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Serotypes of antibiotic resistant *Escherichia coli* isolated from the sewage of Palmerston North (New Zealand)

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SUMMARY

Antibiotic resistant strains of *Escherichia coli* were isolated from the domestic sewage of Palmerston North. The ability of these strains to transfer the drug resistance markers to two different recipient *E. coli* strains was assessed. The 'O' and 'H' serotypes of all strains were determined and attempts at correlation with the drug resistance studies were made. Some typically 'human' serotypes were found as well as a number of rarely described ones. No correlation between serotype and resistance pattern was observed.

INTRODUCTION

Escherichia coli are virtually ubiquitously distributed in the intestines of man and the warm-blooded animals, where they comprise about 1% of the total bacterial flora (Mitsuoka & Hayakawa, 1972). Therefore sewage is likely to contain *E. coli* in large numbers from these sources. Since the establishment of the internationally accepted serotyping scheme for *E. coli* by Kauffmann (1947), it has been realized that certain 'O' groups of *E. coli* may be associated with cases of infantile gastroenteritis and similar diseases in young animals. More recently it has been demonstrated that certain *E. coli* 'OH' serotypes are more likely to carry genes for the production of enterotoxins (Ørskov *et al.* 1976). In some instances enterotoxigenicity has been shown to be plasmid-borne (Wachsmuth, Falkow & Ryder, 1976; Gyles, So & Falkow, 1974). As it appears that 'OH' serotypes of *E. coli* may vary in their host-specificity (Bettelheim, 1978) it is considered important to assess the occurrence of the various serotypes in the environment.

The resistance of potential bacterial pathogens to many of the anti-microbial agents in current use appears to be increasing on a global level. The present study was undertaken in order to assess the range and frequency of resistance to a series of standard anti-microbial agents in use in Palmerston North among strains of *E. coli* which could be isolated from the sewage. The ability of the strains to

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transfer their resistances was also assessed and the 'OH' serotyping of these strains has been undertaken. As Palmerston North is an urban centre within a rural environment, the sources of the *E. coli* in the sewage would be expected to vary.

MATERIALS AND METHODS

Isolation of strains

Three samples of sewage were collected from the sewage treatment plant in Palmerston North in December 1974, May 1975 and October 1975 respectively. For the purpose of recording the results, the three batches were labelled sequentially as batch A, B and C. All sampling was performed midweek at approximately the same time of day in order to minimize variations.

The sewage was serially diluted and spread in 0·1 ml volumes on MacConkey agar or on MacConkey agar supplemented with antibiotics. The antibiotics used were ampicillin, tetracycline, streptomycin and chloramphenicol at concentrations of 25 μg/ml (Linton *et al.* 1974; Sturtevant, Cassell & Feary, 1971). When combinations of antibiotics were used each antibiotic was used at a concentration of only 10 μg/ml.

Lactose-fermenting strains were considered to be *E. coli* on the basis of their ability to produce gas at 44·5 °C in brilliant green bile broth, their ability to produce indole and their inability to utilize citrate or produce hydrogen sulphide.

Determination of antibiotic resistance patterns and ability to transfer antibiotic resistance

E. coli strains isolated were tested for their resistance to the following eight antibiotic preparations: the combination drug sulphamethoxazole/trimethoprim available as septrin (Sep), sulphafurazole (Sf), kanamycin (K), chloramphenicol (C), ampicillin (A), tetracycline (T), streptomycin (S) and nalidixic acid (Nal). The abbreviations in parentheses will be used to designate the antibiotic resistance patterns throughout this paper. Septrin was used as well as sulphafurazole because at that time it was one of the most frequently used antibiotic preparations issued by the pharmacists of Palmerston North for minor infections and it was claimed that organisms did not develop resistance to it. The Kirby Bauer disk diffusion method as recommended by the W.H.O. Expert Committee on Antibiotics (1961) was used to study the resistance patterns of the test strains.

As none of the isolates were found to be resistant to nalidixic acid, it was possible to use recipient strains for transfer studies, *E. coli* K12 and *E. coli* C, which were chromosomally resistant to nalidixic acid. After overnight incubation at 37 °C, the donor-recipient mixtures were swabbed onto MacConkey agar plates containing nalidixic acid and the selected antibiotic for which resistance transfer was being studied. In order to determine any additional resistance markers which might have been transferred, several colonies were picked from each plate and tested by the Kirby Bauer disk diffusion method against all antibiotics to which the donor strain had been resistant.

Serotyping of E. coli *strains*

All strains were tested against all the internationally accepted *E. coli* 'O' antisera from O1 to O163; the methods used were based on those described by Ewing *et al.* (1956). In order to reduce the amounts of serum required microtitre trays were used for all 'O' agglutinations. All strains were passaged repeatedly through semisolid media until fully motile suspensions, as observed microscopically, were obtained. These were then tested against the internationally accepted 'H' sera from H1 to H56 by the methods of Chandler & Bettelheim (1974). Strains which failed to show any motility after repeated subculture were considered non-motile and designated H −.

