



ORIGINAL ARTICLE

Comparison of Antimicrobial Resistance in *Escherichia coli* Strains Isolated From Healthy Poultry and Swine Farm Workers Using Antibiotics in Korea

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Abstract

Objectives: The aim of this study is to compare the antibiotic resistance of *Escherichia coli* isolates from faecal samples of workers who often use antibiotics.

Methods: A total of 163 *E coli* strains isolated from faecal samples of livestock workers (poultry and swine farm workers) and restaurant workers in the same regions as a control group were analyzed by agar disc diffusion to determine their susceptibility patterns to 16 antimicrobial agents.

Results: Most of the tested isolates showed high antimicrobial resistance to ampicillin and tetracycline. The isolates showed higher resistance to cephalothin than other antibiotics among the cephems. Among the aminoglycosides, the resistance to gentamicin and tobramycin occurred at higher frequencies compared with resistance to amikacin and netilmicin. Our data indicated that faecal *E coli* isolates of livestock workers showed higher antibiotic resistances than nonlivestock workers (restaurant workers), especially cephalothin, gentamicin, and tobramycin ($p < 0.05$). Moreover, the rates of the livestock workers in the association of multidrug resistance were also higher than the rates of the restaurant workers.

Conclusion: This study implies that usage of antibiotics may contribute to the prevalence of antibiotic resistance in commensal *E coli* strains of humans.

1. Introduction

Antibiotic usage is possibly the most important factor that promotes the emergence, selection, and dissemination

of antibiotic-resistant microorganisms [1]. This acquired resistance occurs not only in pathogenic bacteria but also in the endogenous flora of exposed individuals or populations [2]. These resistant bacteria may colonize the

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human intestinal tract and may also contribute resistance genes to human endogenous flora [3].

In intensively reared food animals, antibiotics may be administered to whole flocks rather than individual animals, and antimicrobial agents may be continuously fed to food animals such as broilers and turkeys as antimicrobial growth promoters. Many studies on antibiotic resistance of food-born microorganisms in food producing animals in relation with the consumption of antibiotics have been published [4–6]. The long-term use of antimicrobials for therapy and growth promotion in animals selects for drug resistance in *Escherichia coli* [7]. However, the antibiotic resistance of microorganisms isolated from humans who often use antibiotics for feeding food-producing animals is not well known.

Therefore, the aim of this study is to investigate the prevalence of antibiotic resistance in faecal *E coli* isolates from healthy workers who often use antibiotics and compare these data with isolates obtained from healthy persons in the same regions as control group.

2. Materials and Methods

2.1. Collection of faecal samples and isolation of *E coli* from the samples

Faecal samples were collected from healthy persons, i.e., 30 from poultry farm workers and 31 from swine farm workers that often use antibiotics. For the comparison, 31 specimens were collected from healthy persons that work in the restaurants of the same regions (Table 1). The samples were placed in sterile plastic specimen tubes on ice, transported to our laboratory, and plated onto a MacConkey agar directly or, occasionally, after enrichment in trypticase soy broth (TSB) containing vancomycin (Sigma Chemical Co., St. Louis, MO, USA). Candidate colonies were then plated in trypticase soy agar medium and biochemically characterized using the API20E system (Biomerieux, Marcy l'Etoile, France). For individual samples, one or two *E coli* isolates were selected randomly for the purpose of determining susceptibility.

2.2. Antimicrobial susceptibility testing

Susceptibility testing was conducted by disk diffusion according to the guidelines of the Clinical and Laboratory Standards Institute [8]. Antimicrobial susceptibility was determined by agar disk diffusion (Kirby-Bauer method) using Mueller–Hinton agar (Difco, MI, USA). The following antibiotics were tested: ampicillin (AM)/sulbactam (SAM), AM, tetracycline (TE), aztreonam (ATM), cefotetan (CTT), cefepime (FEP), cefoxitin (FOX), cefotaxime (CTX), tobramycin (NN), trimethoprim/sulfamethoxazole (SXT/TM), cephalothin (CF), imipenem (IPM), gentamicin (GM), amikacin (AN), piperacillin/tazobactam (TZP), and netilmicin (NET). *E coli* ATCC 25922 and *E coli* ATCC 35218 were used as quality controls.

2.3. Statistic analysis

The antimicrobial susceptibility data are expressed as percentages or frequency of the avian or human isolates. A one-way analysis of variance or χ^2 statistics was used to estimate overall difference between the percentages or frequencies of resistance between avian and human *E coli* isolates. In all cases, $p < 0.05$ was regarded as statistically significant.

3. Results

3.1. Collected faecal samples and isolated *E coli* strains from the samples

As shown in Table 1, many faecal samples were collected from people ages 30–50 in each group. In the workers that often use antibiotics, more samples were collected from men than women, while there were more samples of women than men in the restaurant workers. A total of 163 *E coli* isolates were obtained from the faecal samples, as shown in Materials and Methods section, of which 112 isolates were derived from live-stock workers (44 isolates from poultry farm workers and 68 from swine farm workers) and 51 isolates from restaurant workers.

