Vital Surveillances

One Health Approach of Enterococcal Population Structure and Antibacterial Resistance Along the Food Chain — Four PLADs, China, 2015–2022

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ABSTRACT

Introduction: Enterococci are considered opportunistic pathogens. However, they can serve as a reservoir of antibacterial resistance (ABR) traits and transfer these to humans through the food chain.

Methods: Antibiotic susceptibility testing and multilocus sequence typing were used to characterize the ABR and population structure of 488 enterococcal isolates recovered along the food chain from four provincial-level administrative divisions (PLADs) in China.

Results: *Enterococcus faecalis* (*E. faecalis*) was the dominant species cultured from pig farms and retail meat, while *Enterococcus faecium* (*E. faecium*) and *Enterococcus casseliflavus* were dominant in patients with diarrhea and retail fruits and vegetables, respectively. Approximately 67% of all *Enterococcus* isolates were multidrug-resistant (MDR). *E. faecium* resistance to ampicillin and penicillin was significantly higher than that of *E. faecalis*. The *E. faecalis* isolates exhibited substantially heterologous sequence types (STs), whereas *E. faecium* isolates were clearly divided into clonal complex (CC) CC17 and CC94 clades. *E. faecium* isolates were mainly detected in hospitalized children and were identified as the hospital-associated CC17 clade with ampicillin and penicillin resistance. Notably, *E. faecalis* ST16 and ST65 and *E. faecium* ST60 and ST94 detected in patients with diarrhea were also detected in farm and food samples, indicating that these STs should be closely monitored. The community-lineage *E. faecium* CC94 clade was detected in patients with diarrhea, implying that community isolates might find their way into hospitals.

Conclusion: This study highlights the One Health challenges posed by enterococci important to human health and the need to implement integrated preventive measures for their control.

Over the past two decades, enterococci have emerged as important multidrug-resistant (MDR) pathogens, responsible for an increasing number of nosocomial and community-acquired invasive infections worldwide (*1*). The injudicious use of antibiotics in food animal production and human disease treatment has contributed to the spread of MDR enterococci in food chains (*2*). *Enterococcus faecalis* (*E. faecalis*) and *Enterococcus faecium* (*E. faecium*) are the two most prevalent enterococcal species identified in human infections (*3*). Other species, such as *Enterococcus casseliflavus* (*E. casseliflavus*), *Enterococcus durans*, *Enterococcus gallinarum* (*E.gallinarum*), and *Enterococcus hirae* (*E. hirae*), can also infect humans with concurrent hematological malignancies, neutropenia, and prior corticosteroid treatment (*4*). Identifying enterococci at the species and molecular levels is clinically relevant due to the antibacterial resistance (ABR) profiles of different isolates. *E. faecium* exhibits higher rates of ampicillin and vancomycin resistance than *E. faecalis* (*5*). *E. gallinarum* and *E. casseliflavus* intrinsically exhibit low-level vancomycin resistance (*6*). *E. faecalis* and *E. faecium* hospital-adapted sequence type (ST) and clonal complex (CC) strains (e.g., *E. faecalis* ST6 and CC2, and *E. faecium* CC17), and community-adapted lineages (e.g., *E. faecium* CC94 and *E. faecalis* ST16) have been identified (*7*).

The One Health approach requires integrated analysis of important bacteria from animal, food, environmental, and human sources to characterize their populations and resistance phenotypes. Given the complexity of enterococcal ABR, highlighting the One Health approach's role in addressing this challenge is particularly important (*8*). Therefore, this study aimed to understand the population structure of MDR enterococcal isolates and their potential transmission risks along the food chain in China.

METHOD

Sample Collection and *Enterococcus* Detection and Identification

This study was performed in accordance with protocols approved by the Ethics Committee of the State Key Laboratory of the China National Centre for Food Safety Risk Assessment (CFSA).

