Incidence of Antibiotic-Resistant *Escherichia coli* Associated with Frozen Chicken Carcasses and Characterization of Conjugative R Plasmids Derived from Such Strains

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Escherichia coli were isolated at concentrations of about 10^2 /ml from the fluid obtained after thawing each of five frozen chicken carcasses. Between 13 and 89% of the *E. coli* were resistant to mercury(II) or to at least one of eight antibiotics tested. Multiple resistance was more common than single resistance, and resistances to tetracycline, streptomycin, sulfathiazole, or chloramphenicol were more frequently encountered than was resistance to ampicillin or mercury(II). Resistance to kanamycin, gentamicin, or trimethoprim occurred rarely. Upwards of 30% of the *E. coli* apparently carried conjugative plasmids and could transfer at least one of their resistance determinants to *E. coli* K-12. Twenty-five conjugative R plasmids obtained in this way were characterized within strain K-12 by a variety of phenotypic criteria. Twenty could be assigned to the incompatibility groups Inc X, Inc N, Inc F_I, or Inc I_a.

Antibiotic-resistant *Escherichia coli* can be isolated from many domestic animals used by humans as a food source, and their occurrence is believed to be correlated with the use of antimicrobial agents for the purpose of growth promotion in animal husbandry (18, 26). It has been recognized that these bacteria may be a potential health risk, since they could be spread to humans via the food chain (18, 26). In this regard, certain meats such as poultry are particularly important in that they are contaminated with relatively large numbers of resistant *E. coli* representing a wide variety of O-serotypes, including many that also occur naturally as colonizers of the human intestinal tract (18). However, considerably less information is available on the types of R plasmids carried by such resistant *E. coli* and whether or not these plasmids are similar to those derived from human *E. coli* isolates. Therefore the aims of this study were, first, to determine the incidence and varieties of antibiotic-resistant *E. coli* contaminating frozen chicken carcasses purchased from retail stores in Melbourne, and second, to characterize the conjugative R plasmids derived from such strains particularly with respect to their incompatibility properties.

MATERIALS AND METHODS

Strains, plasmids, and bacteriophages. The bacteria used were either *E. coli* isolated from chicken carcasses or genetically marked derivatives of *E. coli* K-12. The latter were UB1301 (prototrophic, Rif^r

[11]); UB281 (*met pro* Nal^r [4]) and its *recA* derivative UB2914, obtained from S. Jenkins, Department of Bacteriology, Bristol University, Bristol, England; and C600 (*thr leu thi lac*), obtained from B. Egan, Department of Biochemistry, University of Adelaide, Adelaide, Australia. Sublines of UB1301, UB281, and C600 carrying various plasmids (Tables 1 and 2) were also used. In all experimental procedures, bacteria in exponential-growth phase in nutrient broth were used.

The plasmids used are listed in Tables 1 and 2. RP1 was described previously (11); the remaining plasmids were obtained from N. Datta of the Royal Postgraduate Medical School, Hammersmith Hospital, London, England, via B. Davey of the Department of Microbiology, Melbourne University, Melbourne, Australia. All the plasmids are conjugative and are prototypes of particular incompatibility groups (7, 14). Table 2 lists the plasmids characterized in this study. Abbreviations for chromosomal alleles are those recommended by Demerec et al. (8), and abbreviations for plasmid and chromosomal-resistance determinants are those recommended by Novick et al. (20).

The phages used were the donor-specific phages PRR1 (21), Ike (16), and PR4 (5, 23). The virulent mutants Plvir (obtained from D. MacPhee, Department of Microbiology, La Trobe University) and λ cl857 (obtained from B. Egan, Department of Biochemistry University of Adelaide) were also used.

Media and antibiotics. Nutrient broth, nutrient agar (NA), and minimal medium were as previously described (24). Oxoid MacConkey agar (no. CM7b) was used. Soft overlay agar for phage assays contained tryptone (0.5%; Difco Laboratories), yeast extract (0.5%; Difco), agar (0.7%; Difco), and NaCl (0.5%). This was supplemented with 30 mmol of CaCl₂ per ml and 50 mmol of MgSO₄ per ml before use.

