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Original article

Antibiotic resistance in *Escherichia coli* isolates obtained from animals, foods and humans in Spain

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Abstract

Antibiotic resistance was investigated in 474 *Escherichia coli* isolates recovered from animal faeces (broilers, pigs, pets, bulls and horses), human faeces (patients and healthy volunteers) and food products of animal origin. *E. coli* isolates (3260) recovered from human significant infectious samples were also included. There was a high frequency of nalidixic acid, ciprofloxacin and gentamicin resistance in *E. coli* isolates from broilers (88, 38 and 40%, respectively), and from foods (53, 13 and 17%). High levels of resistance to trimethoprim–sulphamethoxazole and tetracycline have been found in *E. coli* isolates from broilers, pigs and foods. These data raise important questions about the potential impact of antibiotic use in animals and the possible entry of resistant pathogens into the food chain.

Keywords: Antibiotic; Resistance; *Escherichia coli*; Human; Animal; Food

1. Introduction

Antibiotics are used for therapeutic and prophylactic purposes in animals and humans, and some of them have also been used as growth promoters to improve animal production. Antibiotics with similar structure are being used in medical and veterinary practice. The correlation between intensive use of antimicrobial agents and development of resistant bacteria is well documented for pathogenic bacteria [1], but there is less information about the impact on commensal bacteria that commonly colonise the intestinal tract of humans and animals. Endogenous bacterial flora may play an important role as acceptor and donor of transmissible drug resistance genes [2,3]. *Escherichia coli* is commonly found in the intestinal tract of humans and animals [3,4] and can also be implicated in human and animal infectious diseases [1]. Animal food products are an important source of *E. coli* as faecal contamination of carcasses at the slaughterhouse is frequent. These microorganisms and their possible resistance determinants may be transmitted to humans if these foods are improperly cooked or otherwise mishandled. The level of antibiotic resistance in *E. coli* represents a useful indicator of the resistance dissemination in bacterial populations. There are some reports in which antibiotic susceptibility of *E. coli* isolates from healthy humans [5–7] or animals [3,8–11] have been studied, but in few cases comparative results have been shown [7,12] or isolates from foods analysed.

The objective of our study was to determine and compare antibiotic resistance frequencies in *E. coli* isolates recovered from faecal samples of humans and animals, as well as from food products of animal origin.

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2. Materials and methods

Isolates of *E. coli* included in this study were obtained over the period 1997–1999 from human and animal faecal samples and from foods of animal origin. Human faecal samples were collected from: in- and out-patients (235 samples, named as 'human faeces-P') of San Millán Hospital (La Rioja, Spain); and healthy volunteers who had not been treated with antibiotics for at least 3 months preceding sampling (40 samples, named as 'human faeces-HV'). Faecal samples of healthy animals were taken from food producing animals (40 broilers and 74 pigs, collected during evisceration in slaughterhouses) and other animals (28 dogs, 5 cats, 35 fighting bulls and 5 horses). Food products of animal origin (69 samples) obtained from 14 local supermarkets and poultry shops in La Rioja (Spain) were also analysed (Table 1). All faecal and food samples were analysed as follows: 0.5 g of the sample was suspended in sterile saline solution (dilution 1/10 w/v) and 25 μl were plated onto non-supplemented Levine agar plates and incubated at 37 °C for 24 h. One suspected *E. coli* colony from each sample (on the basis of colony size and morphology) was selected for identification [13] and further studies. A total of 474 *E. coli* isolates were recovered from all these samples (Table 1).

E. coli isolates obtained during 1999 from human clinical samples (urine, blood, wound, etc.) in the routine work at the San Millán Hospital (La Rioja, Spain) were also included in this study. These isolates (n = 3260) were designated *E. coli* from 'human infectious samples'.

