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Distribution of R Plasmids Among the O-Antigen Types of *Escherichia coli* Isolated from Various Clinical Sources

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A total of 655 *Escherichia coli* strains from various clinical sources were O-antigen typed, and the serological properties were correlated with R-plasmid carriage. Of the 655 strains, 224 were resistant to one or more antibiotics and 148 of these carried R plasmids. The distribution of O-antigen types among the susceptible strains was similar to that reported previously by others in England. The O-antigen types among the R^+ strains showed some similarities to those found in susceptible strains, but O-antigen types O8, O9, O17, O18 and, perhaps, O101 and O107 were more common in the R^+ than in the susceptible series. There is therefore some overlap between the O-antigen types of *E. coli* prevalent in R^+ strains from clinical cases and from human and animal fecal sources.

Previous studies in this laboratory (6, 7) have shown that *Escherichia coli* strains isolated from human fecal samples and carrying R plasmids are not randomly distributed among the various O-antigen types. Three O-antigen types, O8, O9, and O101, accounted for about 20% of the total number of human strains that carry transferable resistance factors, and the same types also accounted for 24% of all resistant *E. coli* strains isolated from calves.

Although there have been several studies on the distribution of *E. coli* O serotypes in human urinary tract infections, they either do not correlate the O-antigen types with R-plasmid carriage (1, 5, 9) or have been carried out on a limited scale (3), and as yet there is little information on the distribution of R plasmids in strains of *E. coli* isolated from the urinary tract.

In this survey we have typed 655 *E. coli* isolates from clinical specimens sent for culture either to the Bristol Royal Infirmary or to the Public Health Laboratory in Bristol. As found in the survey on human and animal fecal strains (6, 7), R plasmids were not randomly distributed among all O-antigen types. In this series, O4, O6, O8, O17, and O18 were prevalent, thus showing some overlap with the types found previously among the fecal strains from man and from calves.

MATERIALS AND METHODS

Source of strains. Of a total of 655 typed *E. coli* isolates, the majority (561) were isolated from patients with urinary tract infection as defined by the incidence of 10^5 or more bacteria per ml of fresh urine. Of the isolates, 214 came from non-hospitalized patients and the remainder came from those already admitted to hospital, even though some of them may have acquired the strains outside the hospital. A smaller number of *E. coli* strains (94) were isolated from other sources as detailed in Table 1. The "miscellaneous" group includes a number of cultures that yielded "significant" numbers of *E. coli*, but the organisms could not unequivocally be identified as the cause of the infection. The clinical history was available for most of the patients included in this survey.

O-antigen typing. The great majority of specimens yielded a pure culture of *E. coli*. A single colony picked from the agar on which each specimen had been cultured was used to determine the O-antigen type. A total of 147 O sera were available for typing. The method used has been described previously (6).

Tests for the presence of R plasmids. The presence of transmissible R plasmids was demonstrated by showing transfer to a standard *E. coli* K-12 recipient (*E. coli* UB1139) as described previously (6). Alternatively, the presence of a tetracycline (Tc)-resistant marker was taken to denote the presence of a nontransmissible plasmid (6, 8).

Susceptibility test. Resistance to the following antibiotics was determined by using disks impregnated with antibiotics as follows: ampicillin (Ap), 25 μg; tetracycline (Tc), 25 μg; streptomycin (Sm), 10 μg; sulfafurazole (Su), 500 μg; chloramphenicol (Cm), 50 μg; nitrofurantoin, 200 μg; nalidixic acid, 30 μg; and colistin sulfate, 10 μg. The methods of identification, storage, and determination of transfer properties were as previously described (6).

RESULTS

Of the 561 urinary *E. coli* strains studied, 337 were susceptible to all antibiotics used.

TABLE 1. *Source of the 655 E. coli isolates that were O-antigen typed*

Cultures from:	Patients	
	Hospital	General practice
Urine	347	214
Blood	24	
Cerebrospinal fluid	1	
Wound swabs and abscesses	46	
Miscellaneous sources[a]	23	
Total	441	214

[a] This group includes a number of cultures that yielded "significant" numbers of *E. coli*, but the organisms could not unequivocally be identified as the cause of the infection.

Figure 1 shows the distribution of O-antigen types among the susceptible strains. O-antigen type O6 was the most common (13.3%), and O1, O2, O4, O7, O18, O22, and O75 were present at frequencies above 4.0%. Isolates not typable with the available O antisera amounted to 12.4% of the total, and 8.6% were autoagglutinable. The distribution was similar to that found by others from similar sources (5).

