J Clin Pathol 1982;35:1361–1365

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

GN WATSON, MF STRINGER, RJ GILBERT, DE MAHONY*

From the Food Hygiene Laboratory, Central Public Health Laboratory, London NW9 5HT, and the ** Department of Microbiology, Faculty of Medicine, Dalhousie University, Halifax, Nova Scotia, Canada*

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.[1] Confirmation of such incidents may necessitate the preparation of new antisera.

Paine and Cherniak[2] 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,[3] but to date no workable scheme exists.

A typing scheme using the bacteriocins of *C perfringens* was first proposed in 1974,[4] although there are earlier reports of the occurrence of bacteriocins and their properties.[5–7] A number of reports[4 8–11] 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[12] 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.[4] 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.[13] The plates were inoculated with 20 µl drops of up to 10 different bacteriocins using a calibrated pipette

Accepted for publication 10 June 1982

J Clin Pathol: first published as 10.1136/jcp.35.12.1361 on 1 December 1982. Downloaded from http://jcp.bmj.com/ on January 29, 2023 by guest. Protected by copyright.

(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.

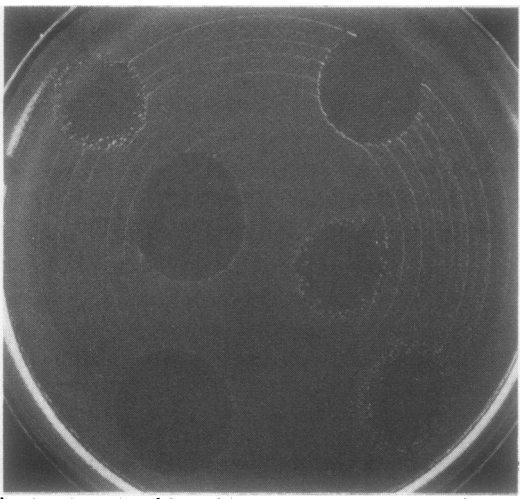

Fig. 1 *A strain of* C perfringens *susceptible to six of ten bacteriocins.*

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[14] 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

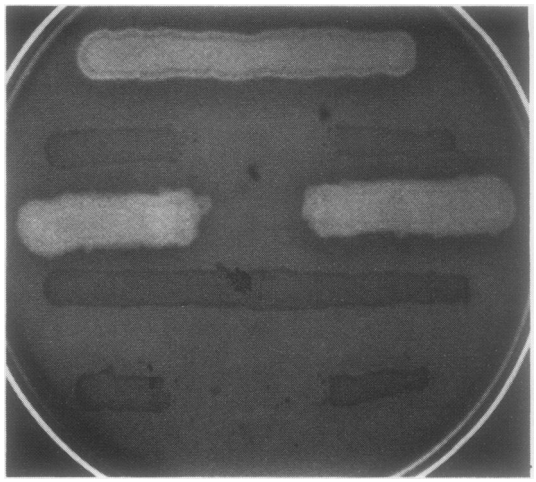

Fig. 2 *Detection of a bacteriocin active against three of five reference indicator strains.*

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

J Clin Pathol: first published as 10.1136/jcp.35.12.1361 on 1 December 1982. Downloaded from http://jcp.bmj.com/ on January 29, 2023 by guest. Protected by copyright.

Table 2 *Bacteriocin typing patterns among 26 unrelated strains of the same serotype*

Reaction differences allowed	*No of distinct patterns*
0	26
1	26
2	19
3	15
4	8
5	5

1; two other patients carried different serotypes (65 and NT).

If no reaction differences were tolerated, four bacteriocin sensitivity patterns were observed with the main cluster containing all but one of the serotype 1 strains (Table 3). If one reaction difference was allowed, the main cluster contained all but one strain (serotype 65) which remained distinct until four differences were tolerated.

OUTBREAK C

One hundred and seventy of 547 hospital patients suffered from diarrhoea after eating cold roast lamb. *Clostridium perfringens* serotype 41 was isolated from both the food and the faeces of 13 of 15 patients investigated, and two patients yielded a non-typable strain. A further three patients died—serotype 41 was isolated from all at post mortem.

