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Enterotoxin A and B production in strains of *Staphylococcus aureus* isolated from human beings and foods

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SUMMARY

The production of enterotoxin A and B by strains of *Staphylococcus aureus* isolated from nasal swabs of healthy carriers, from lesions of hospital patients and from foods unconnected with outbreaks of food-poisoning was investigated. Sixty-six strains of *S. aureus* were obtained from human beings, two produced enterotoxin A, 45 produced enterotoxin B, seven produced enterotoxins A + B. Thirty-six strains were isolated from 111 samples of food, one produced enterotoxin A, 16 produced enterotoxin B. The relative incidence of A, B and A + B enterotoxigenicity was assessed.

INTRODUCTION

Staphylococcal food-poisoning is caused by the ingestion of preformed enterotoxin. So far, six enterotoxins have been identified, called A, B, C, D, E and F, the most common being A and B (Payne & Wood, 1974). It has long been accepted that the majority of enterotoxigenic staphylococci are also coagulase positive, although the isolation of enterotoxigenic coagulase-negative strains has been reported (Thatcher & Simon, 1956; Omori & Kato, 1959; Bergdoll, Weiss & Muster, 1967). The number of coagulase positive staphylococci which produce enterotoxins has been variously estimated to be 40·2 % (Casman *et al.* 1967), 74 % (Muller *et al.* 1973) or 95 % (Terayama *et al.* 1972) for strains isolated from clinical specimens and 96·2 % (Casman *et al.* 1967) or 44 % (Toshach & Thorsteinson, 1972) for strains isolated from food products connected with outbreaks of food-poisoning.

So far, there is little information on the occurrence of enterotoxigenicity in strains isolated from foods not associated with food poisoning. The purpose of this investigation was to study the distribution of the production of enterotoxins A and B among strains of coagulase positive *Staphylococcus aureus* isolated from nasal swabs of healthy carriers, from lesions of hospital patients and from samples of foods unconnected with outbreaks of food-poisoning.

MATERIALS AND METHODS

The strains examined were confirmed as *S. aureus* according to Bergey's Manual (1975). Sixty-six strains were isolated from human beings and 36 strains from 111

samples of foods (milk, whipped cream, sausages, frankfurters). Cultures for enterotoxin production were grown using the technique devised by Caserio & Valcamonica (1974) described here: strains were incubated in L tubes (16·5 × 12 cm; diameter 2 cm) with 10 ml of Brain Heart Infusion Broth (Difco) for 48 h at 37 °C in a shaking water bath. Cultures were then centrifuged to precipitate bacteria and the supernatant fluid containing the antigen was removed; 0·1 ml of Thimerosal (Sigma) was added to the supernatant which was then dialysed in cellophane sacs in running water for 4 h. The fluids were concentrated to 1:10 volume in polyethylene glycol 20000 (Fluka) at 4 °C and stored at −20 °C. The presence of enterotoxin was established by means of the micro-double-diffusion-agar-slide technique modified by Caserio & Valcamonica (1974). In this modification the slide was replaced by a standard plexiglass chamber (2·5 × 3·5 cm). This area was filled with 0·8 ml of agar (Special agar Noble 1·2%, Veronal 0·8%, NaCl 0·85%, Thimerosal 0·01%, pH 7·4) at 70 °C and immediately covered by a plexiglass template with six funnel-shaped holes surrounding a seventh central hole. The reactants are placed in the wells. The presence of enterotoxin is shown by a line of precipitation joining with a reference line of precipitation formed by the interaction of antibody to standard enterotoxin and the culture supernatant of a standard strain of *S. aureus* known to produce enterotoxin A or B or A + B.

The apparatus was incubated in a moist chamber at 30 °C for 72 h; a staining method was used to enhance the lines of precipitation and increase the sensitivity of the test.

