BioOne COMPLETE

Antimicrobial Resistance and Production of Toxins in Escherichia coli Strains from Wild Ruminants and the Alpine Marmot

Authors: Caprioli, A., Donelli, G., Falbo, V., Passi, C., Pagano, A., et al.

Source: Journal of Wildlife Diseases, 27(2) : 324-327

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-27.2.324

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

Downloaded From: https://bioone.org/journals/Journal-of-Wildlife-Diseases on 19 Apr 2024
Terms of Use: https://bioone.org/terms-of-use

Journal of Wildlife Diseases, 27(2), 1991, pp. 324–327
© Wildlife Disease Association 1991

ANTIMICROBIAL RESISTANCE AND PRODUCTION OF TOXINS IN *ESCHERICHIA COLI* STRAINS FROM WILD RUMINANTS AND THE ALPINE MARMOT

A. Caprioli,[1] G. Donelli,[1] V. Falbo,[1] C. Passi,[1] A. Pagano,[3] and A. Mantovani,[2] [1] Laboratorio di Ultrastrutture and [2] Laboratorio di Parassitologia, Istituto Superiore di Sanità, Rome, Italy; [3] Istituto di Igiene, Università di Milano, Milano, Italy

ABSTRACT: *Escherichia coli* strains isolated from 81 fecal samples from red deer (*Cervus elaphus*), roe deer (*Capreoulus capreoulus*), chamois (*Rupicapra rupicapra*) and alpine marmot (*Marmota marmota*) living in the Stelvio National Park, Italy, were examined for antimicrobial resistance and production of toxic factors. Direct plating of specimens on media containing antimicrobial drugs allowed us to isolate resistant strains of *E. coli* from 10 of 59 (17%) specimens examined by this technique. Nine of 31 specimens from red deer (29%) contained resistant strains. Different animals were likely colonized by the same resistant strain of *E. coli*. Conjugative R plasmids were found in four strains isolated from the marmot, roe deer and chamois. A strain from red deer produced heat-stable enterotoxin and another strain produced both hemolysin and cytotoxic necrotizing factor. A marmot isolate produced hemolysin alone. No strains were found to produce heat-labile enterotoxin or verotoxins.

Key words: *Escherichia coli*, antimicrobial resistance, toxins, bacteriology, survey, red deer, *Cervus elaphus*, roe deer, *Capreoulus capreoulus*, chamois, *Rupicapra rupicapra*, alpine marmot, *Marmota marmota*.

While the occurrence of antimicrobial resistance in microflora of farm animals and its association with the use of antibiotics in therapy and in feeds have been studied extensively (Smith and Halls, 1966; Linton, 1977), little is known about the occurrence of this phenomenon in the bacterial flora of wild animals. Van Dijck and Van devoorde (1979) did not find any antimicrobial resistance in *Escherichia coli* strains isolated from wild boars (*Sus scrofa*) of a zoological park and free living in a forest in the Ardennes mountains (Germany). A study conducted by Huber et al. (1971) in Illinois (USA) revealed the presence of R plasmids in <2% of the *Escherichia coli* strains from 78 animals belonging to 17 different species. A higher prevalence of antimicrobial resistance has been reported in wild ruminants and boars in the Republic of South Africa where 9% were colonized by drug-resistant bacteria (Maré, 1968).

In a previous study conducted on fecal samples from wild mammals living in the Stelvio National Park (Italy) we observed antimicrobial-resistant strains of *E. coli* in 17 of 121 fecal specimens examined (Pagano et al., 1985). We report here the results of a second survey from the same area in order to better define the occurrence of drug resistant bacteria and R plasmids in the fecal flora of different species of wild mammals living in the Park. The *E. coli* strains isolated were examined also for the production of toxic factors known to be associated with disease in farm animals: enterotoxins (Wray and Morris, 1985), verotoxins (Sherwood et al., 1985), hemolysins (Gregory, 1962) and cytotoxic necrotizing factor (CNF), a cytotoxin observed in human isolates of *E. coli* from enteritis and urinary tract infections (Caprioli et al., 1983, 1989) and recently reported in strains from piglet and calf diarrhea (Gonzales and Blanco, 1985; De Rycke et al. 1987; Blanco et al., 1988).

The Stelvio National Park is located in the central Alps, and borders with Austria and Switzerland. It extends to over 95,000 hectacres, and is regularly frequented by tourists. The study was conducted in the area of Zebrù Valley (46°27′ to 46°30′N, 10°26′ to 10°34′E).

