

## A case-control study investigating an outbreak of *Bacillus cereus* food poisoning in a hotel in Malta

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**ABSTRACT:** *Bacillus cereus* food poisoning is an intoxication with a short incubation period, characterised predominantly by sudden onset of nausea and vomiting in some cases and in others by abdominal colic, severe watery diarrhoea and tenesmus. The illness generally persists no longer than 24 hours and is rarely fatal. In February 1996 the Department of Public Health investigated an outbreak of food poisoning involving at least 92 persons among local and foreign guests at a local hotel. *B. cereus* was implicated as a cause of the outbreak. A case-control study was performed on 61 cases and 80 controls from among hotel residents using a detailed questionnaire. Consumption of rice at a hotel lunch was associated with subsequent development of symptoms (OR =2.97, 95% CI 1.34 - 6.77). The food-specific attack rate for rice was 0.53 (P =0.0034). *B. cereus* ( $7.5 \times 10^3$  organisms /g) was isolated from leftover samples of boiled rice and from the stools of three patients.

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### Introduction

*B. cereus*, an aerobic, gram-positive bacterium, is a motile rod producing heat resistant spores. Its toxins include a heat and acid labile enterotoxin and a heat resistant emetic toxin. These toxins are responsible for the two distinct types of food borne gastro-enteritis; the diarrhoeal and emetic form respectively<sup>1,2</sup>. A small number of cases are known to exhibit symptoms of both types of illness, probably as a result of the production of both types of toxin by the particular strain ingested. The diarrhoeal form typically has an incubation period of 6-24 hours and is associated with ingestion of proteinaceous foods; the emetic form has a shorter incubation period of 1 - 6 hours, and is mostly associated with consumption of farinaceous foods such as cooked rice<sup>3,4</sup>. Both toxins are pre-formed in foods; enterotoxin can also be formed in the gut<sup>1</sup>. Fever is usually uncommon with either syndrome<sup>4,5</sup>.

*B. cereus* is a ubiquitous organism of soil commonly found at low insignificant levels in raw, dried and processed food including cereal products, spices, dried foods, milk and dairy products and infant foods<sup>1,6,7</sup>. *B. cereus* has an optimum growth temperature of 28-35°C (min. 4-5°C; max. 48°C) and a generation time of between 18 and 27 minutes<sup>3</sup>. Transmission usually occurs by ingestion of contaminated food that has been stored at ambient temperatures after cooking. This allows multiplication of the vegetative forms of the organism and production of the heat-stable toxin that can survive brief heating<sup>8</sup>. A typical example of this is stir frying of cooked rice held at room temperature before re-heating; fried rice is a leading cause of *B. cereus* emetic-

type of food poisoning in the U.S. A variety of mishandled foods has been implicated in outbreaks associated with diarrhoea<sup>5</sup>.

*B. cereus* food poisoning can be conclusively confirmed by the isolation of the microbe at levels greater than  $10^5$  per gram from epidemiologically implicated food<sup>1,4</sup>. However, the mere presence of the toxin-producer in cooked implicated food, may be evidence of causality. Summary results by Kramer and Gilbert (1989) of a large number of outbreaks caused by *B. cereus* show that a diarrhoeal syndrome was caused by levels of *B. cereus* of between  $1.2 \times 10^3$ - $10^8$  organisms per gram and a similar analysis of 107 incidents of the emetic syndrome in the United Kingdom showed that numbers of *B. cereus* present in the food (mainly rice), varied from  $1.0 \times 10^3$  -  $5.0 \times 10^{10}$  organisms per gram<sup>2</sup>.

### Outbreak alert

On the morning of the 26 February 1996 the Department of Public Health (DPH) received a telephone alert of a case of suspected food poisoning (a formal notification was received later from a private laboratory which reported isolation of *B. cereus* in a stool specimen from the index case). The person initially reporting the case indicated that there may have been further similar cases occurring among participants in a retreat held at a local hotel during the previous weekend (23 to 25 February 1996).

The cases were promptly verified by the Medical Officer of the Disease Surveillance Branch and immediate investigation of the outbreak moreover

revealed that similar symptoms occurred in a few tourists also resident at the hotel at this time.

One food handler, a bartender at the hotel, later reported abdominal cramps, fever, diarrhoea and vomiting developing within hours of consuming timpana (baked macaroni) for lunch from the hotel on the 26 February 1996.

Onset of symptoms (nausea, diarrhoea, abdominal cramps and fever) in the index case was on the morning of the 25 February at 08:30. The earliest onset time reported by the other cases was on the evening of the 23 February (two cases) and the latest reported for a single case was 27 February (early morning). The majority of cases occurred on the 25 and 26 February. Symptoms were self-limiting, lasting one to two days. No follow-up study of long-term sequelae was conducted. None of the cases required hospitalisation.

