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Plasmid studies of *Salmonella typhimurium* phage type 179 resistant to ampicillin, tetracycline, sulphonamides and trimethoprim

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SUMMARY

Sixteen strains of *Salmonella typhimurium* phage type 179 were referred to the National Health Institute, Wellington, New Zealand, from 1977 to 1979. This phage type had not been observed here before 1977. All strains were resistant to ampicillin, several were also resistant to tetracycline, and several were resistant to ampicillin, tetracycline, sulphafurazole and trimethoprim. All resistances could be transferred to *Escherichia coli* K 12. Plasmids from these strains and their transconjugants were characterized by agarose gel electrophoresis. It appears that resistance to sulphafurazole and trimethoprim is carried on a plasmid with a molecular weight of 5·2 Mdal and that resistance to ampicillin and tetracycline is carried on a plasmid with a molecular weight of approximately 60 Mdal.

INTRODUCTION

During the period 1977–79, 16 strains of *Salmonella typhimurium* phage type 179 were referred to the National Health Institute, Wellington, New Zealand, the national reference centre for *Salmonella*. These were from human sources and were all found to be resistant to ampicillin. Four were also resistant to tetracycline, and eight were resistant to ampicillin, tetracycline, sulphafurazole and trimethoprim. No other strains of phage type 179 have been referred to the National Health Institute. Only one other trimethoprim-resistant *S. typhimurium* has been received here. This was a phage type 122 *S. typhimurium* isolated in 1977 that was resistant to ampicillin, tetracycline, chloramphenicol, kanamycin, streptomycin and sulphafurazole as well as to trimethoprim.

This report describes the isolation and characterization of plasmids contained in strains of *S. typhimurium* phage type 179 isolated in New Zealand.

MATERIALS AND METHODS

Of the 16 strains of *S. typhimurium* phage type 179 received at the National Health Institute two have since died. All were isolated from human faeces. Two were received from each of two people and three of the remaining isolates were from members of the same family. The rest were from apparently unrelated sources.

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Table 1. Salmonella typhimurium *phage type* 179 *strains used in this study*

Number	Date of isolation	Place of isolation	Antimicrobial resistances	Molecular weight of plasmids present (Mdal)
1123	July 1977	Auckland	ApTc	60, 2·3, 2·1
1162	July 1977	Auckland	ApTc	60, 2·3, 2·1
1163	July 1977	Auckland	ApTc	60, 2·3, 2·1
600*	September 1977	Auckland	Ap	60
2194*	September 1977	Christchurch	Ap	60
2932	December 1977	Rotorua	Ap	60
435†	April 1978	Auckland	ApTcSuTp	60, 5·2, 2·1, 1·3
456†	May 1978	Auckland	ApTcSuTp	60, 5·2, 2·1, 1·3
647‡	August 1978	Whangarei	ApTcSuTp	60, 5·2, 2·1, 1·3
666	September 1978	Auckland	ApTcSuTp	60, 8·7, 5·2, 2·1, 1·3
850§	October 1978	Auckland	ApTcSuTp	60, 5·2, 2·1, 1·3
851§	November 1978	Auckland	ApTcSuTp	60, 5·2, 2·1, 1·3
853§	November 1978	Auckland	ApTcSuTp	60, 5·2, 2·1, 1·3
512‖	July 1979	Ashburton	ApTc	60, 2·3, 2·1, 1·4, 1·3

* Cultures 600 and 2194 were isolated from the same patient who travelled from Auckland to Christchurch between isolations.

† Cultures 435 and 456 were from the same patient.

‡ Patient had recently visited Fiji.

§ Cultures 850, 851 and 853 were from members of the same family.

‖ Patient had recently visited South East Asia.

Two patients had recently been overseas before becoming ill: one to Fiji and the other to South-East Asia. The strains used in this study are listed in Table 1. The type strain for phage type 179, which was obtained from the Hospital Cross Infection Laboratory at the National Health Institute, was found to be fully sensitive to all antimicrobials used in the study.