TABLE 1. *Plasmids used*^a

Plasmid	Phenotype	Plasmid	Phenotype
Ra1	Su Tc Inc A	R446b	Sm Tc Inc M
R40a	Ap Km Su Inc C	N3	Sm/Sp Su Tc Hg Dps(Ike) Res ⁺ Mod ⁺ (RII) Inc N
R386	Tc Inc FI	R46-1	Ap Su Tc Dps (Ike) Uv Inc N
R1drd16	Km Inc FII	R16	Ap Sm Su Tc Inc O
R124	Tc Inc FIV	RP1	Cb Km/Nm Tc Dps (PRR1, PR4) Inc P
R27	Tc Inc H	R478	Cm Km Tc Inc S
R144drd3	Km Inc L _c	Rtst	Km Inc T
R621a	Tc Inc I _y	R753	Ap Cm Sm Su Inc V
R391	Hg Km/Nm Inc J	R388	Su Tp Dps (PR4) Inc W
R387	Cm Sm Inc K	R6K	Ap Sm Inc X
R471	Ap Km Inc L		

^a Symbols for plasmid-determined phenotypes follow those recommended by Novick et al. (20). Resistance determinants: Ap, ampicillin; Cm, chloramphenicol; Km, kanamycin; Nm, neomycin; Sm, streptomycin; Sp, spectinomycin; Su, sulfathiazole; Tc, tetracycline; Hg, mercuric ions; Uv, UV radiation. Dps refers to sensitivity to donor-specific phage; Inc refers to the plasmid incompatibility type; Res Mod refers to restriction-modification specificity.

Amino acid supplements to minimal medium were used at a final concentration of 20 µg/ml. Antibiotic supplements to NA and minimal medium (micrograms per milliliter) were: ampicillin, 20; chloramphenicol, 5; kanamycin, 25; nalidixic acid, 20; rifampin, 150; and spectinomycin, 25. Sulfathiazole (1.5 mg/ml) was used in NA supplemented with lysed, defibrinated horse blood (5%, vol/vol), and the plates were stored at 4°C for 24 h before use. Trimethoprim was used in minimal medium at 20 µg/ml, and mercuric chloride was used in NA at 18 µg/ml.

Conjugation and determination of incompatibility type. Equal volumes of donor and recipient bacteria in NA were mixed and maintained at 37°C for an appropriate time period. The mixture was then diluted, and portions were spread on medium containing the selective agent, either mercuric chloride or an antibiotic, and a contraselective agent, either nalidixic acid or rifampin. Experiments involving transfer of the Tp⁺ determinant were carried out on the appropriate minimal medium containing trimethoprim. Transconjugants that appeared after 24 to 48 h of incubation at 37°C were counted. When incompatibility was being tested, the transconjugants isolated from R⁺ × R⁺ matings were purified through two single-colony isolations (ca. 50 generations) on the medium on which they were first isolated, then tested for their resistance phenotype to determine whether the initially resident plasmid was still carried by the transconjugant.

Procedures involving phages. Estimation of the efficiency of plating (EOP) of P1 or λ was carried out by the overlay assay method. P1 was always assayed on UB281 and its R⁺ derivatives, and λ was assayed on C600 and its R⁺ derivatives. Determination of the resistance or sensitivity of bacteria to various donor-specific phages was done by placing a drop of phage (ca. 10⁷ plaque-forming units per ml) onto the surface of NA previously seeded with the strain to be tested. Evidence of lysis was determined after incubation of the plates at 37°C for 8 h.

Response to UV radiation. Volumes (0.01 ml each) of a 10-fold dilution series of the bacteria to be tested were spotted to the surface of NA plates. After drying, different replica plates were exposed to 28

ergs/s per mm² of ultraviolet (UV) radiation for 0 to 60 s, then incubated in the dark at 37°C overnight. The rate of kill of the bacteria could be determined from the viable count of bacteria surviving each time period of radiation.

Isolation of *E. coli* from thawed fluid. Frozen chicken carcasses sealed in polyethylene bags were purchased from various retail stores. The thawed fluid that collected within the bags after the carcasses were left at room temperature for approximately 4 h was collected with sterile syringes. The fluid was serially diluted in saline, and portions were plated onto NA and MacConkey agar. After overnight growth at 37°C, the presumptive enteric bacterial count was determined from the numbers of colonies appearing on MacConkey agar. Very small colonies that were probably fecal streptococci were not counted. The total bacterial count was determined from the viable count on NA. Putative *E. coli* were selected from among the lactose-fermenting colonies that were either smooth, convex colonies with entire edges and 1 to 1.5 mm in diameter or rough, flat colonies with crenated edges and 2 to 2.5 mm in diameter. A total of 100 colonies of these two types derived from carcass 1 were tested by the analytical profile index system and found to be *E. coli*. It was assumed therefore that colonies of these types selected from the other samples were also *E. coli*.

Determination of resistance phenotype. Colonies of *E. coli* from MacConkey agar were subcultured by point inoculation onto fresh MacConkey medium (50 inocula per plate) to prepare "master" plates. After overnight incubation at 37°C, the growth was replicated, via squares of sterile velveteen, to appropriate media containing the various antimicrobial agents. After incubation, colonies that were resistant to any of these agents were purified on the same medium to confirm their resistance phenotype.

