JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 1997, p. 2184–2185 Vol. 35, No. 8
0095-1137/97/$04.00+0

Prevalence of Bacterial Resistance to Quinolones and Other Antimicrobials among Avian *Escherichia coli* Strains Isolated from Septicemic and Healthy Chickens in Spain

JESUS E. BLANCO, MIGUEL BLANCO, AZUCENA MORA, AND JORGE BLANCO*

Reference Laboratory of E. coli*, Department of Microbiology and Parasitology, Veterinary Faculty, University of Santiago de Compostela, 27002 Lugo, Spain*

Received 11 October 1996/Accepted 30 April 1997

Antimicrobial therapy is an important tool in reducing the enormous losses in the poultry industry caused by *Escherichia coli* infections (colibacillosis). However, resistance to existing antimicrobials is widespread and of concern to poultry veterinarians. Antimicrobial resistance testing of 468 avian *E. coli* strains isolated in Spain showed very high levels of resistance to trimethoprim-sulfamethoxazole (67%) and the new fluoroquinolones (13 to 24%). As these antimicrobial agents may cause cross-resistance with human enteric pathogens, prudent use of them in veterinary medicine is highly recommended.

Escherichia coli is a major pathogen of worldwide importance in commercially produced poultry, contributing significantly to economic losses in both chickens and turkeys (11). Colibacillosis begins, in general, with an infection of the upper respiratory tract, followed by septicemia (17). *E. coli* is commonly found in the intestinal tracts of animals, but usually only certain pathogenic serotypes that show virulence factors (adhesive ability, aerobactin production, serum resistance, and presence of the ColV plasmid) cause disease conditions (5, 9). Antimicrobial therapy is an important tool in reducing both the incidence and mortality associated with avian colibacillosis (7, 10, 18). However, resistance to existing antimicrobials is widespread and of concern to poultry veterinarians (1–4, 10, 12–14). The fluoroquinolones are a new class of antimicrobials that exhibit excellent activity against gram-negative bacilli (8, 16), although their use in poultry may be inappropriate because of cross-resistance with fluoroquinolones used for treatment of important human enteric infections (6, 8, 15). The aim of this study was to evaluate the frequency of resistance to quinolones and other groups of antimicrobial agents in avian *E. coli* strains.

A total of 301 *E. coli* strains isolated from clinically affected broiler chickens and 167 strains from healthy controls were collected in numerous commercial hatcheries in the province of Orense (Galicia, Spain) during 1992 and 1993. All strains isolated from diseased chickens came from confirmed cases of colisepticemia in which bacteria were obtained in profuse culture from both heart and liver tissues. Fecal strains were isolated from the cloacal content of healthy chickens. The antimicrobial resistance of the strains to different antibacterial agents was determined by the standard disk diffusion method in Mueller-Hinton agar with disks provided by Difco (Detroit, Mich.) and BioMérieux (Marcy l'Etoile, France).

The resistance frequencies (RF) for each antimicrobial agent tested are shown in Table 1. These RF are generally high and permit the division of the antibiotics into four groups. The first group includes the antibiotics to which there were very high levels of resistance (RF from 67 to 94%). These are streptomycin, tetracycline, sulfadiazine, and trimethoprim-sulfamethoxazole. The second group includes the antibiotics to which there were medium to high levels of resistance (36 to 46%). These are ampicillin, mezlocillin, piperacillin, nitrofurantoin, nalidixic acid, and pipemidic acid. The third group contains the antibiotics to which there were moderate levels of

