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SHORT COMMUNICATION

Antimicrobial Resistance in *Enterococci* Isolates from pet Dogs in Xi'an, China

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ABSTRACT

To evaluate the prevalence of antimicrobial resistant bacteria in pets, 101 *Enterococci* culture isolates, as the indicator bacteria, were isolated from clinically healthy pet dogs in Xi'an, China and examined for antimicrobial resistance. Tetracycline resistance was found in all *Enterococci* isolates (100.0%), followed by erythromycin (89.1%), ciprofloxacin (56.4%), rifampicin (32.7%), gentamicin (26.7%), penicillin (22.7%) and ciprofloxacin (21.8%). Almost 95% strains were found with multiple drug resistance. Our data suggested that *Enterococci* were prevalent amongst pet dogs and resistant to various antimicrobials, indicating the potential risk to humans and the necessity to establish comprehensive antimicrobial resistance monitoring systems worldwide especially in China.

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INTRODUCTION

The increasing population and changing status of companion animals in society may result in increasing resistant microorganism transmission between pets and humans. Antimicrobial improper and overuses in companion animals may accelerate the emergence of bacterial resistance among pet animals and afterwards transfer to humans. Programs on analyzing and monitoring of antimicrobial usage and antimicrobial resistance (AMR) in humans and production animals have been carried out in various countries, but the information concerning pet animals are rarely included (EARSS Annual Report, 2008; Chen *et al*., 2011). In China, National Antimicrobial Resistance Investigation Net has been established by Ministry of Health aimed to investigate antimicrobial resistance among clinical isolates from Intensive Care Unit (ICU), in 2005 (Chen *et al*., 2011). Moreover, a legal veterinary drug retrieval system (http://sysy.sms.mofcom.gov.cn/) based on the veterinary drugs approved in the US, European Union, Australia and China has also been established since 2006, detailed information such as activity compound, treated animals and symptoms, dose and method of administration, withdrawal period and residues of these drugs are involved, guiding to the appropriate drug use in veterinary medicine. The present study aimed to investigate the levels of antimicrobial resistance in intestinal flora of clinically healthy pet dogs, and to assess the possible role of the dog flora as a reservoir for antimicrobial resistance.

MATERIALS AND METHODS

Fecal samples from 80 clinically healthy dogs of 7 kennels in Xi'an, Shaanxi province, China were collected in January, 2011. Samples were placed into sterile containers and stored at 4°C until processed within 24h of collection.

Samples were diluted with 0.9% NaCl (w/v) to 10^{-4} dilution and further inoculated on Pfizer Selective *Enterococci* (PSE) agar (Qingdao Hope Bio-Technology Co., Ltd, China) for isolation of *Enterococci*. After 36h incubation at 37°C, colonies with typical enterococcal morphology were selected and seeded on Tryptone soya agar plates and incubated overnight at 37°C. Each isolate was identified by means of Gram staining, catalase test, glucose and maltose fermentation, high salt, pH and temperature tolerance test and confirmed by Bergey's Manual of Determinative Bacteriology (Bergey *et al*., 1994). All isolates were stored at -20°C in Tryptone soya broth containing 10% glycerol.

Antimicrobial susceptibility tests of the 101 *Enterococci* isolates were performed by the disk diffusion method. Antimicrobial susceptibility discs approximately 6mm in diameter was used, the disk potency of the individual antibiotic was as follows: penicillin (10 µg), tetracycline (30 µg), rifampicin (5 µg), vancomycin (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), erythromycin (15 µg), furadantin (30 µg), fosfomycin (200 µg), and gentamicin (120 µg). They were chosen for their common use in both veterinary and human infections. Susceptibility results were interpreted using Clinical and Laboratory Standards Institute (CLSI, 2008) criteria. Intermediate resistances were allotted to the resistance category. *Enterococcus faecalis* ATCC 29212 was used as a quality control. Multidrug-resistant (MDR) isolates were defined if they exhibited resistance to drugs of ≥2 antimicrobial types. All statistical analyses were performed using Microsoft Excel (Excel 2010, Microsoft Corp, Redmond, United States).

