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A comparative study of clinical and food isolates of *Listeria monocytogenes* and related species

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SUMMARY

Ninety-six isolates of presumptive or confirmed *Listeria monocytogenes* were obtained from local clinical (30 isolates) or food laboratories (66 isolates). Minimal biochemical analysis identified only 80% of these isolates as *L. monocytogenes* the remaining included *L. seeligeri*, 1%, or the non-haemolytic *L. innocua*, 19%. The 27 clinical and 50 food isolates, mainly from meat products, frozen confectionaries, and cheeses, confirmed as *L. monocytogenes* were compared biochemically and serologically. Twenty-one isolates, including some strains of *L. innocua* and *L. seeligeri*, were examined for pathogenicity in immunocompromized mice and 44 typed using bacterial restriction endonuclease DNA analysis (BRENDA). Only isolates of *L. monocytogenes* were found to be pathogenic. Biovar-typing of the isolates was unreliable and provided poor discrimination. Serogroups 1/2 and 4 predominated among clinical and food isolates and BRENDA provided better discrimination among isolates. Ten stable and reproducible restriction patterns were observed among the *Listeria* sp. isolates studied. Overall, a combination of techniques gave the best discrimination and indicated their potential for use as epidemiological tools.

INTRODUCTION

Listeria monocytogenes is a Gram-positive, non-sporulating, facultative, intracellular parasite that is globally distributed [1]. This organism has been isolated from silage [2], natural vegetation [3], soil [4], wild and domestic animals [5] and water [6]. It also is reported to occur in foods such as raw milk [7], soft cheeses [8], raw chickens [9], pork meat [10], and minced beef [11].

Although a rare human pathogen, mortality from listeric infection may be as high as 30% [12]. The primary manifestations of listeric infection in humans are meningitis, abortion, and perinatal septicaemia, with infants, pregnant women and immunocompromized individuals at risk [13].

In the northern hemisphere, *L. monocytogenes* is emerging as a significant public health problem as demonstrated by six recent food-associated outbreaks of listeriosis in North America and Europe [12, 14–18]. Although outbreaks of listeriosis have yet to be documented in Australia, sporadic cases of listeriosis have

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occurred and *L. monocytogenes* has been isolated from Australian food products [19]. The significance of this organism in Australian foods and processing plants in relation to public health is unknown.

The purpose of this study was to investigate the relationship between Australian isolates of *L. monocytogenes* from clinical cases and a variety of food sources (meat, cheese, ice cream, fish, raw milk, environment) using conventional biochemical, serological and mouse pathogenicity tests and to evaluate the use of bacterial restriction endonuclease DNA analysis (BRENDA) for the subdivision of phenotypically similar isolates. The potential of BRENDA for use as a highly discriminatory typing system in epidemiological studies of listeriosis was also examined.

METHODS

Bacterial strains and growth media

The bacterial strains studied included the following: 27 human (H1–27) and 50 food (F1–50) isolates of *L. monocytogenes*; 2 human (H28, 29) and 16 food (F51–66) isolates of *L. innocua*; one human (H30) isolate of *L. seeligeri*. The sources of the *L. monocytogenes* are shown in Tables 2 and 3. Full clinical history was not available for all isolates although most were from clinical listeriosis. The strain of *L. seeligeri* was cultured from a central lobe abscess of a 57-year-old male alcoholic. Strains related epidemiologically include mother:baby pairs H14, 15; H20, 21 and food isolates from the same sample for example: F10, 13; F11, 12 from meat; F27, 28; F29, 30; F35, 36; F37, 38; F42, 43, from frozen confectionaries; F48, 49 from goats' milk; and F23, 44 from ice cream and the factory environment. *Listeria* sp. strains were minimally passaged following receipt and were grown aerobically at 37 °C on tryptone glucose yeast extract agar (TGYA) which consisted of 0·5% (w/v) tryptone (Oxoid), 0·1% (w/v) glucose, 0·6% (w/v) yeast extract (Oxoid) and 1·5% (w/v) bacteriological agar (Oxoid). Agar was omitted from the formulation when tryptone glucose yeast extract broth (TGYB) was required. *Staphylococcus aureus* UQM 1736, *Rhodococcus equi* UQM 702 and *Streptococcus agalactiae* UQM 1737, obtained from the University of Queensland, Department of Microbiology Culture Collection, were used for the CAMP test.