RESULTS

Antibiotic resistance of isolated E. coli

The number of antibiotic preparations to which the strains were resistant are shown in Table 1.

The antibiotics to which these strains were resistant are listed in Table 2.

A great variety of resistance patterns were observed, of which the most common was Sep, Sf, C, A, T, S; this was observed in 64 strains. The pattern Sf, C, A, T, S was found in 23 strains, the pattern Sep, Sf, K, C, A, T, S in 11 strains and patterns Sep, Sf, A, T, S; Sep, Sf, C, A, S; Sep, Sf, C, A, T; A, T; Sf, C, A, S and Sf, C, A, T in 5 to 10 strains each.

Serotypes of isolated E. coli

The results of typing the 181 strains of *E. coli* with both the standard 'O' and 'H' antisera are summarized in Table 3.

Although in some cases all strains of a given 'O' type carried the same 'H' antigen, this was certainly not true in all cases. The number of fully identified 'OH' serotypes is given in Table 4.

The relationship of antibiotic resistance patterns to the serotype

In general, where a given 'O' group is found associated with a number of 'H' antigens, it is also associated with a number of different antibiotic resistance patterns. This variation is demonstrated in Table 5.

A similar variability occurs among the unidentified 'O' types. The antibiotypes found associated with the more frequently observed 'H' types of 'O' ungroupable strains are listed in Table 6.

The ability of strains to transfer antibiotic resistance, and their relationship to serotype

Thirty-nine different transfer patterns were observed. Complete transfer of all resistance markers to recipient was observed in 87 out of 135 of those isolates which showed transfer of one or more resistance markers.

The nature of the selective medium was important in the detection of different

Table 1. *Multiple resistance among* 181 *strains*

No. of antibiotics	No. of strains resistant to the corresponding no. of antibiotics
0	0
1	6
2	14
3	11
4	25
5	45
6	68
7	12
Total	181

Table 2. *Antibiotic preparations to which the strains of* E. coli *were resistant*

Antibiotic	No. of strains resistant
Sulphafurazole	162
Tetracycline	155
Ampicillin	164
Chloramphenicol	125
Septrin	111
Streptomycin	151
Kanamycin	16

ransfer patterns. In nine strains, colonies selected on ampicillin/nalidixic acid plates showed transfer of the ampicillin marker alone, despite the multiple resistance of the donor strains. Some strains produced a variety of transfer patterns while others did not transfer any resistance markers, despite having the same sero- and antibiotype. An example of this is the three OX8.H15:Sep, Sf, A, T, S, K; only two were observed to transfer the resistance to all six antibiotic preparations while the third did not. There was, also present among the strains studied, a strain with the same antibiotic resistance pattern and 'H' antigen as the OX8 strains, but its 'O' antigen was untypable; this strain was also able to transfer all its resistance markers.

Of the 13 strains with the characters O153.H18:Sep, Sf, C, A, T, S, 12 transferred all six antibiotic resistance markers, but the thirteenth transferred only five and was not observed to transfer the resistance to tetracycline.

Although in some cases selection on different antibiotic-containing media altered the markers which were recovered in the exconjugants, this did not apply in the two cases cited above. In general, colonies picked from ampicillin-containing plates demonstrated transfer of ampicillin resistance alone, whereas recombinant colonies picked from media containing chloramphenicol, streptomycin or tetracycline carried the multiple resistance of the donor strains.

The efficiency of transfer into the two recipient strains (*E. coli* C; *E. coli* K12) was observed to vary; transfer of resistance markers to *E. coli* K12 was observed with 118 strains while only 71 were observed to transfer resistance markers to *E. coli* C.

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Table 3. *'O' and 'H' types of* E. coli *isolated*

'O' type	No. of strains	'H' type	No. of strains
O1	1	H1	7
O2	13	H2	4
O4	1	H4	16
O6	15	H5	7
O7	1	H6	10
O15	18	H7	4
O18ab	2	H9	1
O20	1	H10	13
O21	3	H11	13
O23	3	H15	6
O25	1	H18	38
O27	1	H19	2
O50	1	H21	1
O75	2	H26	1
O91	1	H30	3
O99	3	H31	24
O149	3	H33	9
O153	13	H39	1
O162	1	H45	1
O163	1	H48	2
OX8*	3	H55	1
Typable 'O' strains	88	Typable 'H' strains	164
Untypable smooth strains	79	Untypable motile strains	2
Rough strains	14	Non-motile strains	15
Total	181		181

* OX8 is a serotype which has not yet been given an internationally accepted 'O' no. (Ørskov, personal communication)