We collected 694 samples from various sites and sources along the food chain (animal, food, environment, and human) representing the enterococcal population during 2015–2022. Briefly, the samples were collected from livestock (n=224), the farm environment (n=145), retail meat (n=91), retail fruits and vegetables (n=100), the urban environment (n=47), and humans (n=87) in Beijing Municipality, and Hubei, Henan, and Jilin Provinces. The samples collected from Henan and Jilin Provinces covered the entire food chain, including the community population and inpatients with diarrhea. The sample locations, types, and numbers are shown in Supplementary Table S1 (available at https://weekly.chinacdc.cn/). The sample collection, *Enterococcus* detection, and identification methods are presented in the Supplementary Material (available at https://weekly.chinacdc.cn/).

Antibacterial Susceptibility Testing

Antibacterial susceptibility to a panel of agents was determined by broth microdilution and interpreted according to the Clinical & Laboratory Standards Institute (CLSI) interpretive criteria. The minimum inhibitory concentration (MIC) of 10 antibacterial compounds was tested: ampicillin, penicillin, erythromycin, ciprofloxacin, daptomycin, vancomycin, tetracycline, chloramphenicol, high-level gentamicin (HLGA), and high-level streptomycin (HLSA). An isolate was defined as MDR if it exhibited resistance to 3 or more antibacterial compounds of different classes.

E. faecium and *E. faecalis* Multilocus ST (MLST) and CC Data Analysis

Standard *E. faecium* (*9*) and *E. faecalis* (*10*) MLST schemes were performed. The CCs of *E. faecium* and *E. faecalis* were annotated as previously described (*7*).

Statistical Analysis

Statistical analysis was performed using SPSS Statistics for Windows, version 17.0 (SPSS Inc., Chicago, IL, USA). Categorical variables were compared using Pearson's chi-squared and Fisher's exact tests. Statistical significance was set at P<0.05.

RESULTS

Prevalence of Enterococci and Species Diversity in the Food Chain

As shown in Supplementary Figure S1A (available at https://weekly.chinacdc.cn/) and Supplementary Table S1, most samples (488/694, 70.3%) were positive for enterococci. Enterococci were isolated from most human (65/87, 74.7%) and pig (184/224, 82.1%) samples. The prevalence of enterococci in urban environmental samples (8/47, 17.0%) was significantly lower than in other sample categories. Only 50.0% (20/40) of the fruit samples were positive for enterococci, significantly lower than the vegetable (32/37, 86.5%) and salad (19/23, 82.6%) samples.

Supplementary Figure S1B and Supplementary Table S2 (available at https://weekly.chinacdc.cn/) show the distribution of *Enterococcus* species across the various sample sources. Eight *Enterococcus* species were identified among the 488 isolates. The most common species was *E. faecalis* (358/488, 73.4%), followed by *E. faecium* (69/488, 14.1%) and *E. casseliflavus* (26/488, 5.3%).

The detection rate of *E. faecalis* was significantly higher than that of *E. faecium* in all sample categories (Supplementary Figure S1C). *E. casseliflavus* comprised 45.0% (9/20) of fruit enterococci, while *E. faecium* comprised 57.9% (11/19) of salad enterococci.

ABR of *Enterococcus* Species

The resistance levels against a panel of 10 antibacterial compounds are shown in Figure 1A and Supplementary Table S3 (available at https://weekly.chinacdc.cn/). Resistance to tetracycline (78.3%) and erythromycin (75.4%) was common, while low resistance levels were noted for daptomycin (1.6%) and vancomycin (1.6%). A proportion of *Enterococcus* isolates (67.0%) were defined as MDR bacteria. *E. faecium* resistance to ampicillin and penicillin was significantly higher than that of *E. faecalis*.

E. faecalis from livestock and the farm environment exhibited the lowest susceptibility to antibiotics, followed by multiple *Enterococcus* species from humans and retail meat. Isolates from retail fruits and

