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IncH Plasmids in *Escherichia coli* Strains Isolated from Broiler Chicken Carcasses

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Plasmids conferring tellurite resistance were transferred at low temperature (27°C) from *Escherichia coli* strains isolated from chicken carcasses at the time of slaughter and after storage. They belonged to group IncH, as evidenced by their large molecular weight and incompatibility with plasmid pIP233. *E. coli* strains contaminating chickens meat can thus represent a source of IncH plasmids in the food chain of humans.

Plasmid-mediated resistance to antibiotics is commonly encountered in enteric bacteria isolated from the flora of monogastric farm animals (10), especially poultry (4, 11). Resistant strains from the gut contents readily contaminate broiler chicken carcasses at slaughter (1, 2, 15). Resistant *Escherichia coli* strains of avian origin contaminating chicken carcasses were found to be able to colonize the intestine of a human volunteer and to transfer their drug-resistance plasmids to resident strains in this new habitat (8). Plasmids belonging to a variety of incompatibility groups have been found in resistant *E. coli* strains isolated from poultry feces (5, 11) or from chicken carcasses (2), but the presence of IncH plasmids has not been reported in such strains. IncH plasmids are commonly considered to be associated with *Salmonella* spp. but have occasionally been found in *E. coli* strains from humans (14) or animals (9, 18). The presence of this group of plasmids in *E. coli* strains contaminating poultry carcasses could, however, be of particular significance for public health, considering its frequency in *Salmonella* spp. and its transferability in natural environments (19). IncH plasmids share two particular phenotypic properties: thermosensitivity of their conjugative transfer (17) and resistance to tellurium compounds (12, 16). We have used these two phenotypic traits to screen possible carriers of IncH plasmids among trimethoprim (Tmp)-resistant *E. coli* strains isolated in the course of a study on the bacterial contamination of poultry carcasses.

Samples were taken from eviscerated broiler chicken carcasses in a commercial slaughterhouse by the method of Lahellec and Meurier (6). Samples were taken just before packaging or after 8 days of storage under commercial conditions at 4°C in polyethylene bags. Skin areas of 2 cm^2 were streaked with sterile, moistened swabs at three different sites on fresh carcasses (breast, back, and body cavity along the backbone). For chilled, packaged carcasses, two additional sites were sampled (internal surface of the legs and wings). Swabs were agitated in 10 ml of sterile saline, and the medium was submitted to a series of 10-fold dilutions. Samples (0.1 ml) of these dilutions were streaked on citrate-mannitol agar (Difco Laboratories) containing 10 μg of Tmp (Sigma Chemical Co.) per ml. Tellurite (Tel) resistance of colonies growing on this medium was assessed as described by Pohl and Thomas (12), their resistance to antimicrobial drugs was studied by a disk-diffusion test, and the MICs of Tmp for these strains were determined on solid medium. Tmp-resistant strains were tested for conjugative transfer of this marker to *E. coli* K-12 strain J5-3, resistant to sodium azide, at 37 and 27°C (17), and Tel-resistant transconjugants were sought. These transconjugants were further conjugated with a K-12 strain harboring plasmid pIP233 (7). The compatibility of the transferred plasmid with pIP233 was tested by the method of Chabbert et al. (3).

DNA from transconjugants harboring plasmids incompatible with pIP233 was analyzed as described by Portnoy et al. (13). K-12 strains containing plasmids RP4, pIP233, or TP116 (20), and the V517 Macrina strain were similarly used as controls.

Ten carcasses were sampled before storage, and 64 Tmp-resistant isolates, among which 33 were also Tel resistant, were recovered from six of them. Of four carcasses stored for 8 days, only one carried Tmp-resistant strains. Of 10 such strains, 9 were Tel resistant. The majority of Tmp-Tel-resistant strains transferred their resistances at 37°C. Among the 13 strains which did not transfer at this temperature, 6 were able to transfer at 27°C. They were considered potential carriers of IncH plasmids. These six strains resistant to both Tmp and Tel were also resistant to several antibiotics (Table 1). Three had been recovered from internal swabbings of three different fresh carcasses, and three had been recovered from different sampling sites on the same chilled carcass. Except for strain BN1272, which was not resistant

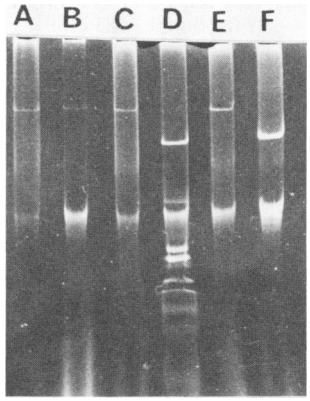

FIG. 1. DNA electrophoresis of transconjugants and reference strains. Lanes: A, pECD 1291; B, pIP 233; C, pECD 1272; D, V 517; E, pECD 1210; F, RP4.

