Preplanned Studies

Prevalence and Risk Factors of Multidrug-Resistant *Enterococcal* Infection in Clinical Dogs and Cats — China, 2018–2021

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Summary

What is already known about this topic?

Enterococcus spp., while naturally occurring as commensal bacteria in the gastrointestinal tract of animals and humans, have emerged as significant opportunistic pathogens in healthcare settings.

What is added by this report?

A comprehensive surveillance study revealed enterococci in 14.39% of clinical samples from dogs and cats across China during 2018–2021. Multidrug-resistant enterococcal infections showed significant correlation with urinary tract catheterization and extended hospitalization periods. Notably, pet-derived *Enterococcus faecalis* isolates demonstrated high genetic similarity with strains isolated from humans, farm animals, and environmental sources.

What are the implications for public health practice?

These findings underscore the critical need for enhanced surveillance of enterococcal infections and implementation of stringent aseptic protocols in veterinary clinical settings. Particular attention should be directed toward linezolid-resistant *Enterococcus faecalis* infections due to their demonstrated potential for transmission between pets and humans.

Enterococci, which naturally colonize the gastrointestinal tract of humans and animals, have emerged as significant healthcare-acquired pathogens (*1*). In veterinary medicine, *Enterococcus* spp. represents the third most prevalent pathogen in dogs and cats according to the China Antimicrobial Resistance Surveillance Network for Pets (CARPet) (*2*). These bacteria serve as reservoirs of antimicrobial resistance (AMR), presenting a public health concern through potential human transmission (*3*–*4*). The emergence of linezolid-resistant enterococci is particularly concerning as it limits therapeutic options. This study revealed enterococci in 14.39% of clinical samples from dogs and cats across China, with *Enterococcus faecium* (*E. faecium*) and *E. faecalis* identified as the predominant species. *E. faecium* exhibited higher resistance rates to daptomycin, rifampin, doxycycline, and amoxicillin-clavulanate compared to *E. faecalis*. Multidrug-resistant enterococcal infections were significantly associated with urinary tract catheterization history and extended hospitalization duration. Whole genome sequencing (WGS) analysis demonstrated genetic similarities between linezolid-resistant *E. faecium* isolates from this study and other pet isolates, while *E. faecalis* showed broader genetic relationships across various sources. To mitigate infection risks, particularly considering the potential for pet-to-human transmission of linezolid-resistant *E. faecalis*, enhanced aseptic practices and reduced hospitalization periods in veterinary clinics are recommended.

This study collected *Enterococcus* spp. from clinical samples of dogs and cats across 20 Chinese provinces and municipalities (2018–2021) through the CARPet surveillance system. Clinical samples and medical records were obtained from diseased animals at regional veterinary hospitals, with veterinarians collecting site-specific infected specimens. Antimicrobial susceptibility testing was conducted following CLSI guidelines (CLSI, VET01) using broth microdilution, with *E. faecalis* ATCC 29212 as the quality control strain. The antimicrobial panel included amoxicillin-clavulanate, doxycycline, azithromycin, rifampin, florfenicol, enrofloxacin, linezolid, vancomycin, and daptomycin. Results were interpreted according to CLSI VET01S and M100 breakpoints. Multidrug-resistant (MDR) isolates were defined as those resistant to three or more antimicrobial classes (*5*). Risk factors for MDR infections were evaluated using univariate analysis and logistic regression SPSS (version 22.0, International Business Machines Corporation, Armonk, USA). WGS of linezolid-resistant *Enterococcus* spp. isolates was performed using the Illumina HiSeq X Ten platform. Draft genomes were assembled using SPAdes and analyzed for sequence types, antimicrobial resistance genes, and virulence genes using SRST2. Phylogenetic analysis compared pet-derived isolates with human, animal, and environmental isolates from China available in the NCBI database (2018–2021). Phylogenetic clusters were determined using fastBAPS software (https://github.com/gtonkinhill/fastbaps). The genome assemblies were deposited under BioProject accession no. PRJNA1039340.