Minimum Inhibitory Concentrations (MIC's) for *S. typhimurium* phage type 179 strains and representative *E. coli* transconjugants were determined for ampicillin (Ap), tetracycline (Tc), chloramphenicol (Cm), cephalothin (Cn), gentamicin (Gm), kanamycin (Km), streptomycin (Sm), sulphafurazole (Su) and trimethoprim (Tp) using an agar dilution method (Ericsson & Sherris, 1971). The abbreviations for antimicrobials given above will be used throughout the text.

Plasmids were transferred from *Salmonella* to *Escherichia coli* K 12 by conjugation with a nalidixic acid-resistant derivative of J5-3 (F^- lac^+ Nx^r) using a method based on that of Schroeder, Terry & Bennett (1968), in which 0·1 ml of overnight donor culture was mixed into a fresh 5 ml of broth (Oxoid Nutrient No. 2) and incubated without shaking for 4 h. Of this donor culture 0·1 ml was then mixed with another 5 ml of broth along with 1 ml of an overnight recipient culture. The mixture was incubated overnight, mixed using a vortex stirrer to break up conjugating pairs, then diluted in saline 1/250 for Ap and ApTc resistant *Salmonella* donors or 1/50 for ApTcSuTp-resistant *Salmonella* donors. The diluted culture (0·2 ml) was then spread on a set of three agar plates, one containing nalidixic acid (20 μg/ml): one containing nalidixic acid (20 μg/ml) and an appropiate antibiotic and one containing no antibiotics. Identical sets of plates were inoculated with an appropriate dilution of overnight cultures of donors and recipient alone

Table. 2 *Resistance transfer from* S. typhimurium *phage type* 179 *strains to* E. coli *K*12

S. typhimurium donor	Donor resistance	Resistances transferred to *E. coli*
600	Ap	Ap
2194	Ap	Ap
2932	Ap	Ap
1123	ApTc	Ap, ApTc
1162	ApTc	ApTc
1163	ApTc	Ap, ApTc
512	ApTc	ApTc
435	ApTcSuTp	ApTc, SuTp, ApTcSuTp
647	ApTcSuTp	ApTc, ApTcSuTp
666	ApTcSuTp	ApTc, SuTp, ApTcSuTp
851	ApTcSuTp	ApTc, SuTp, ApTcSuTp

as a control. For transfer experiments using sulphafurazole and trimethoprim, Wellcotest Agar was used and transconjugants were later checked for lactose fermentation on MacConkey agar. For experiments with other antibiotics MacConkey agar was used in the initial selection plates. The *Salmonella* donor strains had previously been tested and were all found to be sensitive to nalidixic acid and lactose negative. Antibiotic concentrations used in selection plates were – ampicillin 10 μg/ml, tetracycline 10 μg/ml, sulphafurazole 256 μg/ml and trimethoprim 8 μg/ml. Single colonies of transconjugants were plated out on Trypticase Soy Agar and antimicrobial susceptibilities were checked using a disk method (Stokes & Waterworth, 1972).

Plasmid DNA was prepared by the method of Meyers *et al.* (1976). Agarose gel electrophoresis was carried out in a horizontal gel apparatus, similar to that described by McDonell, Simon & Studier (1977), for 4 h at 130 V in 0·7% (w/v) agarose (Sigma A-6877) in borate buffer (Meyers *et al.* 1976). After electrophoresis, gels were stained in ethidium bromide, placed on a shortwave ultraviolet transilluminator screen (Ultraviolet Products Inc., San Gabriel, California 91778) and photographed. Red and yellow filters were attached to the camera and Kodak Tri-X 135 film, ASA 400, at f 2·8 with a 1 s, exposure was used. The relative mobility of plasmid bands was measured from the developed negative using an Autodata Zone Reader (Autodata Scientific Ltd., England) comprising a projector with a measuring attachment linked to a display and print-out. Molecular weights of plasmids were calculated by comparison of their electrophoretic mobilities with the mobilities of plasmids of known molecular weight (Plate 1).