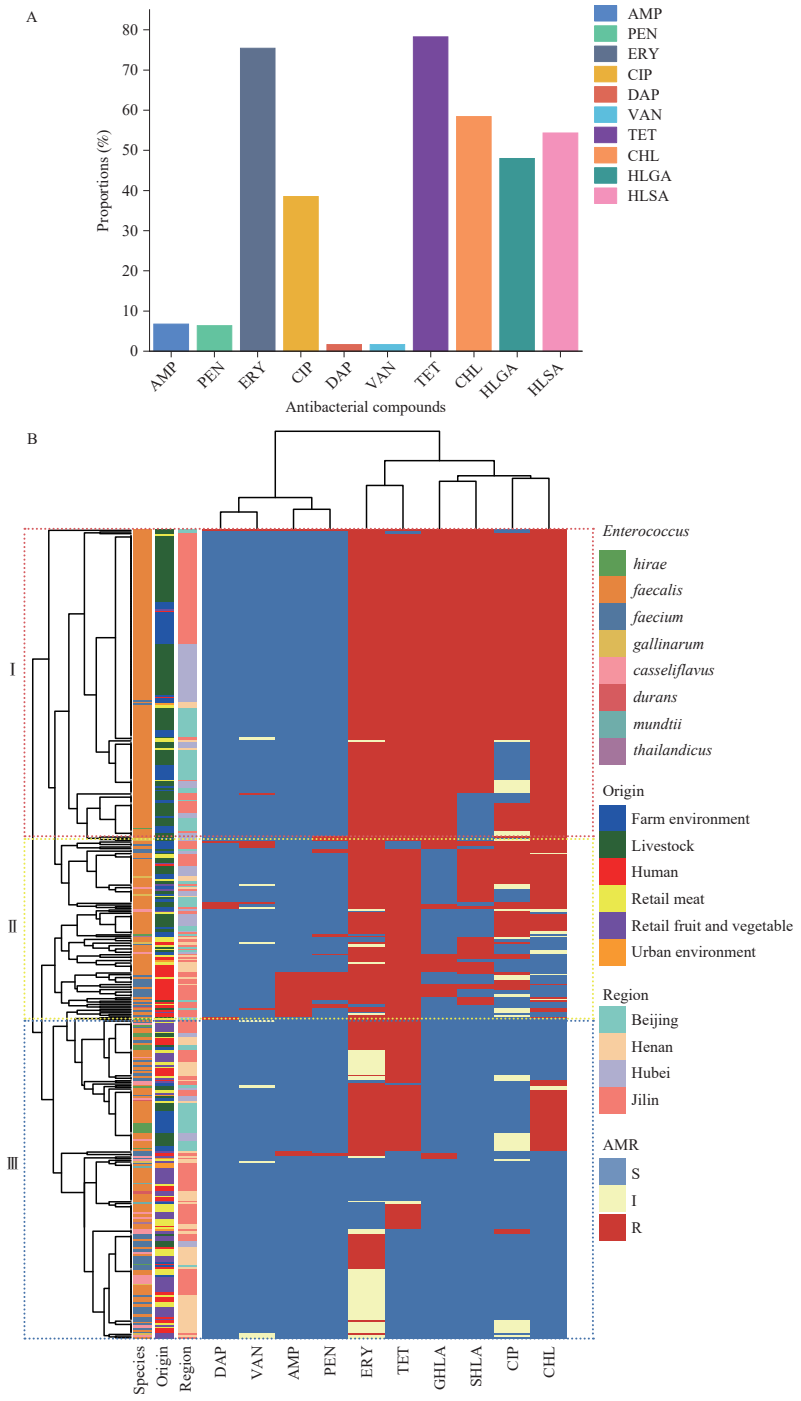

FIGURE 1. The enterococcal resistance levels against a panel of (A) ten antibacterial compounds; (B) hierarchical clustering and antibacterial resistance (ABR) heat map of enterococcal isolates from various sampling sources.

Note: (B) Part I shows that *E. faecalis* from livestock and the farm environment was the least susceptible to antibiotics; (B) Part II shows that multiple *Enterococcus* species were present in humans and retail meat samples; (B) Part III shows that *Enterococcus* isolates from retail fruits and vegetables and urban environments were the most susceptible to the panel of antibacterial compounds tested. Antibacterial susceptibility clusters were constructed using the hclust package in R with complete linkage as the default (https://www.rdocumentation.org/packages/stats/versions/3.6.2/topics/hclust). The ABR heatmaps were drawn using the pheatmap package in R (https://www.rdocumentation.org/packages/pheatmap/versions/1.0.12/topics/pheatmap).

