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Short communication

Antimicrobial resistance observed in *Escherichia coli* strains isolated from fecal samples of cattle and pigs in Korea during 2003–2004

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Abstract

A total of 744 *Escherichia coli* strains isolated from 830 fecal samples of healthy cattle and pigs in all provinces of Korea were examined for resistance to 16 antimicrobials. The most frequently observed resistance in cattle isolates was to tetracycline (30.5%), followed by resistance to streptomycin (20.4%), ampicillin (12.0%) and chlorampenicol (6.9%). Prevalences of resistance to the same four antimicrobials in swine isolates were 96.3%, 66.8%, 66.1%, and 47.6%, respectively. The prevalence of resistance in pigs was much higher than that in cattle, with 98.3% of pig isolates and 37.1% of cattle isolates showing resistance to one or more of the antimicrobial agents tested.

Keywords: *Escherichia coli*; Antimicrobial resistance; Cattle; Pig

1. Introduction

Pathogens resistant to various antimicrobial agents have emerged as a major concern for human and veterinary medicine. To tackle the problem of development and spread of resistance, reliable data on the rates of changes in the susceptibilities to specific antimicrobials of bacteria isolated from both humans and animals are required. It is therefore necessary to develop representative monitoring programs for the objective determination of the local and national extent of antimicrobial resistance (Caprioli et al., 2000; Felmingham, 2002; Salisbury et al., 2002). Only then can effective intervention measures to reduce this risk be identified.

The WHO has recently published its 'global principles for the containment of antimicrobial resistance in animals intended for food' aimed at reducing the overuse of antimicrobials in food animals for protection of human as well as animal health (World Health Organization, 2000). Continued surveillance of the antimicrobial susceptibility profiles of foodborne pathogens, including *Escherichia coli*, has also been strongly recommended to identify emerging antimicrobial-resistant phenotypes within the food production continuum (World Health Organization, 1997). In this context, investigations into the amounts of antimicrobials consumed by livestock and antibiotic resistance of bacteria isolated from food-producing animals and food products were conducted for the first time in 2003 and 2004 in Korea.

E. coli is a common component of the gut flora of food animals that can serve as an indicator for the acquisition of resistance to various antimicrobials by enteric organisms (Aarestrup et al., 1998; Bogaard and Stobberingh, 2000). The use of *E. coli* as the indicator bacteria is also appropriate because changes in the antibiotic resistance of this species may serve as an early warning of the development of resistance by related pathogenic bacteria (Bogaard and Stobberingh, 2000). Many studies on antimicrobial resistance of pathogenic *E. coli* strains isolated from sick animals in Korea have been published (Choi et al., 2002; Han et al., 2002; Kang et al., 2005), but there is little data concerning commensal *E. coli* strains isolated from healthy animals. In this study, we report data on the

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Table 1
Antimicrobial resistance of *Escherichia coli* isolated from cattle and pig

Class	Antimicrobials	Amount/disc (μg)	Diffusion zone breakpoint (mm)	No. of isolates (%)	
				Cattle (n=334)	Pig (n=410)
Penicillins	Ampicillin	10	≤13	40 (12.0)	271 (66.1)
	Amoxicillin/clavulanic acid	20/10	≤13	0 (0)	1 (0.2)
Cephalosporins	Cephalothin	30	≤14	13 (3.9)	30 (7.3)
	Cefazolin	30	≤14	0 (0)	13 (3.2)
	Cefoxitin	30	≤14	2 (0.6)	1 (0.2)
	Cefotaxime	30	≤14	0 (0)	1 (0.2)
	Cefepime	30	≤14	0 (0)	0 (0)
Aminoglycosides	Streptomycin	10	≤11	68 (20.4)	274 (66.8)
	Gentamicin	10	≤12	7 (2.1)	42 (10.2)
	Amikacin	30	≤14	1 (0.3)	0 (0)
Fluoroquinolones	Ciprofloxacin	5	≤15	2 (0.6)	32 (7.8)
	Enrofloxacin	5	≤12	2 (0.6)	33 (8.0)
	Norfloxacin	10	≤10	2 (0.6)	27 (7.1)
Sulfonamides	Trimethoprim/sulfamethoxazole	1.25/23.75	≤10	6 (1.8)	159 (38.8)
Phenicols	Chloramphenicol	30	≤12	23 (6.9)	195 (47.6)
Tetracyclines	Tetracycline	30	≤14	102 (30.5)	395 (96.3)