Phenotypic characterization

The *Listeria* sp. isolates were identified by testing for Gram stain reaction, tumbling and umbrella motility, nitrate reduction, methyl red and Voges–Proskauer reaction, H_2S production (triple sugar iron), catalase and oxidase activity, fermentative metabolism of glucose, aesculin hydrolysis, urease production and haemolysis of horse blood [20]. The CAMP test [21] was used to distinguish *L. innocua* from *L. monocytogenes*. Biotypes and species were determined by the method of Emody and Ralovich [22] based on the ability to ferment, with acid production (no gas), α-methyl-D-mannoside, arabinose, lactose, maltose, mannitol, melibiose, rhamnose, salicin, sorbitol, sucrose, trehalose and xylose.

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Serotyping

Serotyping was performed by rapid slide agglutination using commercially available Bacto-Listeria-O-antiserum (Difco) type 1/2 and type 4, type 3 O-antiserum was prepared according to Seeliger and Höhne [23] using reference strains. A crude unabsorbed rabbit antiserum prepared against O- and H-antigens of *L. monocytogenes* UQM 525 was used as a polyvalent antiserum. Strains were typed into serogroups 1/2, 3, 4 or not typable.

Pathogenicity tests

Mouse pathogenicity was determined using the rapid procedure of Stelma and co-workers [24]. Five carrageenan- (CG; Sigma-type II) treated mice were inoculated intraperitoneally with approximately 10^4 colony-forming units (c.f.u.) of the test strain and observed for up to 3 days. Strains that killed three or more mice were considered to be pathogenic. Three control groups were included: mice treated with CG only (negative control for non-specific deaths), CG plus 10^4 c.f.u. *L. innocua* UQM 3178 (negative control), and CG plus 10^4 c.f.u. *L. monocytogenes* UQM 527 (positive control). Fifteen isolates of *L. monocytogenes* (H4, 14, 15; F1, 6, 8, 14–16, 18, 31, 32, 34, 46, 50), 5 isolates of *L. innocua* (H28, 29; F53, 55, 58), and 1 isolate of *L. seeligeri* (H30) were tested.

Chromosomal DNA preparation

Chromosomal DNA was extracted from 100 ml TGYB cultures and purified by the method of Marmur [25] as modified by Sly and colleagues [26]. Cells were suspended in a lysozyme solution which consisted of 0·14% (w/v) Na_2HPO_4, 20% (w/v) sucrose and 2·5 mg/ml of lysozyme (Sigma) prior to treatment with sodium lauryl sulphate [27]. DNA was extracted from 24 clinical (H1–11, 13–22, 25–27) and 16 food isolates (F6–9, 14–16, 18, 23, 31–35, 44, 50) of *L. monocytogenes*, 1 strain (H30) of *L. seeligeri*, and 3 strains (H29; F53, 57) of *L. innocua*. The concentration and purity (DNA/protein average) of chromosomal DNA was determined spectrophotometrically [28].

Restriction endonuclease digestion of DNA

The mol-percent guanine plus cytosine (mol % G+C) of the DNA of *Listeria* species ranges between 36–39. As the restriction enzyme *Hin*d III (New England BioLabs) recognizes hexameral sequences (A′AGCTT) of comparable mol % G+C to listeria genomic DNA it was expected to cut the DNA frequently and consequently gave good resolution of 6–20 kilobase fragments. Quantities of 5–10 μg of chromosomal DNA were digested with *Hin*d III overnight at 37 °C in a general all purpose buffer [29] and the DNA fragments separated by agarose-gel electrophoresis and visualized by staining with ethidium bromide. Lambda DNA (New England BioLabs) cut with *Hin*d III was used as the restriction fragment size standard in each gel run. Photographs were taken with Polaroid 665 film and a Polaroid MP 4 Land Camera under u.v. (302 nm) illumination (Spectroline Model TR-302 Transilluminator). Each digest was repeated on three occasions so as to ensure that gels were representative of complete, not partial, digests. Undigested DNA from all test isolates were agarose-gel electrophoresed to

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determine the ability of the BRENDA system to detect plasmids which may contribute to minor differences in the restriction pattern. Strain F32 was selected to examine the stability of BRENDA-type during four subcultures before and after animal passage. BRENDA patterns were compared manually.