Abbreviation: AMP=ampicillin; PEN=penicillin; ERY=erythromycin; CIP=ciprofloxacin; DAP=daptomycin; VAN=vancomycin; TET=tetracycline; CHL=chloramphenicol; HLGA=high-level gentamicin; HLSA=high-level streptomycin; S=susceptible; I=intermediate; R=resistant.

vegetables and urban environments were the most susceptible to the tested panel of antibacterial compounds (Figure 1B).

A comparison of ABR enterococcal isolates recovered from humans, food, farms, and the environment is shown in Figure 2A. Approximately half (50.8%, 33/65) of the human *Enterococcus* isolates were MDR. Enterococcal isolates recovered from the diarrheal feces of children were MDR, including resistance to ampicillin and penicillin, unlike those retrieved from other human sample types (Figure 2B). Furthermore, human *E. faecium* was more prone to express an MDR phenotype than *E. faecalis*.

Food (meat, fruits, vegetables, and salads) enterococci exhibited significantly lower resistance to ciprofloxacin (10.5%), HLGA (10.5%), and HLSA (27.8%) than enterococci from other sources. The MDR rate in food enterococci was 20.3% (27/133). Most isolates resistant to ≥5 antibiotics were *E. faecalis*. Most isolates from vegetables, cooked meat, and salads were susceptible to nearly all tested antibiotics (Figure 2C).

Farm enterococci exhibited significantly higher resistance to erythromycin (98.6%) than enterococci from other sources (Figure 2D). Nearly all (93.6%, 264/282) *Enterococcus* isolates from farms were MDR-positive, and most were resistant to four or five antibiotics. However, some *E. faecium* isolated from pig nasal passages, and *E. hirae* isolated from pig feces, were resistant to only one or two antibiotics (erythromycin or tetracycline).

Environmental enterococci exhibited significantly higher resistance to tetracycline, chloramphenicol, HLGA, and HLSA than those isolated from human and food sources. A large proportion (87.7%, 93/106) of environmental enterococci were MDR. Urban environmental and soil enterococcal strains exhibited less antibiotic resistance than pig environmental strains (Figure 2E). *E. hirae* isolated from the pig barn environment was resistant to tetracycline, chloramphenicol, and erythromycin.

ABR of *E. faecalis* and *E. faecium*

E. faecium exhibited significantly higher resistance to ampicillin and penicillin than *E. faecalis* (Figure 3A). The MDR rates among *E. faecalis* and *E. faecium* isolates were 78.2% (280/358) and 36.2% (25/69), respectively. Most MDR *E. faecalis* isolates were from livestock and the farm environment. In contrast, most *E. faecalis* isolates of human and food origin were non-MDR strains (Figure 3B). This finding contrasted with *E. faecium*, in which most isolates from humans were defined as MDR, while most isolates from food and livestock were resistant to no more than two antibiotics (Figure 3C).

STs and CCs of *E. faecalis* and *E. faecium*

The *E. faecalis* isolates showed highly heterologous genotypes, with 95 STs among the 358 *E. faecalis* isolates, including 28 (29.5%) new STs. The main *E. faecalis* STs were ST4 (10.3%), ST86 (7.5%), ST476 (6.2%), and ST330 (5.9%; Figure 4A). Two *E. faecalis* CCs, CC16 (major in ST16) and CC21 (major in ST21), were identified. Three major CC and ST clades (CC21, CC16, and ST69-ST632), covering the isolates from children's diarrheal feces, pigs, meat, vegetables, and fruits, *E. faecalis* ST16 and ST65 had complex sample sources, including animals, food, and patients' diarrheal feces. As shown in Figure 4B, the CC16 clade, ST4, ST16, ST86, ST330, and other isolates were identified as MDR.

The 69 *E. faecium* isolates analyzed were divided into 44 STs, including 21 (47.7%) novel STs. The main *E. faecium* STs included ST94 (11.6%), ST569 (8.7%), and ST296 (7.3%) (Figure 5A). Unlike the *E. faecalis* isolates, which exhibited complex prevalent ST and clade structures, the *E. faecium* isolates were primarily divided into two distinct CC clades, CC17 and CC94. The main isolates in the CC17 clade were recovered from children's diarrheal feces, while CC94 isolates were mainly from retail fruits and vegetables, and pigs. Most isolates in the CC17 clade were MDR, while most isolates in the CC94 clade were non-MDR (Figure 5B). ST60 and ST94 isolates originated from children's diarrheal feces and ready-to-eat food. Notably, despite belonging to the same STs, isolates from clinical patients exhibited MDR phenotypes, while isolates from food were non-MDR.

