Occurrence of *Bacillus cereus* and the Bacteriological Quality of Chinese “Take-Out” Foods

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ABSTRACT

One hundred sixty-five samples of various foods were collected from 24 different Chinese take-out restaurants for bacteriological examination which included enumeration of *Bacillus cereus* by three media, MYP, KG and blood agars. Blood agar was less selective but no quantitative differences in recovery were apparent. Twenty-eight samples (15%) yielded *B. cereus* in excess of 100 per gram, and 20 of these were fried rice (33% positive), which also showed the poorest overall bacteriological quality. Biochemical characterization of 232 isolates of *B. cereus* showed 96% or more positive for catalase, nitrate reduction, beta-haemolysis, subterminal-ellipsoidal spores, aerobic and anaerobic utilization of glucose, Voges-Proskauer, fermentation of glyceral, gelatin hydrolysis, and alkaline peptonization of litmus milk; and a negative reaction in mannitol. Variable results were obtained for motility, fermentation of sucrose and salicine, and starch hydrolysis. Thirty-three isolates were susceptible to 12 of 19 antibiotics tested. and were refrigerated and tested the following morning. For summary purposes, the food samples were grouped into nine general categories (Table 2). The meat-vegetable group represents mainly chow mein and chop suey. Fried rice includes various meat-flavored rice dishes.

Food poisoning outbreaks attributed to *Bacillus cereus* have frequently implicated Chinese foods as the vehicle, especially fried rice (1,4,7,14,15). Gilbert et al. (4) demonstrated that spores of *B. cereus* are able to survive the cooking of rice, and then germinate and multiply readily in both cooked and fried rice. Raevou and Genigeorgis (19) showed that *B. cereus* grew even better in rice than in brain heart infusion broth. There have been very few reported surveys on contamination of foods with *B. cereus*, but those available indicate that the organism is ubiquitous (7,12,18). No reports are available on the incidence of *B. cereus* in Chinese “take-out” foods which have not been immediately implicated in a food poisoning.

Three media are used most commonly for enumeration of *B. cereus*: (a) Blood agar (5,9,20), (b) mannitol-egg yolk-phenol red agar (MYP) (16), and (c) egg yolk-polyoxynin medium (KG) (13). MYP and KG agars have official acceptance in the United States (6,22). Confirmation procedures for presumptive colonies vary widely in the number and type of biochemical reactions utilized.

Over a 2-year period, our laboratory received on five different occasions samples of Chinese foods implicated in food poisoning. Using blood agar for enumeration, a number of these samples showed high counts of *B. cereus* (Table 1). This experience motivated a survey to evaluate three different media for enumeration of *B. cereus*; determining the incidence of *B. cereus* in Chinese “take-out” foods; evaluating the usefulness of various biochemical tests for identification of *B. cereus*; and describing the overall bacteriological quality of Chinese foods.

MATERIALS AND METHODS

Food samples

Samples of foods were collected from 24 different Chinese restaurants in the Metro Toronto area, all providing take-out service, and were delivered to the laboratory for analysis on the same day or were refrigerated and tested the following morning. For summary purposes, the food samples were grouped into nine general categories (Table 2). The meat-vegetable group represents mainly chow mein and chop suey. Fried rice includes various meat-flavored rice dishes.

Bacteriological analysis

Aerobic plate counts and coliform bacteria were determined by standard methods described elsewhere (22). Fecal coliforms were determined by inoculation of EC broth from positive presumptive tubes for total coliforms and incubated at 44.5 C for 22-24 h. *Staphylococcus aureus* was determined with Baird-Parker agar (22). *Clostridium perfringens* with egg yolk-free TSC agar (11,22), and fecal streptococci with KG agar (22). *Salmonella* examinations were completed by pre-enrichment in lactose broth followed by selective enrichment in selenite-cystine broth at 35 C and tetrazolium-brilliant green broth at 43 C.

*Bacillus cereus*

*B. cereus* was recovered by three media using spread plate inoculation and incubation at 30 C: (a) Blood agar (BA), (b) phenol red-egg yolk-polyoxynin agar (MYP) (22), and (c) KG agar (22). Negative plates were held for 48 h before discarding, but all positive samples showed good growth on all media after 24 h incubation.