antimicrobial susceptibility of *E. coli* strains isolated from feces collected from apparently healthy pigs and cattle on 55 feedlots throughout Korea between April 2003 and October 2004.

2. Materials and methods

2.1. Collection of fecal samples

Fresh fecal samples from healthy growing or finishing pigs and adult dairy and beef cattle were collected aseptically on 55 feedlots throughout Korea between April 2003 and October 2004. A total of 830 samples were analyzed in this study, with 447 samples being from 29 pig farms and 383 samples being from 26 cattle farms. About 15 animals were randomly selected for sampling during each visit to a farm. A fecal sample was taken directly from the rectum of each animal. The samples were immediately transported to the laboratory in ice-cooled containers and analyzed within 24 h of collection. Information on the use of antimicrobial agents at the farms where samples were collected for this study was not available.

2.2. Isolation and identification of E. coli

Feces were directly plated on Chromogenic *E. coli*/coliform (CECC) agar (Oxoid Ltd., Basingstoke, Hants, England) and incubated at 37 °C overnight. Purple colonies picked from the CECC agar plates and typical of *E. coli* were streaked onto eosin methylene blue (EMB) agar (Becton Dickinson, Sparks, MD, USA) which were incubated at 37 °C for 18 to 20 h. Colonies with a metallic sheen on EMB agar were picked and streaked onto MacConkey agar (Becton Dickinson). After overnight incubation at 37 °C, one or two typical pink colonies were selected from each MacConkey agar plate and the isolates were tested by the indole, methyl red, Voges–Proskauer, and Simmons citrate (IMVic) tests for confirmation of *E. coli*. API 20 E test strips (bioMerieux Vitek, Hazelwood, MO, USA) were also used to confirm presumptive isolates as *E. coli*.

2.3. Antimicrobial susceptibility testing

The susceptibilities of the *E. coli* isolates to 16 antimicrobials were determined using the standard Kirby–Bauer disk diffusion method (Bauer et al., 1996). The antimicrobials were ampicillin, 10 μg/disc; amoxicillin/clavulanic acid, 20/10 μg/disc; cephalothin, 30 μg/disc; cefazolin, 30 μg/disc; cefoxitin, 30 μg/disc; cefotaxime, 30 μg/disc; cefepime, 30 μg/disc; streptomycin, 10 μg/disc; gentamicin, 10 μg/disc; amikacin, 30 μg/disc; ciprofloxacin, 5 μg/disc; enrofloxacin, 5 μg/disc; norfloxacin, 10 μg/disc; trimethoprim/sulfamethoxazole, 1.25/23.75 μg/disc; chloramphenicol, 30 μg/disc; tetracycline, 30 μg/disc. Cartridges of antimicrobial-containing discs (BBL Sensi-Disks) were obtained from Becton Dickinson. One *E. coli* isolate from each fecal sample was taken for susceptibility testing.

Confirmed *E. coli* isolates were inoculated into Mueller–Hinton (MH) Broth (Becton Dickinson) and incubated for overnight at 37 °C. The suspensions were adjusted to the turbidity of

Table 2
Antimicrobial resistance patterns of *Escherichia coli* isolated from cattle (n=334)

Number of resisted antimicrobials	Number of isolates (%)	Most frequent patterns (no. of isolates)
0	210 (62.9)	–
1	46 (13.3)	TE (34)
2	39 (11.7)	S–TE (27)
3	20 (6.0)	AM–S–TE (13)
4	10 (3.0)	AM–S–C–TE (8)
5	7 (2.1)	AM–GM–S–C–TE (3), AM–S–C–SXT–TE (3)
8	1 (0.3)	AM–GM–S–CIP–ENO–NOR–SXT–TE (1)
9	1 (0.3)	AM–GM–S–CIP–C–ENO–NOR–SXT–TE (1)