Table 1
Source of the samples analysed and *E. coli* strains isolated from them

Samples		*E. coli* isolates
Origin	No.	No.
Faeces of		
Human-P[a]	235	214
Human-HV[b]	40	36
Broilers	40	40
Pigs	74	73
Pets: dogs/cats	28/5	25/3
Bulls/ horses	36/5	32/4
Foods		
Hamburger, sausage and minced chicken	28	16
Skin of chicken	20	16
Caecum of chicken	9	9
Breast of chicken	4	1
Pre-cooked chicken foods	5	3
Turkey products	3	2
Human infectious samples		3260[c]

[a] Human-P: human-patients.
[b] Human-HV: human-healthy volunteers.
[c] These *E. coli* isolates were obtained in the routine work at the Microbiology Laboratory during 1999.

Antimicrobial susceptibility testing was carried out by the agar disk diffusion method [14] on Müeller–Hinton agar plates. The antibiotics tested were the following: nalidixic acid, ciprofloxacin, gentamicin, kanamycin, amikacin, tobramycin, ampicillin, amoxycillin–clavulanic acid, trimethoprim–sulphamethoxazole, ceftazidime, cefotaxime, cefoxitin, imipenem, tetracycline, chloramphenicol and fosfomycin. Susceptibility tests followed NCCLS breakpoints [14].

3. Results and discussion

During the study period, 474 *E. coli* isolates were recovered from 532 samples obtained from human or animal faeces or from food products (Table 1). A high frequency of *E. coli* isolation (47 isolates of 69 samples, 68%) was obtained from supermarket poultry products. This was not surprising as *E. coli* is a normal inhabitant of the chicken intestine [4] and contamination may occur during evisceration.

The results of the in vitro susceptibility testing of *E. coli* isolates of different sources, are shown in Table 2. Higher frequencies of nalidixic acid, ciprofloxacin, gentamicin and kanamycin resistances occurred in *E. coli* isolates from broilers (88, 38, 40 and 38%, respectively) and food products (53, 13, 17 and 40%, respectively), than from the other sources whose ranges of resistance were the following: 0–21, 0–3, 0–7 and 5–20%, respectively (Table 2). Sixteen and 8% of *E. coli* isolates from human clinical samples, were resistant to ciprofloxacin and gentamicin, respectively.

In the period 1992–1993, 7% *E. coli* from healthy chickens in Spain were found to be resistant to ciprofloxacin [9] and a recent study showed a high percentage of intestinal colonisation by ciprofloxacin resistant *E. coli* isolates in healthy chickens [15]. A high prevalence of ciprofloxacin resistance was detected in *E. coli* isolates from turkeys and turkey farmers (49 and 29%, respectively) in contrast to pig or pig farmer faecal samples (1–2%) in The Netherlands [16]. The authors of this last study commented that at that time enrofloxacin (a quinolone similar to ciprofloxacin, used in veterinary medicine) was commonly used for turkeys but not for pigs. A ciprofloxacin resistance level of 8% has also been reported recently in *E. coli* from pig faeces in Spain [17]. The high prevalence of *E. coli* resistant to ciprofloxacin in broilers compared with pig isolates in our study might also reflect a higher use of quinolones for chickens than in pigs or other animals.

A low frequency of ciprofloxacin resistance was found in *E. coli* isolates from human faecal samples and similar results have been reported by other authors [6,18,19]. In another study, the use of selective plates

Table 2
Resistance percentages to 16 antibiotics in *E. coli* isolates of different origins

Origin of samples	*E. coli* no.	Resistance (%)[a] to															
		NALX	CIPX	GEN	KAN	AMK	TOB	AMPC	AMOX/CA	IMIP	CFOX	CTAZ	CTAX	SMX/TMP	TET	CHL	FOS
Faeces of																	
Human-patients	214	11[b]	1	4	15[b]	0	3	39	6	0	1	0	0	19	35	9	1
Human-healthy volunteers	36	6	0	0	6	0	0	31	11	0	0	0	0	3	25	6	0
Broilers	40	88	38	40	38	0	5	58	5	0	2	0	0	65	75	12	0
Pigs	73	14	3	7	20	0	7	29	6	0	0	0	0	48	68	15	2
Pets/bulls/horses	64	8	0	2	5	0	3	31	6	0	0	0	0	19	39	5	0
Human infectious samples	3260	–[c]	16	8	ND[d]	0[e]	6	62	11	0.1	2	0.4	0.5	36	ND	17[f]	4
Foods	47	53	13	17	40	0	6	47	13	0	6	0	0	34	53	8	4

