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Enterotoxin production by strains of *Staphylococcus aureus* isolated from foods and human beings

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SUMMARY

Enterotoxin production by strains of *Staphylococcus aureus* isolated from routine samples of foods and from human beings was investigated. Twenty-one to 26 % of 112 strains isolated from raw meat, sausages and poultry and 32–36 % of 183 strains isolated from cooked foods, e.g. meat, chicken and frozen seafoods, produced enterotoxins A, B, C, D or E. *Staph. aureus* isolated from raw meat and chicken less frequently produced enterotoxins A, B, C or E and more frequently enterotoxin D, than those from cooked meat and seafoods. Of the 113 strains isolated from cheese and raw milk 6–11 % produced enterotoxin and most of these produced enterotoxin D. Only a few strains isolated from foods produced enterotoxin E. Results of enterotoxin tests on *Staph. aureus* from human beings resembled those on strains from cooked foods.

INTRODUCTION

The cause of staphylococcal food poisoning is the enterotoxin produced by a strain of *Staphylococcus aureus* in the implicated food. So far five enterotoxins have been identified, designated A, B, C, D and E. Staphylococci which produce enterotoxins A, or A and D together, have been found in 69–75 % of reported outbreaks of staphylococcal food poisoning in the U.S.A. and the U.K. Strains which produce enterotoxin D alone or together with enterotoxin C have been found in about 10 % of the outbreaks and those which produce enterotoxin B are only rarely found (Casman, Bennett, Dorsey & Issa, 1967; Gilbert & Wieneke, 1973).

The purpose of this investigation was to study the distribution of enterotoxin production among strains of *Staph. aureus* isolated from samples of food unconnected with outbreaks of food poisoning, from admission swabs of patients who entered hospital and from lesions of hospital patients.

MATERIALS AND METHODS

Strains of *Staph. aureus* were isolated from food samples during routine bacteriological examinations at the Food Hygiene Laboratory, Colindale, and at several other Public Health Laboratories. A few strains were isolated by direct culture on phenolphthalein diphosphate agar that contained polymyxin* (PPAP) (Hobbs,

* In the examination of cheese polymyxin was omitted from the medium.

Table 1. *Enterotoxin production by strains of* Staphylococcus aureus *isolated from various sources: % of strains which produce enterotoxin A, B, C, D or E*

Strains isolated from	No. of strains tested	No. of strains which produce entero-toxin A–E	% of strains which produce entero-toxin A–E	% of strains which produce enterotoxin				
				A	B	C	D	E
Frozen raw chicken	50	13	26	—	2·0	6·0	22	—
Raw meat and sausages	62	13	21	1·6	—	1·6	19	—
Cooked chicken	53	17	32	7·6	1·9	17	19	1·9
Cooked meat	50	18	36	10	8·0	14	10	2·0
Frozen cooked prawns, lobster, crab and crayfish	80	28	35	17	8·7	17	3·7	1·2
Salami and pâté	16	3	19	13	—	6·3	—	6·3
Cream and artificial cream mostly in pastries	28	9	32	7·2	7·2	11	11	3·6
Raw milk	50	3	6	—	—	—	6	—
Cheese	63	7	11	1·6	1·6	1·6	9·5	—
Hospital admission swabs	101	38	38	16	7·0	11	7·0	2·0
Lesions among hospital patients	199	91	45	20	14	16	9·5	2·0
Food poisoning out-breaks*	120	113	94	73	1·7	15	40	2·5

* A representative strain from each of 120 separate outbreaks was selected.

Kendall & Gilbert, 1968), but most were isolated through enrichment culture (cooked meat medium that contained a total of 10 % salt) and subculture on PPAP. Phosphatase-positive colonies were tested for coagulase production in 10 % human plasma broth. Coagulase-positive strains were incubated in a sac-culture flask and the culture filtrates were tested for the presence of enterotoxin with the use of the double gel diffusion slide test (Šimkovičová & Gilbert, 1971). Strains of *Staph. aureus* isolated from hospital admission swabs and from lesions of hospital patients were made available to us by Dr M. T. Parker and Mr J. H. Hewitt of the Cross-Infection Reference Laboratory at Colindale.

The culture filtrates were tested immediately after their preparation for the presence of enterotoxins A, B and C. They were then stored at 4° C. and tested for the presence of enterotoxins D and E after a maximum period of 4 years. Enterotoxins A, B and C could still be demonstrated in filtrates stored for 4 years and several of the early filtrates were positive for enterotoxins D and E.

All strains were phage-typed by the Cross-Infection Reference Laboratory at Colindale; the international basic set of 22 phages was used.