RESULTS

Incidence and resistance phenotype of *E. coli* associated with frozen chicken carcasses. The number of presumptive enteric bac-

TABLE 2. Properties of *R* plasmids derived from chicken *E. coli*

Origin of plasmid (sample and iso-late no.)	Plasmid designation	Resistance phenotype	Transfer frequency (selected marker) ^a	Response to lke and PR4 ^b	Response to UV ^c	EOP ^d		Res Mod specificity ^e	Fi (RP1) ^c	Inc type ^d
						Phi (Pl)	Phi (λ)			
1a; 1b	pVS100; pVS101	Ap Cm Tc	1.3×10^{-4} ; 8.0×10^{-4} (Tc)	S	R	10^{-2}	10^{-3}	R(II)	+	N
1c; 1d	pVS106; pVS106	Ap Sm Tc	1.0×10^{-5} ; 1.1×10^{-5} (Sm)	S	R	10^{-2}	10^{-3}	R(II)	+	N
5a; 5b	pVS126; pVS127	Sm Tc	1.5×10^{-5} ; 7.1×10^{-5} (Sm)	S	R	10^{-2}	10^{-3}	R(II)	+	N
5c; 5d	pVS124; pVS125	Sm	1.0×10^{-5} ; 1.5×10^{-5} (Sm)	S	R	10^{-2}	10^{-3}	R(II)	+	N
1e	pVS107	Tc	3.0×10^{-6} (Tc)	R	R	1	1	—	+	L _e
1f; 1g	pVS108; pVS109	Tc	8.0×10^{-3} ; 1.3×10^{-4} (Tc)	R	R	1	1	—	—	L _e
1h	pVS110	Tc	1.5×10^{-4} (Tc)	R	R	1	10^{-3}	Not R(II)	+	FI
3a	pVS111	Su Tp	2.0×10^{-6} (Tp)	R	S	10^{-2}	10^{-3}	R(II)	+	FI
1i	pVS112	Ap Su	2.0×10^{-5} (Ap)	R	S	1	1	—	+	X
1j	pVS113	Sm/Sp Su	6.0×10^{-3} (Sm)	R	S	1	1	—	—	X
1f	pVS115	Ap Cm Tc	2.9×10^{-6} (Ap)	R	S	1	1	—	—	X
5e	pVS129	Sm/Sp Su	1.5×10^{-6} (Sp)	R	S	1	1	—	—	X
1k	pVS117	Ap	7.1×10^{-3} (Ap)	R	S	1	1	—	—	X
2a	pVS116	Ap	6.0×10^{-3} (Ap)	R	S	10^{-2}	1	Not R(II)	—	X
2b	pVS114	Sm Su	1.5×10^{-4} (Sm)	R	S	$<10^{-6}$	$<10^{-6}$	NK	+	X
4a	pVS118	Sm/Sp Su	5.0×10^{-5} (Sm)	R	S	1	1	—	+	NK
4a	pVS121	Ap	1.0×10^{-6} (Ap)	R	S	$<10^{-6}$	1	NK	—	NK
3b	pVS122	Ap Cm Hg Km Sm Su Tc	5.0×10^{-4} (Ap)	R	R	1	1	—	—	NK
5f	pVS130	Km	2.0×10^{-5} (Km)	R	S	1	1	—	—	NK
5g	pVS134	Ap Sm/Sp Su	2.5×10^{-6} (Ap)	R	S	1	1	—	—	NK

^a Determined in 30-min matings in NB by using UB1301R⁺ donors and UB281 as recipient. Frequencies reduced approximately fivefold were obtained by using UB2914 as recipient.

^b See text for details. R, Resistance to phage or to UV; S, sensitivity to phage or to UV; NK, not known.

^c Inhibition of the transfer of RP1 from R⁺ RP1⁺ donors of UB1301 to UB281 as recipient in 30-min matings in NB.