Table 1. Collected faecal samples

| Age groups (y) | Poultry farm workers ($n = 30$) | | Swine farm workers ($n = 31$) | | Healthy persons ($n = 31$) | |
|----------------|-----------------------------------|-------|---------------------------------|-------|------------------------------|-------|
| | Men | Women | Men | Women | Men | Women |
| 1–10 | 0 | 0 | 2 | 0 | 0 | 0 |
| 11–20 | 0 | 0 | 1 | 0 | 0 | 0 |
| 21–30 | 0 | 0 | 1 | 0 | 1 | 1 |
| 31–40 | 1 | 1 | 3 | 1 | 4 | 3 |
| 41–50 | 9 | 9 | 5 | 3 | 1 | 12 |
| 51–60 | 6 | 1 | 7 | 3 | 1 | 5 |
| 61– | 2 | 1 | 2 | 3 | 1 | 2 |
| Total | 18 | 12 | 21 | 10 | 8 | 23 |

3.2. Antibiotic susceptibility of the isolates

Antibiotic resistance rates of each group were shown in Table 2. In the isolates of each group, high antimicrobial resistance to AM and TE was noted in the most tested isolates. In *Enterobacteriaceae*, resistance to AM is mainly due to β -lactamases like bla (TEM-1) and bla (SHV-1) enzymes that hydrolytically cleave the β -lactam ring. Plasmid-encoded derivatives of β -lactamases that show an enhanced spectrum of catalytic activity have been known since the early 1980s [9]. In addition to the large number of extended-spectrum β -lactamase (ESBL)-TEM and -SHV variants, other plasmid-encoded ESBL such as bla (CTX-M) enzymes are now frequently reported [10]. The isolates showed higher resistance to cephalothin than other antibiotics among the cepheids. Most of the *E. coli* isolates were susceptible to CTX, FOX, and ATM. However, in the isolates of swine farmers, 3% showed resistance to cefotaxime, cefoxitin, and aztreonam. Our data showed that CTX-M14 was detected in all cefotaxime resistant isolates (data not shown). TE is a commonly used first line

antibiotic for many domestic animals as a growth promoter or as an infection control agent and is often used before the antibiotic resistance profile of a pathogen has been determined [11–13]. Resistance to tetracycline is plasmid mediated, with a wide variety of genetic determinants. The presence and frequency of tetracycline resistance in *E. coli* in this study agree with findings of other studies on antibiotic resistance in *E. coli* [14,15]. The resistance to SXT/TM was also relatively higher than to other antibiotics. Among the aminoglycosides, the resistance to GM and NN occurred at higher frequencies in comparison with resistance to AN and NET. Aminoglycoside resistance in *E. coli* most often occurs by aminoglycoside-modifying enzymes [16,17] encoded on transmissible plasmids [18]. Alternatively, no isolates showed resistance to FEP, CTT, IPM, AN, and NET.

There was a trend towards higher resistance frequency of the isolates of poultry and swine farm workers than restaurant workers, especially CF, GM, and NN ($p < 0.05$). Among the livestock workers, the

Table 2. Antibiotic resistance rates of the isolates in each group

| Antimicrobial agents | Antibiotic resistances (%) of isolates | | |
|--|--|--------------------|----------------|
| | Poultry farm workers | Swine farm workers | Control groups |
| β-lactams | | | |
| Ampicillin | 93 | 87 | 77 |
| β-lactam/β-lactamase inhibitor combinations | | | |
| Ampicillin-sulbactam | 0 | 16 | 10 |
| Piperacillin/tazobactam | 0 | 13 | 3 |
| Cepheids | | | |
| Cephalothin | 23 | 65* | 26 |
| Cefepime | 0 | 0 | 0 |
| Cefotetan | 0 | 0 | 0 |
| Cefotaxime | 0 | 3 | 3 |
| Cefoxitin | 0 | 3 | 3 |
| Carbapenems | | | |
| Imipenem | 0 | 0 | 0 |
| Aminoglycosides | | | |
| Amikacin | 0 | 0 | 0 |
| Gentamicin | 60* | 58* | 13 |
| Tobramycin | 17 | 32* | 3 |
| Netilmicin | 0 | 0 | 0 |
| Tetracyclines | | | |
| Tetracycline | 97 | 97 | 77 |
| Monobactams | | | |
| Aztreonam | 0 | 3 | 0 |
| Folate pathway inhibitors | | | |
| Trimethoprim-sulfamethoxazole | 73 | 65 | 48 |

* $p < 0.05$.

resistance rates to CF of swine farm workers were significantly higher than poultry farm workers ($p < 0.05$). The findings that the highest prevalence of resistance occurred among swine and poultry farmer isolates suggest that antimicrobial use in these animals may be a factor in the emergence of antimicrobial resistance in the human faecal *E coli* isolates. In a study, faecal *E coli* isolates from swine showed higher levels of antibiotic resistance and multidrug resistance [19].