RESULTS

Tables 1 and 2 give the results of enterotoxin tests on strains of *Staph. aureus* isolated from routine samples of foods, from swabs taken from patients at the time of their admission into hospital and from lesions of hospital patients. For com-

)le 2. *Enterotoxin production by strains of* Staphylococcus aureus *isolated from various sources: number of strains which produce one or more enterotoxins*

Strains isolated		No. of strains which produce enterotoxin A–E	No. of strains which produce enterotoxin												
			A	B	C	D	E	AB	AC	AD	BD	CD	CE	CD	ACE
Frozen raw chic		13	—	1	1	9	—	—	—	—	—	2	—	–	—
Raw meat and s	ges	13	1	—	—	11	—	—	—	—	—	1	—	–	—
Cooked chicken		17	—	1	4	3	1	—	1	3	—	4	—	–	—
Cooked meat		18	3	4	5	1	1	—	—	2	—	2	—	–	—
Frozen cooked p crab and cray	s, lobster,	28	6	6	7	—	—	1	4	1	—	1	—	1	1
Salami and pâte		3	2	—	—	—	—	—	—	—	—	—	1	–	—
Cream and artif mostly in pas	ream	9	2	2	1	2	—	—	—	—	—	1	1	–	—
Raw milk		3	—	—	—	3	—	—	—	—	—	—	—	–	—
Cheese		7	—	—	—	5	—	—	1	—	1	—	—	–	—
Hospital admiss	wabs	38	12	5	9	5	2	2	1	1	—	1	—	–	—
Lesions among l	al patients	91	12	12	26	6	4	15	3	9	1	2	—	1	—
Food poisoning	eaks*	113	53	—	4	8	2	2	3	30	—	10	1	–	—

* A representative strain from each of 120 separate outbreaks was selected.

Table 3. *rotoxin production by strains of* Staphylococcus aureus *of different phage-group isolated from rout* *oods*

Phage-group		No. of strains tested	No. of strains which produce enterotoxin A–E	% of strains which produce enterotoxin A–E	No. of strains which produce enterot												
					A	B	C	D	E	AB	AC	AD	BD	CD	C	CD	ACE
I		65	15	23	2	2	4	3	1	—	—	—	—	3	—	–	—
II		28	3	11	—	2	1	—	—	—	—	—	—	—	—	–	—
III		145	42	29	7	2	2	21	—	—	2	3	1	4	—	–	—
IV		18	2	11	—	—	—	2	—	—	—	—	—	—	—	–	—
I/III		81	20	25	4	2	5	3	—	1	1	3	—	1	—	–	—
II/III I/II/III		7	3		—	1	—	—	—	—	—	—	—	1	—	1	—
I/IV III/IV I/III/IV II/III/IV		21	5	24	—	—	—	4	—	—	—	—	—	1	—	–	—
Miscellaneous (1	187)	4	4		—	—	—	—	—	—	1	—	—	—	:	–	1
Miscellaneous (1	81)	3	1		—	—	1	—	—	—	—	—	—	—	—	–	—
Non-typable		80	16	20	1	5	5	1	1	—	2	—	—	1	—	–	—
Total no. of str		452	111	25	14	14	18	34	2	1	6	6	1	11	:	1	1

parison the results of enterotoxin tests on strains implicated in outbreaks of food poisoning are also given (Gilbert & Wieneke, 1973, and unpublished results).

Twenty-six of 112 strains isolated from raw foods showed enterotoxin production and of these 23 produced enterotoxin D alone or together with C. Of 183 strains from cooked foods, 63 produced one or more enterotoxins. Three enterotoxigenic strains were detected among 16 isolated from salami and pâté. Twenty-eight strains were isolated from cream and pastries filled with cream or artificial cream and nine of these produced enterotoxin. The three enterotoxigenic strains found among 50 isolates from raw milk all produced enterotoxin D. Enterotoxin was produced by seven of 63 strains from cheese and of these six produced D alone or together with B.

The admission swabs yielded 101 strains of which 38 produced one or more enterotoxins. Ninety-one enterotoxigenic strains were detected among 199 strains isolated from lesions.

One hundred and thirteen strains of 120 that were involved in 120 separate outbreaks of staphylococcal food poisoning were found to produce enterotoxin.

Only 11 of 240 enterotoxigenic strains isolated from foods or human sources produced enterotoxin E, alone or together with C or A and C.

The phage-typing patterns of the 452 strains isolated from foods were determined and Table 3 shows the enterotoxin production by staphylococci of different phage groups. Twenty-three–29 % of strains, which lysed with phages of group I or III or with phages of both these groups and 11 % of those in phage group II or IV produced enterotoxin. Four strains lysed by phage 187 (miscellaneous group) were all enterotoxigenic and three of these produced enterotoxin E together with other enterotoxins. Twenty per cent of the non-typable organisms produced enterotoxin. Thirty-nine strains of which 27 were isolated from milk or cheese were lysed by phage 42D (the only phage in group IV). Seven of these were enterotoxigenic and all produced D, alone or together with C.