^d pVS118 was compatible with pVS121, pVS122, pVS130, and members of IncFI, N, O, FI, J, H, L, T, A, V, C, FIV, K, L, X, W, S, and M. pVS121 was compatible with pVS111, pVS113, pVS118, pVS130, and members of IncFI, FII, FIV, L, I, O, J, H, L, T, A, K, W, X, S, N, and M. pVS122 was compatible with pVS111, pVS118, pVS134, pVS113 and members of Inc W. pVS130 was compatible with pVS108, pVS121, pVS118, pVS134 and members of IncFI, FII, FIV, L, N, O, H, L, A, V, K, W, X, and M. pVS134 was compatible with pVS115, pVS122, pVS130 and members of Inc FI, FII, FIV, L, I, N, O, J, H, L, T, A, V, C, K, S, W, and M.

teria in the thawed fluid from five frozen chicken carcasses purchased from different retail stores in Melbourne was determined from the viable count of bacteria on MacConkey agar. About 1,000 lactose-fermenting colonies with the colonial morphology typical of *E. coli* were then selected at random from the five respective dilution series and tested, by the replica plating method, for susceptibility or resistance to various antibacterial agents.

Table 3 shows that in the five samples, *E. coli* occurred in numbers between 1.1×10^2 and 5.0×10^2 bacteria per ml and represented between

TABLE 3. Incidence of susceptible and resistant *E. coli* associated with frozen chicken carcasses

Thawed fluid sample	No. of presumptive enteric bacteria/ml of thawed fluid ($\times 10^3$) ^a	No. of <i>E. coli</i> /ml of thawed fluid ($\times 10^2$) ^b	% Resistant <i>E. coli</i> (no. tested) ^c
1	1.0	3.0	13.7 (750)
2	7.2	3.2	89.8 (1,042)
3	8.0	5.0	65.0 (1,042)
4	4.0	1.1	80.0 (1,042)
5	3.8	3.2	85.2 (613)

^a Determined from the number of colonies formed on MacConkey agar.

^b Determined from the number of lactose-fermenting colonies on MacConkey agar with the colonial morphology typical of *E. coli*.

^c Determined by replica plating.

5 and 30% of the total presumptive enteric bacterial population. The presumptive enteric bacteria in turn represented between 25 to 88% of the total bacteria able to form colonies on NA (4.0×10^3 to 8.0×10^3 bacteria per ml). *E. coli* resistant to mercury(II) or to at least one antibiotic comprised 13.7% of the *E. coli* isolated from sample 1 but between 65 and 90% of the *E. coli* isolated from the remaining samples.

Table 4 shows the incidence of *E. coli* resistant to particular antibacterial agents. Resistance to streptomycin (Sm^r), sulfathiazole (Su^r), and tetracycline (Tc^r) were the most commonly encountered resistance phenotypes and occurred in approximately 10 to 40% of the *E. coli* tested from each sample. Chloramphenicol resistance (Cm^r) was also common (ca. 23%) in the two samples tested. In contrast, the number of *E. coli* resistant to ampicillin (Ap^r) or mercury(II) (Hg^r) varied from a few to 20% in each sample, whereas resistance to kanamycin (Km^r), gentamicin (Gm^r), or trimethoprim (Tp^r) was consistently low (where tested).

Table 5 shows the incidence of *E. coli* resistant to a single antimicrobial agent compared with those resistant to several agents simultaneously. The number of different combinations of resistance phenotypes is also shown. Thus, in sample 1, 18.5% of the *E. coli* exhibited resistance to only one of four antimicrobial agents.

In all samples, resistance to two or more antimicrobial agents was more common than single resistance, and the number of combinations of

TABLE 4. Percentage of *E. coli* resistant to particular antimicrobial agents

Origin of <i>E. coli</i> (sample no.)	No. of <i>E. coli</i> tested	% of <i>E. coli</i> resistant to the following antimicrobial agents ^a								
		Sm	Su	Tc	Cm	Ap	Hg	Km	Gm	Tp
1	750	8.8	17.5	29.9	NT	17.5	16.7	9.6	NT	NT
2	1,042	36.0	36.8	19.5	NT	2.1	5.2	0.4	NT	NT
3	1,042	11.9	32.4	32.7	NT	20.3	2.3	0.1	0.1	0.2
4	1,042	17.0 ^b	40.0	17.0	24.0	1.0	1.0	0	0	0
5	613	14.0 ^c	26.0	22.0	23.0	8.0	0	6.0	0.5	0.5

^a Determined by replica plating. NT, Not tested.

^b Of these bacteria, 47% were Sm^r Sp^r, and 53% were Sm^r Sp^r.

^c Of these bacteria, 58% were Sm^r Sp^r, and 42% were Sm^r Sp^r.