[a] NALX, nalidixic acid; CIPX, ciprofloxacin; GEN, gentamicin; KAN, kanamycin; AMK, amikacin; TOB, tobramycin; AMPC, ampicillin; AMOX/CA, amoxycillin–clavulanic acid; IMIP, imipenem; CFOX, cefoxitin; CTAZ, ceftazidime; CTAX, cefotaxime; SMX/TMP, trimethoprim–sulphamethoxazole; TET, tetracycline; CHL, chloramphenicol; FOS, fosfomycin.
[b] Percentages were calculated with data from 120 E. coli isolates.
[c] Pipemidic acid resistance was 30%.
[d] ND, not determined.
[e] Amikacin resistance was determined in 23 of the *E. coli* isolates.
[f] Chloramphenicol resistance was determined in 386 of the *E. coli* isolates.

supplemented with ciprofloxacin for isolation of resistant *E. coli* strains resulted in finding a high frequency of human faecal colonisation [15]. These results suggest that a high percentage of humans may be colonised in the intestinal tract by ciprofloxacin-resistant *E. coli* isolates. These may be in low numbers only but could be selected by the use of quinolones. An increase in the rate of ciprofloxacin resistance in *E. coli* isolates obtained from human clinical infections has been reported [1,15,20,21]. Ciprofloxacin resistance frequencies of 21.6% have been reported for *E. coli* isolated from complicated urinary tract infections in Spain [22] and Threlfall et al. [1] reported 6% resistant strains in *E. coli* from blood samples in England and Wales. Our study shows that 16% of the *E. coli* isolates from human infectious samples were ciprofloxacin resistant.

In our study, a 40 and a 17% of resistance to gentamicin have been found in *E. coli* isolates from broilers and food of chicken origin. Different frequencies of gentamicin resistance have been reported previously for *E. coli* isolates from chicken: 90% for healthy animals in Saudi Arabia [23] and 8 and 14% for healthy and septicaemic chickens in Spain [9]. By contrast, our study showed a lower incidence of gentamicin resistance in *E. coli* isolates from pigs than from broilers. Low levels of gentamicin resistance has been reported in isolates from pigs [17] although in farms with high-use of antibiotics, the incidence was higher [10]. The difference in ages of the pigs in our study (slaughterhouse) and those analysed by Mathew et al. [10] (sows to pigs of 63 days) could explain the different resistance levels. One-quarter of our gentamicin resistant *E. coli* were also apramycin-resistant. Apramycin is an aminoglycoside, structurally related with gentamicin, with an exclusively veterinary use. This antibiotic could have selected apramycin-resistant *E. coli* strains producing AAC(3)-IV enzyme, that in addition are gentamicin resistant [24,25].

The frequency of gentamicin resistance detected in *E. coli* isolates obtained from human faeces-HV, human faeces-P or human clinical samples were 0, 4 and 8%, respectively. Higher levels of gentamicin resistance (8–38%) have been recently reported in *E. coli* faecal isolates from healthy humans [18,19,23] and from human clinical specimens [23,26].