Table 4. *Number of fully identified 'OH' serotypes found*

'OH' Serotype	No. found	'OH' Serotype	No. found
O1.H7	1	O20.H30	1
O2.H1	1	O21.H5	2
O2.H6	7	O21.H31	1
O2.H18	4	O23.H4	1
O2.H55	1	O23.H18	1
O4.H11	1	O23.H30	1
O6.H1	3	O25.H1	1
O6.H31	11	O27.H31	1
O6.H33	1	O50.H18	1
O7.H4	1	O75.H4	1
O15.H1	1	O75.H5	1
O15.H2	2	O91.H39	1
O15.H4	1	O99.H33	2
O15.H5	1	O149.H48	2
O15.H11	4	O153.H18	13
O15.H18	5	O162.H18	1
O15.H31	2	O163.H31	1
O18a,b.H7	2	OX8.H15	3

Total 84

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Table 5. *Relationship of serotype with antibiotype*

Serotype	Antibiotype	No. of strains
O2.H1	A, T	1
O2.H6	Sep, Sf, C, A, T, S	6
O2.H18	Sep, Sf, C, A, T, S	3
O2.H18	Sf, C, A, T, S,	1
O2.H55	Sep, Sf, A, S	1
O6.H1	A	1
O6.H1	A, T	2
O6.H31	A, T	1
O6.H31	Sep, Sf, A	1
O6.H31	Sf, C, A	1
O6.H31	Sf, C, A, S	1
O6.H31	Sep, Sf, C, A, S	5
O6.H31	Sep, Sf, C, A, T, S	2
O6.H33	A, T	1
O15.H1	A	1
O15.H2	Sf, C, A, T	1
O15.H2	Sf, C, A, T, S	1
O15.H4	Sf, C, A, S	1
O15.H5	Sf, C, A, T, S	1
O15.H11	Sf, C, A, S	1
O15.H11	Sf, C, A, T, S	3
O15.H18	Sf, C, A, S	1
O15.H18	Sf, C, A, T, S	3
O15.H18	Sep, Sf, C, A, T, S	1
O15.H31	Sep, Sf, A	1
O15.H31	Sep, Sf, A, T, S	1
O18a,b.H7	Sf, C, A, T, S	2
O21.H5	Sep, Sf, C, A, T, S	1
O21.H5	Sep, Sf, C, A, T, S, K	1
O21.H31	Sep, Sf, C, A, T, S	1
O23.H4	Sep, Sf C, A, T, S, K	1
O23.H18	Sep, Sf, C, A, T, S	1
O23.H30	Sep, Sf, C, A, T, S	1
O75.H4	A, T	1
O75.H5	Sf, C, A, T, S	1
O99.H33	Sep, Sf, A, T, S	1
O99.H33	Sep, Sf, C, A, T, S	1
O149.H48	T, S	2
O153.H18	Sep, Sf, C, A, T, S	13
OX8.H15	Sep, Sf, A, T, S, K	3

The markers which the strains were able to transfer seemed to vary considerably. Of the six strains with the characteristics O2.H6:Sep, Sf, C, A, T, S, one strain transfered either Sep, Sf, C, A, T, S as a complete unit or T alone; another was observed to transfer only the complete set of markers, while a third transferred only T and the remaining three strains were not observed to transfer any resistance markers. All three strains of O15.H11:Sf, C, A, T, S, were able to transfer the complete set of resistance markers although one strain was observed to transfer Sf, C, A, S in some instances.

On the basis of serotype, antibiotype and transferability a related group of strains can be established, as shown in Table 7.

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Table 6. *Antibiotypes found associated with the various 'H' antigens among the 'O' ungroupable strains*

'H' type	Antibiotype	No. of strains
H4	Sep, Sf, C, A, T, S	6
	Sf, C, A, T, S	1
	Sf, A, T, S	3
	Sf, T, S	1
H5	Sep, Sf, C, A, T, S	3
H6	Sep, Sf, C, A, T, S	2
	Sf, C, A, T, S	1
H10	Sep, Sf, C, A, T, S	2
	Sf, C, A, T, S	6
	Sep, Sf, A, T, S	1
	Sep, Sf, C, S	1
	Sf, A, T, S	1
	Sf, T, S	1
	Sf, A, T	1
H11	Sep, Sf, C, A, T, S	6
	Sep, Sf, A, T, S	1
	A, T, S	1
	A, T	1
H15	Sep, Sf, A, T, S, K	1
	Sep, Sf, A, T, S	1
H18	Sep, Sf, C, A, T, S, K	1
	Sep, Sf, C, A, T, S	5
	Sep, Sf, A, T, S	1
	Sep, Sf, C, A, T	2
	Sf, C, A, T, S	3
H31	Sep, Sf, C, A, T, S	3
	Sep, Sf, C, A, T	1
	Sep, Sf, C, A, S	1
	A, T	1
H33	Sep, Sf, C, A, T, S, K	1
	Sep, Sf, T, S	1
	T	1

DISCUSSION

It is not surprising that multiply drug resistant *E. coli* were isolated from the sewage of Palmerston North, particularly as antibiotics are widely used, both medically and agriculturally. However, for a sewage supply to contain such antibiotic resistant organisms as were found in this study can be considered a problem.