TABLE 5. Number of *E. coli* resistant to one or several antimicrobial agents

Origin of <i>E. coli</i> (sample no.)	No. of resistant <i>E. coli</i> tested	% of <i>E. coli</i> with the following number of resistance determinants ^a						No. of different resistance patterns detected
		1	2	3	4	5	6	
1	103	18.5 (4)	43.7 (10)	19.4 (6)	12.6 (3)	4.9 (2)	0.9 (1)	26
2	936	11.1 (5)	50.5 (9)	35.3 (8)	3.0 (3)	0.1 (1)	0 (0)	26
3	677	6.7 (4)	30.1 (6)	33.8 (5)	28.4 (4)	0.9 (3)	0.1 (1)	23
4	833	27.0 (4)	29.3 (7)	5.8 (13)	37.7 (5)	0.1 (1)	0.1 (1)	30
5	522	32.3 (8)	27.2 (18)	20.9 (28)	15.9 (19)	3.3 (7)	0.4 (2)	82

^a Values in parentheses indicate the number of different resistance patterns.

different phenotypes was 23 to 30 types in each sample. Sample 5 was, however, unusual in that 82 different combinations of resistances were identified.

It can be concluded, therefore, that although *E. coli* occurred in relatively low numbers (1×10^2 to 5×10^2 /ml) in the thawed fluid from five frozen chicken carcasses tested, each sample contained *E. coli* resistant to one or more of the antibiotics currently employed in clinical practice, and in most instances (4 of 5 samples) such resistant bacteria represented the majority population (65 to 90%).

Transfer of resistance determinants from wild-type *E. coli* to *E. coli* K-12. The 103 resistant *E. coli* isolated from sample 1 together with representative resistant isolates from the remaining four samples were tested for their ability to transfer their resistances to a laboratory derivative of *E. coli* K-12, UB1301 (Rif^r). Approximately 28% of the singly or multiply resistant *E. coli* from sample 1 could transfer at least one of their resistance properties to UB1301 (Table 6). This was the only instance in which all the resistant *E. coli* from a particular sample were tested for resistance transfer. Of the remaining *E. coli* isolated from samples 2 to 5, about half or more carried at least one transferable resistance determinant. These bacteria were a more biased sample in that they had been chosen for study either because they displayed the more rarely occurring resistance phenotypes or because they included combinations of resistance properties not encountered in the sample 1 isolates. Transfer of the determinants Tc^r, Su^r, Sm^r, or Ap^r, respectively, was detected in 46 of 129 (35%), 54 of 142 (38%), 45 of 76 (59%), or 29 of 72 (40%) of the strains in which they occurred. Thus, the probability of transfer of any of these determinants was similar. This contrasted with

the transfer of the Hg^r determinant from only 6% (3 of 45) of the strains tested.

It was concluded, therefore, that a minimum of 30% of the resistant *E. coli* associated with frozen chicken carcasses carry at least one resistance determinant that is transferable to *E. coli* K-12. Moreover, since the wild-type *E. coli* that exhibited transferable resistance produced culture supernatants that did not lyse UB1301, it appeared that conjugation, and not transduction, was the process responsible for transfer, and hence that these isolates probably carried conjugative plasmids. Further analysis of the resistant UB1301 derivatives showed that only a proportion of these could transfer their resistance to another K-12 subline—strain UB281 (Nal^r). This suggested that, whereas certain of the wild-type *E. coli* carried conjugative R plasmids, others probably carried a conjugative plasmid that had mobilized a cohabiting, nonconjugative R plasmid to UB1301, but that the conjugative element had subsequently been lost or had never been coinherited. Alternatively, certain of the conjugative R plasmids might have their transfer functions repressed within UB1301 and hence show undetectable levels of transfer under the experimental conditions used. No experiments were carried out to distinguish between the latter two possibilities. Instead, our subsequent analyses were confined to the characterization of 25 conjugative R plasmids that were defined as such by their ability to be (i) transferred to, and stably maintained in, the recombination-deficient strain UB2914 (Table 2), or (ii) transferred between sublines of K-12 and to consistently show 100% linked transfer of their resistance determinants irrespective of which determinant was used as the selected marker. The origin, phenotype, and intra-K-12 transfer frequencies of these various plasmids are shown in Table 2.

Characterization of conjugative plasmids derived from the wild-type *E. coli*. A total of 25 conjugative R plasmids that had been derived from wild-type *E. coli* associated with frozen chicken carcasses were characterized within *E. coli* K-12 by determining their responses in a variety of tests, including the following.