Biochemical tests

Colonies resembling *B. cereus* in any way, and showing any degree of lecinthinase activity on MYP or KG agars, were streaked onto BA for purification and determination of hemolytic activity. Stock cultures were then prepared for further study. All isolates were first examined by gram stain to verify cell and spore morphology and tested for catalase before further biochemical characterization by inoculation of the following media: (a) Nitrate-motility agar (10); (b) lactose-gelatin medium (U1); (c) litmus milk; (d) VP medium (proteose-peptone, 7 g/l; glucose, 5 g/l; NaCl, 5 g/l; pH 6.5-6.8); (e) starch agar (22); (f) phenol red glycerol broth (22); (g) carbohydrate fermentation broth (peptone, 10 g/l; meat extract, 3 g/l; NaCl, 5 g/l; NH₄H₂PO₄, 2.5 g/l; brom cresol...
purple, 0.01 g/1 with 1% of either dextrose, sucrose, salicin or mannitol. All biochemical reactions were determined at 30 C. Nitrates reduction, the Voges-Proskauer test, and starch hydrolyses were recorded after 24 h of incubation. Other tests giving negative reactions were observed for 5 days before recording.

Antibiotic susceptibility

Antibiotic susceptibility was determined by the agar dilution method using the replicator device of Steers et al. (27). Agar concentrations for the antibiotics are given in Table 5. Any isolate giving a growth response of four or less colonies was recorded as susceptible to test antibiotic.

RESULTS

The overall bacteriological quality of the Chinese foods examined in this survey was quite good (Table 2). Thirty-one percent of all samples had aerobic plate counts of < 100/g, and 75% had counts of < 100,000/g. Eighty-five percent of all samples had coliform counts of < 100,000/g (15%); and the presence of fecal coliforms in excess of 1,000/g (11%).

Fifty-one percent of the samples contained Enterobacteriaceae (11%). There was no apparent difference in quantitative recovery was apparent (Table 3). Blood agar, however, sometimes showed a heavy background flora which tended to mask B. cereus colonies and made isolation difficult. Although MYP and KG agars were slightly more selective, background flora on these media was frequently heavy so that purification of presumptive colonies was always necessary before biochemical testing could proceed.

A total of 262 presumptive B. cereus colonies were selected from the three test media for identification. Confirmation rates for the three media were as follows (number confirmed/number fished): BA, 63/68 (92.6%); MYP, 86/97 (88.7%); KG, 83/97 (85.6%). Isolates accepted as B. cereus were gram-positive bacilli having the biochemical characteristics shown in Table 4.

All of the 232 isolates identified as B. cereus were catalase positive, showed beta-hemolysis on blood agar, produced spores which were ellipsoidal and located subterminally, and gave a positive Voges-Proskauer test. Only one isolate failed to ferment glucose aerobically and another gave an acid reaction in mannitol. These abberant results were not confirmed by retesting. Thirty-eight isolates (16.4%) were not motile by the agar-stab technique using nitrate-motility medium (9). Other biochemical reactions with variable results included anaerobic fermentation of glucose (97.8±%); utilization of sucrose (48.7±%), glycerol (94.0±%), and salicin (25.0±%); hydrolysis of starch (37.5±%); nitrate reduction (98.7±%); gelatin hydrolysis (95.7±%); and alkaline peptonization of litmus milk (97.4±%). There was no apparent difference in biotypes between the three isolation media, with the possible exception of a larger percent of starch-hydrolyzing strains from BA (57.1%) compared to MYP (32.6%) and KG (27.7%) agars.

An association between starch-hydrolysis and salicin-utilization was suggested in that 137 starch-negative

**TABLE 1. Foodborne outbreaks implicating Chinese foods containing Bacillus cereus.**

<table>
<thead>
<tr>
<th>Outbreak No.</th>
<th>No. people ill</th>
<th>Symptoms</th>
<th>Incubation time (hours)</th>
<th>Foods received</th>
<th>APC</th>
<th>Coliforms</th>
<th>Fecal Streptococci</th>
<th>B. cereus</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>D, V</td>
<td>7½</td>
<td>Curry fried rice</td>
<td>&gt;3,000,000</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Chicken</td>
<td>&gt;3,000,000</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Chop suey</td>
<td>&gt;3,000,000</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Chop suey</td>
<td>&gt;3,000,000</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Chow mein</td>
<td>&lt;100</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Egg roll</td>
<td>&lt;100</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Beef fried rice</td>
<td>&lt;100</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Sliced pork</td>
<td>&lt;100</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Shrimp</td>
<td>&lt;100</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Spare ribs</td>
<td>&lt;100</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Chicken</td>
<td>&lt;100</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

All isolates negative for Salmonella enterica. a = cramps, D = diarrhea, N = nausea, V = vomiting. bNot from the same meal. cIsolates produced enterotoxin type D. dIsolates negative for enterotoxin types A, B, C, D, E. ND = not done.
isolates were also salicin-negative, and 49 starch-positive isolates were also salicin-positive, representing correlation in 80.2% of the isolates.

