Epidemiol. Infect. (1998), **121**, 49–55. Printed in the United Kingdom

Laboratory investigation and comparison of *Salmonella* Brandenburg cases in New Zealand

J. M. WRIGHT* M. BRETT AND J. BENNETT

National Microbiology Reference Laboratory, ESR Communicable Disease Centre, PO Box 50348, Porirua, New Zealand

(*Accepted 1 November 1997*)

SUMMARY

An apparent increase in the incidence of *S.* Brandenburg in New Zealand, coupled with the possibility that the virulence of the organism may also be changing, prompted this study.

Three typing methods: macro-restriction fragment length polymorphism (MRFLP) profiling using pulsed field gel electrophoresis (PFGE), plasmid profiling and antimicrobial susceptibility profiling were used to determine strain diversity amongst 115 recent and historical isolates of *S.* Brandenburg from both human cases and non-human sources.

Antimicrobial resistance was noted only in three isolates. Plasmids of varying sizes were found in 31 isolates. MRFLP analysis resulted in 13 different patterns. Combining the three sets of typing data yielded 24 composite types. Comparison of composite type, isolation date and geographical location of case allowed the retrospective recognition of seven potential clusters during the 5-year study period. Composite types of 24 (80%) of the non-human isolates tested were indistinguishable from human isolates, suggesting that human infection may be via a number of vehicles.

Although not cost-effective for routine use on all salmonella isolates, the methods used in this study are an important adjunct to serotyping for discrimination within an emerging serotype.

INTRODUCTION

Salmonella Brandenburg was first reported by Kauffmann and Mitsui in 1930 following a case of infection in Germany. The implicated food was raw ham [1]. Worldwide, *S.* Brandenburg is an infrequently occurring serotype. Of the 31 countries reporting to the WHO *Salmonella* surveillance programme in 1987, only five countries reported *S.* Brandenburg among their 15 most commonly detected serotypes. These countries and the reported percentage of *S.* Brandenburg of all human Salmonellae were: Belgium, 2·4%; France, 1·6%; Hungary, 1·7%; Spain, 1·1%; and New Zealand, 1·1% [2]. Of 2919 human isolates serotyped in Scotland in 1993, 7 (0·2%) were identified as *S.* Brandenburg [3]. The Australian *Salmonella* Reference Centre reports that usually only two *S.* Brandenburg isolates are seen at that centre each year; however, 12 isolates were confirmed as *S.* Brandenburg by this centre in the first 4 months of 1995 [4].

Outbreaks of *S.* Brandenburg infection occurred in England in 1963 [5]. The outbreak investigation implicated pork products and the organism was isolated from sausages and other pork products. Switzerland experienced an increase in *S.* Brandenburg from approximately 30 cases annually to 254 cases (6·6% of all human salmonella isolates) in 1992, isolate numbers fell again in 1993 [6].

S. Brandenburg is an infrequent human pathogen in New Zealand, accounting for 142 (1%) of the

* Author for correspondence: J. M. Wright, Pathogen Laboratory, Cawthron Institute, Private Bag 2, Nelson, New Zealand.

Table 1. Salmonella *Brandenburg in New Zealand 1985–95*

Year	Number of human *S.* Brandenburg cases	% *S.* Brandenburg of total human Salmonellosis
1985	28	2·3
1986	11	0·9
1987	12	1·1
1988	10	0·8
1989	7	0·4
1990	26	1·5
1991	2	0·2
1992	6	0·5
1993	7	0·5
1994	33	2·0
1995	31	2·0

14000 salmonellosis case isolates serotyped at the National Microbiology Reference Laboratory in 1985–94. During this 10-year period, the number of isolates confirmed as *S.* Brandenburg per year varied as demonstrated in Table 1. The serotype was also identified from the following non-human sources: meat or bone meal, pigs, cows, goats, poultry, a dog and environmental cultures.

During the first 3 months of 1995, *S.* Brandenburg was isolated from the blood cultures of three patients. Prior to this time, bacteraemia was not recorded to have occurred with this serotype in New Zealand.

A 12-year review of blood culture records at a Canadian hospital [7] revealed that 43 blood cultures (from 21 patients) of a total of 153364 cultures yielded non-(para)typhoidal salmonellae; none of which was *S.* Brandenburg. A case of acute suppurative thyroiditis following *S.* Brandenburg bacteraemia was reported in 1993 [8]. The authors reporting this presentation noted that 'the isolation rate of *S.* brandenburg (*sic*) from all human sources is low and this microorganism is an uncommon agent of bacteremia (*sic*)'.