RESULTS

Phenotypic characterization

All *Listeria* sp. strains tested were positive for Gram stain reaction (rods), tumbling and umbrella motility, catalase, aesculin hydrolysis, methyl-red and Voges–Proskauer tests, and glucose fermentation, but did not reduce nitrate, produce H_2S, hydrolyse urea or show oxidase activity. Strains of *L. innocua*, *L. monocytogenes* and *L. seeligeri* were distinguished biochemically using the tests outlined by Bortolussi and colleagues [20]. Strains of *L. monocytogenes* and *L. innocua* fermented rhamnose and α-methyl-D-mannoside but not xylose. *L. monocytogenes* was haemolytic on horse blood agar and CAMP test with *S. aureus* positive. *L. seeligeri* was distinguished by its inability to ferment rhamnose and α-methyl-D-mannoside and fermentation of xylose. All strains of *L. monocytogenes* tested, uniformly fermented salicin and trehalose but not arabinose. Variable fermentation was observed with sucrose, lactose and sorbitol. Based on the fermentation patterns of these three carbohydrates, the isolates were typed into seven (i–vii) biovars (Table 1). No direct correlation was observed between biovar and any particular serogroup. Biovar (vii) predominated among clinical isolates, 63 %. Biovars (iii) and (vii) accounted for the majority of food isolates, 42 and 38 %, respectively. The reliability of biotyping was, however, poor as fermentation of the carbohydrates was often delayed and weak which made interpretation of some results subjective. Strains F18, 19, 36 and 37 fermented mannitol yet resembled *L. monocytogenes* in every other biochemical test and require further investigation.

Serotyping

With three exceptions, F8, F13 and F27, all strains could be serotyped based on Patersons' antigenic scheme [30] (Table 2). Serogroups 1/2 and 4 were found to predominate amongst clinical isolates, 26 and 74 % respectively. All serogroups were represented among food isolates. Serogroup 1/2 accounted for 52 %, serogroup 3 for 16 % and serogroup 4 for 26 %. No strain of *L. innocua* belonged to serogroup 1/2, serogroup 3 included 19 % of these isolates and serogroup 4, 81 %. *L. seeligeri* was serogroup 4. As each serogroup includes several serovars [31] and *Listeria* species, it could not be assumed that isolates that share a common serogroup represent the same serovar.

Pathogenicity test

All strains of *L. monocytogenes* tested demonstrated 100 % lethality in the mouse bioassay regardless of source, serogroup, or restriction pattern. Strains of *L. innocua* and *L. seeligeri* tested were non-pathogenic.

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Table 1. *Fermentation patterns of food and human isolates of* Listeria monocytogenes*

Biovar	Fermentation of Sucrose	Lactose	Sorbitol	Representative isolates
(i)	−	−	−	F34
(ii)	−	−	+	F6–9, 47
(iii)	−	+	+	H16, 17
				F1–5, 10–13, 16–24, 33, 41, 44
(iv)	+	−	−	F32, 42
(v)	+	−	+	H2, 4, 18
				F29, 48, 49
(vi)	+	+	−	H5, 6, 23, 25, 27
(vii)	+	+	+	H1, 3, 7–15, 19–22, 24, 26
				F14, 15, 25–28, 30, 31, 35–40, 43, 45, 46, 50

* Strains F18, 19, 36 and 37 that fermented mannitol are included.

Table 2. *Serogroups of typable clinical and food isolates of* L. monocytogenes

Source	Serogroup (%) 1/2	3	4
Clinical (n^* = 27)	26	—†	74
Unknown	11	—	29
Blood	11	—	22
Brain	—	—	3
CSF	—	—	8
Faeces	4	—	4
Vagina	—	—	8
Food (n = 50)‡	52	16	26
Meat/fish	14	2	8
Frozen Confectionaries	22	2	8§
Cheese	10	10§	6
Environment/milk	6	2	4

* n, represents the number of isolates.
† —, denotes no representative strains.
‡ Isolates F8, 13 and 27 were not typable.
§ Mannitol-fermenting strains included among these isolates.

