

# The potential of bacteriocin typing in the study of *Clostridium perfringens* food poisoning

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**SUMMARY** A range of 49 bacteriocins was used to type 311 strains of *Clostridium perfringens* isolated from food poisoning outbreaks. Strains of the same serotype within an outbreak showed similar patterns of susceptibility to bacteriocins, whereas strains of different serotype isolated from different sources produced many variations in bacteriocin susceptibility patterns. The 311 strains, along with isolates from a wide range of sources were screened for their ability to produce bacteriocins. A much greater proportion of the strains from food poisoning outbreaks was bacteriocinogenic than were isolates from human and animal infections, various foods and the environment.

*Clostridium perfringens* is recognised as an important agent of food poisoning, and one of the aims in the investigation of outbreaks is to show a relation between isolates from the faeces of most of the patients and from the incriminated food.

Several different techniques for subdividing strains of *C perfringens* have been described. A serological typing scheme is used in the Food Hygiene Laboratory for the investigation of *C perfringens* food poisoning outbreaks. Using a range of 143 antisera, a causative serotype was established in 446 (69%) of the 646 incidents investigated between 1970 and 1980. Of the 8487 strains from these outbreaks, 6957 (82%) could be typed, but in about 5% of cases the majority of strains within an outbreak were serologically non-typable.<sup>1</sup> Confirmation of such incidents may necessitate the preparation of new antisera.

Paine and Cherniak<sup>2</sup> investigated the use of gas-liquid chromatography of capsule preparations to distinguish between strains of *C perfringens*. Qualitative and quantitative differences between the major polysaccharide components separated the four strains studied. The potential for bacteriophage typing of *C perfringens* was reviewed by Mahony,<sup>3</sup> but to date no workable scheme exists.

A typing scheme using the bacteriocins of *C perfringens* was first proposed in 1974,<sup>4</sup> although there are earlier reports of the occurrence of bacteriocins and their properties.<sup>5-7</sup> A number of reports<sup>4,8-11</sup> describe the use of passive bacteriocin typing by

which the sensitivity of strains to a standard range of bacteriocins is tested. Alternatively, active bacteriocin typing involves the study of the range of activity of bacteriocins produced by test strains against a standard set of indicator organisms. This method was employed by Satija and Narayan<sup>12</sup> who suggested a relation between bacteriocin typing pattern and the geographical distribution of food poisoning strains of *C perfringens*.

This paper is the first description of the laboratory investigation of serologically non-typable outbreaks of *C perfringens* food poisoning in which passive bacteriocin typing results have been subsequently confirmed by the preparation of new antisera.

## Material and methods

One hundred and eighty-nine isolates of *Clostridium perfringens* type A associated with seven outbreaks of food poisoning, and 122 representative strains which were the causative serotypes in unrelated food poisoning incidents were typed using a range of 18 bacteriocins prepared at Dalhousie University and a further 31 prepared in the Food Hygiene Laboratory.

The typing method was a modification of the procedure described by Mahony.<sup>4</sup> A tenfold dilution of an overnight culture of each strain in Robertson's cooked meat medium was spread with a swab over the surface of Columbia base blood agar plates (CBA) using a rotary plater (Denley Instruments Ltd, Sussex) as for antibiotic sensitivity testing.<sup>13</sup> The plates were inoculated with 20  $\mu$ l drops of up to 10 different bacteriocins using a calibrated pipette

(Gilson Pipetman P20, Anachem Ltd, Luton). After anaerobic incubation at 37°C for 18 h the zones of inhibition were recorded (Fig. 1). Zones of complete inhibition greater than 6 mm in diameter were scored as positive and less well defined effects as negative. The bacteriocin sensitivity patterns were used to calculate the number of reaction differences between each of the 311 strains from food poisoning outbreaks.

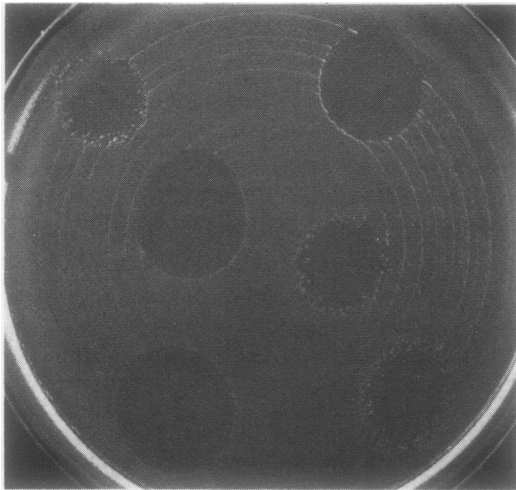


Fig. 1 A strain of *C. perfringens* susceptible to six of ten bacteriocins.

The 311 strains, together with 322 strains from a wide range of sources were screened for their ability to produce bacteriocins. A strip of 0.22 µm membrane filter (Millipore Ltd, London) laid across the surface of a CBA plate was inoculated with a suspension of the test strain according to the method of Riley and Mee<sup>14</sup> and incubated anaerobically at 37°C for 36 h. The filters were discarded and the plates streaked with five reference indicator strains (strain Nos M88, T6, T17, F1726/76, F3600/79) at right angles to the line of original growth. After a further 18 h incubation the plates were examined for inhibition of growth of the indicator strains (Fig. 2).