The possibility that the incidence of *S.* Brandenburg was increasing in New Zealand and that the virulence of the organism may also be changing, prompted the study reported here. Three typing methods: macrorestriction fragment length polymorphism (MRFLP) profiling using pulsed field gel electrophoresis (PFGE), plasmid profiling and antimicrobial susceptibility profiling were used to determine strain diversity amongst recent and historical isolates of *S.* Brandenburg from both human cases and non-human sources.

MATERIALS AND METHODS

Bacterial strains

Salmonellae isolated in clinical laboratories throughout New Zealand are routinely referred to the National Microbiology Reference Laboratory for confirmation and typing. Commercial and animal health laboratories also refer salmonellae for typing; however, these institutions do not routinely refer all isolates. Once typed, isolates are stored on Dorset egg slopes at room temperature.

Viable *S.* Brandenburg isolates referred to the National Microbiology Reference Laboratory between 1 January 1990 and 31 March 1995 were included in the study. Repeat isolations from the same source were not included. Isolates were subcultured from Dorset egg slopes to trypticase soy agar to determine viability. During 1995, the following three techniques were applied to the 115 viable isolates.

Antimicrobial susceptibility testing

Susceptibility testing to determine minimum inhibitory concentration (MIC) was performed by an agar dilution method [9], using a multi-point inoculator (H. I. Clements Pty Ltd., Sydney, Australia). Mueller–Hinton agar was used for all antibiotics except sulphamethoxazole and trimethoprim, for which Mueller–Hinton agar with 5% lysed horse blood was used.

Control strains *Escherichia coli* ATCC 25922 (sensitive control) and *Enterococcus faecalis* ATCC 29212 (sulphamethoxazole, trimethoprim MIC control) were included in each batch of tests.

The MIC was determined as being the lowest concentration of antibiotic that inhibits growth. The MICs for ampicillin, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, streptomycin, sulphamethoxazole, tetracycline and trimethoprim were interpreted by NCCLS interpretive standards [10]. The MICs for nalidixic acid, nitrofurantoin and spectinomycin were interpreted based on the recommended breakpoints of Frost [11].

Plasmid detection

A modification of the method of Kado and Liu [12] was used to extract plasmids. Briefly, a wooden applicator was used to emulsify fresh culture in 150 μl of Kado and Liu lysis buffer (50 mM Tris, 3% SDS,

https://doi.org/10.1017/S0950268898008887 Published online by Cambridge University Press

pH 12·5). After incubation at 56 °C for 45 min, the plasmid extraction was effected by addition of 100 μl of phenol:chloroform (50:50), gentle mixing and centrifuging for 30 min at 4 °C. The extracted layer was electrophoresed in 0·7% agarose at 100 volts for 2·5 h, stained in ethidium bromide and visualized and photographed on a transilluminator. *E. coli* V517, containing eight plasmids [13], was used as both a size marker and as a control organism to indicate the quality of each extraction process.

Macro-restriction fragment length polymorphism (MRFLP) profiling using pulsed field gel electrophoresis (PFGE)

Pulsed field gel electrophoresis (PFGE) was performed by adapting the *E. coli* PFGE method of Bohm and Karch [14] as follows: bacterial strains were grown in nutrient broth (Difco, Detroit, MI, USA) overnight at 37 °C. A 1·5 ml aliquot was then centrifuged, and the deposit resuspended in 1·0 ml 75 mM NaCl-25 mM EDTA. Agarose plugs were prepared from equal volumes of the washed bacterial suspension and 1% molten chromosomal grade agarose (BioRad). Each plug was gently shaken in 1 ml of lysis buffer (50 mM Tris, pH 8·0, 50 mM EDTA, pH 8·0, 1% lauryl sarcosine and 0·1% proteinase K) overnight at 50 °C. The agarose plugs were then washed eight times in 10 mM Tris-1 mM EDTA (TE). The minimum standing time in TE at room temperature between washes was 15 min. Plugs were stored in TE at 4 °C until tested.