BRENDA-typing

The DNA extraction procedure used yielded an average of 100 μg of DNA per 100 ml culture. The DNA/protein ratio averaged 2·1. The restriction pattern of strain F32 was found to remain stable following subculture before and after animal passage. Plasmids were not detected.

Ten different restriction patterns designated *a–j* were observed among the 44 isolates studied. The strains displayed a marked degree of polymorphism evident in the larger fragments (9–20 kb). Restriction patterns *a–h* were unique to strains of *L. monocytogenes* (Fig. 1). Restriction pattern *i* was common to the three strains of *L. innocua* and *j* was assigned to the strain of *L. seeligeri*, H30 (results not

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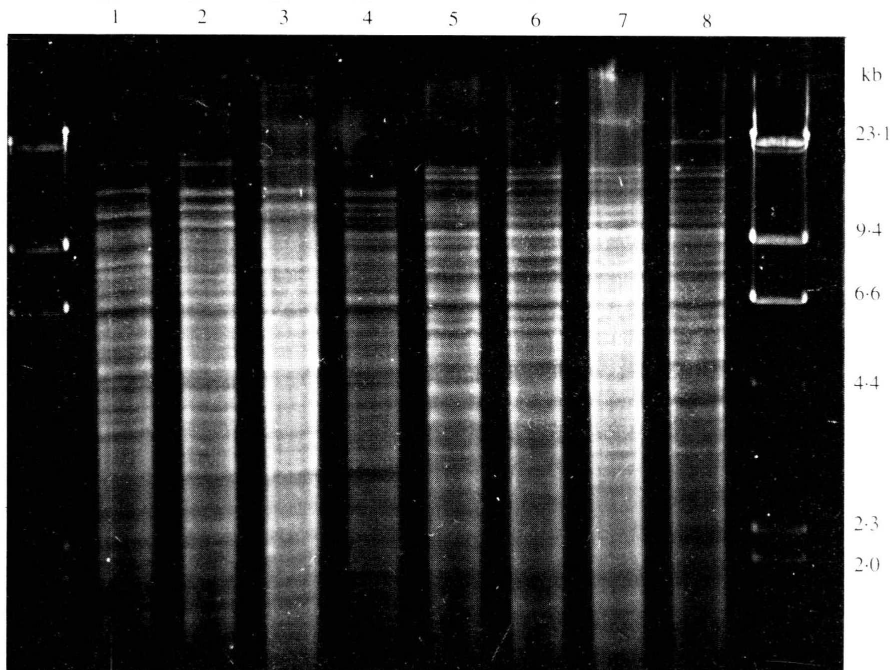

Fig. 1. *Hin*d III digestion of chromosomal DNA isolated from strains of *L. monocytogenes* representing the various BRENDA-types. Lanes: 1, strain H2 (patient, serogroup 1/2, BRENDA – *a*); 2, strain H4 (patient, serogroup 1/2, BRENDA – *b*); 3, strain F31 (cheese, serogroup 4, BRENDA – *c*); 4, strain F34 (cheese, serogroup 3, BRENDA – *d*); 5, strain H9 (patient, serogroup 4, BRENDA – *e*); 6, strain H17 (patient, serogroup 4, BRENDA – *f*); 7, strain F23 (ice cream, serogroup 1/2, BRENDA – *g*); 8, strain F50 (fish, serogroup 4, BRENDA – *h*). The molecular-weight marker is λDNA digested with *Hin*d III.

shown). Restriction fragments of approximately 22·0 and 16·2 kb were common to BRENDA-types *a–d*, fragments of smaller size differed in their band intensity. BRENDA-types *e–h* shared restriction fragments of approximately 21·1, 20·3 and 18·3 kb. A fragment of approximately 25·5 kb was unique to chromosomal DNA from F50 which was the only strain to demonstrate BRENDA-type *h*.