RESULTS

Resistances to ampicillin, tetracycline, sulphafurazole and trimethoprim could all be transferred from the *Salmonella* strains to *E. coli* K 12 (Table 2). With ApTcSuTp resistant *S. typhimurium* the Su and Tp resistances and the Ap and Tc resistances always transferred together. With *S. typhimurium* resistant to Ap and

Table 3. *MIC's of donor* S. typhimurium, *recipient* E. coli *and transconjugant* E. coli

Donors, recipient and transconjugants	Antimicrobial resistance	Range of MIC's (μg/ml)*								
		Ap	Tc	Cm	Cn	Gm	Km	Sm	Su	Tp
Recipient *E. coli*	Nalidixic acid	2	2	4	4	0·25	1	< 1	8	0·25
S. typhimurium	Ap	> 128	1–2	4	4 8	0·25–1	1–2	4–8	32	< 0·12
E coli transconjugants	Ap	> 128	1–2	8	4	0·25	0·5–1	< 1	4–8	0·25
S. typhimurium	ApTc	> 128	> 128	4–8	8	0·5	2–4	4–8	16–32	< 0·12
E. coli transconjugants	ApTc	> 128	64– < 128	8	4	0·12–0·25	0·5–1	< 1	4–8	0·25
S. typhimurium	ApTcSuTp	> 128	> 128	4	8	0·5–1	2–4	8–16	> 1024	64
E. coli transconjugants	ApTcSuTp	> 128	64– > 128	8	4	0·12–0·25	0·5–1	< 1	> 1024	64
E. coli transconjugants	SuTp	2	1–2	8	2–4	0·25	0·5–1	< 1	> 1024	64

* Abbreviations: Ap, Ampicillin; Tc, Tetracycline; Cm, Chloramphenicol; Cn, Cephalothin; Gm, Gentamicin; Km, Kanamycin; Sm, Streptomycin; Su, Sulphafurazole; Tp, Trimethoprim.

Table 4. *Molecular weight of plasmids found in donors and transconjugants*

Donors and transconjugants		Antimicrobial resistance	Molecular weight of plasmids present (Mdal)
S. typhimurium	600	Ap	60
E. coli	600	Ap	60
S. typhimurium	2194	Ap	60
E. coli	2194	Ap	60
S. typhimurium	2932	Ap	60
E. coli	2932	Ap	60
S. typhimurium	1123	ApTc	60, 2·3, 2·1
E. coli	1123	ApTc	60
E. coli	1123	Ap	60
S. typhimurium	1162	ApTc	60
E. coli	1162	ApTc	60
S. typhimurium	1163	ApTc	60, 2·3, 2·1
E. coli	1163	ApTc	60
E. coli	1163	Ap	60
S. typhimurium	512	ApTc	60, 2·3, 2·1, 1·4, 1·3
E. coli	512	ApTc	60, 2·1
S. typhimurium	435	ApTcSuTp	60, 5·2, 2·1, 1·3
E. coli	435	ApTcSuTp	60, 5·2, 2·1
E. coli	435	ApTc	60
E. coli	435	SuTp	5·2, 2·1
S. typhimurium	647	ApTcSuTp	60, 5·2, 2·1, 1·3
E. coli	647	ApTcSuTp	60, 5·2, 2·1
E. coli	647	ApTc	60, 2·1
S. typhimurium	666	ApTcSuTp	60, 8·7, 5·2, 2·1, 1·3
E. coli	666	ApTcSuTp	60, 8·7, 5·2
E. coli	666	ApTc	60
E. coli	666	SuTp	8·7, 5·2, 2·1
S. typhimurium	851	ApTcSuTp	60, 5·2, 2·1, 1·3
E. coli	851	ApTcSuTp	60, 5·2, 2·1
E. coli	851	ApTc	60
E. coli	851	SuTp	5·2, 2·1

Tc only, most transconjugants were found to be ApTc resistant. None were resistant to Tc only, but 2 out of 41 transconjugants selected with ampicillin were resistant to Ap only.