Resistance to at least one antibiotic was found in 224 of the 561 strains. These strains were also O-antigen typed and gave the distribution shown in Fig. 2. Comparison of the distributions of O types among susceptible and resistant strains (Fig. 1 and 2) shows that whereas types O1, O2, O4, O6, O7, O18, O22, and O75 occurred most frequently among the susceptible strains, O2, O4, O6, O8, O9, O17, and O18 were commonest among the resistant.

The resistance patterns among the resistant isolates ranged from single to multiple resistance. The most common multiple patterns were Ap Tc Sm Su and Ap Tc Sm Su Cm. The percentage of strains with one, two, three, four, and five resistance determinants was 29, 29, 24, 11, and 6.6, respectively. Of the resistant *E. coli* strains isolated from urinary tract infections in domiciliary practice, 10% were resistant to Ap and 20% to sulfonamides. About 50% of the resistant urinary tract *E. coli* strains isolated from hospital patients were resistant to those two antibiotics.

The 224 resistant *E. coli* isolates were tested for their carriage of an R plasmid. Of these, 98 (43.7%) were able to transfer one or more of their resistance characters to an R^- strain of *E. coli* in a standard mating test, and 50 others (23.3%) included Tc resistance in their patterns, a character normally taken to indicate the presence of an R plasmid (4, 8). In all, therefore, at least 148 (66%) of the resistant strains examined owed their resistance to plasmid-mediated characters.

Examination of the distribution of R plasmids among the various O-antigen types of the resistant strains showed that types O4, O6, O8, O17, and O18 occurred above a level of 3.5% (Table 2), although the greatest proportion of R^+ strains in the series (12.5%) was among those nontypable with the sera available.

O-antigen types among E. coli isolated from clinical specimens other than urine samples. A total of 94 *E. coli* strains were examined from clinical specimens other than urine samples. Of these, 52 (55%) were susceptible and 42 (45%) were resistant. Of the resistant strains, 24 showed either direct or indirect evidence for the presence of a plasmid. The distribution of the O-antigen types among the R^+ strains from this source showed 8 of 24 strains (33%) to be from O-antigen types O8, O9, O17, O18, and O101 (Table 3).

DISCUSSION

The relative abundance of the various susceptible *E. coli* O-antigen types in the urine samples examined here corresponds closely with information already published by others for urinary tract *E. coli* strains regardless of their antibiotic resistance (see Tables 9.1, 9.2, and 9.3 in the review by Grüneberg et al., ref. 5). In these studies, O-antigen type O6 often comprised more than 10% of the isolates, whereas types O2, O4, O18, and O75 were also common.

The distribution of O-antigen types among the resistant series of strains examined by us differed from that found among the susceptible in a number of important respects. O-antigen types O2, O4, O6, O18, and O75 were all relatively common in both the susceptible and the resistant series, but O8, O9, O17, and O101 were considerably more common among the resistant *E. coli* than among the susceptible (cf. Fig. 1 and 2). This difference was highly significant ($\chi^2_{[2]} = 40$; $P < 0.0005$).

The two O types O8 and O9 are of particular interest in that they have also proved relatively abundant among resistant *E. coli* isolated from human and animal fecal samples (6). Furthermore, O101 strains of *E. coli*, which were also relatively common among the resistant strains from human and animal feces, were only encountered among the resistant strains from urinary tract infections, even though the num-