(i) Response to donor-specific bacteriophages. Derivatives of UB1301 carrying the various conjugative plasmids were tested for their response to the Inc P-specific ribonucleic acid phage, PRR1 (21), the Inc N-specific filamentous phage, Ike (16), and the deoxyribonucleic acid phage PR4, that can lyse bacteria carrying plasmids of either Inc P, Inc N, or Inc W groups (5). All bacteria were resistant to the phages except those carrying plasmids pVS100, pVS101, pVS105, pVS106, or any of pVS124 to pVS127 inclusive (Table 2). These bacteria were

TABLE 6. Transfer of resistance determinants from wild-type *E. coli* to *E. coli* K-12^a

Origin of <i>E. coli</i> (sample no.)	No. of resistant <i>E. coli</i> :		No. of UB1301R ⁺ derivatives transferring R-determinants to UB281 ^c
	Tested	Transferring at least one R-determinant to UB1301 ^b	
1	103	29	19
2	12	6	2
3	10	7	3
4	50	30	8
5	45	19	13

^a Transconjugants were isolated on NA containing rifampin or nalidixic acid, respectively, together with the appropriate antimicrobial agent.

^b Matings were for 2 h.

^c Matings were for 30 min.

lysed by both phages PR4 and Ike, and hence the eight plasmids were probably members of the Inc N group. These eight plasmids comprised four different resistance patterns (Table 2) and were derived from *E. coli* from two of the five samples tested.

(ii) **Response to UV radiation.** The survival of UB281 and its derivatives carrying the various conjugative plasmids were compared after their exposure to UV radiation. Also included for comparison was UB281 carrying the UV-protecting Inc N plasmid, R46-1 (9). A UV dose of 28 ergs/s per mm² for 30 s allowed the various strains to be subdivided into two distinct groups. One group comprised bacteria carrying R46-1 or any of the plasmids pVS100, pVS101, and pVS105 to pVS110 (inclusive), pVS122, and pVS124 to pVS127 (inclusive). These bacteria behaved similarly and showed an enhanced survival rate that was about 100-fold greater than that of the second group that comprised UB281 and its derivatives carrying any of the remaining plasmids (viable count of the former decreased from about 10⁸ bacteria per ml to between 5 × 10⁷ and 4 × 10⁶ bacteria per ml, compared to a decrease to between 3 × 10⁵ and 4 × 10⁶ bacteria per ml for the latter group). The plasmids that conferred a UV-protective effect on UB281 included the eight putative Inc N plasmids identified in the test for response to donor-specific bacteriophages, described above, together with the additional plasmids pVS107, pVS108, pVS109, and pVS122 (Table 2). UV protection has commonly been found associated with Inc N plasmids but is also determined by members of other Inc groups (14).

(iii) **Response to phages P1 and λ.** The EOP of phages P1 and λ on UB281 and its various plasmid-carrying derivatives was determined. Included for comparison was UB281 carrying the Inc N plasmid N3, which is known to determine the RII (or Hsp II) restriction-modification system (3), and as such shows decreased plating of both P1 and λ (Table 2). Of the 25 R⁺ strains studied, 2 show decreased plating of only P1, 1 shows decreased plating of only λ, and 10 show decreased plating of both P1 and λ.

The most common interaction involved plasmid N3, the eight putative Inc N plasmids, and pVS111. Strains carrying these various plasmids plated P1 and λ at an EOP of 10⁻² to 10⁻³. To determine whether the mechanism of these interactions was similar, phages from an RII-modified plaque of P1 and λ (obtained on the N3⁺ strain) were plated back on to UB281 or C600 and their R⁺ derivatives. An EOP of 1 was now obtained on all the strains. This suggests that the RII restriction-modification system is en-

coded by the eight putative Inc N plasmids and by pVS111, and that this is responsible for their interaction with the phages. These findings are consistent with those of Hedges (12), who found that the RII system was commonly encoded by Inc N plasmids.

The interactions between pVS110 and λ, and between pVS116 and P1, involved an EOP decrease of 10⁻² to 10⁻³. These interactions were shown to be due to plasmid-mediated restriction-modification because although R⁺-modified phage plated with equal efficiency on both R⁺ and R⁻ bacteria, it lost its modification specificity after a single cycle of propagation on the R⁻ host. The restriction-modification system involved was clearly not of the RII type (i) because only either P1 or λ was restricted, and (ii) because N3-modified P1 or λ was still restricted by pVS116⁺ and pVS110⁺ bacteria, respectively.

The interactions between one or both phages and the plasmids pVS121 and pVS114 involved the most extreme EOP decrease (<10⁻⁸), but since no residual phage plaquing was obtained, no further analysis was possible.