TE: tetracycline; S: streptomycin; AM: ampicillin; C: chloramphenicol; GM: gentamicin; SXT: trimethoprim/sulfamethoxazole; CIP: ciprofloxacin; ENO: enrofloxacin; NOR: norfloxacin.

a 0.5 McFarland standard and streaked onto MH agar (Becton Dickinson) plates. Antimicrobial discs were placed on the plates, which were incubated aerobically at 35±2 °C for 16 to 18 h. The diameter of inhibition zones surrounding the antimicrobial discs was interpreted according to the National Committee for Clinical Laboratory Standards criteria (NCCLS, 2003). The quality control strain was *E. coli* ATCC 25922.

3. Results

A total of 744 *E. coli* isolates were obtained from the 830 fecal samples with 410 isolates being from pigs and 334 from cattle. No distinction was made between isolates from beef cattle and dairy cows.

The most frequently observed resistance in cattle sample was to tetracycline, followed by resistance to streptomycin, ampicillin and chlorampenicol (Table 1). The order of resistance prevalences in swine was the same, but resistance to each antimicrobial was more prevalent among isolates from pigs than among those from cattle. A large fraction of isolates from pigs but a small fraction of isolates from cattle showed resistances to sulfamethoxazole/trimethoprim. About 8% of swine isolates but <1% of cattle isolates were resistant to fluoroquinolones. Resistance to cephalothin and gentamicin was also observed in isolates from cattle and swine, but few isolates from either species were resistant to amoxicillin/clavulanic acid, cefoxitin, cefepime, cefotaxime, or amikacin. The prevalence of resistance in pigs was much higher than that in cattle as 62.9% of bovine isolates but only 1.7% of pig isolates were sensitive to all the antimicrobial agents tested.

Of all *E. coli* isolates, 10.0% from pigs and 13.7% from cattle were resistant to tetracycline only. Multiple drug resistance also was always associated with tetracycline resistance. The most frequently observed patterns of multi-resistance in cattle (Table 2) and pigs (Table 3) were the combination of tetracycline and streptomycin, and tetracycline–streptomycin–ampicillin–chloramphenicol, respectively. A few isolates from either source were resistant to eight or more antimicrobial agents.

Table 3
Antimicrobial resistance patterns of *Escherichia coli* isolated from pig (n=410)

Number of resisted antimicrobials	Number of isolates (%)	Most frequent patterns (no. of isolates)
0	7 (1.7)	–
1	41 (10.0)	TE (39)
2	70 (17.1)	S–TE (24)
3	83 (20.2)	AM–S–TE (35)
4	102 (24.9)	AM–S–C–TE (43)
5	62 (15.1)	AM–S–SXT–C–TE (35)
6	23 (5.6)	AM–GM–S–SXT–C–TE (6)
7	7 (1.7)	AM–CIP–ENO–NOR–SXT–C–TE (2)
8	9 (2.2)	AM–S–CIP–ENO–NOR–SXT–C–TE(6)
9	4 (1.0)	AM–GM–S–CIP–ENO–NOR–SXT–C–TE (3)
10	1 (0.3)	AM–CF–GM–S–CIP–ENO–NOR–SXT–C–TE (1)
11	1 (0.3)	AM–CZ–CF–S–AmC–FOX–CTX–CIP–SXT–C–TE (1)

TE: tetracycline; S: streptomycin; AM: ampicillin; C: chloramphenicol; SXT: trimethoprim/sulfamethoxazole; GM: gentamicin; CIP: ciprofloxacin; ENO: enrofloxacin; NOR: norfloxacin; CF: cephalothin; CZ: cefazolin; AmC: amoxicillin/clavulanic acid; FOX: cefoxitin; CTX: cefotaxime.