The isolates of *B. cereus* could be grouped into five types based on colonial morphology on blood agar: (a) large colony, round, strong beta-haemolysis; (b) similar to type a but with weak haemolysis; (c) large colony, irregular shape, rhizoid, smaller zone of beta-haemolysis than type a; (d) small colony, otherwise like type a, large zone of beta-haemolysis; and (e) same as type d with very weak beta-haemolysis.

Thirty-three isolates originating from different food samples or from different media and the same food sample, or having some differences in colonial morphology on BA, were tested for antibiotic susceptibility (Table 5). The isolates were susceptible to 12 of 19 antibiotics, the exceptions being colistin (33 resistant), carbenicillin (27 resistant), methicillin (22 resistant), carbenicillin (9 resistant), and ampicillin (7 resistant).
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DISCUSSION

The poorer bacteriological quality of fried rice compared to other Chinese foods examined in this survey, and the greater incidence of B. cereus in this food, is consistent with the epidemiology of B. cereus food poisoning (1,4,14,15). The isolation rates for B. cereus in cooked (10%) and fried (32.8%) rice agree quite well with those found by Gilbert and Parry (3) for routine samples of boiled (10%) and fried (24%) rice. The poor bacteriological quality and the greater incidence of B. cereus in fried rice undoubtedly results from the reported practice of holding cooked rice, which is less frequently implicated in food poisoning, at room temperature before frying and adding other ingredients (1,15). The likelihood of contamination of fried rice with B. cereus is increased by addition of spices, which often contain this organism (8,18).

There seems to be little advantage in the use of MYP or KG agars over blood agar for enumeration of B. cereus, except for their slightly better selectivity. Neither of these media offer essential colonial identification features over BA. The ability of background flora to proliferate on all media requires subculturing for purity before identification can proceed. In the case of foods implicated in outbreaks, and containing a high number of B. cereus, interfering background flora is mostly diluted out and presents less of a problem on BA. BA also has the practical advantage of always being available in public health laboratories, thus eliminating the necessity of stocking a specialized medium or preparing it on short notice to meet infrequent demands.

Seventy-five percent of the B. cereus isolates obtained were unable to utilize salicin, a property which Gilbert and Taylor (5) observed was characteristic of food-poisoning strains in Great Britain and Australia, while routine food isolates were generally able to utilize salicin. They noted, however, that strains from European and American outbreaks fermented salicin.

A rather low percentage (37.5%) of our isolates were able to hydrolyze starch in contrast to some other reports (5). Kim and Goepfert (12), however, found that only 52% of their egg yolk-positive isolates from dried foods were able to hydrolyze starch, and Goepfert (6) reported that 10-50% of strains are positive for this reaction. A longer incubation time than the 24 h used in this study, which conforms with instructions elsewhere (6), may have detected other positive strains. Gordon et al. (8), for example, tested after 3 and 5 days of incubation.

The only antibiotic with uniform resistance between 33 isolates was colistin (polymyxin E), which explains why polymyxin has been the only antibiotic used in selective media such as MYP and KG agars. The only other antibiotic with some degree of resistance in the isolates was penicillin, and then six (19%) were sensitive at the relatively low test concentration of 0.25 units/ml. This is a rather high susceptibility rate considering reports of nearly uniform penicillinase production in B. cereus (17), which supposedly allows the organism to grow on agar with 10 I.U. of penicillin (7), and has encouraged development of selective media with penicillin (2).

This survey substantiates that the presence of B. cereus in fried rice is not uncommon. As is true with many other foodborne disease agents, practical control lies not in elimination from the food but in proper preparation and handling practices which prevent multiplication. We shall undoubtedly continue to see foodborne B. cereus outbreaks involving rice until there are certain changes in preparation procedures as recommended by Gilbert et al. (4).

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