ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 1980, p. 313-315
0066-4804/80/03-0313/03$02.00/0

Vol. 17, No. 3

Incidence of R-Plasmids in Fecal Flora of Healthy Household Dogs

DWIGHT C. HIRSH,[1]* GERALD V. LING,[2] AND ANNETTE L. RUBY[2]

Departments of Veterinary Microbiology[1] and Medicine,[2] School of Veterinary Medicine, University of California, Davis, California 95616

Rectal swabs were taken from healthy household dogs that, insofar as could be determined, had not received antimicrobial drugs. Tetracycline-resistant coliforms comprised 80 to 100% of the total number of coliforms in 61 (65%) of the 94 dogs sampled. The median number of other resistance determinants possessed by these tetracycline-resistant coliforms was 5.1. Of the tetracycline-resistant strains studied, 97% were resistant to streptomycin; 76% were resistant to sulfonamides; 59% were resistant to ampicillin; 59% were resistant to kanamycin/neomycin; and 40% were resistant to chloramphenicol. A total of 64% of the strains was shown to transfer resistance by conjugation or by the aid of the sex factor F. Of the strains transferring resistance, 33% were found to transfer all of their resistance determinants.

The most commonly recognized environmental reservoirs for R-plasmids (or the genes themselves) and the major means by which members of the family *Enterobacteriaceae* may become resistant to antimicrobial agents are the bacteria in the digestive tracts of food-producing animals who are eating medicated feed (9, 14). The family pet may be another potential reservoir for such genetic information. A recent survey indicates that approximately 38% of the households in the United States are dog owners with 1.4 dogs per family unit (1, 7). In some areas, for example, Yolo County in California, as high as 50% (1.5 dogs per family unit) of the households are dog owners (11). With the close association that exists between dogs and human members of a household, cross-contamination of enteric bacteria and their R-plasmids could occur as readily in such a setting as between farm animals and members of farm families (10, 12, 13).

For this reason, we have attempted to determine the relative incidence of R-plasmid-containing enteric bacteria in the digestive tracts of a selected group of normal household dogs who, insofar as was known, had not been treated with antimicrobial drugs. Tetracycline resistance was used as the means of selecting for R-plasmid-containing coliforms since this resistance has been shown to be commonly associated with R-plasmids (5). The results of this investigation have been summarized in this report.

MATERIALS AND METHODS

Animals. The 94 dogs included in this study were from the urban environment surrounding the University of California, Davis, and were patients of the Veterinary Medical Teaching Hospital, University of California, brought to the hospital for sterilization or for other elective surgical procedures. The dogs ranged in age from 1 to 10 years and were of a variety of breeds. As far as the owners could relate, none of the dogs had received antimicrobial agents at any time in their lives.

Sample collection and processing. Samples for culture were collected within 1 h of arrival at the hospital. Samples for culture were obtained with a moistened sterile swab rotated around the wall of the rectum. The swab was immediately placed in a tube containing 5 ml of sterile Ringer lactate solution. The tube was held in a refrigerator before culture preparation (20 min to 2 h). Samples (0.1 ml each) of the contents of this tube, diluted 1:50 and undiluted, were plated onto both plain MacConkey agar and MacConkey agar containing tetracycline (25 μg/ml). From the colony counts made on these plates after 24 h of incubation at 37°C, the percentage of tetracycline-resistant coliforms (coliforms being defined as lactose-positive colonies growing on MacConkey agar) was determined.

Tetracycline-resistant coliforms (one from each specimen or 94) were picked, identified (8), and subcultured on nutrient agar slants, which were paraffin sealed and stored at room temperature.

Susceptibility testing. We tested the tetracycline-resistant strains for susceptibility to ampicillin, streptomycin, kanamycin, neomycin, gentamicin, triple sulfonamides, chloramphenicol, and cephalothin, using the agar overlay modification of the standard disk diffusion assay (2).

Matings and mobilization studies. Procedures used for the single and triparental crosses were those described by Datta and Hedges (6). Samples (0.1 ml each) of the mating mixtures and controls were plated onto MacConkey agar containing tetracycline (25 μg/ml), nalidixic acid (100 μg/ml), and nalidixic acid and tetracycline. Transconjugates growing on MacConkey agar containing tetracycline and nalidixic acid were tested for susceptibility to other antimicrobial agents.