Retreat participants consumed hotel meals between the evening of Friday 23 and Sunday 25 lunch time. Foreign residents at the same hotel also partook of the same meals.

## Methods

- I Immediate investigation
- II Active surveillance
- III Case-control study
- IV Results - epidemiological and microbiological
- V Analysis
- VI Discussion
- VII Recommendations and action taken

### Immediate investigation

On verification of the outbreak, the Disease Surveillance Branch promptly procured lists of retreat participants (233) and foreign residents (224) at the hotel. Members of the Food Safety Branch and the Health Inspectorate from DPH carried out an on-site inspection at the hotel kitchen that same morning (26 February). A large number of deficiencies pertaining to poor kitchen hygiene, food storage and food handling methods were noted and a report drawn up.

A list of food-handlers was compiled; none of these reported gastrointestinal symptoms at that stage. One bartender (see above) who later reported concurrent symptoms was requested to submit stool specimens for culture and to refrain from food handling duties until three consecutive cultures resulted negative. Samples of food leftovers, water, kitchen surface swabs and hand swabs from food handlers were collected and submitted to the Public Health Laboratory (PHL) for microbiological analysis.

### Active surveillance

The immediate investigation was carried out by telephone survey of retreat participants. The interviews were carried out by health inspectors who followed a set of questions regarding meals taken, food items consumed and details of symptoms, if any. Concurrently, foreign hotel guests were interviewed at the hotel by the district health inspector using the same

questionnaire. A total of 197 retreat participants and 49 foreign hotel residents were interviewed in this manner.

Case definition: A case was defined as any person resident at the hotel between Friday 23 February and Monday 26 February, and who developed any one symptom of vomiting, diarrhoea, abdominal cramps, between Friday 23 February and Tuesday 27 February 1996.

A total of 92 such cases were identified as follows: 83 retreat participants and 9 foreign tourists residing at the hotel. The 83 cases were identified from a total of the 197 retreat participants interviewed (the remaining 26 cases could not be traced after several telephone attempts).

Stool specimens were requested from 37 (45%) cases who had symptoms recent to the time of interview. Of these, 21 samples were forwarded to the Public Health Laboratory.

The other nine cases were identified from among 49 foreign hotel residents (present at the hotel at the time) who were interviewed at the hotel that same day. Stool specimens were submitted by 7 (11%) of these residents. The amount and distribution of stool samples was considered adequate for the scope of this investigation.

### Action taken

1. The hotel kitchen was immediately closed down and a supervised clean-up effected. The management was instructed to rectify structural and functional deficiencies noted at inspection. Repeated follow-up inspections and sampling were carried out to assess progress.

2. The symptomatic barman was suspended from food handling duties until three consecutive, spaced, negative stool samples were obtained.

3. 'Unfit for Drinking' signs were set up at the sink in every hotel room. An insanitary water cistern was routed off.

4. Legal action was taken against the hotel licensee by the Department of Public Health for contravening the Food, Drugs and Drinking Water Act<sup>9</sup> by way of deficiencies in food hygiene measures.

### Case-control study

A case-control study was performed using a more detailed questionnaire. This was distributed to 61 cases and 80 controls. Cases were selected by taking the first 61 persons who had come to the attention of the Department of Public Health and who satisfied the case-definition criteria.

The controls (potentially 'at-risk' individuals who remained asymptomatic within the period defined by the case definition) were selected by random sampling using Eptable random number list generator for each category of controls: 39 hotel residents and 41 retreat participants. Unmatched controls were chosen from both groups resident at the hotel during the weekend in question since they were all exposed to the same hotel environment including meals. Data from a total of 141 completed questionnaires was entered using Epinfo 6.04 software.

The null hypothesis stated that there was no association between consumption of hotel meals within the given time period and becoming a case.

## Results

Data on all 141 targeted persons was used in the study.

### Distribution of Cases

Figure 1 shows the distribution of the 61 cases studied by date of onset of symptoms. Most cases had onset of symptoms on Sunday 25 February. The frequency of occurrence of specific symptoms among the cases is shown in Table 1.