(iv) **Transfer inhibition of the Inc P plasmid, RP1.** Each of the conjugative R plasmids was tested for its ability to affect transfer of an Inc P plasmid, RP1, cohabiting the same cell. UB281 carrying RP1 alone could transfer this plasmid to UB1301 at a frequency of 1.2 × 10 per donor per 30 min of broth mating, as determined from the frequency of recovery of Km^r transconjugants. This transfer frequency was unaltered in the presence of 11 of the 25 R plasmids tested, but was reduced 100- to 500-fold by plasmids pVS107, pVS110, pVS111, pVS112, pVS116, and pVS118 (1.6 × 10⁻⁶ to 7.0 × 10⁻⁷/donor), and more than 5,000-fold by plasmids pVS100, pVS101, pVS105, pVS106, and pVS124 to pVS127, inclusive (<5.0 × 10⁻⁹/donor). The latter two groups of plasmids can clearly inhibit the transfer of RP1, an interaction previously described by Olsen and Shipley (22) as effected by certain Inc N, Inc W, and Inc X plasmids.

(v) **Incompatibility type of plasmids.** An attempt was made to assign the 25 conjugative R plasmids to particular incompatibility classes. This involved, first, a series of suitable R⁺ × R⁺ matings to achieve some subgrouping of the test plasmids; for example, pairwise matings between bacteria carrying the putative Inc N plasmids showed that these plasmids were indeed incompatible. A second series of matings involved pairs in which one strain carried the test plasmid, and the other carried one of the prototype plasmids that define a particular Inc group. Twenty prototype plasmids were used (Table 1).

Table 7 summarizes the data on which an

TABLE 7. Assignment of *R* plasmids to incompatibility groups

Plasmid in donor	Phenotype of donor	Plasmid in recipient ^a	Phenotype of recipient	Selected marker	Phenotype of transconjugants ^b	Inc type
N3	Sm ^r Su ^r Tc ^r	pVS100 or pVS101	Ap ^r Cm ^r Tc ^r	Sm ^r	Ap ^r Cm ^r	N
pVS105 or pVS106	Ap ^r Sm ^r Tc ^r	R46-1	Ap ^r Su ^r Tc ^r	Sm ^r	Su ^r	N
pVS124 or pVS125	Sm ^r	R46-1	Ap ^r Su ^r Tc ^r	Sm ^r	Ap ^r Tc ^r Su ^r	N
pVS126 or pVS127	Sm ^r Tc ^r	R46-1	Ap ^r Su ^r Tc ^r	Sm ^r	Ap ^r Su ^r	N
pVS107 or pVS108 or pVS109	Tc ^r	R144 <i>drd3</i>	Km ^r	Tc ^r	Km ^r	I _a
pVS110	Tc ^r	pVS111	Su ^r Tp ^r	Tc ^r	Su ^r Tp ^r	FI
pVS111	Su ^r Tp ^r	R386	Tc ^r	Tp ^r	Su ^r Tc ^r	FI
R6K	Ap ^r Sm ^r	pVS115	Ap ^r Cm ^r Tc ^r	Sm ^r	Cm ^r Tc ^r	X
pVS112	Ap ^r Su ^r	R6K	Ap ^r Sm ^r	Su ^r	Sm ^r	X
pVS113 or pVS129	Sm ^r /Sp ^r Su ^r	R6K	Ap ^r Sm ^r	Sp ^r	Ap ^r	X
pVS114	Sm ^r Su ^r	R6K	Ap ^r Sm ^r	Su ^r	Ap ^r	X
pVS116 or pVS117	Ap ^r	pVS114	Sm ^r Su ^r	Ap ^r	Sm ^r Su ^r	X

^a Donors were R⁺ derivatives of UB281, and recipients were R⁺ derivatives of UB1301. Matings were for 1 h. Transconjugants were isolated on NA containing rifampin and the appropriate antibiotic.

^b Fifteen transconjugants from each mating were tested.

incompatibility type was assigned to 20 of the 25 plasmids studied, although several of these probably represent reisolations of the same plasmid from different *E. coli* strains. It should be noted that in all matings, plasmid incompatibility was also observed when the polarity of the cross was reversed (data not shown). These data confirm that eight of the plasmids are members of Inc N. These originated in *E. coli* from 2 of 25 of the thawed fluid samples tested and, on the basis of resistance phenotype, represent at least four different plasmids. Of 25 plasmids, 7 were Inc X, representing 6 different plasmids on the basis of resistance phenotype (five types) and the difference in the Phi(P1) character of pVS116 and pVS117. These plasmids originated from *E. coli* in 3 of 5 of the samples tested and were more heterogeneous in their various properties than the Inc N plasmids studied. The two Inc FI plasmids each had a different origin and phenotype. The three Inc I_a plasmids were from the same sample but of two phenotypes.