A 2 mm section was cut from each plug for enzyme digestion. Each section was placed in 100 μl of restriction buffer containing 20 units of *Xba*I (Boehringer–Mannheim) and incubated at 37 °C overnight. The digested plugs were inserted into 1% agarose (SeaKem GTG, FMC) and the gel electrophoresed in TBE (0·05 M Tris, 0·05 M borate, 0·1 mM EDTA) at 14 °C in a Chef Mapper apparatus (BioRad). The run time was 22 h, the voltage 200 V (6 cm) and the linearly ramped pulse time 5–50 sec. The gel was stained with ethidium bromide, visualized on a transilluminator and photographed with Polaroid 667 film.

Analysis

Laboratory referral information and study findings were analysed using EpiInfo version 5·01. National salmonellosis demographics were extracted from the national EpiSurv database by the ESR Epidemiology Group. Potential clusters were defined as two or more cases of the same type occurring in the same geographical area within 1 month.

RESULTS

Demographics

Viable isolates totalled 115, including 85 of 88 human isolates. The age of the 84 human cases for whom an age was recorded ranged from 11 days to 82 years; the mean and median ages were 27·3 years and 26 years respectively. Forty cases (49%) were female. The number of viable isolates per month for the study period is shown in Figure 1. Of the 85 human cases, 33 (39%) were from the Waikato area, 28 (33%) were from the Auckland area, 5 (6%) each were from the Bay of Plenty and Otago, and the remaining 14 cases were spread throughout the rest of the country. For the years 1990–4, 43·5% of all human salmonella isolates referred to the National Microbiology Reference Laboratory were from the Auckland/Waikato area. The male:female ratio for all salmonellosis cases notified in New Zealand in the years 1993 and 1994 was 48:52, and the mean and median ages for these cases were 22·3 and 20 years respectively.

The highest numbers of human cases of *S.* Brandenburg were detected during the summer months of December, January, February and March; but this peak did not occur every year.

The 30 non-human isolates studied comprised: meat and/or bone meal (7); porcine (6); dairy environment (5); poultry including environment (4); environment, unspecified (3); bovine (2); canine, caprine and food (1 each).

Antimicrobial resistance patterns

Resistance was noted only in three isolates. An isolate from a human case from 1990, for whom there was no clinical information, was multiply resistant to spectinomycin (MIC > 128 mg/l), streptomycin (MIC 32 mg/l), sulphamethoxazole (MIC 512 mg/l) and tetracycline (MIC 128 mg/l). This resistance pattern was designated R1. A porcine isolate (R2) from 1993 was resistant to the same four antibiotics with the same MIC results and was also resistant to chloramphenicol (MIC 16 mg/l). A porcine isolate (R3) from 1994 was resistant to streptomycin (MIC

https://doi.org/10.1017/S0950268898008887 Published online by Cambridge University Press

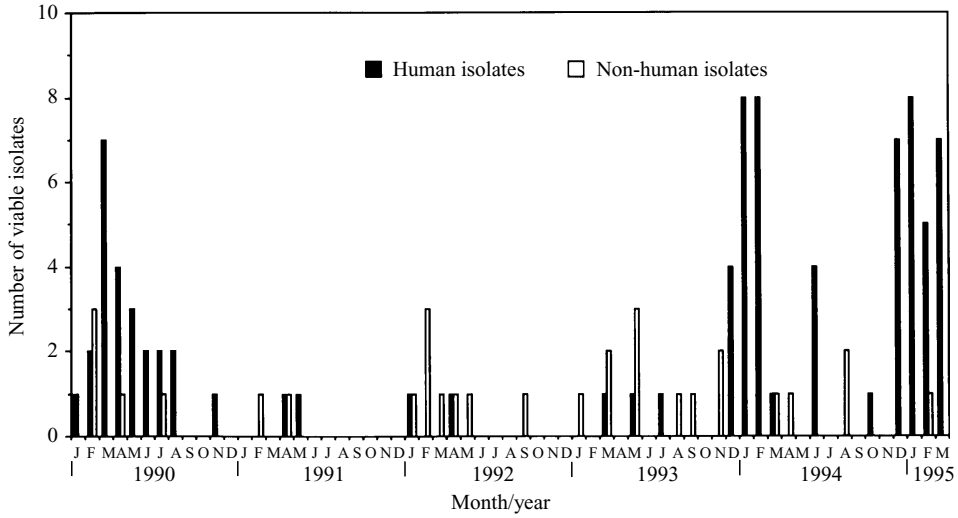

Fig 1. *Salmonella* Brandenburg by month, 1990–5.