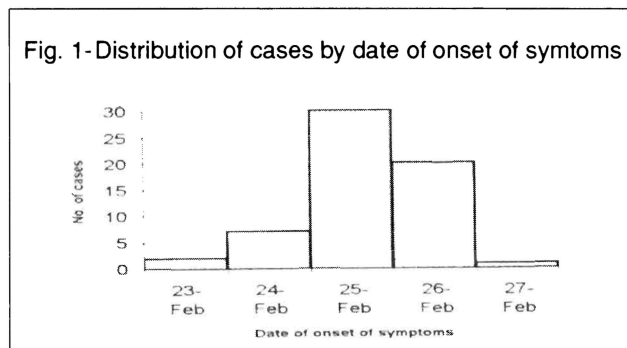


Table 1 - Symptoms reported by affected persons (n=141)

Symptoms	Individuals (% of total)
Abdominal Cramps	49 (52.7%)
Chills	45 (47.4%)
Diarrhoea	36 (38.3%)
Vomiting	33 (34.7%)
Fever	31 (33.0%)

## Microbiology

*B. cereus* ( $7.5 \times 10^5$  organisms per gram) was isolated from a sample of leftovers kept from boiled rice served at lunch on Saturday 24. The organism was also cultured in three (including the index case) out of 21 separate stool specimens submitted by affected individuals.

*Klebsiella* sp. was also cultured from the same rice sample rendering it unfit for human consumption.

*Klebsiella* sp. and faecal streptococci were present on a hand swab taken from a food handler, indicating poor hygienic quality.

Service water samples taken from a number of hotel rooms indicated presence of coliform organisms at levels unfit for drinking purposes.

Follow-up samples from the hotel kitchen (e.g. bolognese sauce) over the following weeks gave isolates of *Klebsiella* sp. indicating the prevailing poor hygienic state.

## Analysis

A chi square test was performed to associate exposure

with becoming a case for all meals shared by retreat participants and foreign residents. (See Table 2). The analysis showed consumption of the meals of Saturday 24 lunch and Sunday 25 lunch to be significantly related to subsequent development of illness. Odds Ratios of 3.11 (95% CI 1.36, 7.38);  $\chi^2 = 8.67$ ,  $P = 0.0032$  for Saturday 24 lunch and of 2.91 (95% CI 1.31, 6.66);  $\chi^2 = 8.16$ ;  $P = 0.0043$  for Sunday lunch were obtained.

Table 2 - Contingency tables for association of illness with consumption of hotel meals

Meals	$\chi^2$	Pvalue
Dinner on Friday 23 Feb (n = 140)	1.30	0.255
Breakfast on Saturday 24 Feb (n = 140)	0.01	0.943
Lunch on Saturday 24 Feb (n = 140)	8.67	0.003*
Dinner on Saturday 24 Feb (n = 141)	0.24	0.621
Breakfast on Sunday 25 Feb (n = 140)	0.46	0.496
Lunch on Sunday 25 Feb (n = 137)	8.16	0.004*

The relationship between ingestion of single food items and becoming a case was also studied. Odds Ratios and  $\chi^2$  were calculated to estimate the strength of association between individual food items consumed and development of illness. There was a significant association for several food items, most of which were served at lunch on Saturday 24 and lunch on Sunday 25 February. Adjustment for confounding risk factors was not performed in the analysis. Food specific attack rates were also calculated for all statistically significantly related food items.

The null hypothesis of no association was rejected for the food items indicated in Table 3.

Table 3 - Odds ratios relating development of illness to consumption of various food items and food specific attack rates (FSAR)

Food Item	Odds Ratio	95 % Confidence Intervals	P value	FSAR	P value
Spaghetti (Fri 23 supper)	3.34	1.54<OR>7.33	< 0.001	0.52	0.003
Rice (Sat 24 lunch)	2.97	1.34<OR>6.74	< 0.004	0.53	0.004
Grilled pork (Sat 24 lunch)	4.13	1.85<OR>9.42	< 0.001	0.50	0.022
Timpana (Sat 24 supper)	2.57	1.22<OR>5.51	0.019	0.56	<0.001
Lasagne (Sun 25 lunch)	2.67	1.22<OR>5.88	0.011	0.57	<0.001
Roast chicken (Sun 25 lunch)	3.14	1.54<OR>6.45	0.001	0.57	<0.002

N.B. only statistically significant items included in table

## Discussion

Very often food poisoning does not result from a single lapse in hygiene, but from a sequence of potentially preventable errors. The susceptible food, once having been contaminated by bacteria (in the case of *B. cereus* this step is difficult to avoid since the organism is present in the soil) needs to be left at 'optimum' temperatures for an appropriate period of time for significant growth to occur.

There is sufficient evidence to conclude that the outbreak of food poisoning episode was caused by a sequence of failures in the preparation of food served at the hotel on Saturday 25 lunch and Sunday 26 lunch. A number of food items are possibly implicated and there is a clear breakdown in kitchen hygiene.

Inadequate temperature control of the boiled rice served at the hotel lunch on the 25 February 1996 (although no details of food preparation were recorded) must have caused the multiplication of *B. cereus* in the cooked rice, which was possibly a primary but not sole cause of the outbreak.

Although no pathogens were isolated from tap water, the high coliform load (assuming cases drank or brushed their teeth with tap water) may have contributed to the overall illness.

