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Serotypes of *Bacillus cereus* from outbreaks of food poisoning and from routine foods

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SUMMARY

A provisional serotyping scheme was used to type cultures of *Bacillus cereus* from 84 outbreaks of food poisoning in seven countries; 283 of the 337 (84%) cultures tested were typable.

In 35 of the 61 outbreaks associated with a vomiting-type syndrome, foods, clinical specimens or both yielded H-serotype 1 only. Type 1 strains together with other serotypes were isolated in seven outbreaks. In 14 outbreaks types 3, 4, 5, 8 or a mixture of serotypes were present. Untypable strains were isolated in five outbreaks.

Two of the nine diarrhoeal-type outbreaks yielded serotype 1 only. Types 2, 6, 8, 9, 10 and a mixture of type 12 and an untypable strain appeared to be responsible for one outbreak each.

Although 16 of the 18 recognized serotypes were present among cultures of *B. cereus* from various routine foods, only 156 of the 400 (39%) isolates tested were typable.

INTRODUCTION

Evidence has accumulated that *Bacillus cereus* is the aetiological agent of two distinct types of food poisoning characterized either by diarrhoea and abdominal pain 8–16 h after ingestion of contaminated food (diarrhoeal-type syndrome) or by nausea and vomiting with an incubation period of only 1–5 h (vomiting-type syndrome).

The diarrhoeal-type syndrome has been reported from several countries, particularly in Northern and Eastern Europe, and a wide range of foods have been implicated including meat and vegetable soups, cooked meat, poultry and vegetables, and dessert dishes and sauces (Hauge, 1950, 1955; Ormay & Novotny, 1969; Goepfert, Spira & Kim, 1972). In most outbreaks large numbers of *B. cereus*, *ca.* 10^6–10^8/g, were isolated from food remnants but rarely from faeces, although in most instances the examination of faecal specimens was not recorded.

The vomiting-type syndrome has been associated almost exclusively with the consumption of cooked rice, usually fried, from Chinese restaurants and 'take-away' shops. In most of the incidents large numbers of *B. cereus*, *ca.* 10^6–10^9/g, were isolated from remnants of cooked rice, faecal specimens or both. Reports of episodes in this country (Public Health Laboratory Service, 1972, 1973, 1976; Mortimer &

McCann, 1974) have been followed by similar accounts in Australia (Dr J. Taplin, personal communication), Canada (Lefebvre, Gregoire, Brabant & Todd, 1973; Mathias, Todd, Szabo & Martin, 1976), Finland (Raevuori, Kiutamo, Niskanen & Salminen, 1976), the Netherlands (Beckers, 1976) and the U.S.A. (U.S. Center for Disease Control, 1976).

Taylor & Gilbert (1975) have described the application of a provisional serotyping scheme to the investigation of *B. cereus* food poisoning. This work has been extended in the present paper and serotyping results of cultures of *B. cereus* from routine samples of food are also presented.

MATERIALS AND METHODS

Strains of *B. cereus* implicated in incidents of food poisoning in this country were received from Public Health Laboratory Service and hospital laboratories. Strains from outbreaks overseas were received from Dr Jennifer Taplin (Australia), Dr E. Todd (Canada), Drs A. Niskanen and M. Raevuori (Finland), Drs H. J. Beckers and M. D. Northolt (Netherlands), Professor S. Hauge and Dr R. Skjekvale (Norway) and Dr J. M. Goepfert (U.S.A.).

Routine samples of uncooked, boiled and fried rice were obtained from Chinese and Indian restaurants and 'take-away' shops in the North London area. Portions, *ca.* 25 g, were incubated in 100 ml. volumes of nutrient and 1% lactose broths for 18 h at 35° C; subcultures were made on 5% defibrinated horse-blood agar. For samples of cooked rice, tenfold dilutions were made in quarter-strength Ringer's solution and colony plate counts made on blood agar with incubation for 48 h at 35° C. The identity of *B. cereus* colonies was confirmed by further studies including subculture on Kendall's B.C. medium (Gilbert & Taylor, 1976) and fermentation tests with glucose, arabinose, mannitol and xylose ammonium salt sugars. Cultures from routine samples of cooked meat and poultry and from dairy produce, mainly pasteurized milk and cream, were obtained from cooked meat media plated on blood agar.