Sixteen different bacteriocin typing patterns were observed among the 22 strains submitted if no reaction differences were tolerated. However, if one difference was allowed two large clusters developed (containing 15 and five strains) which were made up exclusively of serotype 41 isolates. If two reaction differences were allowed, all serotype 41 isolates were included in a single cluster, while the two non-typable strains remained distinct until five differences were tolerated.

OUTBREAK D

Ten diners complained of diarrhoea and abdominal pain following the consumption of undercooked turkey in a restaurant. The six patients investigated all yielded heat-resistant isolates of *C perfringens* which could not be typed serologically. Bacteriocin typing showed no reaction differences and at a later date an antiserum prepared from one of the isolates agglutinated all six strains.[1]

OUTBREAK E

Sixty-four of 813 hospital patients suffered from diarrhoea 12–24 h after consuming rolled roast beef. The meat and the faeces of 15/22 patients investigated yielded isolates of *C perfringens* that were serologically non-typable.

Many bacteriocin typing patterns were observed among the 28 strains submitted and, if no reaction differences were allowed, the largest cluster contained only three strains. If two reaction differences were tolerated, a cluster developed which contained three serologically typable and 15 non-typable strains.

An antiserum prepared from a non-typable strain from the food agglutinated with two other strains from the same source and with one strain from faeces, while a second antiserum prepared from a faecal isolate agglutinated with nine other strains from faeces. All strains reacting with the new antisera were included in the major cluster of 18 strains.

OUTBREAK F

Of 151 diners in a large hotel, 123 suffered abdominal pain and diarrhoea 9–15 h after a meal which included pâté and cheesecake. Isolates of *C perfringens* from both foods, work surfaces and the faeces of the 18 patients investigated could not be typed serologically. A variety of typable strains was also found.

Bacteriocin typing results showed that many of the 75 isolates from foods, surfaces and 16 patients were indistinguishable. An antiserum subsequently prepared from one of these non-typable strains agglutinated only with the other members of a cluster of 65 strains. The remaining isolates showed a range of quite different sensitivity patterns and remained distinct until six reaction differences were allowed.

Table 3 *Outbreak B—an example of sensitivity patterns using 40 bacteriocins*

Source of strain	*Serotype*	*Sensitivity recorded to bacteriocin no:* M433	MP37	M554	M385	M28	MP30	M518	Mr14	M4	M43	M96	M48	ME6	M78	M139	MA2	M446	M384	1	5	6	8	10	14	21	24	25v	27	31x	32	37	40	54	56	62	63	64	70	89	M89
Patients 1–8	1	−	−	−	−	−	−	+	+	+	−	−	−	+	−	+	−	+	+	−	+	+	+	+	+	+	+	+	+	+	−	−	−	+	+	+	+	+	+	−	−
Patient 9	1	−	−	−	−	−	−	+	+	−	−	−	−	+	−	+	−	+	+	−	+	+	+	+	+	+	+	+	+	+	−	−	−	+	+	+	+	+	+	−	−
Patient 10	NT	−	−	−	−	−	−	+	+	+	−	−	−	+	+	+	−	+	+	−	+	+	+	+	+	+	+	+	+	+	−	−	−	+	+	+	+	+	+	−	−
Patient 11	65	−	−	−	−	−	−	+	+	+	−	−	−	+	+	+	−	+	+	−	+	+	+	+	+	+	−	+	+	+	−	−	+	−	−	+	+	+	+	−	−
Roast Pork (two isolates)	1	−	−	−	−	−	−	+	+	+	−	−	−	+	−	+	−	+	+	−	+	+	+	+	+	+	+	+	+	+	−	−	−	+	+	+	+	+	+	−	−

J Clin Pathol: first published as 10.1136/jcp.35.12.1361 on 1 December 1982. Downloaded from http://jcp.bmj.com/ on January 29, 2023 by guest. Protected by copyright.

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[4] described one strain of *C perfringens* which became consistently susceptible to a bacteriocin to which it was previously resistant, while Scott and Mahony[15] 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[16] 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[17] 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.[18] 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.

The authors are greatly indebted to Mr DM Williams of the Public Health Laboratory Service Computer Unit for his assistance in the analysis of results, and

J Clin Pathol: first published as 10.1136/jcp.35.12.1361 on 1 December 1982. Downloaded from http://jcp.bmj.com/ on January 29, 2023 by guest. Protected by copyright.

one author (DEM) acknowledges the support of a National Health Research and Development Grant, Canada, No 6603-1101-54.