Bacterial strains. For single crosses, *Escherichia*

coli K-12 strain 711 (F^-, Nal^r) was used. For triparental crosses, *E. coli* strain JL2830 (Leu, F-*lac*, Nal^s) was used as the intermediate donor.

RESULTS

The percentage of tetracycline-resistant isolates from the 94 dogs is shown in Table 1. Every animal yielded isolates resistant to tetracycline.

All of the tetracycline-resistant isolates picked for susceptibility and transfer studies were proved to be *E. coli*. The susceptibility patterns of these isolates are shown in Table 2. The median number of antimicrobial drugs, including tetracycline, to which these isolates were resistant was 5.1. The frequency with which resistance of these isolates to a single antimicrobial agent was associated with resistance to other antimicrobial agents is shown in Table 3.

A total of 60 (53%) of the strains possessed the ability to transfer resistance in vitro. Of these, 23 (38%) transferred all of the resistance markers observed in the wild strain. Of the remaining 37 isolates, 15 (41%) were shown to possess tetracycline resistance that was "mobilizable" in a triparental cross, and in 10 of the 15, all of the resistance markers were mobilized. A total of 35 (37%) of the tetracycline-resistant *E. coli* strains was not shown to pass resistance either directly or in a triparental cross.

A relationship between autotransmitting resistance and relative incidence of tetracycline resistance in a sample could not be demonstrated. For example, in specimens containing >50% tetracycline-resistant coliforms, 47% of the R-plasmids were shown to be autotransmissible; among specimens containing <50% resistant coliforms, 44% were shown to be autotransmissible.

TABLE 1. *Relative incidence of tetracycline-resistant coliforms in the rectum of healthy household dogs*

Percent resistant coliforms	No. of animals (%)
80–100	65 (69)
20–80	19 (20)
<20	10 (11)

TABLE 2. *Resistance patterns of tetracycline-resistant E. coli isolated from the rectum of healthy household dogs*

No. of strains (%)	Resistance pattern[a]						
	Ap	Cp	Cm	Km(Nm)	Sm	Su	Tc
17 (18)	X		X	X	X	X	X
17 (18)				X	X	X	X
12 (13)	X	X	X	X	X	X	X
9 (10)					X		X
5 (5)	X				X	X	X
5 (5)	X				X		X
5 (5)					X	X	X
2 (2)	X	X			X	X	X
2 (2)	X		X		X	X	X
2 (2)			X		X	X	X
2 (2)				X	X		X
1 (1)	X	X		X	X	X	X
1 (1)	X	X		X	X		X
1 (1)	X			X	X	X	X
1 (1)	X			X	X		X
1 (1)	X	X			X		X
1 (1)	X	X					X
1 (1)			X		X		X
1 (1)	X						X
1 (1)							X

[a] Ap = ampicillin, Cp = cephalothin, Cm = chloramphenicol, Km = kanamycin, Nm = neomycin, Sm = streptomycin, Su = sulphonamides, Tc = tetracycline.

DISCUSSION

The results of this study indicate that normal household dogs shed large numbers of tetracycline-resistant enteric bacteria in their feces in the absence of known exposure to antimicrobial agents. As would be predicted, because of R-plasmid association, tetracycline resistance was found frequently with other resistance determinants. The origin of these organisms, the plasmids, or the source of the selective pressure is of some concern. Possibilities include commercial dog foods containing antibiotic residues in the food-animal parts of which they are composed, or association with other animals on medication (other companion animals or food animals). Studies are currently under way to answer these questions.

In a previous study performed on laboratory dogs, less than 1% of the lactose-fermenting microorganisms were found to be resistant to tetracycline (20 μg/ml) (15). The reasons for the difference between these findings and those reported here are not readily apparent, though the animals in the aforementioned study were known to be fed antibiotic-free food. Further, it was shown that as little as 10 μg of tetracycline per g of food was sufficient to change the flora to one as resistant as we report here.