The methods of Taylor & Gilbert (1975) were used for the preparation of H antigens and antisera to serotypes 1–18. Agglutination tests were carried out in WHO agglutination trays. Doubling dilutions of 0·25 ml volumes of serum were made and equal volumes of antigen suspension added. Trays were incubated at 50° C for 2 h.

RESULTS

Table 1 shows the distribution of serotypes of *B. cereus* from 84 outbreaks of food poisoning in seven countries; 283 of the 337 (84%) cultures tested were typable.

In 35 of the 61 vomiting-type outbreaks, foods, clinical specimens or both yielded serotype 1 only. Type 1 strains together with other serotypes were isolated from foods, clinical specimens or both in seven episodes. In 14 outbreaks serotypes 3, 4, 5 or 8 or a mixture of serotypes were present. Untypable strains were isolated from five outbreaks.

Two of the nine diarrhoeal-type outbreaks yielded serotype 1 only. Types

Table 1. *Distribution of serotypes of* Bacillus cereus *from 84 outbreaks of food poisoning*

Incidents	Country	Source	Serotype(s) isolated	No. of incidents from which serotype(s) isolated
Vomiting-type	Great Britain	Fried or boiled rice, faeces or vomitus or both food and clinical specimens	1	31
			1 and NT	3
			1 and 8	2
			1 and 12	1
			1, 4 and 14	1
			3	2
			5	4
			8	4
			8 and 13	1
			NT	3
	Australia	Fried rice	1	2
	Finland	Boiled rice	1	1
	Netherlands	Indonesian rice dish	1	1
			4	2
			4 and 8	1
			NT	2
Diarrhoeal-type	Canada	Barbecued chicken	6	1
	Netherlands	Indonesian rice dish	8	1
			10	1
		Ox tongue and ragout rolls	12 and NT	1
		Pea soup	NT	1
		Vanilla pudding	9	1
	Norway	Vanilla sauce	1	2
	U.S.A.	Meat loaf	2	1
Miscellaneous*	Finland	?	8	1
		?	12	1
		?	NT	1
	Great Britain	Vanilla slice	1	1
		Cream	8	1
	Netherlands	Ketjap	1	1
		Pudding	8	1
		Rice	NT	1
	Norway	?	1	1
		?	NT	2
	U.S.A.	Buffaloburger	1	1
		Cooked potato	NT	1
		Cooked sprouts	NT	1

NT = not typable.

* Refers to outbreaks where epidemiological information such as incriminated food, incubation period and symptoms was incomplete or indecisive.

Table 2. *Distribution of serotypes among* 400 *isolates of* Bacillus cereus *from various foods*

Serotype	Uncooked rice 100*	Boiled and fried rice 100*	Milk and cream 100*	Cooked meat and poultry 100*
1	3	23	11	15
2	0	0	0	1
3	2	0	2	5
4	0	1	0	0
5	1	6	0	0
6	0	0	0	0
7	0	0	0	0
8	1	6	5	3
9	0	0	0	1
10	0	1	0	0
11	2	1	3	6
12	6	1	1	3
13	2	0	0	0
14	2	3	3	0
15	6	1	2	1
16	1	1	0	1
17	15	1	0	1
18	4	2	0	0
Not typable	55	53	73	63

* Number of isolates.

2, 6, 8, 9, 10 and a mixture of type 12 and an untypable strain appeared to be responsible for one outbreak each. An untypable strain alone was isolated from one incident.

The miscellaneous group of 14 outbreaks refers to cultures where the epidemiological information was incomplete, for example, the incubation period or symptoms were not recorded. Serotypes isolated were type 1 (four outbreaks), type 8 (three outbreaks) and type 12 (one outbreak). Untypable strains were obtained from six outbreaks.