Differences in restriction pattern were noticed among strains of the same serogroup (Table 3). Five BRENDA-types were observed among isolates of serogroup 1/2, and four among serogroup 4. It was apparent that the genetic similarity between some strains with different serogroup antigens was greater than between isolates of the same serogroup. BRENDA-type *f* which was most common to both sets of isolates was found only in serogroup 4 human isolates and serogroup 1/2 or 3 food isolates. Strain F8 which could not be serotyped was also BRENDA-type *f*.

Food and clinical isolates that shared the same serogroup and BRENDA-type could be further differentiated using phenotypic characterization results. Two phenotypes were identified among food and clinical isolates of serogroup 1/2-BRENDA-type *a* [H1, F35, biovar (vii); H2, biovar (v)], four among serogroup 1/2-BRENDA-type *g* isolates [H27, biovar (vi); F23, 44, biovar (iii); H26, biovar

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Table 3. *BRENDA-types of clinical and food isolates of* L. monocytogenes *representing various serogroups*

Source	Serogroup 1/2	3	4
Clinical (n* = 24)			
Unknown	*a*† (2)‡, *b* (1)	—§	*e* (4), *f* (4)
Blood	*d* (2), *g* (1)	—	*e* (2), *f* (2)
Faeces	*g* (1)	—	*f* (1)
Vagina	—	—	*e* (1), *f* (1)
Brain	—	—	*e* (1)
CSF	—	—	*e* (1)
Total	*a* (2), *b* (1), *d* (2), *g* (2)	—	*e* (9), *f* (8)
Food (n = 15)			
Meat	*f* (2), *g* (1)	—	—
Cheese	*a* (1), *f* (2)	*d* (1)	*c* (1)
Ice cream	*f* (2), *g* (1)	—	*e* (2)‖
Fish/environment	*g* (1)	—	*h* (1)
Total	*a* (1), *f* (6), *g* (3)	*d* (1)	*c* (1), *e* (2), *h* (1)

* n, represents the total number of isolates.
† BRENDA-types *a*–*h*.
‡ Figures in parentheses indicate the number of representative isolates.
§ —, no representative isolates studied.
‖ Mannitol-fermenting strain F18 included.

(vii); F6, biovar (ii)], and three within serogroup 4-BRENDA-type *e* organisms [H5, 6, biovar (vi); H7, 9, 10, 13, 19–21; F15, biovar (vii); F18, biovar (iii)].

DISCUSSION

The observations of this comparative study indicate that extreme caution should be taken in extrapolating the significance of the presence of *L. monocytogenes* in Australian foods. Each of the typing methods employed had certain limitations and if used individually may give a misleading impression of the relationship among isolates from human and food. The best discrimination among isolates was achieved using a combination of typing techniques and indicated their potential for use as epidemiological tools especially in an outbreak situation.

BRENDA-typing has been documented as a useful technique for the typing of a broad spectrum of species including, *Leptospira interrogans* [32], *Campylobacter* spp. [33] and *Legionella* sp. strains [34]. In this study, BRENDA permitted the subgrouping of serotypically similar strains of *L. monocytogenes* from food and clinical origin and demonstrated the potential ability to differentiate genotypically the species of *Listeria*. The stability of restriction patterns as demonstrated with leptospira [32] must be examined before BRENDA may be considered as an alternative typing scheme. Strain F32 maintained a stable restriction pattern during four subcultures before and after animal passage. Further evidence of the stability of the typing scheme was provided by the recovery of identical restriction

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patterns from strains H14, 15; H20, 21 which represent vertical transmission of infection from mother to baby.

Although the restriction patterns of the isolates of *L. monocytogenes* studied were similar, minor differences such as band intensity were observed. This phenomenon was reproducible and has been observed in other studies using BRENDA [35]. Bjorvatn and co-workers [36] reported that such variations may result from DNA repetition, variation in the degree of methylation of the restriction sites or the presence of plasmids with high copy number. These workers also associated band intensity with increased virulence in *Neisseria meningitidis* strains.

