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Drug Resistance of Enteric Bacteria

XIII. Distribution of R Factors in *Escherichia coli* Strains Isolated from Livestock

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Escherichia coli strains isolated from 151 swine and 108 fowl, which were kept at the Animal Health Center, Maebashi, Japan, were surveyed for drug resistance and distribution of R factors. All of the swine and 38% of the fowl excreted *E. coli* strains resistant to tetracycline, chloramphenicol, streptomycin, and sulfanilamide, or certain combinations thereof. Among 278 resistant cultures isolated from swine, 13% were found to be resistant to one antibiotic, whereas 87% were resistant to more than one antibiotic. Among these resistant strains, 40% carried R factors which were transferable by the usual conjugal process. The resistance patterns of these R factors included 36% which were singly resistant and 64% which were multiply resistant. Among 54 resistant cultures isolated from fowl, 24% were singly resistant and 76% were multiply resistant. Of the resistant strains from fowl, 22% carried R factors. The resistance patterns of R factors included 50% of the singly resistant type and 50% which were multiply resistant. In spite of feeding with dairy products containing only tetracycline, a high incidence of multiple resistance was observed in the *E. coli* strains and the R factors isolated from these animals.

The introduction of antimicrobial agents has produced great progress in the treatment of infectious diseases. However, the extensive use of these agents has resulted in the emergence of strains of bacteria resistant to them. In previous papers from this and other laboratories, it was reported that R factors are widespread among strains of gram-negative bacteria of clinical significance and that R factors are prevalent throughout the world (4, 10, 14, 17).

The extensive and sometimes indiscriminate use of these invaluable antimicrobial agents in commercial dairy products may create a problem in livestock almost as great as that in human beings, namely, emergence of strains of bacteria resistant to these agents, and, more specifically, strains harboring R factors.

This paper deals with the isolation of R factors from strains of *Escherichia coli* which were isolated from swine and fowl.

MATERIALS AND METHODS

Swine. All swine were 2 to 2.5 months old, and the total number studied was 151. They were kept under hygienic conditions at the Animal Health Center, Maebashi, Japan. They were fed with mothers' milk for about 50 days and thereafter with dairy products (Nihon Dairy Products Corp., Maebashi, Japan) containing 0.1% tetracycline (TC). After weaning, each pig was kept in a separate compartment and great care was taken to maintain hygienic conditions.

Fowl. The chickens were all hatched at the Animal Health Center, and the total number studied was 108. They were all 30 days old, and each chicken was kept in a separate cage. They were fed with dairy products (Nihon Dairy Products Corp.) containing 0.1% TC.

Media. Brain heart infusion (BHI; Eiken, Tokyo) was used as propagating medium for the transfer of R factors. Heart infusion (HI) agar (Eiken, Tokyo) was used for the assay of drug resistance. Semisynthetic medium was used for the determination of resistance to sulfanilamide (SA) and consisted of: medium A (5), 1,000 ml; Casamino Acids, 2.0 g; tryptophan, 10.0 mg; nicotinic acid, 2 mg; thiamine hydrochloride, 10.0 mg; glucose, 2.0 g; and agar, 12.5 g. Bromothymol blue (BTB)-lactose-agar was used for isolation of *E. coli* strains and consisted of 1,000 ml of HI agar, 20.0 g of lactose, and 40 ml of 0.2% BTB. The cultures originated as single lactose-fermenting colonies on the medium used diagnostically, i.e., BTB-lactose-agar, and were thereafter subcultured and identified as *E. coli* by fermentative and biochemical reactions.

Drugs. Chloramphenicol (CM), TC, dihydrostreptomycin (SM), SA, and nalidixic acid (NA) were used. These drugs were used in the following final concentrations: CM, 25 μg/ml; TC, 25 μg/ml;

SM, 25 μg/ml; and SA, 200 μg/ml. Drug resistance was determined by the method described previously (12) and is expressed as the maximal concentration of drug which allowed visible growth of bacteria after 18 hr of incubation at 37 C.

Isolation of E. coli strains resistant to drugs. Fresh feces was collected from each of the pigs and chickens, mixed with 5 volumes of saline, and streaked on the four different plates, i.e., BTB-lactose-agar containing CM, TC. or SM, and semisynthetic medium containing BTB-lactose and SA. After incubation for 18 hr at 37 C, lactose-fermenting colonies which developed on each plate were picked and subjected to three successive single-colony isolations on the medium used diagnostically for isolation of the resistant bacteria. These cultures were thereafter subcultured and identified, by fermentative and biochemical reactions, as *E. coli.* All cultures isolated from each of the four selective media were inoculated on BTB-lactose-agar, incubated at 37 C for 18 hr, and used as a master plate for the determination of drug resistance. The resistance pattern of each colony was determined by replica-plating on the four different plates, i.e., HI agar containing CM, TC, or SM, and semisynthetic medium containing SA. All cultures showing drug resistance were subcultured in cooked-meat medium and kept in a cold room at 4 C. The drug resistance patterns of each culture were determined by the method described previously (12).

Transfer of R factors. E. coli K-12 ML1410, resistant to NA (200 μg/ml), was used as the recipient of the R factors. Equal volumes of overnight BHI cultures of ML1410 and of the tested strain were mixed and incubated at 37 C. After 18 hr of incubation, the mixed culture was streaked on four different plates, i.e., HI agar plates containing NA (50 μg/ml) and TC, NA and CM, NA and SM, and semisynthetic medium containing NA and SA. Three colonies were picked from each selective plate and subjected to three successive single-colony isolations on medium of the same constitution. All colonies thus obtained were inoculated on an HI agar plate and used as a master plate for the determination of drug resistance. Drug resistance and resistance patterns of ML1410, to which R factors were transferred, was determined by replica-plating as described above. All cultures of ML1410 carrying R factors with different resistance patterns were selected and subcultured on HI agar plates. The drug resistance of each culture was again determined by the method described above.