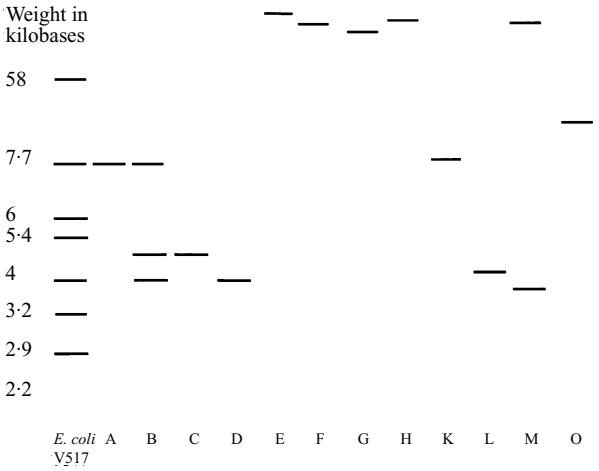

Fig. 2. Plasmid profiles of *S.* Brandenburg isolates.

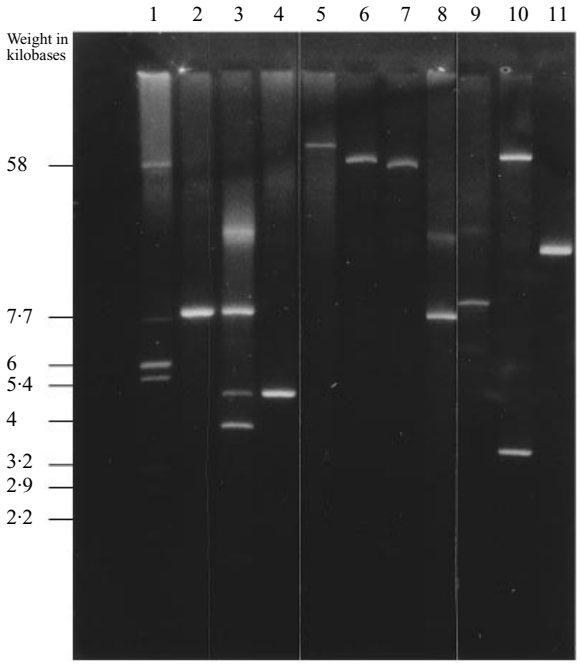

Fig. 3. Electrophoresis results for some *S.* Brandenburg plasmid profiles. Lane 1, *E. coli* V517; Lane 2, profile A; Lane 3, profile B; Lane 4, profile C; Lane 5, profile E; Lane 6, profile F; Lane 7, profile G; Lane 8, profile A; Lane 9, Profile K; Lane 10, profile M; Lane 11, profile O.

32 mg/l) and sulphamethoxazole (MIC 512 mg/l). The remaining 112 isolates exhibited no resistance to any of the 12 antibiotics tested and were designated R0.

Plasmid analysis

Plasmids of varying sizes were found in 31/115 (27%) of isolates. Plasmid profiles were designated types A–H and K–O. Type N was the designation for no plasmids detected. The profiles are diagrammatically represented in Figure 2, and the actual electrophoresis patterns of some types are shown in Figure 3. Excepting type N (84), type A was the most frequently detected (7), followed by type F (6), type B (4), types C and E (3 each) and type D (2). There was one isolate each of the types G, H, K, L, M and O. The profile patterns were reproducible on repeat testing.

MRFLP analysis

MRFLP analysis of 115 isolates resulted in 13 different *Xba*I restriction profiles. These profiles were

https://doi.org/10.1017/S0950268898008887 Published online by Cambridge University Press