References

[1] Stringer MF, Watson GN, Gilbert RJ. *Clostridium perfringens* type A: serological typing and methods for the detection of enterotoxin. In: Corry JEL, Roberts D, Skinner FA, eds. *Methods for the isolation and identification of food poisoning organisms*. Soc Appl Bacteriol Technical Series No 17. London: Academic Press, 1982:111–35.

[2] Paine CM, Cherniak R. Composition of the capsular polysaccharides of *Clostridium perfringens* as a basis for their classification by chemotypes. *Can J Microbiol* 1975;**21**:181–5.

[3] Mahony DE. Bacteriocin, bacteriophage and other epidemiological typing methods for the genus *Clostridium*. In: Bergan T, Norris JR, eds. *Methods in microbiology* Vol 13. London: Academic Press, 1979:1–30.

[4] Mahony DE. Bacteriocin susceptibility of *Clostridium perfringens*: a provisional typing schema. *Appl Microbiol* 1974;**28**:172–6.

[5] Sasarman A, Antohi M. Presence des bactériocines chez le *Clostridium perfringens*. *Arch Roum Pathol Exp Microbiol* 1963;**22**:377–81.

[6] Tubylewicz H. Experimental studies on bacteriocinogeneity in *Clostridium perfringens* type A: 1) Isolation of bacteriocins and their antibacterial spectrum. *Bull Acad Pol Sci (Biol)* 1966;**14**:31–6.

[7] Uchiyama K. Studies on bacteriocin-like substances produced by *Clostridium perfringens*. *Med J Kagoshima Univ* 1966;**18**:131–44.

[8] Bittner J, Antohi M, Vanescu V, Nicolescu M, Badita Gh. *Clostridium perfringens* food-borne disease and bacteriological analysis for strain identification. *Arch Roum Pathol Exp Microbiol* 1979;**39**:95–103.

[9] Moors DC, Haldane EV, Martin RS, Sumarah R. Two episodes of food poisoning due to *Clostridium perfringens*—Nova Scotia. *Canadian Diseases Weekly Report* 1980;**6**:230–1.

[10] Satija KC, Narayan KG. Passive bacteriocin typing of *Clostridium perfringens* type A causing food poisoning for epidemiologic studies. *J Infect Dis* 1980;**142**:899–902.

[11] Mahony DE, Swantee CA. Bacteriocin typing of *Clostridium perfringens* in human feces. *J Clin Microbiol* 1978;**7**:309–9.

[12] Satija KC, Narayan KG. Active bacteriocin typing of food poisoning strains of *Clostridium perfringens*—a new tool for epidemiological investigation. *Int J Zoonoses* 1980;**7**:78–84.

[13] Stokes EJ, Ridgway GL. *Clinical bacteriology*. 5th ed. London: Arnold, 1980:101–3.

[14] Riley TV, Mee BJ. Simple method for detecting *Bacteroides* spp bacteriocin production. *J Clin Microbiol* 1981;**13**:594–5.

[15] Scott HG, Mahony DE. Further development of a bacteriocin typing scheme for *Clostridium perfringens*. *J Appl Bacteriol* 1982; (in press).

[16] Govan JRW. Pyocin typing of *Pseudomonas aeruginosa*. In: Bergan T, Norris JR, eds. *Methods in microbiology* Vol 10. London: Academic Press, 1978:61–91.

[17] Birge EA. *Bacterial and bacteriophage genetics: an introduction*. New York: Springer-Verlag, 1981:236–44.

[18] Parker MT. Phage-typing of *Staphylococcus aureus*. In: Norris JR, Ribbons DW, eds. *Methods in microbiology* Vol 7B. London: Academic Press, 1972:1–28.

Requests for reprints to: GN Watson, Food Hygiene Laboratory, Central Public Health Laboratory, 175 Colindale Avenue, London NW9 5HT, England.

J Clin Pathol: first published as 10.1136/jcp.35.12.1361 on 1 December 1982. Downloaded from http://jcp.bmj.com/ on January 29, 2023 by guest. Protected by copyright.