Fourty-six fecal samples from red deer (*Cervus elaphus*), 13 from roe deer (*Capreolus capreolus*), seven from chamois

Downloaded From: https://bioone.org/journals/Journal-of-Wildlife-Diseases on 19 Apr 2024
Terms of Use: https://bioone.org/terms-of-use

TABLE 1. Antibiotic resistance of *E. coli* strains isolated from feces collected by the first sampling on medium without antimicrobial drugs.

Source of feces	Number of specimens examined	Number of strains studied	Number of resistant strains (R type)*	Resistances transferred to *E. coli* K12
Red deer	15	43	0	
Marmot	6	18	1 (A)	A
Chamois	1	2	1 (T)	T
Total	22	63	2	

* A, ampicillin; T, tetracycline.

TABLE 2. Antibiotic resistance of *E. coli* strains isolated from feces collected by the second sampling on medium containing antimicrobial drugs.

Source of feces	Number of specimens examined	Number of specimens from which drug-resistant strains were isolated	Number of resistant strains isolated
Red deer	31	9	14
Marmot	9	1	1
Roe deer	13	1	1
Chamois	6	0	0
Total	59	11	16

(*Rupicapra rupicapra*) and 15 from alpine marmots (*Marmota marmota*) were collected by two distinct sampling campaigns. The ruminants were free-living and their stools were collected soon after elimination. Fresh fecal marmot samples were collected outside burrows. Specimens were stored in Cary-Blair transport medium and examined within 3 days.

Fifteen fecal samples from red deer, six from marmot and one from chamois were collected by the first sampling and plated on MacConkey agar. Sixty-three strains of *E. coli*, at least two colonies from each specimen, were identified by the API 20 E system (API System S.A., La Balme Les Grottes, France) and tested for antimicrobial susceptibility using the method described by Bauer et al. (1966). The following discs were used: nalidixic acid, 30 μg, cephalothin, 30 μg; chloramphenicol, 30 μg; gentamicin, 10 μg; kanamycin, 30 μg; streptomycin, 10 μg; tetracycline, 30 μg; ampillicin, 10 μg; sulphathiazole, 1.0 mg. Presence of conjugative R plasmids was verified as described by Anderson and Threlfall (1974).

Antibiotic resistant *E. coli* were found in two specimens (Table 1). A strain from the chamois was resistant to tetracycline and one from marmot to ampicillin. In both cases resistance was plasmid medicated.

To increase the recovery rate of resistant strains, 59 additional fecal specimens were collected by a second sampling campaign and directly plated on MacConkey plates supplemented with one of the following antimicrobial agents: nalidixic acid, 60 μg/ml, ampicillin, 20 μg/ml, chloramphenicol, 20 μg/ml, gentamicin, 10 μg/ml, kanamycin, 20 gmg/ml, streptomycin, 20 μg/ml, tetracycline, 10 μg/ml. Table 2 shows the source of the specimens and the results of the fecal cultures.

Sixteen antimicrobial-resistant strains of *E. coli* were isolated from 11 of the 59 specimens examined (17%), with red deer being the species in which these bacteria were most frequently observed (nine of 31 specimens, 29%). Thirteen strains were resistant to several antimicrobial agents (Table 3), while three isolates were resistant to tetracycline only. Transferable resistances were found in the marmot strain, and in the isolate from roe deer. All the 14 isolates from red deer failed to transfer their resistance to the *E. coli* K12 strain used as recipient. Three strains from three different specimens were resistant to tetracycline and 11 strains from six different specimens to streptomycin, sulphathiazole and tetracycline. To verify if different red deer were colonized by the same resistant strain of *E. coli* the plasmid profiles and outer membrane proteins (OMP) electrophoretic patterns of these isolates were studied. Plasmid DNA was isolated according to Birnboim and Doly (1979) and subjected to electrophoresis in 0.8% agarose gels. OMP were extracted and ana-

Downloaded From: https://bioone.org/journals/Journal-of-Wildlife-Diseases on 19 Apr 2024
Terms of Use: https://bioone.org/terms-of-use

TABLE 3. Resistance patterns of *E. coli* strains isolated from feces on media containing antimicrobial drugs.

