
Enterotoxin type											
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											
No.	37	13	41	1	5	1	9	21	21	42	191
%	19·4	6·8	21·5	0·5	2·6	0·5	4·7	11	11	22	100
Total enterotoxin-negative											
No.	14	34	20	0	9	1	17	17	15	56	183
%	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

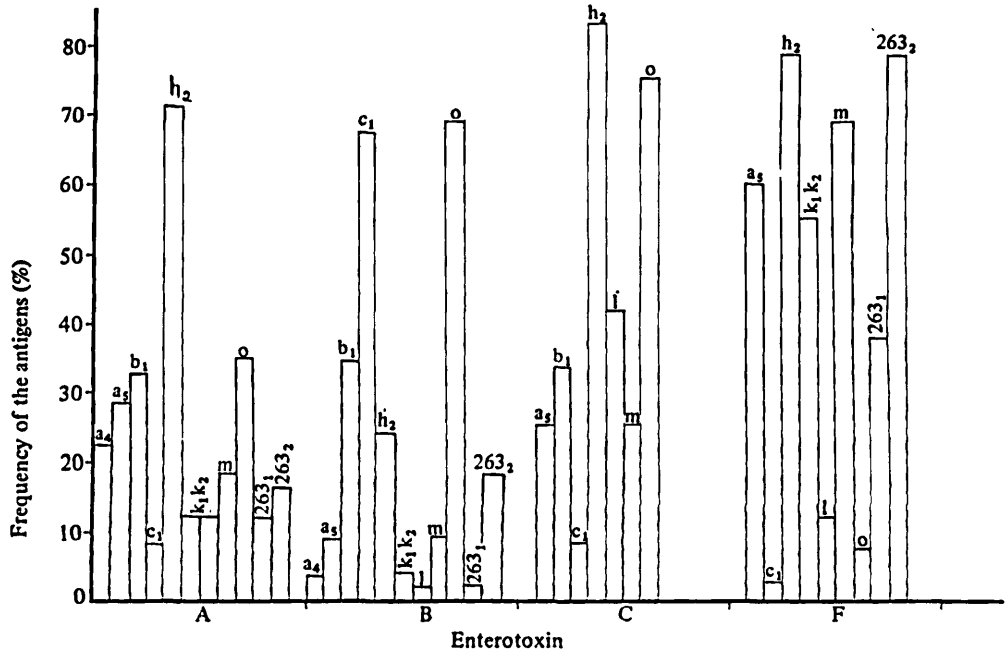

Fig. 1. Occurrence of agglutinogens among the enterotoxigenic strains isolated from clinical materials.

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

	Strains		Enterotoxin type							
Origin	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 a_5, b_1, c_1, h_2, l, m, o with SEC strains and the antigens a_5, c_1, h_2, k_1k_2, l, m, o, 263_1, 263_2, with SEF strains. The most frequent antigens found with SEA were the antigens h_2 (71·4 %) and o (34·7 %), with SEB were c_1 (67·3 %) and o (69·1 %), with SEC were h_2 (83·3 %) and o (75 %), with SEF were h_2 (78·6 %) and 263_2 (78·8 %). The SEC and SEF producer strains were found to be excluded from a_4 antigen whilst this antigen was found at a frequency of 22·5 % with SEA strains. The antigens k_1k_2 (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

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

	Number of strains lysed by phage group								
	I	II	III	II/III	Miscellaneous, 81, 94, 95, 96	Mixed	94/96	Not typable, 100 RTD	Total
Enterotoxin type									
A	—	—	1	1	—	1	—	—	3
B	—	—	—	—	—	—	2	—	2
C	—	—	—	—	2	—	—	1	3
F	1	—	—	—	—	—	—	—	1
Comb. A–C and F	2	—	2	—	—	2	—	3	9
Total enterotoxin positive No.	3	0	3	1	2	3	2	4	18
Total enterotoxin negative No.	0	4	2	0	0	0	1	4	11
Total No.	3	4	5	1	2	3	3	8	29

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 263_2 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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REFERENCES

Altemeier, W. A., Lewis, S. A., Schlievert, P. M., Bergdoll, M. S., Bjornson, H. S., Staneck, J. L. & Crass, B. A. (1982). *Staphylococcus aureus* associated with toxic shock syndrome. *Annals of Internal Medicine* **96**, 978–982.