RESULTS AND DISCUSSION

Faecal samples were diluted and plated on PSE agar plated, after 36h incubation at 37°C, colonies with the brown-black halo were selected. Microscopic examination of the selected isolates showed Gram-positive cocci, and often occurred in pairs or short chains. The pure culture of the isolates on Tryptone soya agar plates presented lacte, small and smooth bacteria colony. The selected *Enterococcus* spp. colonies were catalase negative, ferment glucose and maltose generating acid. They can grow in 6.5% NaCl, 40% bile salts, at pH 9.6, at 45°C and can resist 30min at 60°C. The culture characteristics and biochemical test results of the *Enterococci* isolates were the same with the *Enterococcus faecalis* ATCC 29212.

Of the total 80 samples, 93.7% (75/80) were *Enterococci* positive, while relative lower positive percentages were found in dogs from veterinary clinics in the US in 2007 and dogs from kennels or household in Belgium (De Graef *et al*., 2004; Jackson *et al*., 2009). This difference may be relative to different origins of dogs, different animal feed and living conditions.

A total of 101 *enterococci* cultures were isolated from 75 positive samples. According to the interpretation standard as shown in Table 1, results of susceptibility tests are presented in Fig. 1. Resistance to tetracycline was occurred in all *enterococci* isolates, which is much greater than 32% of *enterococci* isolated from healthy dogs in Denmark in 2006 and 51% of *enterococci* from pets sampled from veterinary clinics in the US in 2007 (Damborg *et al*., 2008; Jackson *et al*., 2009). A high erythromycin resistance of 89.1% was observed, comparable to the *E. faecalis* from healthy pets (91%) in Portugal, which confirmed the wide distribution of erythromycin-resistant *enterococci* mediated by resistance genes *erm*(B) (Jackson *et al*., 2010). *Enterococci* resistant to higher levels (56.4%) of ciprofloxacin, compared to that (32%) isolated from dog samples collected from animal hospital in Italy in 2005 (Ossiprandi *et al*., 2008). The present study found higher resistance to tetracycline, erythromycin and ciprofloxacin in clinically healthy pet dogs than studies from other research groups, indicating worse pet antimicrobial resistance situation in China.

Gentamicin belongs to aminoglycosides, are routinely used in combinations for topical therapy in canine (Guardabassi *et al*., 2004). Relatively common (26.7%) resistance was observed in *enterococci* isolates in China. None of the 101 enterococci isolates was resistant to vancomycin (the last treatment available in serious and multi-drug resistant infections), indicating VRE are rare in dogs, which is consistent with previous study in companion animals (De Graef *et al*., 2004; Leener *et al*., 2005; Delgado *et al*., 2007; Ossiprandi *et al*., 2008).

Table 1: The interpretation standard of antimicrobial susceptibility tests with disc diffusion method of *Enterococci*

Antibiotics	The diameter of the inhibition zone (mm)		
	Resistant zone	Intermediate zone	Susceptible zone
Penicillin	≤14	-	≥15
Tetracycline	≤14	15~18	≥19
Rifampicin	≤16	17~19	≥20
Vancomycin	≤14	15~16	≥17
Chloramphenicol	≤12	13~17	≥18
Ciprofloxacin	≤15	16~20	≥20
Erythromycin	≤13	14~22	≥23
Furadantin	≤14	15~16	≥17
Fosfomycin	≤12	13~15	≥17
Gentamicin	≤ 6	7~9	≥10