Source of feces	Number of strains	Resistance pattern*	Resistances transferred to *E. coli* K12
Marmot	1	S Su T	S Su T
Roe deer	1	A Ce S Su T	A Ce Su T
Red deer	3	T	—
Red deer	11	S Su T	—

* A, ampicillin; Ce, cephalotin; S, streptomycin; Su, sulphatiazole, T, tetracycline.

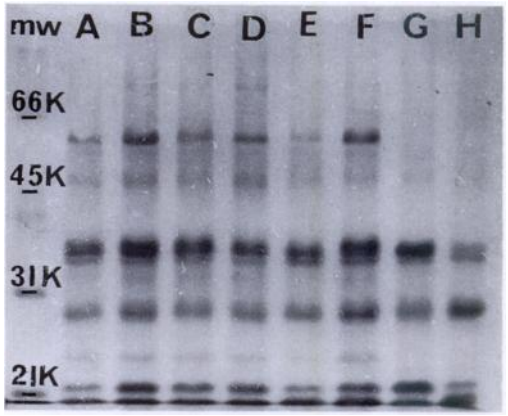

FIGURE 1. Outer membrane protein patterns of antimicrobial resistant *E. coli* strains from red deer. Lanes A to F, strains with R-type S Su T. Lanes G and H, strains with R-type T. MW, molecular weight markers.

lyzed according to the method of Achtman et al. (1983).

The T-resistant isolates showed the same biochemical API pattern and OMP profile and contained two plasmids of 2.8 and 3.6 megadaltons (not shown). The SSuT-resistant strains did not contain plasmids, produced colicin I (Frederiq, 1957) and shared the same API pattern and OMP profile. Figure 1 shows the OMP patterns of some of these strains.

All the *E. coli* isolates were also examined for the production of enterotoxins, hemolysin (Hly) and cytotoxins. The cell culture assays used to detect heat-labile enterotoxin (LT) and cytotoxin production are described elsewhere (Caprioli et al., 1983; Bisicchia et al., 1985). Heat-stable enterotoxin (ST) production was tested in infant mice according to the method of Dean et al. (1972). For Hly production, *E. coli* strains were grown on 5% sheep blood agar plates.

Three strains were found to produce toxic factors. An isolate (serogroup 036) was positive for ST and another one (serogroup 02) for Hly and CNF. Both strains were isolated from red deer and did not show antimicrobial resistance. The ampicillin-resistant marmot strain produced Hly alone. No isolates were found to produce LT or verotoxins.

In conclusion, we found *E. coli* strains producing toxic factors related to disease in farm animals in feces of wild mammals in Stelvio National Park. The *E. coli* strains from wildlife may be resistant to several antimicrobial agents and carry R plasmids, although the intestinal microbiota of free ranging animals is not directly exposed to antibiotics. A possible source of the antimicrobial-resistant *E. coli* observed in these animals might be those humans and domestic animals which carry antibiotic-resistant bacteria. In fact, the area in which the animals live is regularly frequented by tourists; dairy cows, goats and some poultry use the same pastures as wild ruminants although this type of contact may be only occasional for marmots. Local veterinarians and farmers only occasionally use antibiotics, since it is not an area of intensive farming.

It was not possible to determine the sources of the antimicrobial-resistance in wild mammals. Further work is in progress to characterize the R plasmids isolated in this study and to collect other strains of *E. coli* carrying R plasmids from humans and domestic animals living in the same area of the Park. The comparison between the characteristics of these two groups of plasmids would be a useful tool in order to ascertain how the antibiotic resistance spreads in such an environment.

We are grateful to W. Frigo, Director of the National Park of Stelvio, together with his staff for their kind co-operation.

Downloaded From: https://bioone.org/journals/Journal-of-Wildlife-Diseases on 19 Apr 2024
Terms of Use: https://bioone.org/terms-of-use

Serotyping of toxin producing *E. coli* strains was kindly carried out by Ida and Frits Ørskov at the International Escherichia Centre in Copenhagen, Denmark.

LITERATURE CITED

ACHTMAN, M., A. MERCER, B. KUSECEK, A. POHL, M. HEUZENROEDER, W. AARONSON, A. SUTTON, AND R. P. SILVER. 1983. Six widespread bacterial clones among *Escherichia coli* K1 isolates. Infection and Immunity 39: 315–335.

ANDERSON, E. S., AND E. J. THRELFALL. 1974. The characterization of plasmids in the enterobacteria. Journal of Hygiene (Cambridge) 72: 471–487.