Asheshov, E. H., Coe, A. W. & Porthouse, A. (1976). Properties of strains of *Staphylococcus aureus* in the 94, 96 complex. *Journal of Medical Microbiology* **10**, 171–178.

Bergdoll, M. S. (1979). Staphylococcal intoxications. In *Food-born Infections and Intoxications* (ed. H. Riemann and F. L. Bryan), pp. 443–494, New York: Academic Press.

Bergdoll, M. S., Borja, C. R. & Avena, R. M. (1965). Identification of a new enterotoxin as enterotoxin C. *Journal of Bacteriology* **90**, 1481–1485.

Bergdoll, M. S., Borja, C. R., Robbins, R. N. & Weiss, K. F. (1971). Identification of enterotoxin E. *Infection and Immunity* **4**, 593–595.

Bergdoll, M. S., Crass, B., Reiser, R. F., Robbins, R. & Davies, J. P. (1981). A New staphylococcal enterotoxin, enterotoxin F, associated with toxic shock syndrome *Staphylococcus aureus* isolates. *Lancet* i, 1017–1021.

Bergdoll, M. S., Crass, B. A., Reiser, R. F., Robbins, R. N., Lee, A. C. M., Chesney, P. J., Davis, J. P., Vergeront, J. M. & Wand, P. J. (1982). An enterotoxin like protein in *Staphylococcus aureus* strains from patients with toxic shock syndrome. *Annals of Internal Medicine* **96**, 969–971.

Bergdoll, M. S., Huang, I. Y. & Schantz, E. J. (1974). Chemistry of the staphylococcus enterotoxins. *Journal of Agricultural and Food Chemistry* **22**, 9–13.

Bergdoll, M. S., Surgalla, M. J. & Dack, G. M. (1959). Staphylococcal enterotoxin. Identification of a specific precipitating antibody with enterotoxin neutralizing property. *The Journal of Immunology* **83**, 334–338.

Casman, E. P. (1960). Further serological studies of staphylococcal enterotoxin. *Journal of Bacteriology* **79**, 849–856.

Casman, E. P., Bennett, R. W., Dorsey, A. E. & Issa, J. A. (1967). Identification of a fourth staphylococcal enterotoxin, enterotoxin D. *Journal of Bacteriology* **94**, 1875–1882.

De Buyser, M. L. & Janin, F. (1981). Les entérotoxines staphylococciques: détection dans les aliments. *Recherche Médicale Vétérinaire* **157**, 809–818.

De Nooij, M. P., Van Leeuwen, W. J. & Notermans, S. (1982). Enterotoxin production by strains of *Staphylococcus aureus* isolated from clinical and non-clinical specimens with special reference to enterotoxin F and toxic shock syndrome. *Journal of Hygiene* **89**, 499–505.

De Saxe, M. J. & Rosendal, K. (1982). International Committee on Systematic Bacteriology. Subcommittee on Phage-typing of Staphylococci. Minutes of the meeting 2 Sept. 1978. *International Journal of Systematic Bacteriology* **32**, 253–254.

Flandrois, J. P., Fleurette, J. & Behr, H. (1978). Evaluation of *Staphylococcus aureus* serotyping method. *Zentralblatt für Bakteriologie (Mikrobiologie und Hygiene, I. Abteilung, Originale A)* **241**, 279–285.

Fleurette, J. & Brun, Y. (1981). Antigenic properties and susceptibility to antibiotics of strains of *Staphylococcus aureus* in the 94, 96 phage complex. *Zentralblatt für Bakteriologie, Mikrobiologie und Hygiene, I. Abteilung. Originale A* **249**, 24–31.