Table 2: Multidrug resistance patterns in *Enterococci* from pet dogs

Patterns (No. and types of antimicrobials)		No. of resistant strains	Percentage of resistant strains
2	TE-E	15	16.80%
	TE-CIP	2	
3	TE-E-C	5	22.70%
	TE-E-CIP	5	
	TE-E-GM	5	
	TE-E-RA	4	
	TE-E-FT	1	
	TE-GM-RA	1	
	TE-CIP-GM	1	
	TE-E-P	1	
4	TE-E-CIP-RA	10	34.60%
	TE-E-CIP-P	7	
	TE-E-CIP-GM	5	
	TE-E-CIP-C	3	
	TE-E-C-GM	3	
	TE-E-CIP-FT	2	
	TE-E-C-RA	1	
	TE-E-C-P	1	
	TE-CIP-P-RA	1	
	TE-E-P-A	1	
	TE-GM-E-RA	1	
5	TE-E-CIP-P-RA	4	16.80%
	TE-E-CIP-C-RA	3	
	TE-E-CIP-GM-P	3	
	TE-E-CIP-GM-RA	3	
	TE-E-CIP-C-P	2	
	TE-E-CIP-C-FOS	1	
	TE-E-CIP-C-GM	1	
6	TE-E-CIP-C-GM-RA	2	3.96%
	TE-E-CIP-GM-P-RA	2	

Isolates of multidrug resistance patterns were most often identified from pet dogs in Xi'an, China. TE = Tetracycline; E = Erythromycin; CIP = Ciprofloxacin; RA = Rifampicin; GM = Gentamicin; P = Penicillin; C = Chloramphenicol; FOS =Fosfomycin; VA =Vancomycin; FT= Furadantin.

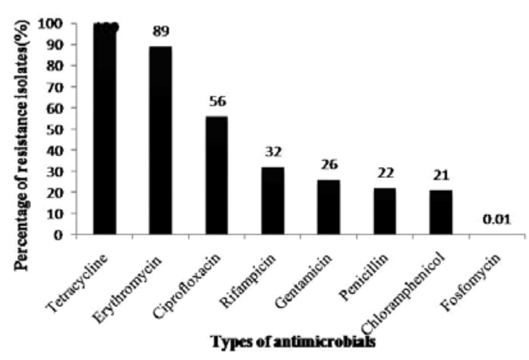

Fig. 1: Antimicrobial resistance pattern of *Enterococci* isolated from pet dogs in Xi'an, China

Fosfomycin has been reported for the treatment of VRE infections and MDR gram-negative bacteria, resistance to fosfomycin was also detected in the present study, but at very low frequency (1.0%).

As shown in Table 2, the MDR cultures among canine *enterococci* were high in this study, almost 95% isolates were resistant to at least two antibiotics (tetracycline and erythromycin), 55.4% of strains were resistant up to three antibiotics, compared with 17.6% of strains resistant to the same number of antibiotics regardless of class in healthy pets from Portugal (PatrÍcia *et al*., 2006). The largest groups of MDR belonged to those composed of three (22.7%) and four (34.6%) different antimicrobials with eight and eleven different drug combinations, respectively; only two different patterns were detected for the double antimicrobial resistance. Only 4.0% isolates (4/101) were resistant to six antimicrobials in two patterns varied by two antimicrobials (ciprofloxacin and penicillin) with tetracycline, erythromycin, ciprofloxacin, gentamicin and rifampicin common among the isolates.

The number of pets in China has rapidly grown, the expending pet population allows more and closer physical contact between owners and their pets, thus creating more opportunities for microorganisms transmission. Quantification of this risk is hard to evaluate at present, because the information on antimicrobial use in companion animals is available only in a few countries, for example in Denmark, an estimation of 2.2 metric ton of antimicrobial substance used in 2009 in companion animals, representing 1.7% of the total national veterinary consumption (129.7 metric ton) (Jensen and Hammerum, 2009).

The present study implied that the antimicrobial resistance situation of *Enterococci* from pets is more serious in China than in many other countries. Further in-depth investigations would be necessary to evaluate the situation of pet antimicrobial resistance in China and its impact on human health. Prevalence and characterization of the antimicrobial resistant *Enterococci* from pets distributed in different areas of China would be focused in the following study.

In order to establish the antimicrobial resistance surveillance system on companion animals, some measures including the establishment of standardization antimicrobials use guidelines in pet animals, collection of relevant information about antibiotics consumption in companion animals and the conduction of surveillance programs on resistant bacteria on companion animals, should be introduced and applied in pet medicine.

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