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The distribution of specific phage types of *Salmonella typhimurium* in chickens in Australia

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SUMMARY

The distribution of specific phage types of *Salmonella typhimurium* within the Australian chicken industry has been studied and documented on an Australia-wide and state-by-state basis. A total of 1799 strains of *S. typhimurium* were obtained from Australia-wide sources and phage typing categorized 1498 of these isolates into 30 distinct phage types, with the remaining 301 strains untypable. Five phage types, 6, 26, 31, 135 and 179, accounted for 76% of the total strains typed, with the remaining 24% of strains being distributed among 25 phage types.

Of the major phage types, type 31 was restricted to Victoria and Western Australia, but the other types were distributed throughout Australia.

In addition, the antibiotic resistance pattern of the various phage types was determined and only five of the 30 phage types showed appreciable levels of resistance.

INTRODUCTION

The chicken meat industry has been developed on intensive rearing programmes and large-scale marketing of products. It relies heavily on consumer confidence in the marketed product and so is extremely sensitive to disease outbreaks in the human population where processed chicken meat is thought to be the source of the disease. One of the most common causes of foodborne infection in man where chicken meat is often implicated is salmonellosis. The importance of poultry meat as a source of *Salmonella* spp. should not be underestimated, in the light of the study in England by Vernon & Tillett (1974), who found that 66 (52%) of 127 general and family outbreaks of human salmonellosis for which a source could be established were attributable to poultry and poultry products.

In Australia, as in most other countries, the most common cause of salmonellosis in man is *Salmonella typhimurium* (Beaton & Taplin, 1981; Barker, Old & Sharp, 1980). A knowledge of the distribution of specific strains of *S. typhimurium* in the chicken community would be of value in understanding the epidemiology of *S. typhimurium* in chickens in Australia and may give an indication as to the role of chicken meat in transfer of salmonellosis to man.

There are now adequate techniques for differentiating *S. typhimurium* strains on the basis of their susceptibility to specific bacteriophages. This technique,

reported by Felix (1956) and Callow (1959), and extended by Anderson *et al.* (1977), can now distinguish 207 definitive phage types of *S. typhimurium*.

This study was initiated to investigate the distribution of specific phage types of *S. typhimurium* in commercial chickens in Australia, and in addition to investigate the antibiotic resistance status of these *S. typhimurium* isolates.

MATERIALS AND METHODS

Cultures

Isolates of *S. typhimurium*, totalling 1799, were either isolated at the Veterinary Research Institute, Parkville from chicken post-mortem material or were forwarded from state veterinary and public health laboratories, or from other laboratories servicing the chicken industry. Isolates were received in the period 1976–81 from all Australian mainland states, New South Wales (779), Victoria (646), Western Australia (206), Queensland (111) and South Australia (57). The isolates were from processed chickens (690), chicken caeca (618), cloacal swabs (64), chicken viscera (42), chicken litter (351), factory swabs (6), chicken feed (6) and unspecified sites (22).

Phage typing

A tube containing 3·5 ml of double-strength nutrient broth was inoculated with the culture to be phage typed and incubated in a shaking water bath at 37 °C for 90 min. This inoculum was flooded on to duplicate double-strength nutrient agar plates (5 mm thick), allowed to absorb into the medium and then the phages at routine test dilution were applied to the test plate using a multi-loop inoculator (Biddulph & Co., Manchester, England). The phage inoculum was allowed to absorb into the plate and the plate incubated overnight at 37·5 °C.

The phage type designations are the definitive types of Anderson *et al.* (1977). Isolates which were resistant to all typing phages were designated as untypable, while isolates which reacted with some typing phages but gave patterns which did not conform with any of the definitive types were called RDNC.

Antibiotic sensitivity testing

The antibiotic sensitivity patterns of all isolates were determined using the Oxoid multodisc diffusion technique. Isolates were tested for resistance to ampicillin (10 μg), streptomycin (25 μg), tetracycline (10 μg), chloramphenicol (10 μg), sulphafurazole (100 μg), trimethoprim (1·25 μg), kanamycin (30 μg) and naladixic acid (30 μg).