The plexiglass chambers were filled with sodium barbital (pH 8·2) for 24 h. This stage was very important: a shorter washing gave much less clear results in these trials. Afterwards, a tannic acid solution (1% in distilled water) was put in the chamber for 5 min; it was then drained off and replaced by distilled water for 3 h. After this time, the line of precipitation was stained for 10 min with solution containing Schwarz starch 1·5%, methanol 30%, acetic acid 10%. Decolorization was then performed for 4–5 h with the mixture: methanol 300 ml, acetic acid 100 ml, distilled water 600 ml.

The formation of a dark blue line of precipitation, mid-way between the two reactant wells and visible to the naked eye required 0·5 μg/ml of enterotoxins A or B. A faint line of precipitation which was just visible was formed at a toxin concentration of 0·3–0·2 μg/ml. Such a test is not strictly quantitative however, even if it is performed with standard enterotoxins.

Standard enterotoxins A and B, their respective antisera and the apparatus for the immunodiffusion test were supplied by Biolife Italiana SPA. The standard strains of *S. aureus* known to produce enterotoxin A, B or A + B were obtained from the Institute de Bacteriologie et de Virologie, Faculté de Medicine, Lausanne.

RESULTS

Tables 1 and 2 give the results of enterotoxin tests on strains of *S. aureus* isolated from swabs of healthy carriers, from lesions of hospital patients and from foods unconnected with outbreaks of food-poisoning. Fifty-four of 66 strains of *S. aureus* isolated from human beings showed enterotoxin production

Table 1. *Enterotoxins A and B production by strains of* S. aureus *isolated from human beings*

Strains isolated from	No. strains *S. aureus* coagulase positive tested	Strains which produce enterotoxin			Total enterotoxigenic strains	
		A	B	A+B	No.	%
Infected wounds, cutaneous and uro-genital infections	15	1	11	3	15	100
Clinical specimens (spt., faeces, urine)	10	—	7	1	8	80
Nasal swabs of healthy carriers	41	1	27	3	31	76
Total	66	2(3%)	45(68%)	7(11%)	54	82

Table 2. *Enterotoxins A and B production by strains of* S. aureus *isolated from foods*

Strains isolated from	No. samples examined	No. strains *S. aureus* coagulase positive tested	Strains which produce enterotoxin			Total enterotoxigenic strains	
			A	B	A+B	No.	%
Raw milk	51	29	—	15	—	15	52
Whipped cream	20	—	—	—	—	—	—
Sausages	30	7	1	1	—	2	29
Frankfurters	10	—	—	—	—	—	—
Total	111	36	1(3%)	16(44%)	—	17	47

(82%) and, of these, two produced enterotoxin A (3%), 45 produced enterotoxin B (68%) and seven enterotoxin A together with B (11%).

Seventeen of 36 strains of *S. aureus* isolated from foods showed enterotoxin production (47%): one produced enterotoxin A and 16 produced enterotoxin B (44%). No strain produced both enterotoxins A and B.

DISCUSSION

The usual biochemical tests (coagulase, phosphatase, DNAase) of staphylococci do not allow us to decide whether they produce enterotoxin. However, they usually form a criterion for the screening of strains to be subjected to further tests. Use of immuno-precipitation tests with specific anti-enterotoxin sera can supply diagnostic information which not even biological tests (the kitten-test, the frog-test, the monkey-test) can provide.

Our results, that 82% of the *S. aureus* strains isolated from human material

produced enterotoxins A or B or both at the same time, would seem to confirm the importance of nasal carriers in the epidemiology of food-poisoning due to staphylococci. The true frequency of carriers is likely to be even greater than that found. Some strains may produce a different enterotoxin from those detected with the sera we used. In the foods we examined, which were unconnected with food-poisoning, 47% of the *S. aureus* proved to be enterotoxigenic; it is worthwhile pointing out that such a positive finding does not necessarily indicate a risk of intoxication from the ingestion of this food. However, such a finding points to lack of hygiene in the production and processing of the food and may mean that better sanitary and hygienic precautions should be taken at the various stages of production.

Staphylococci producing enterotoxin B (86%) were much more frequent than those producing enterotoxin A (4%) in all the materials we examined. However it must be emphasized that no strain was connected with an episode of food-poisoning.

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