From 2018 to 2021, surveillance across 20 Chinese provinces identified *Enterococcus* spp. in 460 (14.39%) of 3,197 clinical samples (2,247 canine and 950 feline). The highest detection rates were observed in ascites (24.00%), hepatobiliary system (22.00%), and urinary tract (18.68%) specimens. Feline samples exhibited a significantly higher detection rate (22.53%) compared to canine samples (10.95%) (Supplementary Figure S1, available at https://weekly.chinacdc.cn/). Among the 477 *Enterococcus* spp. isolates recovered, *E. faecium* (45.49%) and *E. faecalis* (43.40%) were predominant, with *E. gallinarum, E. avium* and others comprising the remainder (Figure 1). While most isolates originated from Beijing (*n*=413), others were distributed across Shanghai, Inner Mongolia, Liaoning, Fujian, Jiangsu, and 13 additional provinces (Supplementary Table S1, available at https://weekly.chinacdc.cn/).

Antimicrobial susceptibility testing revealed significantly higher resistance rates in *E. faecium* compared to *E. faecalis* for amoxicillin-clavulanate (86.75% *vs.* 1.14%), doxycycline (81.33% *vs.* 57.14%), rifampin (77.11% *vs.* 63.43%), and daptomycin (7.83% *vs.* 1.14%) (*P*<0.01) (Tables 1–2). Linezolid resistance was detected in 6.29% of *E. faecalis* and 5.42% of *E. faecium* isolates. All isolates were vancomycin-susceptible except for one intermediate *E. faecium* (Tables 1–2). Multidrug resistance was more prevalent among feline isolates (48.84%) than canine isolates (26.92%), with *E. faecium* exhibiting a substantially higher MDR rate (78.31%) compared to *E. faecalis* (4.57%).

Among 203 enterococcal isolates with complete case information, 76 were classified as MDR. Multivariate analysis initially considered four variables (*P*<0.05): duration of hospitalization, pet species, pet sex, and history of urinary tract catheterization. The final model, after backward selection, retained only two significant predictors: history of urinary tract catheterization (*P*=0.03) and duration of hospitalization (*P*=0.04). These findings indicate that pets with previous urinary tract catheterization or extended hospitalization periods had significantly higher risks of MDR *Enterococcus* infection (Supplementary Figure S2, available at https://weekly.chinacdc.cn/).

Analysis of 19 linezolid-resistant isolates, comprising 11 *E. faecalis* (LREfs) and 8 *E. faecium* (LREfm), revealed the presence of the *optrA* gene in all specimens. The LREfs represented eight distinct sequence types (STs). Phylogenetic analysis, incorporating 81 LREfs from the NCBI database, revealed distribution across six lineages with mixed-source isolates. Notably, seven LREfs showed close genetic relationships (19–94 SNPs) with isolates from humans, pets, farm animals, and plants, suggesting cross-species transmission potential (Figure 2A). The eight LREfm belonged to four known STs, with ST80 being predominant (*n*=4). Phylogenetic analysis of 56 isolates (including 48 from NCBI) identified three distinct lineages, with seven studied isolates clustering in lineage 3. Two isolates (20928 and 21196) from a cat and dog at the same hospital exhibited remarkable genetic similarity, differing by only five SNPs (Figure 2B), indicating clonal spread among pet isolates distinct from other sources.

DISCUSSION

This study provides a comprehensive analysis of enterococcal infections in Chinese veterinary clinics, encompassing diverse clinical samples from dogs and cats. Our findings, supported by CARPet surveillance data, revealed that *Enterococcus* spp. were present in 14.39% of clinical samples, establishing them as the third most prevalent bacterial pathogens in companion animals (*2*). This prevalence aligns with Korean data (19.3%) (*6*) and parallels reports from European countries, where *Enterococcus* spp. rank among the top five clinical pathogens in Spain (*7*), Portugal (*8*), and the Iberian Peninsula (*9*), comprising 5.6%–15.0% of isolates. Similarly, in Chinese human clinical settings, *Enterococcus* spp. account for 8.89% of isolates, ranking fourth (*10*). These parallel findings underscore the significance of *Enterococcus* as a pathogen in both veterinary and human medicine, emphasizing the critical need for enhanced surveillance protocols.