Fleurette, J. & Modjadedy, A. (1976). Attempts to combine and simplify two methods for serotyping of *Staphylococcus aureus: Zentralblatt für Bakteriologie, Parasitenkunde, Infektioskrankheiten und Hygiene, I. Abteilung. Supplement 5*. **5**, 71–80.

Girija, Gupta, U. & Mithal, S. (1980). Enterotoxigenic staphylococci from non-faecal human sources. *The Indian Journal of Medical Research* **71**, 688–691.

Hallander, H. O. (1965). Production of large quantities of enterotoxin B and other staphylococcal toxins on solid media. *Acta Pathologica et Microbiologia Scandinavica*, **63**, 299–305.

Jarvis, A. W. & Lawrence, R. C. (1970) Production of high titers of enterotoxins for the routine testing of staphylococci. *Applied Microbiology* **19**, 698–699.

Melconian, A., Brun, Y. & Fleurette, J. (1982). Prévalence de production d'entérotoxines chez des souches de *Staphylococcus aureus* d'origine clinique. *La Nouvelle Presse Medicale* **11**, 3140.

Minor, T. E. & Marth, E. H. (1972). *Staphylococcus aureus* and staphylococcal food intoxication. II. Enterotoxins and epidemiology. *Journal of Milk and Food Technology* **35**, 21–29.

Mochmann, H., Akatov, A. K., Khatenever, M. L., Richter, U., Kustchko, I. W. & Karsch, W. (1981). Studies on enterotoxin production by strains of Staphylococcus of different origine obtained from USSR. In *Staphylococci and Staphylococcal Infections* (ed. J. Jeljaszewicz), pp. 377–380. *Zentralblatt für Bakteriologie, Microbiologie and Hygiene, I. Abteilung. Supplement 10* Stuttgart, New York: Gustav Fischer Verlag.

Payne, D. N. & Wood, J. M. (1974). The incidence of enterotoxin production in strains of *Staphylococcus aureus* isolated from foods. *Journal of Applied Bacteriology* **37**, 319–325.

Petras, P. & Maskovà, L. (1980). Detection of staphylococcal enterotoxigenicity II. Field strains. *Journal of Hygiene, Epidemiology and Microbiology and Immunology* **24**, 177–182.

Piotrowska, E. & Jòzefczyk, Z. (1976). Toxin-forming potency of clinical strains of *Staphylococcus aureus*. In *Staphylococci and Staphylococcal Diseases* (ed. J. Jeljaszewicz), pp. 577–582. *Zentralblatt für Bakteriologie, Microbiologie und Hygiène, I. Abteilung. Supplement 5*. Stuttgart, New York: Gustav Fisher Verlag.

Reali, D. (1982). .Enterotoxin A and B production in strains of *Staphylococcus aureus* isolated from human beings and foods. *Journal of Hygiene* **88**, 103–106.

Simkovicovà M. & Gilbert, R. J. (1971). Serological detection of enterotoxin food-poisoning strains of *Staphylococcus aureus*. *Journal of Medical Microbiology* **4**, 19–30.

Sourek, J. (1980). Circulation of enterotoxigenic strains of *Staphylococcus aureus* in humans and their environment. *Journal of Hygiene, Epidemiology and Microbiology and Immunology* **24**, 183–191.

Sourek, J., Vymola, F., Trojanova, M., Zelenkova, L., Matejovska, V. & Bergdoll, M. S. (1979). Enterotoxin production by *Staphylococcus aureus* strains isolated from cases of chronic osteomyelitis. *Journal of Clinical Microbiology* **9**, 266–268.

Wieneke, A. A. (1974). Enterotoxin production by strains of *Staphylococcus aures* isolated from foods and human beings. *Journal of Hygiene* **73**, 255–262.

Williams, R. E. O. (1963). Healthy carriage of *Staphylococcus aureus*: its prevalence and importance. *Bacteriological Reviews* **27**, 56–67.