THE OCCURRENCE OF ENTEROTOXIGENIC STRAINS OF *STAPHYLOCOCCUS AUREUS* IN FOODS

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SUMMARY

Enterotoxin production by strains of ***Staphylococcus aureus*** *isolated from foods unconnected with outbreaks of food poisoning was investigated. Twenty-three percent of 217 strains examined produced enterotoxins A, B, C, D or E. Enterotoxin C was found to occur most frequently. Enterotoxin A was not detected alone from any of the strains examined, but occurred together with other enterotoxins. The overall number of strains isolated from raw foods which produced one or more enterotoxins was higher than that for cooked foods. Antibiotic sensitivities were unrelated to enterotoxin production and no correlation could be found between methicillin resistance and enterotoxigenicity.*

INTRODUCTION

Staphylococcal food poisoning is an intoxication resulting from the ingestion of food containing the enterotoxin produced by certain strains of *Staphylococcus aureus*. Six serologically distinct enterotoxins, designated A, B, C, D, E and F, have so far been identified.

It has generally been accepted that enterotoxin production is confined to coagulase-positive staphylococci, although the isolation of enterotoxigenic coagulase-negative strain has been reported. [1,2,3,4] Coagulase-positive staphylococci which produce enterotoxins have been found in 96.2 percent, [5] 92 percent, [6] 44 percent, [7] or 95 percent [8] of the strains isolated from food products involved in food poisoning outbreaks. The occurrence of enterotoxigenicity in staphylococcal strains isolated from foods not associated with food poisoning has been estimated to be 62.5 percent, [9] 24.6 percent, [10] or 47.2 percent [11]

The purpose of this investigation was to study the frequency of enterotoxin production among strains of *S. aureus* isolated from routine food samples unconnected with outbreaks of food poisoning. As Dornbusch *et al* [12] had observed that there was a correlation between enterotoxigenicity and methicillin resistance, we decided to investigate if there was a predilection among the different types of enterotoxin producers to exhibit antibiotic resistance.

MATERIALS AND METHODS

Strains of *S. aureus* were isolated from food samples during routine bacteriological examinations.

The cellophane-over-agar method of Hallander, [13] as applied by Jarvis and Lawrence, [14]

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TABLE I
ENTEROTOXIN PRODUCTION BY *S. AUREUS* STRAINS ISOLATED FROM FOODS

Source	No. of strains tested	No. (%) of enterotoxigenic strains	No. of strains producing enterotoxin (s)								
			A	B	C	D	E	AE	BD	ABE	ACE
Raw meats and meat products	116	28 (24.1)	0	1	11	3	0	7	2	2	2
Frozen cooked prawns	43	7 (16.3)	0	2	0	2	1	0	0	1	1
Raw seafoods	31	11 (35.5)	0	4	6	0	0	0	1	0	0
Cooked meals	27	4 (14.8)	0	1	0	1	0	1	0	0	1
Total	217	50 (23.0)	0	8	17	6	1	8	3	3	4

was used to grow the staphylococci to test for enterotoxin production. Sterile cellophane was placed on Brain Heart Infusion (BHI, Difco) agar in a petri dish and its surface was inoculated with 0.1 ml of an overnight BHI broth culture of staphylococci and spread with a sterile bent glass rod. The plate was incubated for 24 h at 37°C after which the growth was harvested by washing off with 2.5 ml of sterile 0.85% saline and centrifuged, and the supernatent fluid was tested for enterotoxin.

Detection of the enterotoxin in the culture supernatent fluid was achieved by using the optimal sensitivity plate method of Robbins *et al.* [15] The plates used contained 1.2% Noble agar (Difco) in phospate-buffered saline with merthiolate (1 : 10,000). A 3/16-inch thick Plexiglas template was positioned over each plate and seven wells in the agar were cut using cork borers. The antiserum was placed in the center well, the control enterotoxin in the two smaller wells, and the unknown samples in the four larger outer wells. The petri dishes were closed and kept in a humidified plastic box at 37°C overnight after which they were flooded with 0.1 M H_3PO_4 to make the precipitin lines more visible.

Antibiotic sensitivity tests were carried out by a comparative disc-diffusion method. [16] Lawn cultures were prepared on Diagnostic Sensitivity Test (Oxoid) agar containing 5% defibrinated ox blood, using saline suspension of staphylococci. Antibiotic discs (Mast Laboratories) consisting of lincomycin (2 μg), penicillin G (2 units), tetracycline (10 μg), erythromycin (15 μg) and methicillin (10 μg) were placed in the plates which were then incubated overnight at 37°C (30°C for methicillin) and examined for zones of inhibition.