The emergence of antimicrobial resistance in *Enterococcus* spp., whether through genetic mutations or mobile genetic elements, presents significant therapeutic challenges. Our study revealed that over

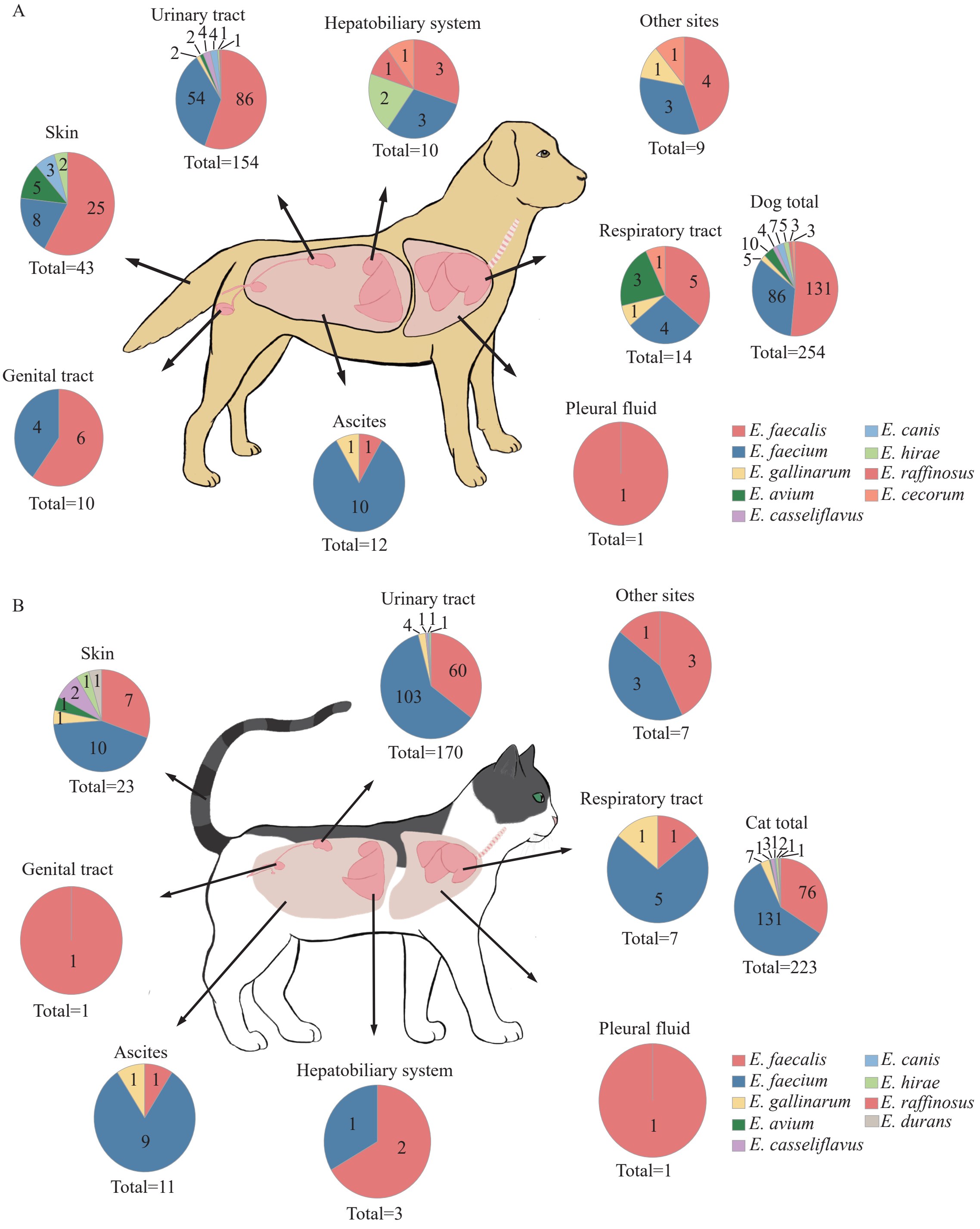

FIGURE 1. Distribution of *Enterococcus (E.)* spp. isolates in various samples. (A) From dogs; (B) From cats.

50% of isolates demonstrated resistance to doxycycline or exhibited elevated MICs for azithromycin and enrofloxacin, consistent with findings (43.2%–99.0%) from Japan (*11*), Australia (*12*), the UK (*13*), and Sweden (*14*). While enrofloxacin remains exclusive in veterinary medicine, both doxycycline and azithromycin are crucial antimicrobials in human and veterinary treatment of urinary and respiratory infections. Of particular concern, 86.75% of *E. faecium* isolates showed resistance to amoxicillin-clavulanate, a primary therapeutic option in both veterinary and human medicine. Encouragingly, resistance rates to last-resort antimicrobials — vancomycin, linezolid, and daptomycin — remained low (0–6.29%), preserving their efficacy against multidrug-resistant infections. These findings

TABLE 1. Minimal inhibitory concentrations and resistance rates of canine and feline *Enterococcus faecalis* isolates.