The ranges of MIC observed in the various groups of *S. typhimurium* strains, *E. coli* transconjugants and the *E. coli* recipient are shown in Table 3.

Molecular weights of plasmids contained by the *S. typhimurium* strains and their various *E. coli* transconjugants, as calculated from gel electrophoresis results, are given in Table 4. A typical agarose gel of the electrophoresis of cleared lysates is shown in Plate 1. From the results it can be seen that all Ap and ApTc resistant strains and transconjugants have in common a plasmid band corresponding to approximately 60×10^6 daltons (60 Mdal). Those strains and transconjugants which are ApTcSuTp-resistant have in common the 60 Mdal band and an additional 5·2 Mdal plasmid band.

Those transconjugants which are SuTp resistant only do not contain a 60 Mdal plasmid but contain the 5·2 Mdal plasmid and a smaller 2·1 Mdal plasmid. However, the 2·1 Mdal plasmid is also present in some strains which are ApTc resistant only and is absent from some transconjugants that are ApTcSuTp-resistant and thus is not consistent with any particular resistance pattern. Plasmids of 1·3, 1·4 and 2·3 Mdal found in some of the *S. typhimurium* strains were not transferred to any of the *E. coli* transconjugants examined.

It was noted that *S. typhimurium* strain 666 showed powerful inhibition of the *E. coli* K 12 recipient. In comparison with other strains, approximately tenfold less transconjugants were observed. When the recipient was swabbed on a MacConkey agar plate to form a lawn and *Salmonella* strain 666 streaked across it, a definite zone of inhibition of the *E. coli* was observed. Strain 666, as well as some of its transconjugants, was also found to contain a plasmid corresponding to 8·7 Mdal not observed in other strains (Plate 1, Table 4).

DISCUSSION

S. typhimurium phage type 179 is the most common phage type isolated in Australia (Australia Communicable Diseases Intelligence Bulletin, 1979), but remains rare in New Zealand despite extensive travel between the two countries. In Australia the type was found first in dressed poultry, then in humans. Strains isolated there are ampicillin-resistant and often resistant to tetracycline (J. Taplin, personal communication); thus the appearance in New Zealand of phage type 179 resistant to ampicillin and tetracycline is not altogether surprising. More unexpected was the appearance of phage type 179 *S. typhimurium* resistant to sulphonamides and trimethoprim in addition to ampicillin and tetracycline. In April 1978, trimethoprim resistance, linked with resistance to sulphonamides, appeared for the first time in New Zealand *Shigella* strains, the strains being received initially from Auckland. The first *S. typhimurium* phage type 179 resistant to trimethoprim and sulphonamides was also isolated from Auckland in April 1978, suggesting that the introduction of a trimethoprim resistance marker into the local genetic pool must have occurred about this time.

The MIC for trimethoprim in the trimethoprim-resistant *Salmonella* used in the present study was 64 μg per ml. This is low in comparison with that of transferable trimethoprim resistance observed previously (Datta & Hedges, 1972; Finlayson & Jackson, 1978), where the MIC of trimethoprim was greater than 1000 μg per ml. Nevertheless, trimethoprim resistance was able to be transferred from the *Salmonella* strains to *E. coli* K 12 and it appears that it is carried, along with sulphonamide resistance, on a 5·2 Mdal plasmid. Datta & Hedges (1972) have observed linked sulphonamide and trimethoprim resistance to be carried on a plasmid belonging to compatibility group W in enterobacteria causing urinary tract infection.