Only 5 plasmids could not be assigned to a particular Inc group because their resistance phenotypes precluded matings in certain R⁺ × R⁺ combinations. This was a particular problem in the case of pVS122 which carries seven resistance determinants. However, various combinations of pairwise matings between pVS118⁺, pVS121⁺, pVS130⁺, and pVS134⁺ bacteria showed that these plasmids were all compatible with each other and with many of the prototype plasmids (Table 2). Overall, therefore, 24 of 25 plasmids that could be tested for their incompatibility type represented a maximum of eight incompatibility types, of which four were groups Inc N, Inc I_a, Inc FI, and Inc X.

DISCUSSION

This study has confirmed previous findings that many of the *E. coli* that are excreted by chickens or that contaminate commercially slaughtered or frozen chicken carcasses are resistant to a variety of the antibiotics used in clinical practice and that, more often than not, the bacteria are multiply resistant (17-19). We observed many different combinations of resistance phenotypes that included Cm and Ap resistance in addition to the Sm, Tc, and Su resistances detected by Linton et al. (19) and Howe et al. (13). This probably accounts for the significant numbers of bacteria that displayed even quadruple resistances (3 to 37%), although the numbers with five or six resistance determinants were very few. Tp or Gm resistance occurred rarely, but all of the resistances except that for gentamicin were subsequently found associated with conjugative R plasmids. We presume that the occurrence of resistant *E. coli* is due, at least in part, to the use of antimicrobial agents in the rearing of poultry, and certainly the antibiotics benzyl penicillin, chlortetracycline, oxytetracycline, and sulfonamides, in addition to several others, can be used in animal feeds in this country for the specific purpose of growth promotion (2).

More than 30% of the *E. coli* from each carcass examined could transfer at least one of their resistance determinants to *E. coli* K-12, and the probability of transfer of any of the determinants Tc^r, Su^r, Sm^r, or Ap^r was similar (35 to 59%). This contrasted with a transfer of the Hg^r determinant from only 3 of 45 (6%) strains tested. It might be that mercury(II) resistance is commonly due to a chromosomal mutation, or alter-

natively that the expression of some Hg^r determinants is poor in *E. coli* K-12, thereby reducing detection of their transfer.

It was thought likely that the majority of transfer events were due to the activity of conjugative plasmids, and this was confirmed by the subsequent recovery and partial characterization of 25 conjugative R plasmids from the wild-type strains. However, about half of the resistance determinants transferred from the wild-type strains to strain K-12 were nontransferable in subsequent intra-K-12 matings. Although this aspect was not further investigated, it seemed likely that the original transfer had been the result of mobilization of a nonconjugative R plasmid by a cohabiting conjugative plasmid in the same cell. Certainly, the likelihood that many of the wild-type strains harbored more than one R plasmid was evidenced by the recovery of the plasmid pairs pVS118 and pVS121 (total of three resistances), and pVS108 and pVS115 (total of four resistances) from the same bacterium, and by the fact that, although many strains displayed multiple resistances, only 1 of the 25 conjugative R plasmids studied carried greater than three resistance determinants.

The 25 conjugative R plasmids characterized carried single or multiple resistance to combinations of the Tc^r, Ap^r, Sm^r, Sm^r/Sp^r, Cm^r, Tp^r, and Hg^r determinants, and 20 of these plasmids were distinguishable by at least one of the criteria tested. The remaining five were probably duplicate isolations of the same plasmid from different *E. coli*. Of the plasmids, 20 were members of either the Inc X, Inc N, Inc I_a, or Inc FI groups. In a similar study carried out in Melbourne, Grant and Pittard (10) identified members of groups Inc I_a, Inc FII, Inc P, and Inc N from *E. coli* from calves, pigs, and chickens; the actual derivation of the plasmid-carrying strains was not stated. In other studies, Inc I_a, Inc H, and Inc P plasmids have been detected in *E. coli* from Danish pigs (15), from pigeons in Japan (25), and from breeding hens in France (6), respectively. Moreover, Δ and Δ-like plasmids (Inc I_a) were common in *Salmonella typhimurium* type 29 from human and bovine origin in England in the 1960s (1). The Inc FI and Inc X plasmids detected in this study have not been previously reported in Melbourne. Four of the five plasmids that could not be assigned to an Inc group were compatible with each other and with all the prototype plasmids against which they could be tested (Table 2). Thus, a minimum of 4, and a maximum of 8, plasmid types have been detected in this survey and include the broad host range Inc N plasmids that have been detected naturally in 13 different enteric species (14) and the Inc I_a plasmids that appear to be most common in *Salmonella* species (7, 14). It

seems, therefore, that the handling of poultry could constitute a potential health hazard not only because these animals carry many antibiotic-resistant *E. coli* of diverse O-serotypes, but also because these bacteria may harbor a range of different conjugative R plasmids including those with incompatibility types previously encountered in clinically isolated bacteria of human origin (7).

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