Antimicrobial agents	**Number of *Enterococcal faecalis* corresponding to MIC (mg/L)**											**No. of resistant isolates (%)**
	0.064	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	
Amoxicillin-clavulanate (2:1)			6	22	126	16	3	–	–	2		2 (1.14)
Doxycycline		12	8	4	2	1	4	44	76	24		100 (57.14)
Azithromycin			1	–	–	3	14	30	8	119		–
Florfenicol					6	42	93	20	–	–	14	–
Enrofloxacin		–	1	32	91	12	–	1	38			–
Rifampin			6	4	18	36	54	49	8			111 (63.43)
Vancomycin				2	121	49	3	–	–	–	–	0 (0)
Linezolid				4	40	111	9	11	–	–		11 (6.29)
Daptomycin				27	74	52	20	1	1	–		2 (1.14)

Note: The gray-shaded areas indicate untested antimicrobial concentrations. For isolates showing no growth at any concentration, the lowest MIC value was assigned. For isolates exhibiting growth at all tested concentrations, the next higher MIC value above the highest tested concentration was assigned (black numbers on gray background). MIC values for amoxicillin-clavulanic acid (2:1) are reported as amoxicillin MIC values. Color coding indicates susceptibility categories: susceptible (green), intermediate (yellow), and resistant (red).
Abbreviation: MIC=minimum inhibitory concentration.

TABLE 2. Minimal inhibitory concentrations and resistance rates of canine and feline *Enterococcus faecium* isolates.

Antimicrobial agents	**Number of *Enterococcal faecium* corresponding to MIC (mg/L)**											**No. of resistant isolates (%)**
	0.064	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	
Amoxicillin-clavulanate (2:1)			2	8	5	1	4	2	2	142		144 (86.75)
Doxycycline		12	16	1	1	2	–	–	39	95		134 (80.72）
Azithromycin			1	1	–	–	3	10	10	141		–
Florfenicol					1	14	94	38	2	4	13	–
Enrofloxacin		–	–	–	5	6	3	6	146			–
Rifampin			22	1	3	12	45	58	25			128 (77.11)
Vancomycin				24	117	13	11	1	–	–	–	0 (0)
Linezolid				–	19	118	21	3	5			8 (4.82)
Daptomycin				9	15	60	69	8	5			13 (1.81)

Note: The gray-shaded areas indicate untested antimicrobial concentrations. For isolates showing no growth at any concentration, the lowest MIC value was assigned. For isolates exhibiting growth at all tested concentrations, the next higher MIC value above the highest tested concentration was assigned (black numbers on gray background). MIC values for amoxicillin-clavulanic acid (2:1) are reported as amoxicillin MIC values. Color coding indicates susceptibility categories: susceptible (green), intermediate (yellow), and resistant (red).
Abbreviation: MIC=minimum inhibitory concentration.

emphasize the necessity for systematic antimicrobial susceptibility testing and resistance monitoring in veterinary practice.

Extended hospitalization significantly increases the risk of MDR enterococcal infections in companion animals, potentially through environmental transmission within hospital settings. Previous studies have demonstrated the potential for resistant *Enterococcus* spp. clone transmission within veterinary facilities through multiple vectors, including infected dogs, their owners, veterinary personnel, and hospital environmental surfaces (*6*). Similarly, pets requiring urinary tract catheterization exhibit elevated risks of MDR infections, potentially due to suboptimal aseptic technique during catheter placement and maintenance. The increased risk of healthcare-associated infections through invasive procedures is well-documented in veterinary medicine, emphasizing the critical importance of implementing rigorous cleaning and disinfection protocols to prevent MDR pathogen transmission (*15*).