The frequencies of ampicillin resistance among *E. coli* isolates from broilers and foods were 58 and 47%, respectively, with fewer isolated from the other origins (29–39%) (Table 2). All studied isolates from these groups were susceptible to imipenem, ceftazidime or cefotaxime and only 34 of the 474 isolates were resistant to amoxycillin–clavulanic acid (20% of those resistant to ampicillin). The level of ampicillin resistance in *E. coli* from human clinical specimens (62%) was similar to other studies at [19,22,23], whereas resistance rates to imipenem, cefotaxime and ceftazidime were lower than 1%. High frequencies of ampicillin resistance have also been reported previously in isolates from healthy chickens in Saudi Arabia (89%) and from healthy chickens and pigs in Spain (66–72%) [9,17,23]. Eighteen per cent of ampicillin resistance was found in *E. coli* isolated from faecal samples of veterinarians [5], 54% in poultry workers [23] and 47–60% (depending on the age) in healthy population in Shangai [19].

The highest frequencies of trimethoprim–sulphamethoxazole, tetracycline and chloramphenicol resistance were observed in isolates from broilers (65, 75 and 12%, respectively) and pigs (48, 68 and 15%), and lower frequencies were detected from the other sources (ranges of resistance: 3–19, 25–39 and 5–9%, respectively) (Table 2). In the group of *E. coli* isolates obtained from human clinical specimens there was a high level of chloramphenicol resistance (17%). Frequencies in the range of 59 to 92% for trimethoprim–sulphamethoxazole resistance have been reported previously for healthy and sick chickens [9,23] and dogs [8]. High rates of tetracycline resistance have also been reported by other authors in faecal samples of healthy and sick chickens (94–99%), healthy pigs (77–95%), healthy dogs or with diarrhoea (60%) [8–10,17,23]. Lower frequencies of tetracycline resistance have been detected in this study in *E. coli* isolates from human faecal samples of healthy volunteers (25%) or patients (35%), than in isolates from non-human origin. Similar frequencies were detected in healthy volunteers in The Netherlands [18] and in clinical isolates in England and Wales [1].

Frequencies of 5–17% of chloramphenicol resistance have been found in the *E. coli* isolates in our study. Resistance frequencies of 29–59% have been reported for *E. coli* isolates from healthy chickens and pigs in Spain [9,17] and lower values (0–13%) in faecal *E. coli* isolates of healthy pigs in other European countries [3,11]. Resistances of 4–73% in *E. coli* isolates from healthy volunteers have been reported [5,6,18,19,23]. A resistance rate of 10% has been reported for *E. coli* isolates from blood and cerebrospinal fluid samples [1], and of 28% in faecal samples of human patients in Saudi Arabia [23].

The percentage of *E. coli* isolates with resistance to up to six different antibiotics (nalidixic acid, gentamicin, ampicillin, trimethoprim–sulphamethoxazole, tetracycline, and chloramphenicol) is shown in Fig. 1. 70.3% of *E. coli* isolates from human faeces (either from patients or healthy volunteers) showed resistance to none or only one of the six antibiotics, and only 6.3% of the isolates of this group were resistant to ≥ 4 antibiotics. In contrast, 50% of *E. coli* isolates from broilers were resistant to ≥ 4 antibiotics, and only 2.5% were susceptible to all antibiotics. *E. coli* isolates from pets, bulls and horses had a similar distribution to human strains. Isolates from food products or pigs

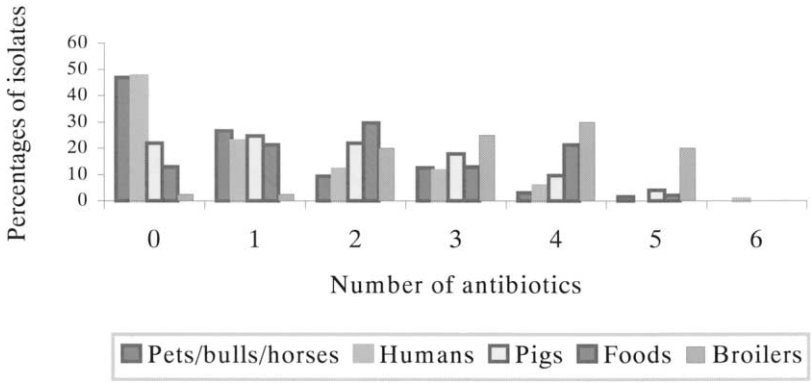

Fig. 1. Resistance of *E. coli* isolates of different origins to a variety of antibiotics (the antibiotics considered were: nalidixic acid, ampicillin, tetractycline, gentamicin, trimethoprim–sulphamethoxazole, chloramphenicol). *X*-axis represents the number of antibiotics to which isolates are resistant.