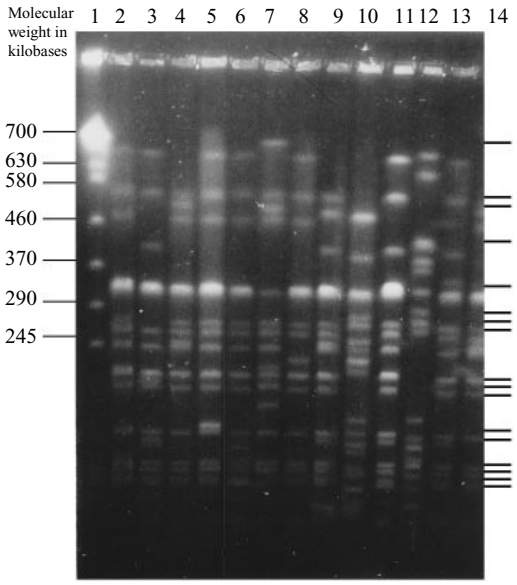

Fig. 4. *Xba*I restriction profiles for *S*. Brandenburg isolates. Lane 1, yeast molecular weight marker; Lane 2, type 1; Lane 3, type 2; Lane 4, type 3; Lane 5, type 4; Lane 6, type 5; Lane 7, type 6; Lane 8, type 7; Lane 9, type 8; Lane 10, type 9; Lane 11, type 10; Lane 12, type 11; Lane 13, type 12; Lane 14 (diagrammatic representation), type 13.

designated types 1–13 and are depicted in Figure 4. The most frequently detected profile was type 1 (82 isolates), followed by type 3 (16 isolates), and types 2, 4 and 10 (3 isolates each). The remaining types each included one isolate only. The profiles were reproducible on repeat testing.

Composite types

For each of the three methods used in this study, the majority of isolates conformed to one type: 97% did not exhibit antimicrobial resistance (R0); plasmids were not detected in 73% (N); and 71% were MRFLP type 1. Combining the three sets of typing data yielded 24 composite types. Composite type 1 N R0 included 71 isolates (62%) and was found throughout the 5 years studied. The number and distribution of composite types are shown in Table 2.

Clusters

Potential clusters of the same composite type detected within a limited time period and geographical area included: four cases of 1 N R0 from the Waikato area, June–August 1990; 15 cases of 1 N R0 in the Waikato area, December 1993–February 1994; 11 cases of 1 N R0 in the Waikato/Auckland area, December 1994–January 1995 (two of which were bacteraemic); three cases of 3 A R0 from the Waikato area February–March 1995 (one of which was bacteraemic); four cases of 3 B R0 from the Waikato/Bay of Plenty area in March 1995; three cases of 4 E R0 from the Waikato/Hawkes Bay area in February 1994; two cases of 10 N R0 from the Canterbury area March–April 1990.

DISCUSSION

S. Brandenburg is relatively uncommon in New Zealand, but during 1994 and 1995, the incidence increased. This serotype infects all age groups and, although spread throughout New Zealand, cases are predominantly located in the central and upper North Island. This predominance is greater than for all Salmonellae. For the years 1990–4, 43·5% of all salmonella isolates were referred from the Auckland/Waikato area, compared with 72% of *S*. Brandenburg. The male/female ratio of *S*. Brandenburg cases is similar to all salmonellosis cases notified in New Zealand in the years 1993 and 1994 (49:51, versus 48:52). However, the mean and median ages of *S*. Brandenburg cases (27·3 and 26 years respectively) are slightly higher than for all salmonellosis (22·3 and 20 years respectively).

Antimicrobial resistance is uncommon among New Zealand isolates of salmonella [15], and the results reported here indicate that *S*. Brandenburg is no exception. Two of the three resistant strains were from porcine sources, indicating that pigs have the potential to carry, and therefore transmit, antimicrobial resistant *S*. Brandenburg.

Plasmid carriage was detected in 27% of New Zealand *S*. Brandenburg strains. Serotype-specific plasmids have been reported for seven *Salmonella* serotypes [16], but there are few records of plasmid carriage by *S*. Brandenburg. A Swiss study of 32 *S*. Brandenburg isolates found only two were plasmid-bearing and both exhibited antimicrobial resistance [6]. Plasmid carriage in New Zealand strains did not correlate with antimicrobial resistance. The variety of plasmids detected in the New Zealand *S*. Brandenburg strains indicates that although some plasmids of a similar size were detectable across several isolates, none was found throughout the sample population and therefore no type specific plasmid could be defined. In *S*. Typhimurium, serotype-specific plasmid carriage has been associated with increased virulence and bacteraemia [17]. This does not appear to be the case with New Zealand *S*. Brandenburg bacteraemia