Table 2 shows the distribution of serotypes among 400 cultures of *B. cereus* from various routine foods. Sixteen of the 18 recognized serotypes were isolated although for all foods at least 53 % of the cultures were not typable. Type 1 was the most common serotype isolated. For boiled and fried rice 23 % of the cultures were type 1 compared to only 3 % for uncooked rice. In contrast, 15 % of the cultures from uncooked rice were type 17 compared with only 1 % for cooked rice. Several food samples, particularly uncooked rice, yielded multiple serotypes.

DISCUSSION

Feeding trials in rhesus monkeys, tests for fluid accumulation in ligated ileal sections of young rabbits and assays for adenylate cyclase activity have provided evidence that the diarrhoeal and vomiting-type syndromes of *B. cereus* food poisoning are associated with the production of two distinct toxins (Melling,

Capel, Turnbull & Gilbert, 1976; Turnbull, 1976). When these toxins have been purified, immunological methods may be developed for their detection in foods.

Previous data (Taylor & Gilbert, 1975) have indicated that certain serotypes tend to be associated with the two distinct syndromes of food poisoning, and the present results lend support to this finding. Of the 18 H serotypes of *B. cereus* described in this study, five (types 1, 3, 4, 5 and 8) were most commonly associated with the vomiting-type syndrome. One of the five serotypes was found, either alone or in combination with others, in 56 of the 61 outbreaks; all 61 were associated with the consumption of cooked rice. Type 1 was the most common serotype isolated from 42 of 61 (69%) outbreaks.

Serotypes 2, 6, 9, 10 and 12 were isolated from separate diarrhoeal-type outbreaks associated with a variety of foods. However, types 1 and 8 were implicated also in diarrhoeal-type outbreaks and thus serotyping cannot by itself be used for distinguishing cultures from the two food-poisoning syndromes. Gas chromatographic analysis of the fatty-acid composition of *B. cereus* has been used in the epidemiological investigation of outbreaks (Raevuori *et al.* 1976) and this merits further study with more strains.

B. cereus has been isolated in several countries from a wide variety of foods and is responsible for a spoilage problem in milk and cream. In this laboratory the organism has been isolated from 98 of 108 (91%) samples of uncooked rice and can be considered as part of the normal flora. The storage of boiled or fried rice at ambient temperature provides excellent conditions for the germination and outgrowth of *B. cereus* spores that have survived the boiling and frying process, and this has led to numerous outbreaks of food poisoning (Gilbert, Stringer & Peace, 1974; Public Health Laboratory Service, 1976). During the period 1971–6 *B. cereus* was isolated from 26 of 252 (10%) routine samples of boiled rice and 48 of 204 (24%) routine samples of fried rice examined in this laboratory.

Factors such as heat resistance or growth rate may be selective for certain serotypes during the cooking and storage of food. This is a possible explanation for the variation in isolation rates of serotypes 1 and 17 in routine samples of uncooked and cooked rice. The importance of growth environment and conditions is possibly reflected in the particular involvement of cooked rice from Chinese restaurants in the vomiting-type outbreaks of food poisoning.

There are few reports implicating milk and cream in outbreaks of *B. cereus* food poisoning. Serotype 1 from a vanilla slice (Pinegar & Buxton, 1977) and type 8 from pasteurized cream (McSwiggan, Gilbert & Fowler, 1975) have been suspected as the causal agent in two incidents in this country. The results of serotyping cultures from routine samples of dairy produce and cooked meat and poultry indicate that a variety of types can be obtained including most of those associated with the two distinct syndromes of food poisoning. The significance of *B. cereus* and of specific serotypes in food is difficult to assess. Low numbers, $< 100/g$, are probably of little significance but with large numbers, especially $> 10^5/g$, there is a definite risk that an outbreak of food poisoning may occur.

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