occupy an intermediate position although isolates from food products show more frequently multiresistance than those from pigs. Al-Ghamdi et al. [23] have also found that *E. coli* isolates from healthy chickens frequently show antibiotic multiresistance.

All but two chloramphenicol resistant *E. coli* isolates obtained either from human or animal faecal samples, or from food products, also showed tetracycline resistance. The percentages of isolates resistant to both ampicillin and gentamicin among our *E. coli* were as follows: 22% in broilers (56% of these isolates were ciprofloxacin resistant), 13% in foods, 7% in pigs, 3% in human faeces-P, 2% in pets/bulls/horses and 0% in human faeces-HV. Nalidixic acid and tetracycline resistance occurred in 65% of isolates of broilers, 34% of foods, 11% of pigs, 10% of human faeces-P, 7% of pets, 3% of human faeces-HV, but no bulls/horses.

In conclusion, differences in antibiotic resistance frequencies were detected in *E. coli* isolates, from different sources (human, broiler, pig, pet, food, etc.). These differences could reflect the specific use of antibiotics in these groups. The high prevalence of quinolone and gentamicin resistance in *E. coli* isolates from broilers and the high level of trimethoprim–sulphamethoxazole and tetracycline resistance in *E. coli* from broilers and pigs is worrying. The increasing prevalence of resistance in the isolates of human origin may have important therapeutic implications. More restrictive policies on the use of antibiotics in animals may result in an improvement of the current situation.

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References

[1] Threlfall EJ, Cheasty T, Graham A, Rowe B. Antibiotic resistance in *Escherichia coli* isolated from blood and cerebrospinal fluid: a 6-year study of isolates from patients in England and Wales. Int J Antimicrob Agents 1998;9:201–5.
[2] Davies J. Inactivation of antibiotics and the dissemination of resistance genes. Science 1994;264:375–82.
[3] Sunde M, Fossum K, Solberg A, Sørum H. Antibiotic resistance in *Escherichia coli* of the normal intestinal flora of swine. Microb Drug Resist 1998;4:289–99.
[4] Tannock GW. Normal Microflora. An Introduction to Microbes Inhabiting the Human Body. London: Chapman and Hall, 1995:37–47.
[5] Bongers JH, Franssen F, Elbers ARW, Tielen MJM. Antimicrobial resistance of *Escherichia coli* isolates from the faecal flora of veterinarians with different professional specialties. Vet Q 1995;17:146–9.
[6] London N, Nijsten R, van den Bogaard A, Stobberingh E. Carriage of antibiotic-resistant *Escherichia coli* by healthy volunteers during a 15-week period. Infection 1994;22:187–92.
[7] Nijsten R, London N, van den Bogaard A, Stobberingh E. Antibiotic resistance among *Escherichia coli* isolated from faecal samples of pig farmers and pigs. J Antimicrob Chemother 1996;37:1131–40.
[8] Adesiyun AA, Campbell M, Kaminjolo JS. Prevalence of bacterial enteropathogens in pet dogs in Trinidad. J Vet Med 1997;B44:19–27.
[9] Blanco JE, Blanco M, Mora A, Blanco J. Prevalence of bacterial resistance to quinolones and other antimicrobials among avian *Escherichia coli* strains isolated from septicemic and healthy chickens in Spain. J Clin Microbiol 1997;35:2184–5.
[10] Mathew AG, Saxton AM, Upchurch WG, Chattin SE. Multiple antibiotic resistance patterns of *Escherichia coli* isolates from swine farms. Appl Environ Microbiol 1999;65:2770–2.
[11] Nijsten R, London N, van den Bogaard A, Stobberingh E. Antibiotic resistance of enterobacteriaceae isolated from the faecal flora of fattening pigs. Vet Q 1993;15:152–6.
[12] Van den Bogaard AE. Antimicrobial resistance. Relation to human and animal exposure to antibiotics. J Antimicrob Chemother 1997;40:453–61.
[13] Murray PR. Pocket guide to clinical Microbiology. In: American Society for Microbiology. ASM, Washington, 1996.
[14] National Committee for Clinical Laboratory Standards, 2000. Performance standards for antimicrobial disk susceptibility tests, 7th ed. Approved standard M2-A7. National Committee for Clinical Laboratory Standards, Wayne, PA.