Table 2. *Number and distribution of composite types of* S. *Brandenburg*

Composite type	Number (%)	Isolation dates	Geographical location of human cases*	Source
1 N R0	71 (61·7%)	Jan. 90–Mar. 95	WK (26), AK (17), CB/SC (3), Wn (1), BP (2), OT/SO (4), NM (1), TK (1)	Human, porcine, poultry, meat/bone meal, environment, bovine, caprine
3 A R0	7 (6·1%)	Mar. 93–Mar. 95	OT (2), WK (3), CB (1)	Human, canine
1 F R0	6 (5·2%)	Feb. 90–Dec. 94	AK (4)	Human, porcine, dairy, environment
3 B R0	4 (3·5%)	Mar. 95	WK (2), BP (2)	Human
3 C R0	3 (2·6%)	Feb.–Apr. 90		Environment
4 E R0	3	Feb. 94	WK (2), HB (1)	Human
2 N R0	3	Nov. 93–Dec. 94	AK (1)	Meat/bone meal, food, human
10 N R0	2 (1·7%)	Mar.–Apr. 90	CB (2)	Human
1 G R0	1 (0·9%)	Mar. 94	WK	Human
1 H R0	1	Apr. 90	OT	Human
1 K R3	1	Aug. 94		Porcine
1 L R0	1	Jun. 94	AK	Human
1 N R1	1	Nov. 90	MW	Human
3 D R0	1	Feb. 95	BP	Human
3 N R0	1	Sep. 93		Animal food
5 M R2	1	Aug. 93		Porcine
6 N R0	1	Sep. 92		Bovine
7 N R0	1	Jan. 92	AK	Human
8 N R0	1	Jan. 92		Dairy environment
9 O R0	1	Feb. 90	AK	Human
10 D R0	1	Mar. 90	AK	Human
11 N R0	1	Apr. 90	AK	Human
12 N R0	1	Feb. 95	AK	Human
13 N R0	1	Feb. 92		Environmental

* AK, Auckland; WK, Waikato; BP, Bay of Plenty; OT, Otago; SO, Southland; CB, Canterbury; SC, South Canterbury; NM, Nelson/Marlborough; MW, Manawatu; TK, Taranaki; WN, Wellington.

isolates as plasmids were not detectable in two of the three blood culture isolates. However, the numbers are small, thus making generalizations difficult. Plasmid profiling has been used successfully to discriminate within some *Salmonella* serotypes [18], but the usefulness of this technique depends on the number and variety of plasmids occurring within the sample group. In this instance, plasmid profiling has allowed some discrimination; but, as the majority of isolates did not carry plasmids, this method is of limited value as a sole tool for additional discrimination within New Zealand isolates of *Salmonella* Brandenburg.

MRFLP allowed the recognition of 13 genotypes. A Swiss study used MRFLP to discriminate *S.* Brandenburg isolates and designated 10 types, some of which were country-specific [6]. Overseas travel was not recorded on the laboratory request forms for any of the New Zealand cases, indicating that all 13 genotypes may be New Zealand-derived. Unfortunately, although the Swiss investigators used *Xba*1 in their MRFLP studies, the pulse time used was different from that used here and the New Zealand and Swiss profiles are therefore not readily comparable.

In isolation, each of the three methods provided inadequate discrimination of strains for epidemiological purposes. However, when the results of the three methods were used together, 24 composite types were defined and greater discrimination was possible. More than 60% of the isolates belonged to type 1 N R0, indicating that this is a dominant clonal type which was isolated throughout the study period. Thus linkages between cases of 1 N R0 cannot be confirmed by laboratory testing alone, as apparent clusters may occur by chance. For this reason, clusters suggested here can only be 'potential'. Other strains which were genomically different and/or plasmid bearing emerged during the period studied. It would appear that some genomic types are more likely to carry plasmids. Of

the 16 strains identified as MRFLP type 3, 15 were found to carry one or more plasmids.

Composite type profiles show that the 3 strains isolated from blood cultures are indistinguishable from other strains isolated from diarrhoeal patients during the same time period. Thus it would appear that severity of illness in these three cases was not due to a newly emergent strain.

A number of non-human *S*. Brandenburg sources have been confirmed. Composite types of isolates from 24 (80 %) non-human sources were indistinguishable from human isolates, thus suggesting that human infection may be via a number of vehicles. This suggestion concurs with Swiss findings that multiple food sources acted as vehicles for an outbreak strain [6].