DISCUSSION

The production of enterotoxins A, B, C or E was less frequent among strains isolated from raw foods, e.g. chicken, meat and milk, and from Cheddar cheese, than among those from cooked foods, e.g. chicken, meat and seafoods and from human beings. This difference between strains from raw foods, cooked foods and human beings is probably due to the fact that raw foods are likely to carry staphylococci mainly from the animal environment and cooked foods from the human environment; Casman *et al.* (1967) and Hájek & Maršálek (1973) found that production of enterotoxins A, B and C was less common among strains isolated from animals than among those from human beings.

Enterotoxin D production was more often found among strains isolated from raw meat and raw chicken than among those from cooked meat, cooked seafood and human beings. Staphylococci from cooked chicken resembled those from raw foods in this respect except that A or C were usually produced at the same time. It may be that enterotoxin D is more frequently produced by animal strains than by human strains.

Enterotoxigenic strains isolated from cheese and milk mainly produced enterotoxin D. Casman *et al.* (1967) also found that enterotoxin D production was more frequent among strains isolated from milk than production of A, B or C.

Enterotoxin A production occurred more often among the enterotoxigenic strains implicated in outbreaks of food poisoning, than among those isolated from foods or human beings unconnected with outbreaks. The frequency of enterotoxin B, C, D or E production was also different in staphylococci from outbreaks of food poisoning and in those from cooked foods and human beings. Thus, it appears that factors other than the presence of an enterotoxigenic staphylococcus in a food, play an important role in the development of enterotoxin in the food.

The results in Table 1 are similar to those of Casman *et al.* (1967), Untermann & Sinell (1970), Jarvis & Lawrence (1970), Terplan, Zaadhof & Bobeth (1971), Untermann (1972) and Hájek & Maršálek (1973).

Zak, Jeljaszewicz & Stochmal (1971) found that 75 % of strains isolated from faeces (mainly connected with diarrhoea) produced enterotoxin A, B or C or combinations of these. Terayama, Igarashi, Ushioda & Zen-Yoji (1972) detected enterotoxin production in 83 % of strains isolated from foods and 95 % of those from nasal and finger swabs and faeces from healthy human beings; the organisms were tested for the production of enterotoxins A–D only. Müller *et al.* (1973) reported that 74 % of strains isolated from nasal swabs of human beings and from clinical specimens produced enterotoxin when the organisms were tested for A, B and C and that 65 % of strains from the faeces of healthy human beings produced enterotoxin when tested for A, B, C, D or E; 25 % of the faecal strains produced enterotoxin E, alone or together with other enterotoxins.

The phage-typing pattern of a staphylococcus does not tell whether or not the organism produces an enterotoxin. Nevertheless in outbreaks of food poisoning phage-typing patterns are useful in the search for the implicated strains, which are in most cases lysed by phages of group III or I/III. Outbreaks are mainly caused by enterotoxins A, D, A + D or C + D and strains that were isolated from human beings and produced similar enterotoxins also yielded a high number (more than 75 %) that were lysed by phages of group III or I/III (M. T. Parker and J. H. Hewitt, to be published). The same results were obtained with strains isolated from routine samples of food, except for strains which produced enterotoxins C + D; only about half of these were lysed by phages of group III or I/III. The phage-typing pattern is also useful in the correlation of staphylococci isolated from foods, clinical specimens and food handlers in outbreaks of food poisoning.

Seven enterotoxigenic strains isolated from foods were lysed by phage 42D (the only phage in Group IV) or by this phage together with phages from other groups, and all produced enterotoxin D. One such strain isolated from a case of food poisoning also produced enterotoxin D. Of two similar strains isolated from lesions of hospital patients, however, one produced enterotoxin B and the other C. Toshach & Thorsteinson (1972) found one strain lysed by phage 42D (and also by phages of group III) that produced enterotoxin A.

Six strains* isolated from routine samples of food, three from foods implicated

* Two of these strains are not included in Table 3.

in outbreaks of food poisoning, three from hospital admission swabs, one from faeces and one from a lesion were lysed only by phage 187 (miscellaneous group). Thirteen of these produced enterotoxin (2 A, 5 (A+C), 1 C, 1 E, 3 (C+E) and 1 (A+C+E)). Toshach and Thorsteinson (1972) reported two strains implicated in two outbreaks of food poisoning that were lysed by phage 187 and both produced enterotoxin A+C. The number of strains tested is, however, too small to state whether there is a definite relationship between the lysis by phage 187 and the ability to produce enterotoxin.

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