4. Discussion

The prevalence of antimicrobial-resistant bacteria in hospitalized humans and animals in Korea is higher than in other industrialized countries (Chong and Lee, 2000; Han et al., 2002). The findings of this study were comparable with those observations, as commensal *E. coli* from food animals showed a high prevalence of resistance to antimicrobials commonly used with livestock, such as tetracycline, streptomycin, and ampicillin. Nearly all the *E. coli* isolates from pigs were resistant to at least one antibiotic tested. A higher prevalence of resistance in *E. coli* isolates from pigs than in those from cattle was also observed in Germany (Guerra et al., 2003) and Japan (Asai et al., 2005). This may reflect the generally more extensive use of antimicrobials as feed additives and for treatment with pigs than with cattle. Indeed, the national survey of annual antimicrobial consumption in Korean livestock conducted in 2003 and 2004 along with this study demonstrated that much greater amounts of antimicrobials had been used with pigs than with cattle (Korean Food Drug Administration, 2003, 2004).

The most frequently observed antimicrobial resistance in *E. coli* isolates was to tetracycline, which agreed with the findings in other countries (Bogaard and Stobberingh, 2000; Teshager et al., 2000; Sayah et al., 2005), although the prevalence was much higher in Korea. This result is understandable as tetracycline has long been the antimicrobial most commonly used with Korean livestock, accounting for over 50% of the total amount of antimicrobial consumptions (Korean Food Drug Administration, 2003, 2004). A correlation between the amount of antimicrobial consumed by animals and the prevalence of antimicrobial resistance was also demonstrated in the case of streptomycin (Sunde et al., 1998), which was the most common aminoglycoside antimicrobial used in Korea. The prevalence of resistance to streptomycin was greater than the prevalence of resistance to other antimicrobial of this class. Although no resistance to amikacin was observed, the resistance to gentamicin found in this study also reflects the seriousness of the antibiotic resistance problem in Korea compared to other countries (Kijima-Tanaka et al., 2003; Bywater et al., 2004; Sayah et al., 2005).

Despite the ban on the use of chloramphenicol with food animals in Korea since 1992, the prevalence of resistance to this antimicrobial was high. This may be partly due to the co-selection of resistant determinants as a result of the use of other antimicrobials as well as cross-resistance to this antimicrobial induced by its derivative, florfenicol (Arcangioli et al., 2000). Florfenicol is still commonly used for treatment of respiratory diseases of pigs in Korea. Resistance to quinolones was also relatively high in Korea compared to other countries (Bywater et al., 2004; Sayah et al., 2005).

Most *E. coli* strains that were resistant to fluoroquinolones were also resistant to the three antimicrobials of this class that were tested in this study. Chromosomal mutation confers resistance to fluoroquinolones and development of resistance to one agent results in cross-resistance to other fluoroquinolones (Hooper, 1999). Fluoroquinolones are one of the most valuable antimicrobial classes for treatment of human infection, and many countries are increasingly reducing its use with food-producing animals (Huotari et al., 2003). However, no such regulation exists in Korea. Urgent introduction of measures to curb the use of these antimicrobials within Korea would be desirable.

Resistance to cephalosporins including the first-generation cephalosporin, cephalothin, was also found in some isolates from both animals. Although only a few isolates were resistant to the 2nd-, 3rd-, or 4th-generation cephalosporins, careful monitoring of resistance to these antimicrobials is needed in the future.

Multidrug resistance in *E. coli* isolated from cattle and pigs was higher in Korea than in the USA, where 12.2% and 51.7% of *E. coli* isolated from cattle and pigs, respectively, were resistant to more than one antimicrobial (Sayah et al., 2005). All the multidrug resistance combinations included tetracycline resistance, which suggests that *E. coli* strains resistant to tetracycline have an enhanced ability to become resistant to other antimicrobials (Sayah et al., 2005).

The high prevalence of antimicrobial resistance among strains of commensal *E. coli* from Korean livestock highlights the urgent need for measures to regulate the use of antimicrobials with food-producing animals in Korea.

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