TABLE 1. Antibiotic resistance of avian *E. coli* strains

Antibiotic (amt in disk[a])	No. (%) of resistant strains[b]		
	Septicemic chickens	Healthy chickens	Total
Ampicillin (10)	104 (35)	111 (66)	215 (46)
Amoxicillin-clavulanic acid (30)	2 (0.7)	3 (2)	5 (1)
Mezlocillin (75)	93 (31)	87 (52)	180 (38)
Piperacillin (100)	91 (30)	78 (47)	169 (36)
Cephalothin (30)	47 (16)	19 (11)	66 (14)
Cefoxitin (30)	0 (0)	0 (0)	0 (0)
Cefotaxime (30)	0 (0)	0 (0)	0 (0)
Streptomycin (10)	235 (78)	135 (81)	370 (79)
Neomycin (30)	43 (14)	44 (26)	87 (19)
Kanamycin (30)	56 (19)	49 (29)	105 (22)
Gentamicin (10)	41 (14)	14 (8)	55 (12)
Tobramycin (10)	23 (8)	6 (4)	29 (6)
Amikacin (30)	0 (0)	0 (0)	0 (0)
Tetracycline (30)	283 (94)	159 (95)	442 (94)
Colistin (10)	0 (0)	0 (0)	0 (0)
Polymyxin B (300 U)	0 (0)	0 (0)	0 (0)
Chloramphenicol (30)	75 (25)	48 (29)	123 (26)
Sulfadiazine (300)	264 (88)	145 (87)	409 (87)
Trimethoprim (5)	197 (65)	131 (78)	328 (70)
Trimethoprim-sulfamethoxazole (25)	189 (63)	127 (76)	316 (67)
Nitrofurantoin (300)	146 (49)	74 (44)	220 (47)
Nalidixic acid (30)	144 (48)	61 (37)	205 (44)
Pipemidic acid (30)	122 (41)	51 (31)	173 (37)
Norfloxacin (10)	50 (17)	11 (7)	61 (13)
Flumequine (30)	72 (24)	19 (11)	91 (19)
Ofloxacin (5)	54 (18)	13 (8)	67 (14)
Ciprofloxacin (5)	50 (17)	11 (7)	61 (13)
Pefloxacin (5)	84 (28)	27 (16)	111 (24)

[a] Amounts are given in micrograms, except where indicated.

[b] Total incidences of resistance of strains from septicemic (n = 301) and healthy (n = 167) chickens were 2,465 and 1,423, respectively (total for both groups, 3,888). Incidences of resistance per strain were 8.2 and 8.5 for septicemic and healthy groups, respectively (average for both groups, 8.3).

* Corresponding author. Mailing address: Laboratorio de Referencia de *E. coli*, Departamento de Microbiología y Parasítología, Facultad de Veterinaria, 27002 Lugo, Spain. Phone: 34/82/252303. Fax: 34/82/252195. E-mail: jba@Lugo.wsc.es.

resistance (12 to 26%). These are cephalothin, neomycin, kanamycin, gentamicin, chloramphenicol, and the five fluoroquinolones assayed (norfloxacin, flumequine, ofloxacin, ciprofloxacin, and pefloxacin). The fourth group (IV) contains the antibiotics to which there were low levels of resistance (0 to 6%). These are amoxicillin-clavulanic acid, cefoxitin, cefotaxime, tobramycin, amikacin, colistin, and polymyxin B.

In vitro antimicrobial susceptibility testing of veterinary pathogens can provide valuable guidance to the veterinarian in the choice of appropriate chemotherapy. In vitro antibiotic sensitivity results obtained in our study agreed with several previous reports (1, 2, 4, 10, 14), which have indicated increasing incidences of antibiotic-resistant *E. coli* strains isolated from chickens with colibacillosis. However, the high percentage of *E. coli* strains that were resistant to trimethoprim-sulfamethoxazole (67%) and to the new fluoroquinolones (13 to 24%) in our study was surprising. Similar results were found by Amara et al. (2) in Morocco, although the poultry industry in that African, Mediterranean country is relatively young. Our field observations indicate that the abusive and anarchic use of antibiotics is probably the cause of the high percentages of resistance detected in Spanish avian *E. coli* strains. Since the use of cotrimoxazole and fluoroquinolones in poultry may cause cross-resistance with human enteric pathogens (especially with *Salmonella* and *Campylobacter* spp.), prudent use of these antimicrobial agents in avian species is highly recommended.

This study was supported by grants from the Xunta de Galicia (26102A94 and 26101B94), from the Comisión Interministerial de Ciencia y Tecnología (AGF92-0570), and from the Fondo de Investigación Sanitaria (FIS 94/1056). J.E.B. and A.M. acknowledge the DGICYT of the Spanish Ministerio de Educación y Ciencia, Xunta de Galicia, and the Universidad de Santiago de Compostela for their research fellowships.

We thank Cristina Prado, Marta Rio, and Lucía Fernández for skillful technical assistance.