DISCUSSION

Although enterococci are considered opportunistic pathogens, they can be reservoirs of ABR and transfer resistance to humans through the food chain (*11*). This study demonstrated that enterococci were abundant across most parts of the food chain in China, particularly in animal farms and humans. The most dominant species identified was *E. faecalis*, followed by

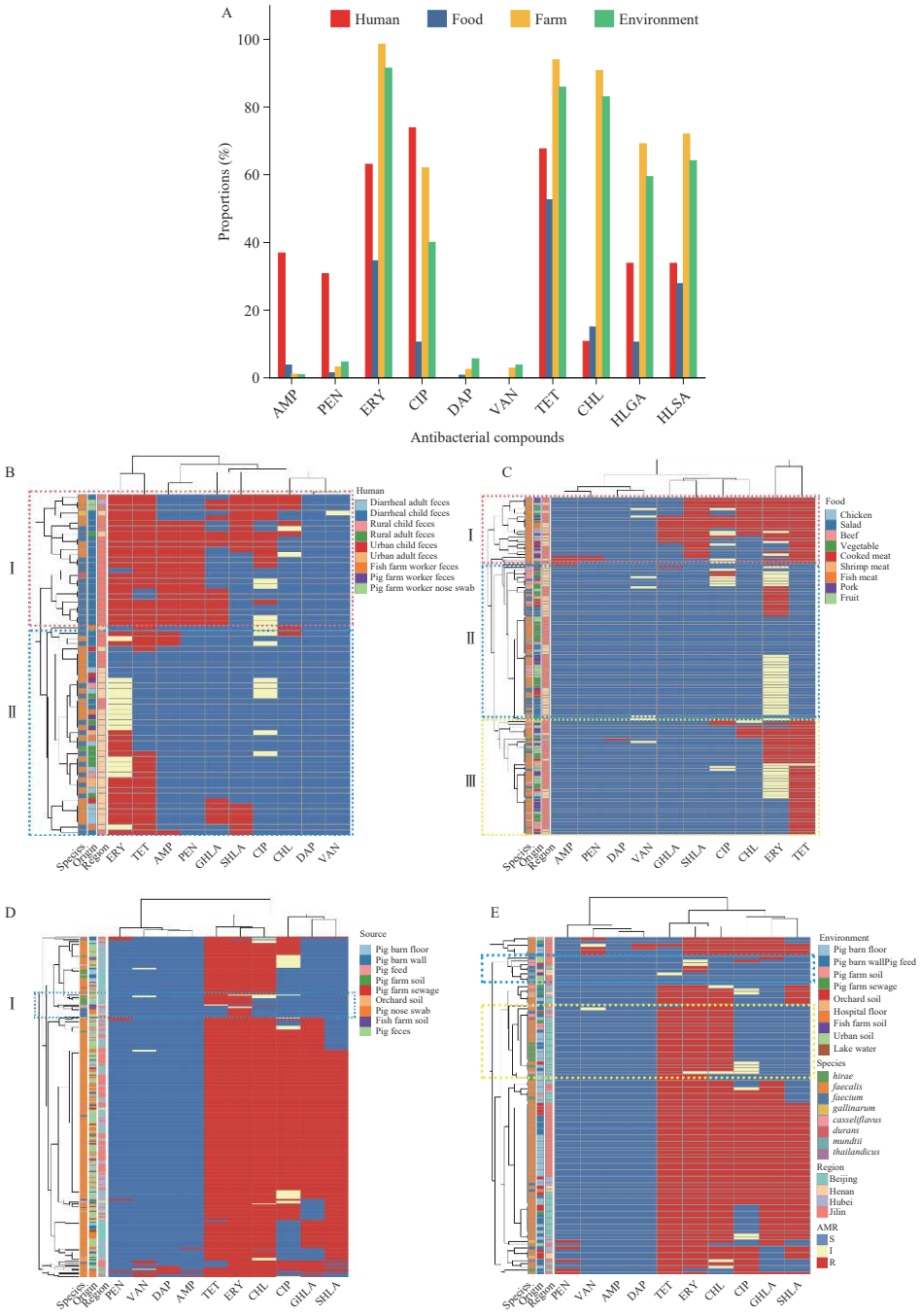

FIGURE 2. Comparison of the resistance levels of enterococci recovered from human, food, farm, and environmental sources against (A) a panel of ten antibacterial compounds. Hierarchical clustering and antibacterial resistance heat map of enterococcal isolates recovered from (B) human, (C) food, (D) farm, (E) and environmental sources.

Note: Enterococcal isolates recovered from the diarrheal feces of children were multidrug-resistant, unlike those retrieved from other human sample types (B, Part Ⅰ and Ⅱ). Most *E. faecalis* isolates from animal food samples (chicken, pork, and beef) were resistant to ≥5 antibiotics (C, Part Ⅰ); most isolates from vegetables, cooked meat, and salads were sensitive to nearly all tested antibiotics (C, Part Ⅱ); most isolates from Jilin Province were *E. faecalis* and resistant to tetracycline (TET; C, Part Ⅲ). In farm samples (D, Part Ⅰ), some *E. faecium* isolates recovered from pig nasal passages, and *E. hirae* isolated from pig feces were resistant to only one or two antibiotics (ERY or/and TET). Urban environmental and soil *enterococcal* strains showed less antibiotic resistance than those recovered from the pig environment (E, Part I); *E. hirae* recovered from the pig barn environment had the resistance profile of TET-CHL-ERY (E, Part Ⅱ).

Abbreviation: AMP=ampicillin; PEN=penicillin; ERY=erythromycin; CIP=ciprofloxacin; DAP=daptomycin; VAN=vancomycin; TET=tetracycline; CHL=chloramphenicol; HLGA=high-level gentamicin; HLSA=high-level streptomycin; S=susceptible; I=intermediate; R=resistant.