BAUER, A. W., W. M. M. KIRBY, J. C. SHERRIS, AND M. TURCK. 1966. Antibiotic susceptibility testing by a standardized single disc method. American Journal of Clinical Pathology 45: 493–496.

BIRNBOIM, H. C., AND J. DOLY. 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acid Research 7: 1513–1523.

BISICCHIA, R., R. CIAMMARUGHI, A. CAPRIOLI, V. FALBO, AND F. M. RUGGERI. 1985. Toxin production and haemagglutination in strains of *Escherichia coli* from diarrhoea in Brescia, Italy. Journal of Hygiene (Cambridge) 95: 353–361.

BLANCO, J., E. A. GONZALES, S. GARCIA, M. BLANCO, B. REGUEIRO, AND I. BERNARDEZ. 1988. Production of toxins by *Escherichia coli* strains isolated from calves with diarrhoea in Galicia (North Western Spain). Veterinary Microbiology 18: 297–311.

CAPRIOLI, A., V. FALBO, L. G. RODA, F. M. RUGGERI, AND C. ZONA. 1983. Partial purification and characterization of an *Escherichia coli* toxic factor that induces morphological cell alterations. Infection and Immunity 39: 1300–1306.

———, ———, F. M. RUGGERI, F. MINELLI, I. ØRSKOV, AND G. DONELLI. 1989. Relationship between cytotoxic necrotizing factor production and serotype in hemolytic *Escherichia coli*. Journal of Clinical Microbiology 27: 759–761.

DEAN, A. G., Y. C. CHING, R. G. WILLIAMS, AND L. B. HARDEN. 1972. Test for *E. coli* enterotoxin using infant mice: Application in a study of diarrhoea in children in Honolulu. Journal of Infectious Diseases 125: 407–411.

DE RYCKE, J., J. F. GUILLOT, AND R. BOIVIN. 1987. Cytotoxins in non-enterotoxigenic strains of *Escherichia coli* isolated from feces of diarrheic calves. Veterinary Microbiology 15: 137–150.

FREDERIQ, P. 1957. Colicins. Annual Reviews in Microbiology 11: 7–22.

GONZALES, E. A., AND J. BLANCO. 1985. Production of cytotoxin VT in enteropathogenic and non-enteropathogenic *Escherichia coli* strains of porcine origin. FEMS Microbiology Letters 26: 127–130.

GREGORY, D. W. 1962. Hemolytic *Escherichia coli* enteritis of weanling pigs. American Journal of Veterinary Research 141: 947–949.

HUBER, W. G., D. KORICA, T. P. NEAL, P. R. SCHNURRENBERGER, AND R. J. MARTIN. 1971. Antibiotic sensitivity patterns and R factors in domestic and wild animals. Archives of Environmental Health 22: 561–567.

LINTON, A. H. 1977. Antibiotic resistance: The present situation reviewed. Veterinary Records 100: 354–360.

MARÉ, I. J. 1968. Incidence of R factors among gram negative bacteria in drug-free human and animal communities. Nature (London) 220: 1046–1047.

PAGANO, A., G. NARDI, C. BONACCORSO, V. FALBO, C. PASSI, V. SANGUINETTI, AND A. MANTOVANI. 1985. Faecal bacteria of wild ruminants and the alpine marmot. Veterinary Research Communications 9: 227–232.

SHERWOOD, D., D. R. SNODGRASS, AND A. D. O'BRIEN. 1985. Shiga-like toxin production from *Escherichia coli* associated with calf diarrhea. Veterinary Records 116: 217.

SMITH, H. W., AND S. HALLS. 1966. Observation of infective drug resistance in Britain. British Medical Journal 1: 266–269.

VAN DIJCK, P. J., AND H. VAN DEVOORDE. 1979. Course of antibiotic sensitivities in *E. coli* and *Staphylococcus aureus* from animals. Zentralblatt für Bakteriologie, Mikrobiologie und Hygiene (I° Abstract Original B) 169: 519–529.

WRAY, C., AND J. A. MORRIS. 1985. Aspects of colibacillosis in farm animals. Journal of Hygiene (Cambridge) 95: 577–593.

Received for publication 22 January 1990.

Downloaded From: https://bioone.org/journals/Journal-of-Wildlife-Diseases on 19 Apr 2024
Terms of Use: https://bioone.org/terms-of-use