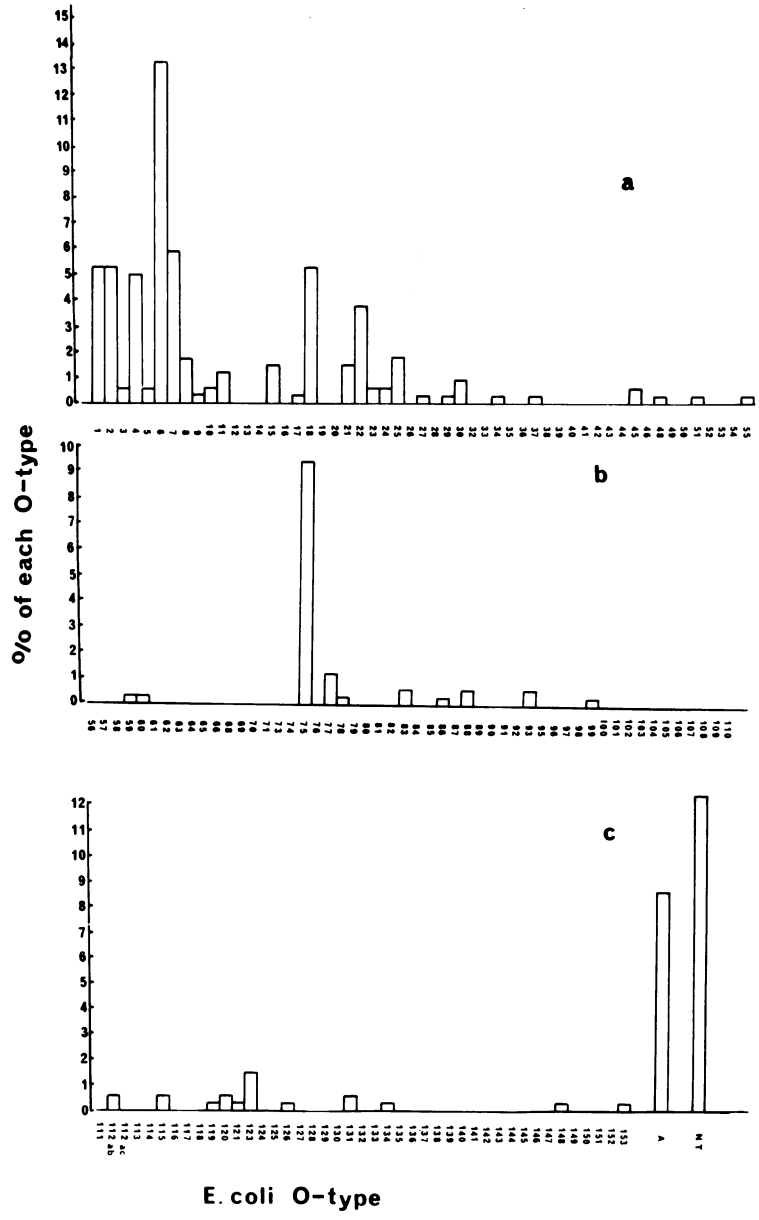

FIG. 1. *Distribution of O-antigen types among 337 susceptible E. coli strains isolated from urinary tract infections. (a) O-antigen types 1-55; (b) types 56-110; (c) types 111-153, together with autoagglutinable (A) and nontypable (NT) strains.*

ber of the isolates of this particular O-antigen type was few in the series studied here.

No significant difference was found in the distribution of *E. coli* O-antigen types among the hospital isolates and those from domiciliary practice ($\chi^2_{[1]} = 0.005$; $P < 0.995$).

In all, 224 of the strains examined in this survey were resistant to antibiotics, and about 148 (66%) of these showed positive evidence for the presence of R plasmids, either by the demonstration of transfer in the standard tests or by the presence of the Tc resistance marker. Examination of the distribution of O antigen types among the strains known to be carrying plasmids, as opposed to the resistant bacteria considered as a whole, showed few differences.