The presence of MDR bacteria in companion animals, including colistin-resistant and ESBL-producing *E. coli* (*16*) and methicillin-resistant staphylococci (*17–18*), represents a substantial transmission risk to pet owners. Companion animals can serve as reservoirs for antibiotic-resistant enterococci, facilitating their dissemination across human, animal, and environmental interfaces. Our genomic analyses revealed that LREfs demonstrate high

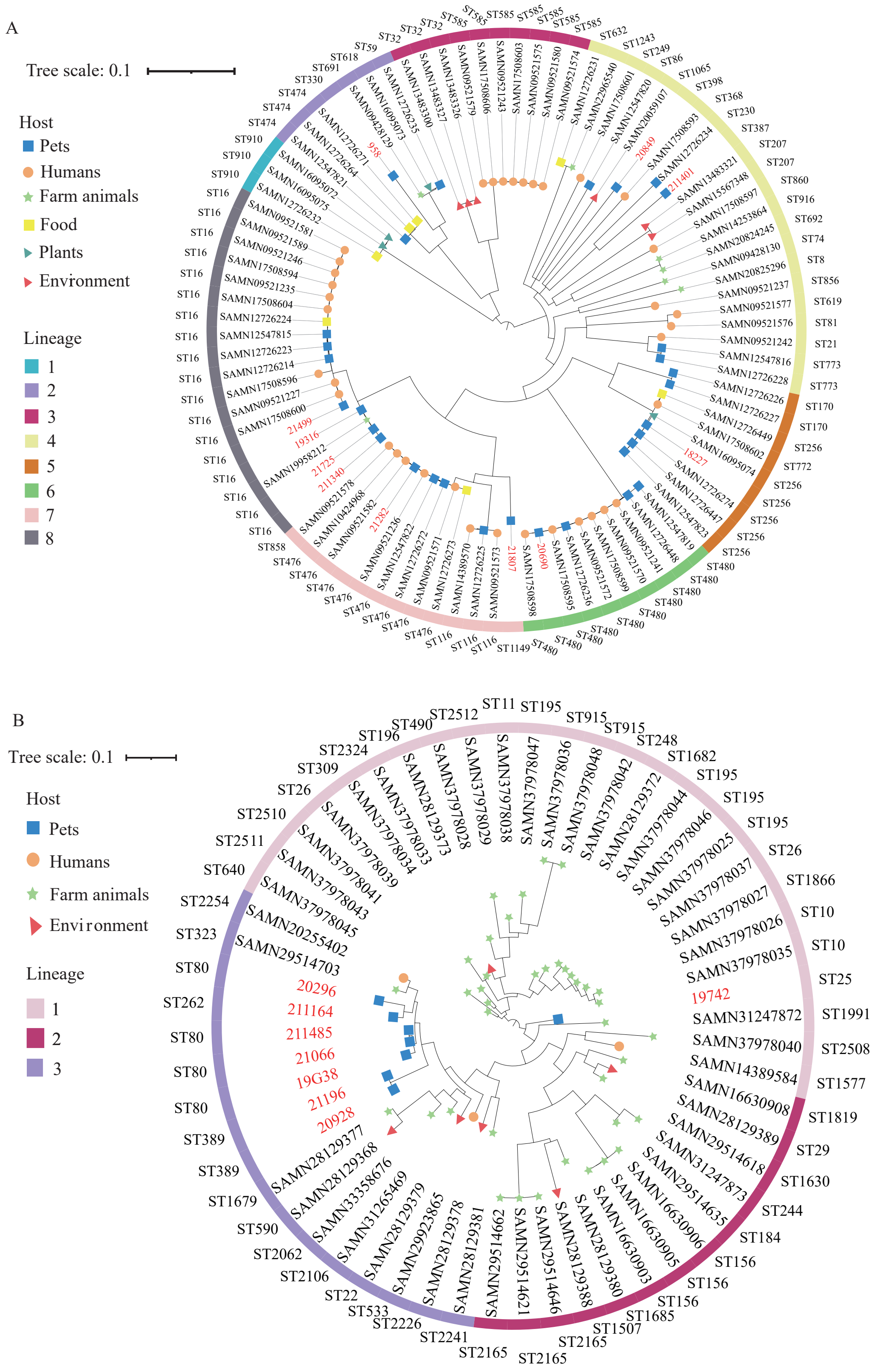

FIGURE 2. Phylogenetic tree of (A) linezolid-resistant *Enterococcus faecalis* and (B) linezolid-resistant *Enterococcus faecium* from pets in this study and humans, pets, farm animals, food, environments, and plants available from NCBI based on the core genome analysis.

genetic similarity across human, animal, and environmental isolates, whereas LREfm exhibits closer genetic relationships primarily among pet isolates. This pattern suggests that LREfs have achieved broader host adaptation and dissemination compared to LREfm, indicating potentially higher transmission risks between pets and their owners.