REFERENCES

1. **Allan, B. J., J. Van den Hurk, and A. A. Potter.** 1993. Characterization of *Escherichia coli* isolated from cases of avian colibacillosis. Can. J. Vet. Res. **57:**146–151.
2. **Amara, A., Z. Ziani, and K. Bouzoubaa.** 1995. Antibioresistance of *Escherichia coli* strains isolated in Morocco from chickens with colibacillosis. Vet. Microbiol. **43:**325–330.
3. **Chulasiri, M., and O. Suthienkul.** 1989. Antimicrobial resistance of *Escherichia coli* isolated from chickens. Vet. Microbiol. **21:**189–194.
4. **Cloud, S. S., J. K. Rosenberger, P. A. Fries, R. A. Wilson, and E. M. Odor.** 1985. In vitro and in vivo characterization of avian *Escherichia coli*. Serotypes, metabolic activity, and antibiotic sensitivity. Avian Dis. **29:**1084–1093.
5. **Dho-Moulin, M.** 1993. Les *Escherichia coli* pathogènes des volailles. Ann. Med. Vet. **137:**353–357.
6. **Endtz, H. P., G. J. Ruijis, and B. van Klingeren.** 1991. Quinolone resistance in *Campylobacter* isolated from man and poultry following introduction of fluoroquinolones in veterinary medicine. J. Antimicrob. Chemother. **27:**199–211.
7. **Freed, M., J. P. Clarke, T. L. Bowersock, W. G. van Alstine, J. M. Balog, and P. Y. Hester.** 1993. Effect of spectinomycin on *Escherichia coli* infection in 1-day-old ducklings. Avian Dis. **37:**763–766.
8. **García-Rodríguez, J. A., M. J. Fresnadillo, M. I. García, E. García-Sánchez, J. E. García Sánchez, I. Trujillano, and the Spanish Study Group on Quinolone Resistance.** 1995. Multicenter Spanish study of ciprofloxacin susceptibility in gram-negative bacteria. Eur. J. Clin. Microbiol. Infect. Dis. **14:**456–459.
9. **González, E. A., J. Blanco, S. B. Baloda, G. Fröman, M. Dho, J. P. Lafont, and T. Wadström.** 1990. Virulent *Escherichia coli* strains for chicks bind fibronectin and type II collagen. Microbios **62:**113–127.
10. **Goren, E.** 1990. Colibacillose bij pluimvee: etiologie, pathologie en therapie. Pluimvee Symposium. Upjohn-Nederland, Doorn, The Netherlands.
11. **Gross, W. G.** 1994. Diseases due to *Escherichia coli* in poultry, p. 237–259. *In* C. L. Gyles (ed.), *Escherichia coli* in domestic animals and humans. CAB International, Wallingford, United Kingdom.
12. **Hinton, M., S. K. Lim, and A. H. Linton.** 1987. The influence of antibacterial agents on the complexity of the *Escherichia coli* flora of chickens. FEMS Microbiol. Lett. **41:**169–173.
13. **Nakamura, M., H. Yoshimura, and T. Koeda.** 1981. Fluctuation of drug-resistant *Escherichia coli* strains in chickens. Jpn. J. Vet. Sci. **43:**481–490.
14. **Peighambari, S. M., J. P. Vaillancourt, R. A. Wilson, and C. L. Gyles.** 1995. Characteristics of *Escherichia coli* isolates from avian cellulitis. Avian Dis. **39:**116–124.
15. **Piddock, L. J. V., C. Wray, and I. McLaren.** 1990. Quinolone resistance in *Salmonella* spp.: veterinary pointers. Lancet **336:**125.
16. **Raemdonck, D. L., A. C. Tanner, S. T. Tolling, and S. L. Michener.** 1992. In vitro susceptibility of avian *Escherichia coli* and *Pasteurella multocida* to danofloxacin and five other antimicrobials. Avian Dis. **36:**964–967.
17. **Sojka, W. J., and R. B. A. Carnaghan.** 1961. *Escherichia coli* infection in poultry. Res. Vet. Sci. **2:**340–352.
18. **Watts, J. L., S. A. Salmon, R. J. Yancey, B. Nersessian, and Z. V. Kounev.** 1993. Minimum inhibitory concentrations of bacteria isolated from septicemia and airsacculitis in ducks. J. Vet. Diagn. Invest. **5:**625–628.