## Results

In general, strains of different serotype showed many variations in sensitivity to bacteriocins. If more reaction differences were tolerated before distinguishing between strains, the number of distinct sensitivity patterns decreased, although no major clusters of strains developed (Table 1). Strains of the same serotype from different outbreaks also showed

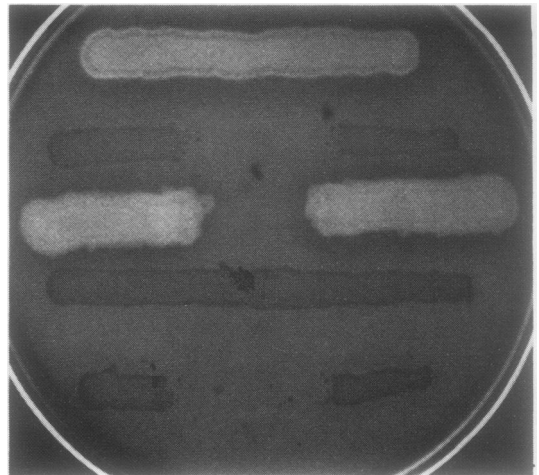


Fig. 2 Detection of a bacteriocin active against three of five reference indicator strains.

large numbers of reaction differences. For instance, 26 of the 129 isolates that were serotype 3/4 displayed distinct bacteriocin typing patterns (Table 2). Within outbreaks, however, strains of the same serotype showed similar sensitivity patterns.

### OUTBREAK A

*Clostridium perfringens* serotype TW22 was isolated from roast beef and from 12 patients. If no reaction differences were tolerated in bacteriocin sensitivity, three patterns could be observed with the main cluster containing nine strains. The 13 strains were indistinguishable if one reaction difference was allowed.

Bacteriocin production could be demonstrated by all of the strains. No differences were detected in the range of activity of these bacteriocins nor were any of the bacteriocins active against the producing strains.

### OUTBREAK B

Residents of a nursing home experienced abdominal pain and diarrhoea following the consumption of roast pork. The meat and faecal specimens from 9/11 patients investigated yielded *C. perfringens* serotype

Table 1 Bacteriocin typing patterns among 129 strains representing 44 serotypes

Reaction differences allowed	No of distinct patterns
0	117
1	91
2	60
3	45
4	28
5	20



Table 4 Formation of clusters within outbreaks and a group of unrelated strains as a result of bacteriocin typing

Outbreak	Causative serotype	Number of strains of causative serotype	Percentage of strains included in largest cluster with reaction differences			
			0	1	2	3
A	TW22	13 (13*)	69.2	100	—	—
B	1	11 (13)	90.9	100	—	—
C	41	20 (22)	20	75	100	—
D	NT	6 (6)	100	—	—	—
E	NT	10 (28)	20	30	100	—
F	NT	65 (75)	92.3	95.4	100	—
G	NT	25 (32)	84	100	—	—
Unrelated strains	3/4	26	3.8	3.8	11.5	23.1

\*No of strains in outbreak.

#### OUTBREAK G

A serologically non-typable strain of *C perfringens* was isolated from 10 patients who experienced diarrhoea and abdominal pain after an evening meal in a hotel.

If one bacteriocin reaction difference was allowed, a large cluster of strains was observed which contained only serologically non-typable strains from nine of the patients. An antiserum prepared from one of these isolates agglutinated all but one of the strains in this group. Three other strains (serotypes 25, 65/69 and NT) showed distinct sensitivity patterns and failed to react with the new serum.

A summary of reaction differences between strains within incidents is given in Table 4.

Of the 129 unrelated strains implicated in food poisoning outbreaks, 102 (79.1%) produced bacteriocins. However, bacteriocin production could be demonstrated in only 58 (18%) of 322 isolates from the faeces of healthy persons, human and animal infections, various foods and the environment.

#### Discussion

In the development and application of a bacteriocin typing scheme it is necessary to consider the differing sensitivity patterns of unrelated strains and of strains within outbreaks. A small number of bacteriocin typing patterns may be observed among strains of the same serotype within a single outbreak, and these strains readily cluster together when a small number of reaction differences are allowed. This is in marked contrast to the results with strains of different serotype or with those of the same serotype isolated from different sources. Unrelated strains such as these display quite distinct bacteriocin typing patterns and show little tendency to form clusters until relatively large numbers of reaction differences are allowed.

Reports of changes in typing patterns on repeated subculture are rare. Mahony<sup>4</sup> described one strain of *C perfringens* which became consistently susceptible to a bacteriocin to which it was previously resistant, while Scott and Mahony<sup>15</sup> have subsequently shown a 73% reproducibility when 60 strains were typed on three occasions. The reproducibility increased to 89% if one reaction difference was allowed. Govan<sup>16</sup> found that 15 (5.7%) of 260 strains of *Pseudomonas aeruginosa* showed minor changes in bacteriocin type when stored for periods of up to three years. Birge<sup>17</sup> suggested that changes in bacteriocin production and sensitivity may be effected by mutation or by the loss or acquisition of plasmids.

In the development of the phage-typing scheme for *Staphylococcus aureus* it was shown that when groups of cultures from a common source were compared, variations occurred even when great care was taken to standardise techniques.<sup>18</sup> Our results suggest that a small number of reaction differences should be tolerated in bacteriocin typing patterns before it is concluded that two strains are unrelated.

A high proportion (79.1%) of the isolates from food poisoning incidents was capable of bacteriocin production. Of the 26 isolates which were of serotype 3/4 (the type most frequently implicated in outbreaks), 24 (92.3%) were bacteriocinogenic, indicating the possibility of a relationship between this property and the ability to cause food poisoning.

The absence of a complete correlation between bacteriocin type and serotype suggests that bacteriocin typing may be used to subdivide serological types. The technique may be a valuable complement to serotyping in the laboratory confirmation of *C perfringens* food poisoning outbreaks, especially when the causative strain is serologically non-typable.

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