Whatever the selective pressure, the fact remains that the normal household dogs evaluated in this study excreted large numbers of R-plasmid-possessing enteric bacteria. If these findings are representative of the canine pet population, R-plasmid-containing bacteria would have ample opportunity to contaminate the digestive tracts of human contacts. Such contacts may allow for the exchange of genetic information between ingested canine flora and resident human enteric flora. Such an exchange could add

TABLE 3. *Frequency of occurrence of resistance to a single antimicrobial agent in combination with resistance to other antimicrobial agents*

Antimicrobial agent[a]	No. of strains	Frequency of resistance[a] (%)					
		Ap	Cp	Cm	Km(Nm)	Sm	Su
Ap	55 (59)[b]	—[c]	42	64	60	95	78
Cp	25 (27)	92	—	60	56	96	92
Cm	38 (40)	92	39	—	71	97	95
Km (Nm)	55 (59)	60	25	49	—	96	89
Sm	91 (97)	57	26	41	58	—	78
Su	71 (76)	61	32	51	69	100	—

[a] Abbreviations are the same as those in Table 2, footnote *b*.
[b] Number in parentheses represents the percentage of the total number of tetracycline-resistant strains.

to the already undesirably high level of resistance genes in the human digestive tract (3, 4).

ACKNOWLEDGMENTS

The authors gratefully acknowledge the assistance of Rebecca Hall, Kimberly Nixon, Patricia Peterson, Mary Zimmerman, Nancy Steward, Richard Smith, and Faye Umeda.

LITERATURE CITED

1. **American Humane Association.** 1971. Animal Control Survey. American Humane Association, Denver.
2. **Barry, A. L., F. Garcia, and L. D. Thrupp.** 1970. An improved single disk method for testing the antibiotic susceptibility of rapidly growing pathogens. Am. J. Clin. Pathol. **53**:149–158.
3. **Burton, G. C., D. C. Hirsh, D. C. Blenden, and J. L. Zeigler.** 1974. The effects of tetracycline on the establishment of *Escherichia coli* of animal origin, and in vivo transfer of antibiotic resistance, in the intestinal tract of man, p. 241–253. *In* F. A. Skinner and J. G. Carr (ed.), The normal microbial flora of man. Academic Press Inc., London.
4. **Datta, N.** 1965. Drug resistance and R-factors in the bowel bacteria of London patients before and after admission to hospital. Br. Med. J. **2**:407–411.
5. **Datta, N.** 1971. R-factors in *Escherichia coli*. Ann. N. Y. Acad. Sci. **182**:59–64.
6. **Datta, N., and R. W. Hedges.** 1972. Host ranges of R-factors. J. Gen. Microbiol. **70**:453–460.
7. **Djerassi, C., A. Israel, and W. Jochle.** 1973. Planned parenthood for pets. Bull. At. Sci. **29**:10–19.
8. **Edwards, P. R., and W. H. Ewing.** 1972. Identification of the enterobacteriaceae, 3rd ed. Burgess Publishing Co., Minneapolis.
9. **Falkow, S.** 1975. Infectious multiple drug resistance. Pion, London.
10. **Fein, D., G. Burton, R. Tsutakawa, and D. Blenden.** 1974. Matching of antibiotic resistance patterns of *Escherichia coli* of farm families and their animals. J. Infect. Dis. **130**:274–279.
11. **Franti, C. E., and J. F. Kraus.** 1970. Aspects of pet ownership in Yolo County, California. J. Am. Vet. Med. Assoc. **164**:166–171.
12. **Levy, S. B., G. B. FitzGerald, and A. B. Macone.** 1976. Spread of antibiotic-resistant plasmids from chicken to chicken and from chicken to man. Nature (London) **260**:40–42.
13. **Linton, K. B., P. A. Lee, W. A. Richmond, W. A. Gillespie, J. J. Rowland, and V. N. Baker.** 1972. Antibiotic resistance and transmissible R-factors in the intestinal coliform flora of healthy adults and children in an urban and rural community. J. Hyg. **70**:99–104.
14. **Mercer, H. D., D. Pocurull, S. Gaines, S. Wilson, and J. V. Bennett.** 1971. Characteristics of antimicrobial resistance of *Escherichia coli* from animals: relationship to veterinary and management uses of antimicrobial agents. Appl. Microbiol. **22**:700–705.
15. **Rollins, L. D., S. A. Gaines, D. W. Pocurull, and H. D. Mercer.** 1975. Animal model for determining the no-effect level of an antimicrobial drug on drug resistance in the lactose-fermenting enteric flora. Antimicrob. Agents Chemother. **7**:661–665.