[15] Garau J, Xercavins M, Rodríguez-Carballeira M, et al. Emergence and dissemination of quinolone-resistant *Escherichia coli* in the community. Antimicrob Agents Chemother 1999;43:2736–41.

[16] Van den Bogaard A, London N, Driessen C, Stobberingh E. Prog. Abstr. 37th Intersci. Conf. Antimicrob. Agents Chemother., 1997; abstr. C-137, Toronto.

[17] Teshager T, Herrero IA, Porrero MC, Garde J, Moreno MA, Dominguez L. Surveillance of antimicrobial resistance in Escherichia coli strains isolated from pigs at spanish slaughterhouses. Int J Antimicrob Agents 2000;15:137–42.

[18] Bonten M, Stobberingh E, Philips J, Houben A. High prevalence of antibiotic resistant *Escherichia coli* in faecal samples of students in the south-east of The Netherlands. J Antimicrob Chemother 1990;26:585–92.

[19] Zhang X-L, Wang F, Zhu D-M, et al. The carriage of *Escherichia coli* resistant to antibiotics in healthy populations in Shanghai. Biomed Environ Sci 1998;11:314–20.

[20] Aguiar JM, Chacon J, Cantón R, Baquero F. The emergence of highly fluoroquinolone-resistant *Escherichia coli* in community-acquired urinary tract infections. J Antimicrob Chemother 1992;29:349–50.

[21] Bauernfeind A, Abele-Horn M, Emmerling P, Jungwith R. Multiclonal emergence of ciprofloxacin-resistant clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae*. Antimicrob Chemother J 1994;34:1075–6.

[22] Oteo J, Aracil B, Hoyo JF, Perianes J, Gómez-Garcés JL, Alós JI. Do the quinolones still constitute valid empirical therapy for community-acquired urinary tract infections in Spain? Clin Microbiol Infect 1999;5:654–6.

[23] Al-Ghamdi MS, El-Morsy F, Al-Mustafa ZH, Al-Ramadhan M, and. Hanif M. Antibiotic resistance of *Escherichia coli* isolated from poultry workers, patients and chicken in the eastern province of Saudi Arabia. Trop Med Int Health 1999;4:278–83.

[24] Chalus-Dancla E, Martel JL, Carlier C, Lafont JP, Courvalin P. Emergence of aminoglycoside 3-*N*-acetyltransferase IV in *Escherichia coli* and *Salmonella typhimurium* isolated from animals in France. Antimicrob Agents Chemother 1986;29:239–43.

[25] Salauze D, Otal I, Gómez-Lus R, Davies J. Aminoglycoside acetyltransferase 3-IV (aacC4) and hygromycin B 4-I phosphotransferase (hphB) in bacteria isolated from human and animal sources. Antimicrob Agents Chemother 1990;34:1915–20.

[26] SENTRY Participants Group, Schmitz F-J, Verhoef J, Fluit AC. Prevalence of aminoglycoside resistance in 20 European university hospitals participating in the European SENTRY Antimicrobial Surveillance Programme. Eur J Clin Microbiol Infect Dis 1999;18:414–21.