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TABLE 1. Plasmids and their origins

Wild-type *E. coli*			Transconjugants	
Strain	Resistance phenotypes[a]	Origin	Plasmid	Resistance phenotypes[a]
BN1136	Ap Sm Cm Tc Su Tmp Tel	Chilled carcass no. 4 (breast)	pECD1136	Sm Cm Tc Su Tmp Tel
BN1210	Ap Sm Cm Tc Su Tmp Tel	Chilled carcass no. 4 (wing)	pECD1210	Sm Cm Tc Su Tmp Tel
BN1213	Ap Sm Cm Tc Su Tmp Tel	Chilled carcass no. 4 (leg)	pECD1213	Sm Cm Tc Su Tmp Tel
BN1272	Sm Km Cm Tc Su Tmp Tel	Fresh carcass no. 8 (internal)	pECD1272	Cm Tc Su Tmp Tel
BN1276	Ap Sm Cm Tc Su Tmp Tel	Fresh carcass no. 9 (internal)	pECD1276	Sm Cm Tc Su Tmp Tel
BN1291	Ap Sm Cm Tc Su Tmp Tel	Fresh carcass no. 10 (internal)	pECD1291	Sm Cm Tc Su Tmp Tel

[a] Antibiotic resistances: Ap, ampicillin; Sm, streptomycin; Cm, chloramphenicol; Tc, tetracycline; Su, sulfonamide; Tmp, trimethoprim; Km, kanamycin; Tel, tellurite.

to ampicillin, all exhibited the same resistance pattern. All the resistances, except resistance to ampicillin, were transferred to *E. coli* K-12 strains by selection on Tmp. All the transconjugants had also acquired Tel resistance. Tmp MICs were in all cases greater than 1,024 μg/ml.

Electrophoretic patterns obtained with the transconjugants revealed that they all showed the same single band corresponding to a plasmid of large molecular weight (Fig. 1 and 2). By comparison with the migration distances of reference plasmids pIP233, TP116 (not shown), and RP4 and of the higher band of strain V517, this molecular weight can be estimated at about 150×10^6. The six plasmids (pECD1136, pECD1210, pECD1213, pECD1272, pECD1276, and pECD1291) exhibited a strong incompatibility with pIP233.

These plasmids share a number of properties assigning them to group IncH: Tel resistance, thermosensitive transfer, high molecular weight close to those of pIP233 and TP116, and incompatibility with pIP233. As demonstrated by Pohl and Thomas (12), the use of this set of properties should lead to a rapid detection of IncH plasmids in a bacterial population. However, these properties must be used in conjunction, since Tel resistance does not seem to be specifically related to group IncH. In an independent study of 602 *E. coli* strains isolated in the same conditions from a poultry flock, 40% were Tel resistant, and in several cases this resistance was not mediated by IncH plasmids (E. Chaslus-Dancla, unpublished results).

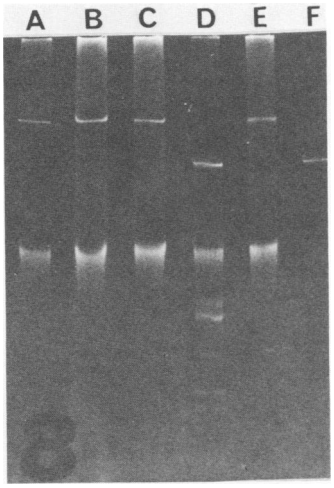

FIG. 2. DNA electrophoresis of transconjugants and reference strains. Lanes: A, pECD 1136; B, pIP 233; C, pECD 1276; D, V 517; E, pECD 1213; F, RP4.

Tel resistance nevertheless enables us to suggest that our IncH plasmids belong to subgroups HI2 or HI3, since no such resistance has been reported from HI1 plasmids (20). However, all members of the H complex are readily harbored by *Salmonella* spp., and their demonstration in strains contaminating human food thus provides additional evidence that broiler chicken carcasses may represent a reservoir of *E. coli* strains harboring undesirable plasmids in the food chain of humans. The present study has made use of enrichment methods which do not allow us to quantify the importance of the contamination by such plasmids. We cannot assume that the origin of the contaminating strains was either the flora of the animals or contamination from an external source at slaughter.

However, our results stress the importance of a thorough surveillance of the plasmid content of bacteria isolated from animal products.

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