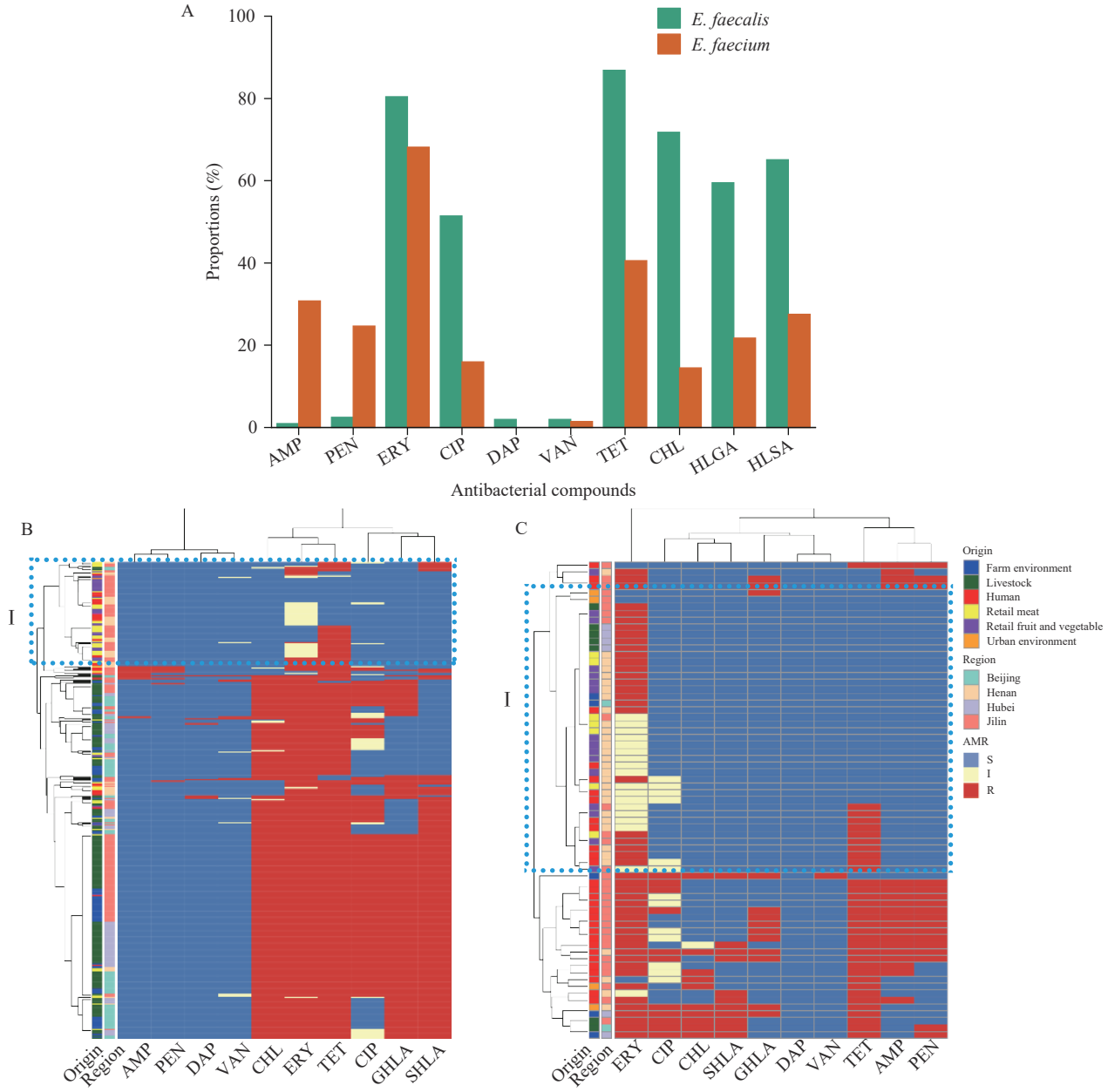

FIGURE 3. Comparison of *E. faecalis* and *E. faecium* resistance levels against (A) a panel of ten antibacterial compounds. (B) Hierarchical clustering and antibacterial resistance heat maps of *E. faecalis* and (C) *E. faecium* isolated from all tested samples.
Note: Most *E. faecalis* isolates of human and food origin were non-MDR strains (B, Part I). Most isolates from food and livestock were resistant to no more than two antibiotics (C, Part I).
Abbreviation: AMP=ampicillin; PEN=penicillin; ERY=erythromycin; CIP=ciprofloxacin; DAP=daptomycin; VAN=vancomycin; TET=tetracycline; CHL=chloramphenicol; HLGA=high-level gentamicin; HLSA=high-level streptomycin; S=susceptible; I=intermediate; R=resistant.

E. faecium and *E. casseliflavus*. *E. faecium* CC17 isolates were dominant in children's diarrheal feces, whereas *E. casseliflavus* was the dominant species in retail vegetables and fruits.

A high level of MDR enterococci was found among food-producing animals and the animal farm environment, possibly because antibiotics are widely used as animal growth promoters in China. *Enterococci* in commercial food animal production and human feces could contaminate the food chain during processing or through the composting of these wastes for use as biofertilizers on farms (*12*). It has been