While epidemiological relationships cannot be reliably determined with the limited number of isolates examined in this study it is interesting to notice that a strain of *L. monocytogenes* isolated locally from ice cream (F15) was identical to strains isolated from local cases of human listeriosis (H9, 13). Maternal infection was shown by identical isolates present in mother: baby pairs (H14, 15; H20, 21). Also, the possibility of environmental contamination of food products was demonstrated by identical strains found in an ice-cream isolate (F23) and from the factory environment (F44). This indicated the potential for this typing scheme in the investigation of food-processing environments.

The typing methods used in this study identified differences between food isolates that originated from the same sample. F29 and 30 from a chocolate coated ice confectionary belonged to different serogroups (1/2 and 3, respectively) and biovars [(v) and (vii), respectively]. Similar observations were made with F35 [serogroup 1/2. biovar (vii)] and F36 [serogroup 3, biovar (vii), mannitol fermenter] from Bresse cheese; F37 [serogroup 3, biovar (vii), mannitol fermenter] and F38 [serogroup 1/2, biovar (vii)] also from a Breese cheese sample; F42 [serogroup 1/2, biovar (iv)] and F43 [serogroup 3, biovar (vii)] from Brie cheese; and F48 [serogroup 1/2, biovar (v)] and F49 [serogroup 3, biovar (v)] from raw goats' milk. These findings stress that during the investigation of a suspected foodborne outbreak of listeriosis multiple isolates from the incriminated product need to be examined to reliably determine the epidemiological relationships.

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REFERENCES

1. Seeliger HPR. Listeriosis. 2nd ed. New York: S Karger, 1961.
2. Gray ML. Silage feeding and listeriosis. J Am Vet Med Assoc 1960; **136**: 205–8.
3. Welshimer HJ. Isolation of *Listeria monocytogenes* from vegetation. J Bacteriol 1968; **95**: 300–3.

https://doi.org/10.1017/S0950268800047853 Published online by Cambridge University Press