In conclusion, the use of multiple typing methods has allowed discrimination within an uncommon *Salmonella* serotype. The results have enabled retrospective detection of potential clusters, comparison of isolates from bacteraemic and diarrhoeal patients and comparison of isolates from human and non-human sources. The methods used for this study are not cost effective for routine use on salmonella isolates, but can provide an important adjunct to serotyping if a particular type appears to be emerging in the future.

ACKNOWLEDGEMENTS

This work was performed under contract to the Ministry of Health. We acknowledge their support for this study and thank them for granting permission to publish the findings.

The following laboratory workers made invaluable contributions to this study: Scott Beatson and Taryrn Shapcott (antimicrobial susceptibility testing), Maora Sweeney (genomic DNA extraction and data entry).

Yvonne Galloway provided information on New Zealand salmonellosis notifications, and Michael Baker, Carolyn Nicol, Gail Meekin, Helen Heffernan and Diana Martin provided helpful comments on the content of this report.

REFERENCES

1. Kauffmann F, Mitsui C. Zwei neue Paratyphustypen mit bisher unbekanntem Phasenwechsel. Z Hyg Infektionskr 1930; **111**: 740.
2. Rowe B. Salmonella surveillance: Reports received from Centres participating in the WHO programme, 1987. London: WHO Collaborating Centre for Phage Typing and Resistance of Enterobacteria, Central Public Health Laboratory, 1987.
3. Communicable Diseases in Scotland: Annual Summary of Salmonellosis, 1993. Glasgow; Scottish Centre for Infection and Environmental Health. ISSN 0264-1542.
4. Murray C, Davos D. Australian Salmonella Reference Centre monthly report – April 1995. Adelaide: Institute of Medical and Veterinary Science, 1995.
5. Jones HB, Farkas G, Ghosh A, Hobbs BC. *Salmonella brandenburg*: an epidemiological study. Monthly Bull Minist Health 1964; **23**: 162–77.
6. Baquar N, Burnene A, Stanley J. Comparative evaluation of molecular typing of strains from a national epidemic due to *Salmonella brandenburg* by rRNA gene and IS*200* probes and pulsed-field gel electrophoresis. J Clin Microbiol 1994; **32**: 1876–80.
7. Roberts FJ. Nontyphoidal, nonparatyphoidal Salmonella septicemia in adults. Eur J Clin Microbiol Infect Dis 1993; **12**: 205–8.
8. Chiovato L, Canale G, Maccerini D, Falcone V, Pacini F, Pinchera A. *Salmonella brandenburg*: a novel cause of acute suppurative thyroiditis. Acta Endocrinol 1993; **128**: 439–42.
9. National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: approved standard, 3rd ed. Villanova, Pennsylvania: NCCLS, 1993. NCCLS document M7-A3.
10. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial susceptibility testing; fifth informational supplement. Villanova, Pennsylvania: NCCLS, 1994. NCCLS document M100-S5.
11. Frost JA. Testing for resistance to antimicrobial drugs. In: Chart H, ed. Methods in practical laboratory bacteriology. Boca Raton, FL: CRC Press, 1994: 107–21.
12. Kado CI, Liu S-T. Rapid procedure for the detection and isolation of large and small plasmids. J Bacteriol 1981; **145**: 1365–73.
13. Macrina FL, Kopecko D, Jones KR, Ayers DJ, McCowen SM. A multiple plasmid containing *Escherichia coli* strain: convenient source of size reference plasmid molecules. Plasmid 1978; **1**: 417–20.
14. Bohm H, Karch H. DNA fingerprinting of *Escherichia coli* O157:H7 strains by pulsed field gel electrophoresis. J Clin Microbiol 1992; **30**: 2169–72.
15. Heffernan H. Antibiotic resistance among salmonella from human and other sources in New Zealand. Epidemiol Infect 1991; **106**: 17–23.
16. Helmuth R, Stephan R, Bunge C, Hoog B, Steinbeck A, Bulling E. Epidemiology of virulence-associated plasmids and outer membrane proteins within seven common *Salmonella* serotypes. Infect Immun 1985; **48**: 175–82.
17. Fierer J, Krause M, Tauxe R, Guiney D. *Salmonella typhimurium* bacteremia: association with the virulence plasmid. J Infect Dis 1992; **166**: 639–42.
18. Trefall EJ, Powell NG, Rowe B. Differentiation of salmonellas by molecular methods. PHLS Microbiol Dig 1994; **11**: 199–202.