RESULTS

Distribution of S. typhimurium *phage types Australia-wide and state-by-state*

The distribution of phage types of *S. typhimurium* isolated from chickens and chicken material has been determined for Australia and for individual Australian states (Table 1). Of 1799 strains analysed from Australia-wide sources 1498 were successfully phage typed and divided into 30 types. The commonest types, representing greater than 5% of the total, were 6, 26, 31, 135 and 179.

Table 1. *The distribution of phage types of* Salmonella typhimurium *isolated from chickens in Australia*, 1976–1981

	Number of cultures isolated					
		State-by-state distribution				
Phage type	Australia (n = 1779)*	N.S.W.† (n = 779)	Vic. (n = 646)	W.A. (n = 206)	Qld (n = 111)	S.A. (n = 57)
4	1	—	—	—	1	—
6	113	74	20	16	1	2
8	2	2	—	—	—	—
9	18	5	9	1	—	3
12	4	1	3	—	—	—
12a	51	26	12	4	9	—
16	3	—	3	—	—	—
22	6	—	1	2	3	—
23	6	4	—	2	—	—
25	18	2	6	10	—	—
26	126	86	9	28	—	3
27	9	4	1	—	4	—
29	1	—	1	—	—	—
31	215	—	191	24	—	—
35	1	—	1	—	—	—
55	5	3	2	—	—	—
90	2	—	1	1	—	—
101	36	32	1	3	—	—
108	18	4	—	—	14	—
116	1	1	—	—	—	—
120	2	2	—	—	—	—
124	1	1	—	—	—	—
135	329	99	192	8	8	22
141	38	18	—	—	20	—
145	55	28	7	16	2	2
170	73	50	8	—	1	14
174	1	1	—	—	—	—
179	357	205	44	60	39	9
182	1	—	—	—	1	—
185	5	4	1	—	—	—
‡RDNC	26	14	7	3	1	1
Rough	22	18	2	1	1	—
Untypable	253	95	124	27	6	1

* Total number of strains analysed for the area.

† N.S.W., New South Wales; Vic., Victoria; W.A., Western Australia; Qld, Queensland; S.A., South Australia.

‡ RDNC, culture sensitive to some typing phages but does not conform to any recognized phage type.

The distribution of phage types within various states revealed significant variation. The most common strains (> 10%) in New South Wales were 179, 135 and 26; in Victoria, 135 and 31; in Western Australia 179, 26 and 31; in Queensland 179, 141 and 108; and in South Australia 135, 170 and 179.

Table 2. *Antibiotic resistance characteristics of selected phage types of* Salmonella typhimurium *isolated from chickens in Australia*

Phage type	Number of isolates	Percentage showing antibiotic resistance	Antibiotics* to which strains show resistance
6	113	71	†Tet (99), Strep (1)
26	126	6·3	Tet (100), Strep (12·5), Sul (12·5)
31	215	4·2	Tet (66), Amp (22), Sul (22), Strep (11)
101	36	72	Sul (96), Tet (4)
135	329	4·5	Tet (66), Sul (26), Strep (26), Amp (6)
145	55	56	Tet (100), Amp (10)
179	357	99	Amp (100), Sul (13), Tet (11), Strep (7)
185	5	100	Sul (100), Amp (60), Strep (60)

* Numbers in parentheses are the percentage of 'resistant' phage types resistant to the aforementioned antibiotic.

† Sul, Sulphonamide; Strep, Streptomycin; Amp, Ampicillin; Tet, Tetracycline.

Antibiotic resistance characteristics of S. typhimurium *phage types from Australia-wide sources*

The antibiotic resistance characteristics of all the phage types were determined. Two-thirds of the total strains showed no antibiotic resistance and in only five of the 30 phage types did more than 50% of the strains show antibiotic resistance. These five phage types were 179, 6, 145, 101 and 185. The resistance characteristics of these strains, along with those of the major phage types 135, 31 and 26 are shown in Table 2.

DISCUSSION

Analyses were performed on 1799 strains of *S. typhimurium* isolated from chicken products and derived from sources throughout Australia. Phage typing of the isolates categorized 1498 of the isolates into 30 distinct phage types, but the remaining 301 strains were either resistant to all typing phages (untypable) or did not conform to any definititive phage type (RDNC).

