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Prevalence of *Bacillus cereus* in the faeces of healthy adults

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SUMMARY

In a survey designed to determine the prevalence of *Bacillus cereus* in the faeces of healthy persons, the organism was found in low numbers in 100 (14%) of single faecal specimens from 711 adults in the general population. In addition, in an attempt at assessing the changes in the *B. cereus* distribution within the faecal flora of the individual, weekly faecal specimens were submitted over a seven-week period by 18 members of staff of two laboratories. The total isolation rate was again 14%, with 15 serotypes represented. In four individuals *B. cereus* was isolated in two consecutive weeks and in all cases the isolates were of different serotypes. Excretion was never recorded for more than two consecutive weeks. These findings probably reflect the intake of *B. cereus* in the individual's diet.

INTRODUCTION

The first well documented accounts of *Bacillus cereus* food poisoning were presented by Hauge (1950, 1955). Since then many outbreaks have been reported and the subject has been well reviewed (Goepfert, Spira & Kim, 1972; Gilbert & Taylor, 1976). It is now established that *B. cereus* causes two distinct types of food poisoning, characterized respectively by diarrhoea with abdominal pain 8–16 h or nausea and vomiting 1–5 h after consumption of food contaminated with large numbers of *B. cereus*. Incidents reported from Great Britain have been of the vomiting type and mainly associated with the consumption of fried rice from Chinese restaurants and 'take-away' shops. In these incidents, large numbers of *B. cereus* (*ca.* $10^6 - 10^9$/g) were usually present in remnants of cooked rice and in faecal specimens from the patients (PHLS, 1972, 1973; Mortimer & McCann, 1974; Gilbert & Taylor, 1976). The outbreaks reported from Europe and America have been largely of the diarrhoeal type and a wide variety of food vehicles including meat and vegetable soups, cooked meat and poultry, puddings, dried milk, vanilla sauce and corn starch were implicated. In these *B. cereus* was found in large numbers in the food remnants, but in most instances the examination of faecal specimens was not recorded.

Apart from the food poisoning cases, there are very few data on the carriage of *B. cereus* in human faeces. This investigation was undertaken to assess the frequency of excretion of *B. cereus* and the distribution of serotypes in a sample of the British population.

MATERIALS AND METHODS

Samples

Faecal specimens from two groups were examined. The first consisted of specimens from 711 adults of either sex in several regions of the country, obtained for surveillance purposes unrelated to the present study and stored either frozen or freeze dried. Frozen samples were thawed at 4 °C before examination. The second group consisted of 18 members of staff from two Colindale laboratories who submitted faecal specimens weekly. At the end of the study these persons consumed a Chinese 'take-away' meal, consisting of fried rice and sweet and sour vegetables. Two portions of fried rice and mixed vegetables were examined for *B. cereus*. Each participant submitted one faecal specimen before and two specimens within 48 h after the meal.

Culture method

Samples of 0·5 g of faeces were incubated at 37 °C in 3 ml of nutrient broth. Subcultures were made after 24 and 48 h on blood agar and the mannitol egg yolk polymyxin agar (MYPA) of Mossel, Koopman & Jongerius (1967). Plates were examined after 24 h incubation at 37 °C. Typical or suspect colonies were screened on Kendall's BC medium (Gilbert & Taylor, 1976) and plated on blood agar for purity. Biochemical confirmation was followed by serotyping using the scheme of Taylor & Gilbert (1975) extended with a further five serotypes.

Direct cultures on blood agar and MYPA were made from the portions of fried rice and mixed vegetables from the Chinese meal. Colony counts of *B. cereus* were made immediately after consumption of the meal and again after overnight incubation at room temperature. In addition, food samples were tested by the enrichment method also. Twenty-five g of food were added to 100 ml of nutrient broth, incubated at 37 °C for 24 h and plated on blood agar and MYPA as before.

RESULTS AND DISCUSSION

Of a total of 711 frozen or freeze-dried faecal samples obtained from adults in the general population, 100 (14%) were positive for *B. cereus* (Table 1). Fifteen serotypes were represented among the 100 strains but 52% were non-typable (Table 2). The distribution of serotypes was similar to that recorded by Gilbert & Parry (1977) in various foods and probably reflects the fact that transient carriage of *B. cereus* in the gut is directly related to the diet of the individual. Examination of fresh specimens from the laboratory staff gave similar results; 24 of 171 samples (14%) were positive for *B. cereus* (Table 1). In the examination of samples obtained at intervals over a seven-week period, *B. cereus* was isolated three times from one participant, twice from five participants, once from eight participants and not at all from the remaining four. Excretion was never recorded for more than two consecutive weeks in one individual and on the four occasions this occurred the isolations were of a different serotype.

One sample of fried rice from the meal contained 500 *B. cereus*/g which after overnight incubation at room temperature increased to 5×10^5/g. *B. cereus* was

Table 1. *Prevalence of* Bacillus cereus *in faecal specimens*

Nature of specimen	Source	Faeces examined	*B. cereus* isolated	%.
Frozen	Adults in general	480	67	13·9
Freeze dried	population	231	33	14·2
Fresh	Laboratory staff	171*	24	14·0

* Specimens from 18 members of staff from two laboratories submitted at intervals over 7 weeks.

Table 2. *Distribution of serotypes among* 100 *isolations of* Bacillus cereus *from faecal specimens of adults in the general population*

Serotype	No. of times isolated	Serotype	No. of times isolated
1	7	17	4
3	2	18	8
5	1	19	1
8	3	20	4
10	1	21	3
11	3	22	7
12	1	23	1
15	2	NT	52

NT = not typable.

Table 3. *Comparative isolation of* Bacillus cereus *from faecal specimens using two plating media*

No. of samples examined	No. positive for *B. cereus* on: Blood agar 24 h	Blood agar 48 h	Blood agar Total	MYPA medium 24 h	MYPA medium 48 h	MYPA medium Total
595	63	9	72	59	7	66

isolated from the second sample of fried rice and from the mixed vegetables by enrichment culture only. While serotype 2 and a non-typable strain were isolated from the fried rice and a non-typable strain from the mixed vegetables, types 1 and 22 respectively were found in the only two positive faecal samples after the meal (these individuals had been negative for *B. cereus* before the meal). It is evident that the Chinese meal did not produce any increased excretion of *B. cereus*.

No advantage was found in heating the nutrient broth suspension of faeces at 60 or 80 °C for 15 min before incubation. The importance of nutrient broth enrichment culture was evident from the finding that, in the examination of 200 of the samples by both direct and enrichment methods, only three samples were positive on direct plating whereas 30 were positive after enrichment. In contrast, faeces from patients in the first few hours after an attack of food poisoning have large numbers of organisms and nutrient broth enrichment is unnecessary (Mortimer & McCann, 1974). It appeared that nutrient broth, although a non-selective medium, was suitable for the isolation of *B. cereus* present in small numbers in faeces of

healthy persons. MYPA developed for the isolation of *B. cereus* from food, was not found to have any advantage over blood agar. The relative efficiency of the plating media is presented in Table 3. Subcultures after 48 h incubation yielded nine additional isolates.

In view of the wide distribution of *B. cereus* in nature and in various foods including milk, rice, other cereals, spices, and meat and poultry, these organisms are inevitably ingested from time to time in small numbers and contribute to the transitory intestinal flora. A higher isolation rate might be expected in ethnic groups whose diet is mainly rice and other cereals.

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