Study limitations include the restricted whole genome sequencing analysis of only linezolid-resistant isolates, which constrains our ability to comprehensively evaluate transmission patterns. Furthermore, the geographical distribution of samples was notably skewed, with 86.58% of pet-derived enterococci originating from Beijing, highlighting the need for broader surveillance across China to accurately assess national prevalence patterns.

This investigation establishes *E. faecium* and *E. faecalis* as the predominant enterococcal species in Chinese veterinary clinical settings. The study demonstrates that multidrug-resistant enterococcal infections correlate significantly with urinary catheterization procedures and extended hospitalization periods. To mitigate MDR transmission, we recommend implementing dedicated isolation facilities for infected animals, establishing rigorous cleaning and disinfection protocols, and ensuring thorough sterilization of medical instruments. Additionally, enhanced veterinary staff training in aseptic techniques and evidence-based antimicrobial selection, guided by pathogen identification and susceptibility testing, is crucial. Sustained surveillance efforts are essential to prevent the bidirectional transmission of MDR organisms between companion animals and humans.

Conflicts of interest: No conflicts of interest.

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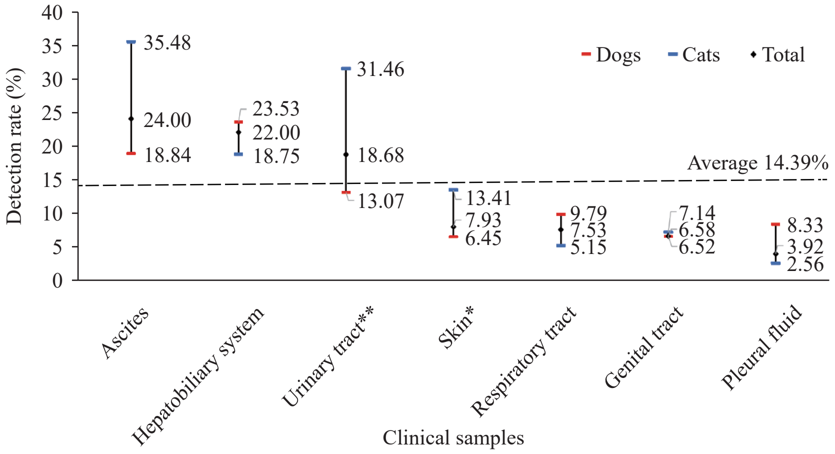
SUPPLEMENTARY MATERIAL