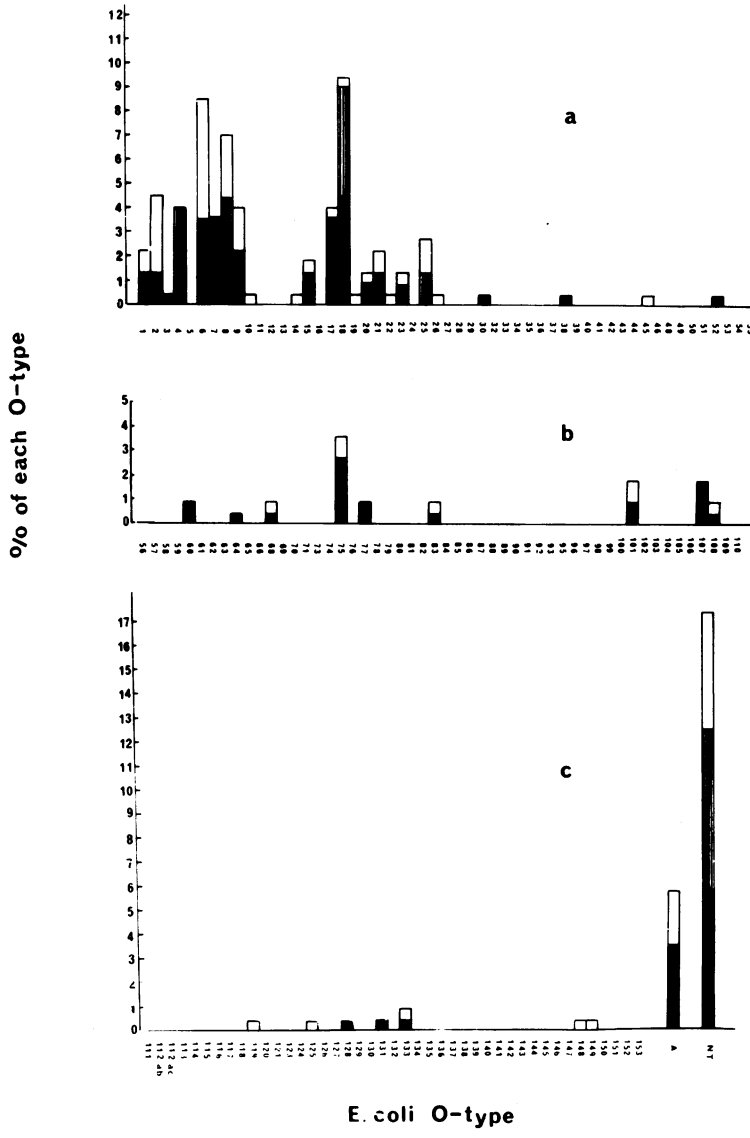

FIG. 2. *Distribution of O-antigen types among 224 antibiotic-resistant E. coli strains isolated from urinary tract infections. (a) O-antigen types 1-55; (b) types 56-110; (c) types 111-153. The various types of resistance are shown on the diagram as follows: solid black, transferable resistance demonstrable; vertical hatching, nontransferable but with a pattern including tetracycline resistance; open, nontransferable resistance with a pattern not including tetracycline resistance.*

O-antigen types O4, O6, O8, O17, and O18 were relatively abundant in the R^+ series, with the differences from the susceptible series being most marked in types again centering on O4, O8, O9, O17, O18 and, perhaps, O101 and O107.

The number of strains studied here from clinical sources other than urine samples is few and the data must be treated with caution. Nevertheless, it is interesting that O types O8, O9, O17, O18, O101, and O107 are prevalent among plasmid-carrying bacteria isolated from these sources, and this serves to highlight the importance of these O-antigen types in resistant *E. coli* infections of clinical importance.

There has been much discussion as to whether resistant *E. coli* in the gut of man and of farm animals could be distinguished from strains isolated from human urinary tract infections (2). Although the information reported here is no proof that the resistant *E. coli* strains

TABLE 2. *Analysis of the various patterns of antibiotic resistance within O-antigen types O4, O6, O8, O17, and O18 compared with others isolated from urine cultures*

O-antigen type	R plasmid demonstrated by transfer (i)	Tc resistance but transfer not demonstrated (ii)	R^+ and Tc-resistant strains (i + ii)	Resistance not shown to be plasmid[a] (iii)	Total resistant strains (i + ii + iii)
O4	7	2	9 (4.0)[b]		9
O6	5	3	8 (3.5)	11	19
O8	7	3	10 (4.4)	6	16
O17	8		8 (3.5)	1	9
O18	10	10	20 (8.9)	1	21
Total	37 (16.5%)	18 (8.0%)	55 24.5	19	74
All other types	61 (27.2%)	32 (14.2%)	93 41.5	77	170
Total	98	50	148 66.0	96	244

[a] Some of these strains may carry nontransmissible R plasmids other than those specifying resistance to tetracycline.

[b] Numbers in parentheses are percentages.

TABLE 3. *Analysis of the various patterns of antibiotic resistance within the various E. coli O-antigen types isolated from clinical sources other than urine*

O-antigen type	Total	No. of strains			
		Resistant	Susceptible	Plasmid carrying	Resistance not shown to be plasmid mediated[a]
All	94	42	52	24	18
O8	3	2	1	2	
O9	3	3		2	1
O17	2	1	1	1	
O18	3	1	2	1	
O101	2	2		2	
Others	81	33	48	16	17

[a] See footnote *a*, Table 2.

in these various ecological situations are part of the same pool of organisms, it is certainly impossible to exclude resistant fecal strains of human or animal origin as possible sources of resistant urinary tract infection on the basis of O-antigen type alone.

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