RESULTS

This survey disclosed that all of the swine and 38% of the fowl excreted *E. coli* strains resistant to TC, CM, SM, and SA. However, none of the swine or fowl excreted *E. coli* strains resistant to these drugs before feeding with dairy products containing TC. The isolation frequency of resistance to each of these drugs is shown in Table 1. Resistance to TC, SM, and SA was isolated most frequently; resistance to CM was rare in comparison with *Shigella* and with other gram-negative bacteria isolated from human subjects (10, 14).

TABLE 1. *Isolation frequency of resistance to TC, CM, SM, and SA in Escherichia coli strains isolated from swine and fowl*[a]

Resistant to	Strains from swine		Strains from fowl	
	No.	%	No.	%
TC	233	84	39	72
CM	11	4	1	1.9
SM	249	90	33	61
SA	180	65	40	75

[a] TC, tetracycline; CM, chloramphenicol; SM, dihydrostreptomycin; SA, sulfanilamide.

The resistance patterns of the *E. coli* strains are shown in Table 2. Among the resistant strains from swine and fowl, 13 and 24%, respectively, were singly resistant, with the others being multiply resistant. Multiple resistance included 4% quadruple, 47% triple, and 32% double resistance in fowl. The multiply resistant strains were isolated at high frequency, even though the animals were fed with TC alone.

Among the resistant strains from the swine and fowl, 40 and 22%, respectively, carried R factors which were transferable by the conjugal process; 50% of 151 pigs and 11% of 108 chickens excreted *E. coli* strains carrying R factors.

R factors carrying multiple resistance patterns were isolated at high frequency (Table 3).

DISCUSSION

R factors, which were discovered in Japan (1, 9, 13, 16), have since been isolated in England (3, 4), West Germany (7), and in the United States (17). A previous paper (11) from this laboratory showed that 1.4% of 1,145 healthy

TABLE 2. *Resistance patterns of Escherichia coli strains isolated from swine and fowl*

Resistance pattern	Strains from swine		Strains from fowl	
	No.	%	No.	%
TC, CM, SM, SA	11	4	0	0
TC, SM, SA	131	47	18	33
TC, SM	68	24	5	9
TC, SA	11	4	9	17
SM, SA	21	8	8	15
CM, SA	0	0	1	2
SM	18	7	2	4
TC	12	4	7	13
SA	6	2	4	7

TABLE 3. *Resistance patterns of R factors isolated from Escherichia coli strains*

Resistance pattern	R factors in strains from swine		R factors in strains from fowl	
	No.	%	No.	%
TC, CM, SM, SA	11	10	0	0
TC, SM, SA	31	28	2	17
TC, SM	35	31	3	25
SM, SA	5	4	0	0
CM, SA	0	0	1	8
TC	6	5	5	42
SM	22	20	0	0
SA	2	2	1	8

human subjects carried multiply resistant *E. coli* strains. Another survey in 1960 disclosed that 1.3% of healthy human subjects carried multiply resistant *E. coli* (14). In contrast, 58.9% of inpatients treated with CM, and 20.5% of inpatients with tuberculosis and treated with SM, carried multiply resistant *E. coli* (11). Of 93 drug-resistant *E. coli* strains isolated from tuberculous patients, 53.7% were multiply resistant, i.e., resistant to TC, CM, SM, SA; TC, SM, SA; or CM, SM, SA.

In public health, the most important enteric bacterium in England and Europe is *Salmonella typhimurium*, of both animal and human origin (2, 3). Among 1,700 cultures of *S. typhimurium*, 500 were of human and 1,200 of animal origin; the overwhelming majority of animal cultures of *S. typhimurium* were isolated from calves, and many of the human infections with salmonellae were directly related to bovine disease (2).

A recent survey from this laboratory revealed that, of 2,650 *Shigella* strains isolated from patients in 1965, 58.4% were antibiotic-resistant and 95.0% of these resistant strains were multiply resistant. Of these resistant strains, 81% harbored R factors which were transmissible by cell-to-cell contact (14).

This laboratory has also investigated the drug resistance and biological properties of many other *Enterobacteriaceae* from clinical sources. The drug-resistant strains included 84.2% of the *E. coli*, 93.0% of the *Klebsiella*, and 90.0% of the *Proteus* strains; the strains carrying R factors included 84.0% of the *Proteus* cultures. Similarly, surveys for drug resistance and distribution of R factors in Europe and in the United States have shown that R factors are widely distributed (10, 14, 17).

The fact that R factors render drug-resistant a wide range of gram-negative bacilli (6) is of great importance in public health and animal husbandry.

Drug resistance and isolation of R factors in *S. typhimurium* were first reported in England, in strains of both human and animal origin (2). The present survey also disclosed that all swine and 38% of fowl fed with dairy products containing TC alone excreted multiply resistant *E. coli* strains, and that many of them were infectious. In Japan it is known that inpatients often excrete multiply resistant *Shigella* or *E. coli* strains after treatment with a single drug such as TC or CM (15).

As for swine, there is a possibility that they are infected with resistant strains excreted from the mothers during suckling, although resistant cultures were not isolated unless dairy products containing the drug were used as feed. A study of the emergence of resistant cultures after feeding fowl with drugs is now under way, because animal husbandry of fowl is very easy before and after hatching.

Isolation frequencies of R factors which are transmissible by cell-to-cell contact have been shown to be very high among *Shigella* and other gram-negative bacilli, but the distribution of nontransferable R factors has not yet been investigated.

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