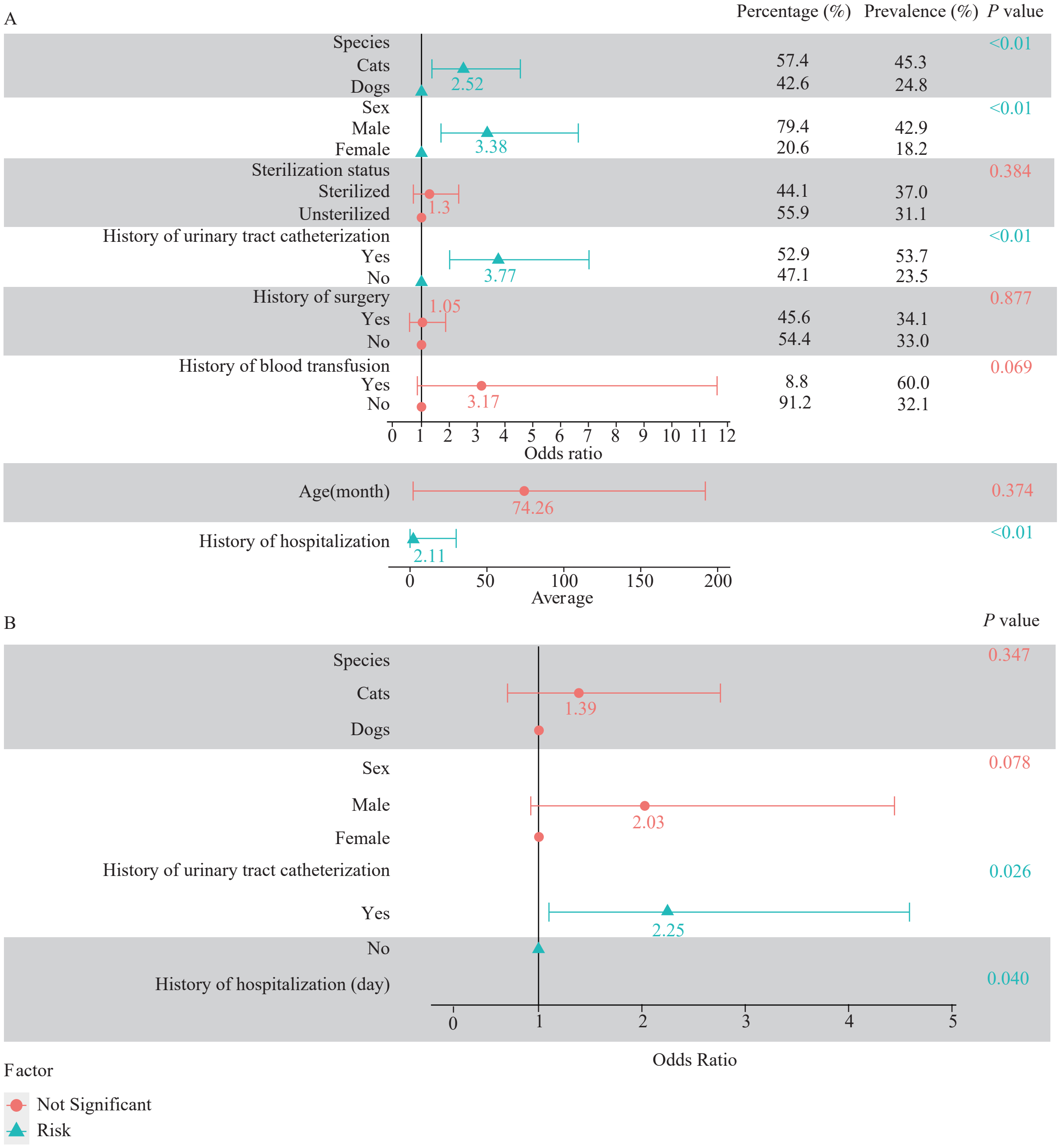


SUPPLEMENTARY FIGURE S1. Detection rates of *Enterococcus* spp. isolates from different clinical samples from dogs and cats in animal hospitals from 2018 to 2021 in China.

Note: The blue bar represents the detection rate of cat samples; the red bar represents the detection rate of dog samples; and the black dot represents the combined rate of total samples of dogs and cats. The dashed line indicates the average prevalence of *Enterococcus* isolates among dogs and cats. Statistical differences in detection rates between dogs and cats are indicated by asterisks.

* *P*<0.01.

** *P*<0.001.



SUPPLEMENTARY FIGURE S2. Forest plots of (A) univariate and (B) multivariate analyses of the risk factors associated with MDR *Enterococcus* spp. infections in dogs and cats.

Abbreviation: MDR=multidrug resistance.

SUPPLEMENTARY TABLE S1. Distribution of *Enterococcus* spp. isolates across 20 provincial-level administrative divisions in China.

Source	**Beijing**		**Shanghai**		**Inner Mongolia**		**Liaoning**		**Fujian**		**Jiangsu**		**Shandong**		**Chongqing**		**Anhui**		**Henan**	
	Dog	**Cat**	**Dog**	**Cat**	**Dog**	**Cat**	**Dog**	**Cat**	**Dog**	**Cat**	**Dog**	**Cat**	**Dog**	**Cat**	**Dog**	**Cat**	**Dog**	**Cat**	**Dog**	**Cat**
Urinary tract	130	139	3	12	2	5	3	2	2	2	3	2	1	1		2	2		2	
Skin	39	21		1	1					1			1		1					
Ascites	13	11																		
Respiratory tract	14	7																		
Hepatobiliary system	9	3	1																	
Genital tract	9	1					1													
Pleural fluid	1	1																		
Other sites	8	7																		

Source	**Hunan**		**Jiangxi**		**Hebei**		**Guangdong**		**Hubei**		**Shanxi**		**Guangxi**		**Guizhou**		**Hainan**		**Zhejiang**	
	Dog	**Cat**	**Dog**	**Cat**	**Dog**	**Cat**	**Dog**	**Cat**	**Dog**	**Cat**	**Dog**	**Cat**	**Dog**	**Cat**	**Dog**	**Cat**	**Dog**	**Cat**	**Dog**	**Cat**
Urinary tract	1	1			2		1	0	1	0	1	0	0	1	0	1	0	1	0	1
Skin			1																	
Other sites			1																	