RESULTS

Of the 217 strains of *S. aureus* examined, 50 (23 percent) produced one or more enterotoxins. Most strains produced only one type of enterotoxin but others produced up to three enterotoxins simultaneously. Table I shows the distribution of enterotoxins A - E among the strains tested. Thirty-two strains (14.7 percent) produced only a single enterotoxin; of these 8 strains produced enterotoxin B, 17 produced C, 6 produced D and 1 produced E. Eighteen strains (8.3 percent) produced more than one enterotoxin; 8 strains produced A with E, 3 produced B with D, 3 produced A, B and E and 4 produced A, C and E.

The strains of *S. aureus* used in this study were isolated from two main groups; those isolated from raw foods (meats and meat products and seafoods) and those from cooked foods (frozen prawns and cooked meals) (Table I). For raw foods, the relative frequency of strains producing one or more enterotoxins was higher than that for cooked foods: 35.5 percent for raw seafoods and 24.1 percent for raw meats and meat products as compared to 16.3 percent for frozen cooked prawns and 14.8 percent for cooked meals. Enterotoxin C alone, or with other enterotoxins, predominated in strains from raw foods. Enterotoxin A was not detected alone from any of the enterotoxigenic strains tested, but occurred together with E, B and E or C and E.

Table II gives the results of antibiotic sensitivity tests on enterotoxigenic strains of *S. aureus*. Seventy-four percent, 78 percent and 90 percent of the strains tested were sensitive to penicillin G, tetracycline and erythromycin, respectively. All the strains were sensitive to lincomycin and methicillin.

DISCUSSION

The occurrence of enterotoxigenicity in *S. aureus* strains isolated from foods not associated with food poisoning has been studied by a number of workers.

TABLE II
ANTIBIOTIC SENSITIVITIES OF ENTEROTOXIGENIC STRAINS OF *S. AUREUS*

Enterotoxin (s) produced	No. of strains tested	No. of strains sensitive to				
		Lincomycin	Penicillin G	Tetracycline	Erythromycin	Methicillin
B	8	8	7	8	8	8
C	17	17	15	13	16	17
D	6	6	4	5	4	6
E	1	1	1	1	1	1
AE	8	8	5	5	6	8
BD	3	3	3	3	3	3
ABE	3	3	1	2	3	3
ACE	4	4	1	2	4	4
Total	50	50 (100%)	37 (74%)	39 (78%)	45 (90%)	50 (100%)

In England, Wieneke [10] found that 24.6 percent of the strains from a range of raw and cooked foods produced enterotoxin while Payne and Wood [9] found that 62.5 percent of the strains from meat and dairy products produced enterotoxin. Reali [11] in Italy found that 47.2 percent of the strains were enterotoxigenic when he examined 36 strains isolated from 111 samples of milk and meat products. However, he tested the strains for enterotoxins A and B only. In the present study in which the strains were isolated from raw and cooked foods, 23 percent of the strains produced enterotoxin. This value is comparable to that obtained by Wieneke. [10] Enterotoxins A, D and B occurred most frequently in staphylococcal strains examined by Payne and Wood, [9] Wieneke [10] and Reali, [11] respectively whereas enterotoxin C was the most commonly occurring enterotoxin in our survey.

The production of enterotoxins A - E was less frequent among strains isolated from cooked foods than among those from raw foods. It was suggested by Wieneke [10] that cooked foods are more likely to carry staphylococci mainly from the human environment and raw foods from the animal environment. Enterotoxin C production was more often found among strains isolated from raw foods than among those from cooked foods. This finding may indicate that enterotoxin C is more frequently produced by animal strains than by human strains.

In this study, 23 percent of the staphylococcal strains isolated from foods unconnected with food poisoning were found to be enterotoxigenic. The ingestion of these foods does not necessarily indicate a risk of intoxication. Such a finding, however, reflects the lack of hygiene in the production and processing of the foods. The need to practise better sanitary and hygienic procedures at the various stages of production should thus be emphasized.

No correlation was apparent between the antibiotic sensitivities of the staphylococcal strains and their enterotoxigenicity. Most of the enterotoxigenic strains tested were sensitive to penicillin G, tetracycline and erythromycin and none of them were resistant to lincomycin and methicillin. This is contrary to the reported correlation between enterotoxigenicity and methicillin resistance as observed by Dornbusch *et al.* [12]

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