Resistance to ampicillin in the strains studied here is carried on a plasmid with a molecular weight of approximately 60 Mdal. Plasmid studies of strains and transconjugants resistant to ampicillin and tetracycline showed that they had in

common a plasmid also of molecular weight 60 Mdal. In plasmid transfer studies with ApTc-resistant strains (Table 2), no transconjugants were obtained that were resistant to tetracycline alone and only two out of 41 transconjugants were resistant to ampicillin alone. These two transconjugants also contained a plasmid with a molecular weight of approximately 60 Mdal. Thus it would appear that the ampicillin and tetracycline resistances are carried on the same plasmid of approximately 60 Mdal. The two transconjugants resistant to ampicillin alone probably contain the same plasmid, from which the tetracycline resistance genes have been deleted.

The loss of this length of DNA might only produce an insignificant change in the relative mobility of the plasmid on electrophoresis because of the logarithmic nature of the relation between mobility and molecular weight. Loss of the tetracycline resistance marker from plasmids has been observed previously (Foster, 1975). Foster, Howe & Richmond (1975) have suggested that this predisposition of the tetracycline transposon towards translocation and deletion may be explained by the presence of two sequences of DNA on either side of the transposon that are inverted repetitions, the two sequences being the insertion sequence IS 3.

The observation that no transconjugants resistant to tetracycline alone were found negates the possibility of there being two plasmids in ApTc resistant *Salmonella* – one bearing genes for resistance to ampicillin and the other genes for resistance to tetracycline.

The inhibition observed between *S. typhimurium* 666 and *E. coli* K 12 may be related to incompatibility phenomena. Such effects between strains of *S. typhimurium* and in particular between strains of phage type 179 and strains of other phage types found in New Zealand have been described previously (Bettelheim, 1978). The phage type 179 strains were found to belong to a single incompatibility group. However, no observations were made concerning inhibition of other enterobacteria by *S. typhimurium* and it is not known whether the phenomenon is related to plasmid carriage.

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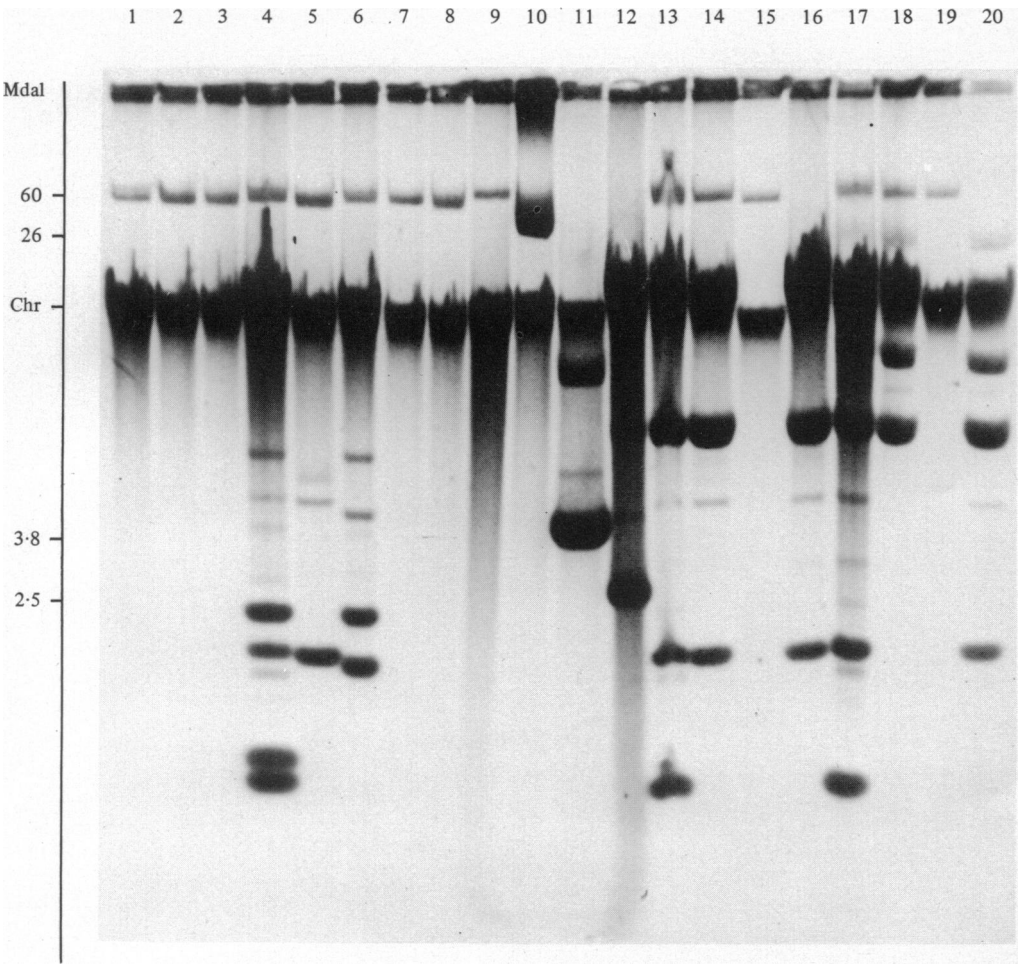
EXPLANATION OF PLATE 1