Of those strains successfully typed, phage types 179 and 135 each accounted for 24% and 22% respectively of the typed strains and, in conjuction with three other phage types, 31, 26 and 6, accounted for 76% of the total strains typed. The remaining 24% were distributed among 25 phage types with 17 of those phage types accounting for only 3·4% of the typable isolates. This grouping of phage types at these percentages appears to be unique to the Australian chicken community. Anderson *et al.* (1978), when studying a series of 2092 cultures of *S. typhimurium* from human, animal and other sources from 57 countries, found that those phage types that we have found as common in chickens were also present in varying percentages in his study, with the exception of phage type 26, which he did not isolate. Indeed, phage type 135 was present at 4% and this he cited as a common type, whereas phage type 179 was represented by only a single isolate. Our results confirm that there is a small nucleus of *S. typhimurium* phage types that are widely spread within chickens in Australia and goes some way to confirm the hypothesis of Anderson (1971) that in any country the association of strains of a particular phage type is a well-established clone within that host. The five major phage types

are excellent examples of this phenomenon, with close association being illustrated on either an Australia-wide or a state-wide basis, with phage types 179, 135, 26, and 6 being widely distributed throughout Australia, but phage type 31 being restricted to Victoria and Western Australia.

A detailed knowledge of the particular phage types of *S. typhimurium* commonly found in chickens would be useful to public health personnel endeavouring to identify sources of gastroenteritis in humans caused by *S. typhimurium*. This information may help to provisionally clear, or alternatively, incriminate chicken meat as the possible source of these outbreaks.

All of the major phage types of *S. typhimurium* isolated from chickens have been recorded as causing gastroenteritis in humans. However, many of the other strains of *S. typhimurium* isolated from human sources are different phage types from those that have been isolated from chicken sources during this survey (Anon, 1980).

The picture of antibiotic resistance within specific phage types demonstrates that some phage types appear to contain consistent antibiotic resistance characters. Ampicillin resistance appears to be a consistent character of phage type 179, with 354 of 357 strains having this feature. However, the absence of ampicillin resistance in most other *S. typhimurium* phage types suggests that this resistance capacity is not readily transferred to other *Salmonella* spp.

There is evidence that the transmission of antibiotic resistance plasmids within strains leads to changes in the phage type of the recipient strain (Anderson, 1966; Anderson *et al.* 1973; Lamont, 1976) and so care must be taken in analysing the distribution of phage types of *S. typhimurium* when strains possess specific antibiotic resistance characters. Fortunately, we have found the level of antibiotic resistance in *S. typhimurium* to be low and so the chance of resistance transfer within *Salmonella* spp. is also probably quite low. Whilst there is no evidence to suggest extensive plasmid transfer of antibiotic resistance within the chicken salmonella population, the potential remains for the development and spread of resistant strains along the lines outlined by Threlfall, Ward & Rowe (1978). It does appear, however, that the acquisition of resistance plasmids does not change the virulence of the recipient organism and only gives it a selective advantage in the presence of the corresponding antibiotic (Smith & Tucker, 1979). Threlfall, Ward & Rowe (1978) have postulated that the selective pressures to produce multi-resistant strains of *S. typhimurium* in cattle in Britain have been due to the use of anti-bacterial drugs to treat bovine salmonellosis. Consequently, the use of high levels of anti-bacterial drugs to control disease in chickens needs to be carefully monitored to ensure that such a selective development of multi-resistant strains does not evolve.

The widespread resistance to tetracycline of a number of phage types may indicate that tetracycline resistance is plasmid borne. This same phenomenon may also apply to sulphonamide resistance, since virtually all of the strains that showed any multiple resistance to antibiotics included resistance to tetracycline or sulphonamides or both. More work is necessary to determine if this potential transfer of resistance plasmids between *Salmonella* spp. or from *Salmonella* spp. to other enteric organisms may pose a threat to the Australian chicken industry, or be a source of multiple-resistant salmonella that could be transferred to the human population.

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