Multiple causality may explain why the distribution of disease is not typical of a point source outbreak. The presence of fever which is not a common feature of *B. cereus* food poisoning, in 31 cases (33 %) and the fact that *B. cereus* levels in the rice were below those generally associated with definite food poisoning, further suggest that *B. cereus* may not have been the sole cause of the outbreak.

On the other hand, significant evidence of association between ingestion of rice and illness is substantiated by the following:

1. concurrent clustering of reported symptoms of abdominal cramps, diarrhoea and vomiting in a number of cases significantly linked to consumption of common food at the 5 % level (Odds Ratio of for consumption of boiled rice 2.97 (CI 1.34, 6.77),  $\chi^2=8.58$  ;  $P=0.0034$ ).

2. isolation of *B. cereus* organisms from the boiled rice (albeit counts of  $7.5 \times 10^3$  per gram)

3. isolation of *B. cereus* organisms from stool specimens of three human cases.

Other food items which achieved statistical significance at analysis (Table 3) may have acted as confounders. Bias arising from preference by same subject for certain foods must be considered. Multiple causality due to cross-contamination due to poor general food hygiene is, however, more likely given that the food specific attack rates (FSAR) were significant for a number of items (See Table 3).

The presence of faecal coliforms and *Klebsiella* counts in hand swabs, rice and follow-up food samples taken from the hotel kitchen adds weight to this consideration, as does the development of symptoms on the 26 February in the bartender following consumption of hotel food. The report on the poor hygienic state and structural and functional deficiencies on the premises adds further support.

## Recommendations

The mainstay of consumer protection from such food poisoning outbreaks is the production of 'safe food' through tighter prevention and control measures. One cannot underestimate the importance of improved and continuing education of food handlers. Basic principles of safe food handling should be emphasised<sup>10</sup> with particular attention to effective temperature control of cooked food. Keeping cooked food at temperatures above 60°C prior to consumption minimises germination of spores and multiplication of bacteria. Storage at room temperature and reheating should be avoided as much as possible. Refrigeration of any leftover food must be immediate and kept to a minimum<sup>5</sup>.

A more diffuse introduction of Good Manufacturing Practices (GMP) coupled with process control based on Hazard Analysis Critical Control Point (HACCP) locally is strongly recommended.

1. There is a definite need for better education of food handlers on basic food handling practices. The further institution of compulsory training programmes (possibly subsidised) should be considered. Alternatively food handlers should be required to produce certification evidence of such basic training prior to approval for employment.

The training of catering managers in food safety is also a priority. This will enable more effective staff supervision to ensure safe food-handling practices.

2. More frequent and regular inspections and follow-up by Food Safety Branch of high risk catering and manufacturing establishments with internal audit of inspection reports are indicated. The problem of limited personnel on the Food Safety Branch may be overcome through further in-training of health inspectors.

3. The slow introduction of the concept of Good Manufacturing Procedure (GMP) and control processes through Hazard Analysis Critical Control Point (HACCP) approach toward 'zero defect manufacture'. The latter technique, developed in the 1970s, assesses the flow of food through the process and provides a mechanism to monitor these operations frequently and determine points that are critical for the control of foodborne disease hazards. The critical control point (identified from a flow chart during the HACCP evaluation and analysis) is an operation or step by which preventive or control measures that will eliminate, prevent, or minimise a hazard(s) that has occurred prior to this point can be exercised. It is clearly a valuable technique for process control of microbial hazards through the identification and elimination of bacteriological growth. This method has been found to be more efficient and cost-effective than more traditional methods, however for such a system to achieve its objectives, a large number of people must be educated and trained<sup>11</sup>.

## Conclusion

A total of 91 guests (83 of 233 Maltese retreat participants and 8 of 224 foreign residents) at a local hotel during the 23 and 25 February 1996 suffered gastrointestinal symptoms following ingestion of meals



at the hotel.

A case-control study of 141 individuals 'at-risk' showed an association between consumption of hotel meals on Saturday 24 February (particularly boiled rice) and Sunday 25 February and becoming ill.

*B. cereus* was cultivated from stools of three cases and was also identified in low counts in leftovers of boiled rice served at the hotel lunch on Saturday 24 February 1996. The null hypothesis between consumption of rice at the hotel lunch on Saturday 24 and becoming a case was rejected.

The presence of *Klebsiella* organisms and faecal streptococci on culture from a hand swab from a food-handler at the hotel confirms the almost certain contribution of poor hygienic standards of the establishment to this food poisoning event. Furthermore, the high coliform load in the tanks supplying hotel tap water adds to the probability that there were other microbiological agents involved.

Such outbreaks of food poisoning only highlight the necessity of reinforcement and further implementation of more effective control measures through better educational training of food handlers and more frequent routine and spot checks are in order.

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