Kanamycin is a restricted drug and the number of *E. coli* strains which were resistant to this antibiotic was low; it is likely that the restricted use of the drug has kept the selective pressure in the environment for kanamycin resistance at a low level and this has delayed the appearance of the kanamycin resistance marker in significant numbers.

As yet, there are no established figures available of those serotypes of *E. coli* which are most prevalent in New Zealand in various situations; it is therefore

difficult at this stage to assess the significance of the distribution of the various 'O' types or 'H' types. While the frequent occurrence of O2 and O6 with four or three different 'H' types respectively may indicate that these 'O' types may play as important a role ecologically in New Zealand as they do in the rest of the world, it cannot yet be fully confirmed. It is also significant that 'O' types O1, O4, O7 and O75 were not commonly encountered in this study. While these types may be the predominant 'human' types in Western Europe and North America, different distributions have been noticed when other parts of the world were examined (Wong & Bettelheim, 1977). Whether the large number and variety of O15 serotypes implies that this is an important 'O' group in this part of the world or whether it is an animal type cannot yet be ascertained. As all the O153 strains carried the same H18 antigen, were resistant to the same antibiotics and, with one exception, transferred them all, it can be considered most likely that they represent a clone. However, for such a number of representatives of one clone to be isolated among such a mixture of strains may reflect that these strains may be particularly widespread in this environment.

That 13 strains out of 181 should be virtually identical with respect to 'O' type, 'H' type, antibiotype and transferability highlights the great mixture of types which the remaining strains represent. Table 5 shows this remarkable diversity. Thus within one serotype there are, in the case of O6.H31, as many as six different antibiotypes. Similar diversity exists even among 'O' groups less well represented, such as O21, O23, O75 and O99 (Table 5). Although it is more difficult to discuss those strains whose 'O' type could not be determined (Table 6), it nevertheless appears that there is similar diversity among those. If this assumption is correct, then there are probably a large number of new 'O' types to be designated in New Zealand. It is noteworthy that very many strains could not be 'O' typed whereas most could be 'H' typed (Table 3).

It has been suggested in previous epidemiological studies that serological variation may occur among *E. coli* (Bettelheim & Taylor, 1969; O'Farrell & Bettelheim, 1976) and in a recent study on *E. coli* in a maternity ward (Bettelheim *et al.* 1974) a group of serologically related strains with varying biotypes was presented, suggesting that under the various environmental pressures certain characteristic markers may alter. Apart from the well-known resistance transfer factors, other enzymic markers can also be transferred (Shinebaum *et al.* 1977). It thus does not seem unreasonable to postulate that some antigenic markers might similarly be transferable. Particularly noteworthy in this study is the occurrence of certain 'H' antigens such as H18 which is associated with O2, O15, O23, O153 and O162 as well as 'O' untypable and rough strains. The antigen H31, which is associated with O6, O15, O21, O27 and O163 as well as 'O' untypable and rough strains may be another example. If there is such a thing as 'H' antigen transfer, then the occurrence of a variety of 'H' antigens with one 'O' antigen may suggest 'O' antigen transfer. The 'family' of strains listed in Table 7 may be such an example.

A study of this kind illustrates how varied the *E. coli* types in sewage can be, and suggests how markers of any type may be variable and may interact with each

Table 7. *Varieties of related strains on the basis of serotype, antibiotype and antibiotic resistance transferability*

Serotype	Antibiotype	Resistances transferred
O2.H18	Sep, Sf, C, A, T, S	Sep, Sf, C, A, T, S
O2.H18	Sep, Sf, C, A, T, S	C, A
O2.H18	Sep, Sf, C, A, T, S	A
O2.H18	Sep, Sf, C, A, T, S	A S
O15.H18	Sf, C, A, T, S	C, A
O15.H11	Sf, C, A, S	A
O15.H11	Sf, C, A, T, S	Sf, C, A, T, S
O4.H11	Sep, Sf, C, A, T, S	A Sf, C, T, S

other. If in such an environment plasmids carrying genes for enterotoxigenicity were introduced, then this study demonstrates how easily these could become incorporated into strains of a variety of serotypes, some of which may have the specific ability to colonize the human bowel, or that of man's domestic animals. If, additionally, these strains have picked up antibiotic resistance markers, then potentially dangerous strains can emerge from these environments. Only the restricted use of an antibiotic, as in the case of kanamycin, will ensure that the selection pressure, which causes the emergence of multiple resistance organisms, will be reduced.

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