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Antimicrobial resistance in *Escherichia coli* isolates from food animals, animal food products and companion animals in China

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ABSTRACT

One thousand and thirty *Escherichia coli* isolates from food animals, animals-derived foods, and companion animals between 2007 and 2008 in Southern China were used to investigate their antimicrobial susceptibility to 14 different antimicrobials by the standard agar dilution method. More than 70% of isolates showed resistance to tetracycline, trimethoprim–sulphamethoxazole, nalidixic acid, and ampicillin. In general, resistance was less frequent in companion animal isolates vs food animals isolates, but cephalosporin and amikacin resistance was more frequent in companion animal isolates, 42.6% to 56.2% vs 14.1% to 24.3%, and 28.5% vs 18.8%, respectively, which was most likely due to the common use of these antimicrobials as treatment in pet animals. Fluroquinolones resistance was high in all animal isolates (>50%). Food products showed lowest resistance among isolates from these three resources. PFGE analysis indicated that a majority of multidrug-resistant *E. coli* isolates showed unique, unrelated PFGE profiles and were unlikely to be the spread of a specific clone. This study provides useful information about the prevalence of antimicrobial resistance in *E. coli* isolated from animals and food products in China and provided evidence of the linkage of the use of antimicrobials in animals and its selection of antimicrobial resistance in bacterial isolates. The data from this study further warns the prudent use of antimicrobials in food and pet animals to reduce the risks of transmission of antimicrobial resistance zoonotic pathogen to humans.

1. Introduction

The appearance of antibiotic resistance among bacteria from animals has raised considerable concern due to the potential for transfer of resistant pathogens and commensal bacteria to the human population (Van den Bogaard and Stobberingh, 2000; Schwarz et al., 2001). *Escherichia coli* are the most prevalent enteric bacteria in animals and humans, and are also an important zoonotic agent, which can be implicated in animal and human infectious diseases (Costa et al., 2008). For this reason, the level of antibiotic resistance in commensal *E. coli* is considered to be a good indicator of the selection pressure exerted by antibiotic use and for resistance problems to be expected in pathogenic bacteria (Van den Bogaard and Stobberingh, 2000; Sáenz et al., 2001).

Many studies on the prevalence of antimicrobial resistance in *E. coli* isolates from farm animals and pets have been performed in other countries (Lanz et al., 2003; Lim et al., 2007; Pedersen et al., 2007; Costa et al., 2008;

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Damborg et al., 2008; Enne et al., 2008). However, only a few scattered resistance surveillance studies with limited number of *E. coli* isolates from farm animals have been published in China (Yang et al., 2004; Dai et al., 2008). And these data showed that the prevalence of antimicrobial resistance in *E. coli* isolates from farm animals in China is higher than other countries. But there is a little data on the prevalence of antimicrobial resistance in *E. coli* isolates from companion animals in China. Considering the shared environment of humans and companion animals, transfer of resistant bacteria or mobile resistant determinant between companion animals and humans is more likely to occur and has been indicated in some studies (Simjee et al., 2002; Guardabassi et al., 2004). Moreover, the use of antimicrobials in companion animals has received little attention worldwide, especially in China. The aim of our present study is to examine the susceptibility of *E. coli* isolates collected from farm animals, animal-derived foods, and companion animals in South China from 2007 to 2008. To investigate the linkage between the use of antimicrobials and its selection of antimicrobial resistance in *E. coli* isolates, the usages of antimicrobials in companion animals was also examined. This is the first report on such topic in China.

2. Materials and methods

2.1. Sampling and Escherichia coli isolation

E. coli strains of farm animal origin were randomly collected from feces of healthy animals from different farms located in Southern China, and feces or liver samples of sick animals submitted to the Veterinary Research Institute of Guangdong Academy of Agricultural Sciences and Foshan University for diagnosis between January 2007 and October 2008. No more than ten samples were taken from the same farm of origin. Strains from food samples were randomly collected from fresh or chilled chickens and pork at 12 convenient open markets and supermarkets in six cities of Southern China during November 2007 to July 2008. One or two types of meat were sampled at each sampling site. *E. coli* isolates of companion animal origin were randomly collected from feces of healthy or diseased dogs and cats at 9 small animal hospitals in Guangzhou during November 2007 to June 2008.

All samples were seeded on MacConkey agar plates and incubated at 37 °C for 24 h. One suspected colony with typical *E. coli* morphology and size was selected from each sample, and then identified by classical biochemical methods and confirmed by the API 20E system (bioMérieux, France).

2.2. Collection of information on antimicrobial treatment in pets

Information on the usage of antimicrobial agents in pets within last 12 months, where the *E. coli* was isolated and used in this study, was obtained from the owner or medical record.

2.3. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was carried out by the agar dilution method on Mueller–Hinton agar plates as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2008a,b). The following antimicrobials were tested: ampicillin, cephazolin, ceftriaxone, kanamycin, gentamicin, amikacin, chloramphenicol, tetracycline, nalidixic acid, norfloxacin, ciprofloxacin, enrofloxacin, levofloxacin, and trimethoprim–sulphamethoxazole. *E. coli* ATCC 25922 was used as quality control strains. The isolates were classified as susceptible or resistant according to the interpretative standards recommended by CLSI (CLSI, 2008a,b). Isolates with intermediate susceptibility were considered as susceptible. When breakpoints were unavailable for *E. coli* isolates of animal origin, they were referred to human CLSL document or other specific bacteria from animals, but the clinical breakpoints adopted from human medicine do not be allowed to predict therapeutic success or failure in animals. For the Results and Discussion, We used the terminology of Knezevic and Petrovic (2008): very high rate of resistance (>75% resistant isolates); high rate (50–75%); moderate rate (30–50%); low rate (10–30%); and very low resistance rate (0–10%). The χ^2-test was performed using the Statistical Package for the Social Sciences (SPSS version 15.0; SPSS, Chicago, IL, USA) to analyze differences between the frequencies of resistance among isolates obtained from different sources.

2.4. Pulsed-field gel electrophoresis (PFGE)

To study the clonal transmission of multidrug-resistant *E. coli*, chromosomal DNA of 170 (100 from food animals and 70 from pets) randomly selected *E. coli* isolates resistant to more than 7 antimicrobials were digested with the restriction enzyme XbaI and then subjected to PFGE analysis using the CHEF-MAPPER System (Bio-Rad Laboratories, Hercules, CA, USA) as described by Gautom (1997). The gels were run at 6.0 V/cm with an initial/final switch time of 0.5 s/60 s and an angle of 120° at 14 °C for 22 h. A bacteriophage lambda DNA ladder consisting of 48.5 kb concatemers was used as a size marker. The results were interpreted according to the criteria of Tenover et al. (1995).

3. Results

A total of 1030 *E. coli* isolates were recovered from 1376 samples from animals or animal food products including 608 *E. coli* isolates from food animals (216 pigs, 187 chickens, 151 ducks, 25 geese, and 29 pigeons) in 67 different farms in Southern China, 178 from animal food products (29 from chicken and 149 from pork) and 244 from pet animals (187 dogs and 57 cats).

3.1. Comparisons of antimicrobial resistance in isolates of different origins

The results of the in vitro susceptibility testing of all *E. coli* isolates from different sources were shown in Table 1. There was a very high frequency (78.9–96.7%) of tetracycline, nalidixic acid, trimethoprim–sulphamethoxazole