4. Botzler RG, Cowan, AB, Wetzoer TF. Survival of *Listeria monocytogenes* in soil and water. J Wildl Dis 1974; **10**: 204–13.
5. Weis J, Seeliger HPR. Incidence of *Listeria monocytogenes* in nature. Appl Microbiol 1975; **30**: 29–32.
6. Geuenich H, Muller HE, Schretten-brunner A, Seeliger HPR. The occurrence of different *Listeria* species in municipal waste water. Zentralbl Bakteriol Hyg B 1985; **181**: 563–5.
7. Lovett J, Francis DW, Hunt JM. *Listeria monocytogenes* in raw milk: detection, incidence and pathogenicity. J Food Protect 1987; **50**: 188–92.
8. Beckers HJ, Soentoro PSS, Delgou-van Asch EHM. The occurrence of *Listeria monocytogenes* in soft cheeses and raw milk and its resistance to heat. Int J Food Microbiol 1987; **4**: 249–256.
9. Pini PN, Gilbert RJ. The occurrence in the U.K. of *Listeria* species in raw chickens and soft cheeses. Int J Food Microbiol 1988; **6**: 317–26.
10 Skovgaard N, Norrung B. Detection of *Listeria* spp. in faeces of Danish pigs and minced pork meat. Int J Food Microbiol 1989; **8**: 59–63.
11 Johnson JL, Doyle MP, Cassens RG. Survival of *Listeria monocytogenes* in ground beef. Int J Food Microbiol 1988; **6**: 243–7.
12. Ho JL, Shands KN, Friedland G, Eckind P, Fraser W. An outbreak of type 4b *Listeria monocytogenes* infection involving patients from eight Boston hospitals. Arch Intern Med 1986; **146**: 520–4.
13. Gantz NM, Myerowitz RL, Medeiros AA, Carrera, GF, Wilson RE, O'Brien B. Listeriosis in immunosuppressed patients. Am J Med 1975; **58**: 637–43.
14. Schlech WF, Lavigne PM, Bortolussi RA, et al. Epidemic listeriosis – evidence for transmission by food. N Eng J Med 1983; **308**: 203–6.
15. Fleming DW, Cochi SL, MacDonald KL, et al. Pasteurized milk as a vehicle of infection in an outbreak of listeriosis. N Eng J Med 1985; **312**: 404–8.
16. James SM, Fannin SL, Agee BA, et al. Listeriosis outbreaks associated with Mexican-style cheese – California. MMWR 1985; **34**: 357–9.
17. Anonymous. Foodborne listeriosis – Switzerland. J Food Protect 1988; **51**: 425.
18. Kvenbery JE. Outbreaks of listeriosis/*Listeria* contaminated foods. Micro Sci 1988; **5**: 355–8.
19. Sutherland PS. *Listeria monocytogenes*. In: Buckle KA, ed. Foodborne microorganisms of public health significance. Sydney: Australian Institute of Food Science and Technology Ltd, 1989.
20. Bortolussi R, Schlech WF, Albritton WL. *Listeria*. In: Lennette EH, Balows A, Hausler Jr WJ, Shadomy HJ, eds. Manual of clinical microbiology, 4th ed. Washington, D.C.: American Society of Microbiology, 1985: 205–8.
21. Christie R, Atkins NE, Munch-Peterson E. A note on a lytic phenomenon shown by group B streptococci. Aust J Exp Med 1944; **22**: 197–200.
22. Emody L, Ralovich B. Ability of *Listeria monocytogenes* strains to attack carbohydrates and related compounds. Acta Microbiol Hung 1972; **19**: 287–91.
23. Seeliger HPR, Höhne K. Serotyping of *Listeria monocytogenes* and related species. In Bergan, T, Norris JR, eds. Methods in microbiology, vol. 13. London: Academic Press, 1969: 31–49.
24. Stelma GN, Reyes AL, Peeler JT, et al. Pathogenicity test for *Listeria monocytogenes* using immunocompromised mice. J Clin Microbiol 1987; **25**: 2085–9.
25. Marmur J. A procedure for the isolation of DNA from microorganisms. J Mol Biol 1961; **3**: 208–18.
26. Sly LI, Blackall LL, Kraat PC, Tian-Shen T, Sangkhobol V. The use of second derivative plots for the determination of mol % guanine plus cytosine of DNA by the thermal denaturation methods. J Micro Methods 1986; **5**; 139–56.
27. Mengaud J, Vicente M, Chenevert J, et al. Expression in *Escherichia coli* and sequence analysis of the listerolysin O determinant of *Listeria monocytogenes*. Infect Immun 1988; **56**: 766–72.
28. Maniatis T, Fritsch EF, Sambrook J. Molecular cloning – a laboratory manual. New York: Cold Spring Harbor Laboratory, 1982.
29. O'Farrell, PH, Kutter E, Nakanishi M. A restriction map of the bacteriophage T4 genome. MGG 1980; **179**: 421–35.
30. Paterson JS. The antigenic structure of organisms of the genus *Listerella*. J Pathol Bacteriol 1940; **51**: 427–36.

https://doi.org/10.1017/S0950268800047853 Published online by Cambridge University Press

31. Lamont RJ, Postlethwaite R, MacGowan AP. *Listeria monocytogenes* and its role in human infection. J Infect 1988; **17**: 7–28.
32. Marshall RB, Wilton BE, Robinson AJ. Identification of *Leptospira* serovars by restriction endonuclease analysis. J Med Microbiol 1981; **14**: 163–6.
33. Chevrier D, Larzul D, Megraud F, Guesdon J. Identification and classification of *Campylobacter* strains by using nonradioactive DNA probes. J Clin Microbiol 1989; **27**: 321–6.
34. Ketel RJ. Similar DNA restriction endonuclease profiles in strains of *Legionella pneumophila* from different serogroups. J Clin Microbiol 1988; **26**: 1838–41.
35. Renaud F, Freney J, Etienne J, et al. Restriction endonuclease analysis of *Staphylococcus epidermidis* DNA may be a useful epidemiological marker. J Clin Microbiol 1988; **26**: 1729–34.
36. Bjorvatn B, Lund V, Kristiansen B, Korsnes L, Spanne O, Linqvist, B. Applications of restriction endonuclease fingerprinting of chromosomal DNA of *Neisseria meningitidis*. J Clin Microbiol 1984; **19**: 763–5.

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