3.3. Multiresistance patterns

The percentage of multiple resistance patterns in *E coli* isolates of each group is given in Figure 1. Multi-drug resistance was defined as resistance exhibited to two or more antimicrobials.

Multidrug resistance was found in *E coli* from both groups, but was higher in frequency and proportion in livestock workers. A total of 78% (40 of 163 strains) of restaurant workers expressed resistance to two or more antimicrobials. Approximately 16% of the isolates showed resistance to four or more antibiotics. The resistance most frequently observed pattern in this group was resistance to AM/TE (41.2%) and AM/TE/SXT (41.2%). Among the isolates of livestock workers, 93% (63 of 68 strains) of swine farm workers and 91% (40 of 44 strains) of poultry farm workers exhibited resistance to two or more antimicrobials. The rates of antibiotic resistance to four or more antibiotics of livestock workers were higher than the rates of restaurant workers (66% of swine farm workers, 43% of poultry workers and 16% of restaurant workers). The resistance most frequently observed pattern was resistance to AM/TE (41.2%) in the poultry farm workers and AM//CF/GM/TE/SXT (41.2%) in the swine farm workers. The highest number of resistant antibiotics was nine (SAM/AM/CF/FOX/CTX/NN/GM/TE/SXT) in an isolate of a swine farm worker.

4. Discussion

The poultry and swine industry is a significant economic force for providing food in the world.

However, antimicrobial resistance of intestinal bacteria isolated from these food animals due to antibiotic usage is an increasing global problem in these livestock environments [7,20,21].

However, the information of the antibiotic resistance of faecal *E coli* isolates of livestock workers has been not well known. Therefore, in this study, the prevalence of antibiotic resistance in faecal *E coli* isolates from healthy poultry and swine farm workers was examined.

The resistance pattern most frequently observed in the isolates was resistance to AM in combination with TE (data not shown). A study has reported that swine farm workers were at higher risk of exhibiting multi-drug-resistant *E coli* than nonswine workers [19]. The higher levels of multidrug resistance in the swine farm workers might be attributed to several factors: (1) the prophylactic/subtherapeutic use of several antimicrobial agents in feed at the swine farms, and (2) the intensive farm management practices on swine farms that may facilitate the transmission, propagation, and maintenance of the antibiotic resistant bacterial populations in both the swine hosts and the farm environment.

The microbial ecosystem of humans, animal and food are undoubtedly inextricably connected. Our data indicated that faecal *E coli* isolates from livestock workers showed higher antibiotic resistances than nonlivestock workers, although it is difficult to pinpoint the origin of the antimicrobial resistance that we observed. One possibility of these antibiotic resistances is that resistant bacteria may be readily transferred from food animals to humans because antimicrobial resistant bacteria from food animals may colonize the human population via the contact through occupational exposure, or waste runoff from animal production facilities [22–24].

In conclusion, the data obtained in this study indicate that usage of antibiotics contribute to the prevalence of antibiotic resistance in commensal *E coli* strains of humans. However, further study on the ecology of resistance with respect to genetic exchange and interaction with members of the microbial community is

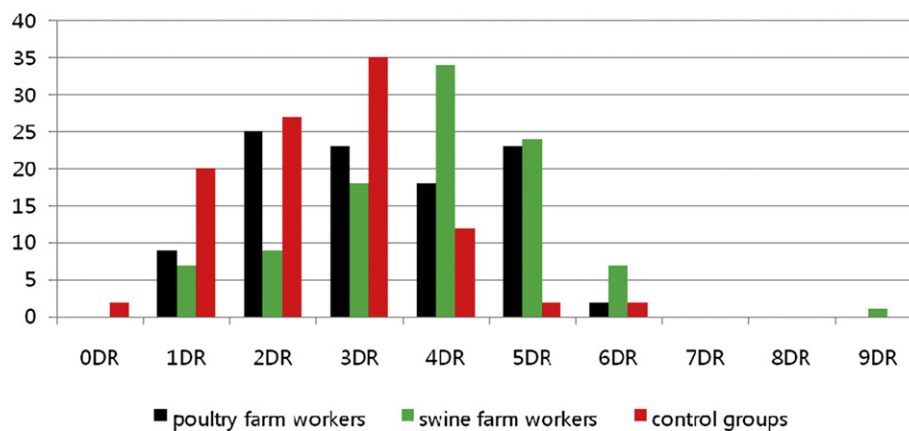


Figure 1. Antibiotic multiresistance patterns of *E coli* strain in each group. DR = drug resistance.

necessary. The information gathered from these types of studies may help us manage the evolution of antimicrobial resistance in the future.

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