Electrophoresis in 0·7 % agarose of DNA from strains of *S. typhimurium* phage type 179 and *E. coli* transconjugants. Chr indicates the banding position of chromosomal DNA. Paler bands trailing plasmids of lower molecular weight are open circular and linear DNA (Willshaw, Smith & Anderson, 1979).

1. *S. typhimurium* 2194, Ap resistant; 60 Mdal plasmid.
2. *E. coli* K 12 transconjugant of 2194, Ap resistant; 60 Mdal plasmid.
3. *E. coli* K 12 transconjugant of 2932, Ap resistant; 60 Mdal plasmid.
4. *S. typhimurium* 512, ApTc resistant; 60, 2·3, 2·1, 1·4, 1·3 Mdal plasmids.
5. *E. coli* K 12 transconjugant of 512, ApTc resistant; 60, 2·1 Mdal plasmids.
6. *S. typhimurium* 1123, ApTc resistant; 60, 2·3, 2·1 Mdal plasmids.
7. *E. coli* K 12 transconjugant of 1123, ApTc resistant; 60 Mdal plasmid.
8. *E. coli* K 12 transconjugant of 1123, Ap resistant; 60 Mdal plasmid.
9. Molecular weight standard R100-1; 60 Mdal plasmid.
10. Molecular weight standard R6 K; 26 Mdal plasmid.
11. Molecular weight standard PMB 9; 3·8 Mdal plasmid.
12. Molecular weight standard PBR 322; 2·5 Mdal plasmid.
13. *S. typhimurium* 851, ApTcSuTp resistant; 60, 5·2, 2·1, 1·3 Mdal plasmids.
14. *E. coli* K 12 transconjugant of 851, ApTcSuTp resistant; 60, 5·2, 2·1 Mdal plasmids.
15. *E. coli* K 12 transconjugant of 851, ApTc resistant; 60 Mdal plasmid.
16. *E. coli* K 12 transconjugant of 851, SuTp resistant; 5·2, 2·1 Mdal plasmids.
17. *S. typhimurium* 666, ApTcSuTp resistant; 60, 8·7, 5·2, 2·1, 1·3 Mdal plasmids.
18. *E. coli* K 12 transconjugant of 666, ApTcSuTp resistant; 60, 8·7, 5·2 Mdal plasmids.
19. *E. coli* K 12 transconjugant of 666, ApTc resistant; 60 Mdal plasmid.
20. *E. coli* K 12 transconjugant of 666, SuTp resistant; 8·7, 5·2, 2·1 Mdal plasmids.


1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Mdal
60
26
Chr
3·8
2·5