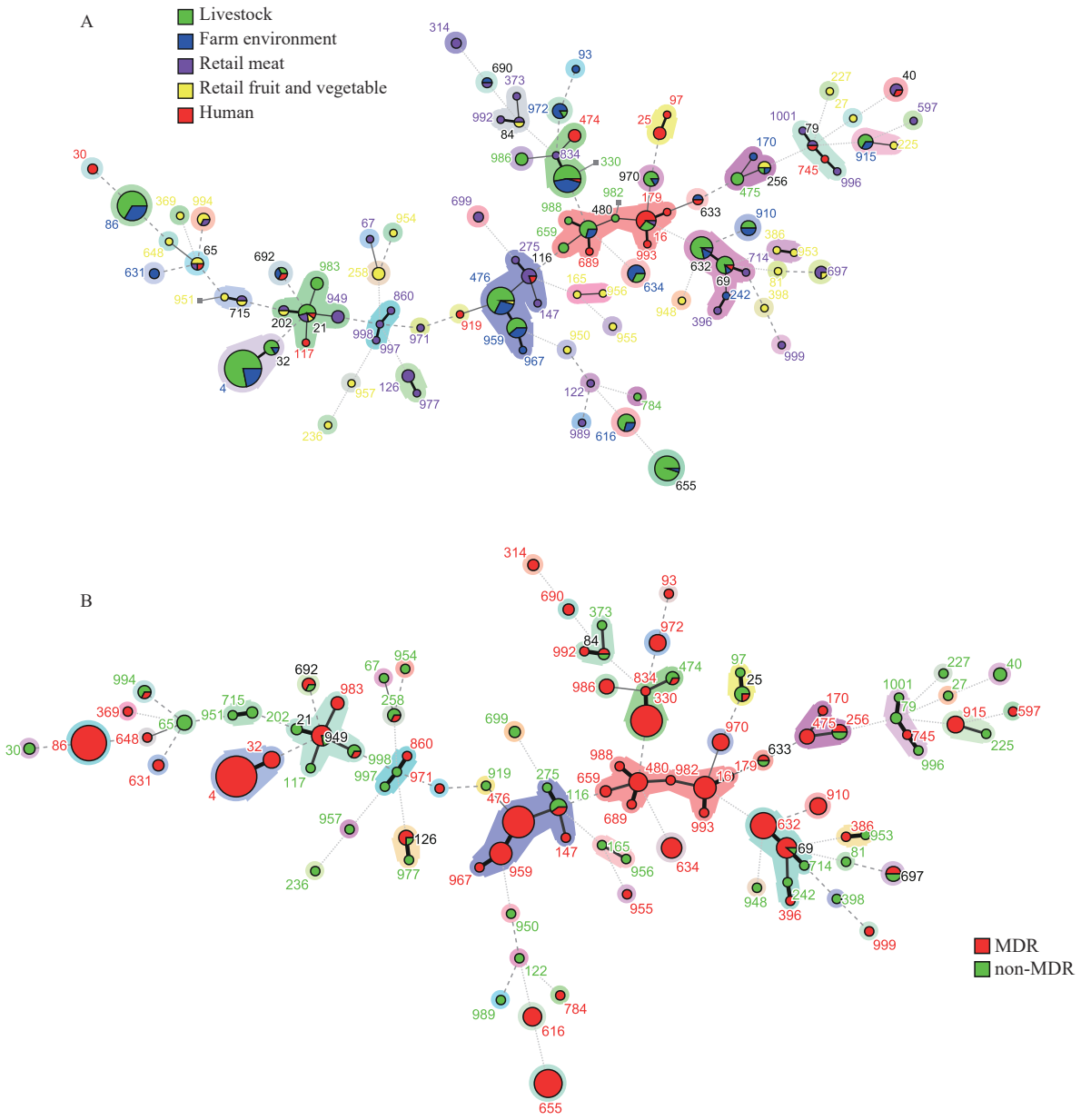

FIGURE 4. A minimum spanning tree of (A) *E. faecalis* STs, (B) and MDR isolate distribution as determined by multilocus sequence typing.
Note: The node size reflects the number of isolates included in that particular clade.
Abbreviation: ST=sequence type; CC=clonal complex; MDR=multidrug resistance.

reported that many *E. faecium* bloodstream infections were of gastrointestinal origin, raising the possibility that food might be a vehicle for such bacteria (*13*). Our data showed that farms and foods were highly contaminated by enterococci and that the most dominant species was *E. faecalis*, followed by *E. faecium*. Farm, food, and environmental enterococci exhibited high resistance to tetracycline, erythromycin, and HLSA. Notably, *E. faecalis* ST16 and ST65 and *E. faecium* ST60 and ST94 were found in isolates from farms, food, and patients' diarrheal feces, suggesting potential pathogen transfer along the food chain.

To treat enterococcal infections, first-choice antibiotics are typically β-lactam-based compounds and aminoglycosides. Second-choice antibiotics include glycopeptides, especially vancomycin (*14*). However, in this study, most enterococci isolated from children's diarrheal feces belonged to the *E. faecium* hospital-lineage CC17 clade, exhibiting high resistance to ampicillin, penicillin, HLGA, and HLSA, which

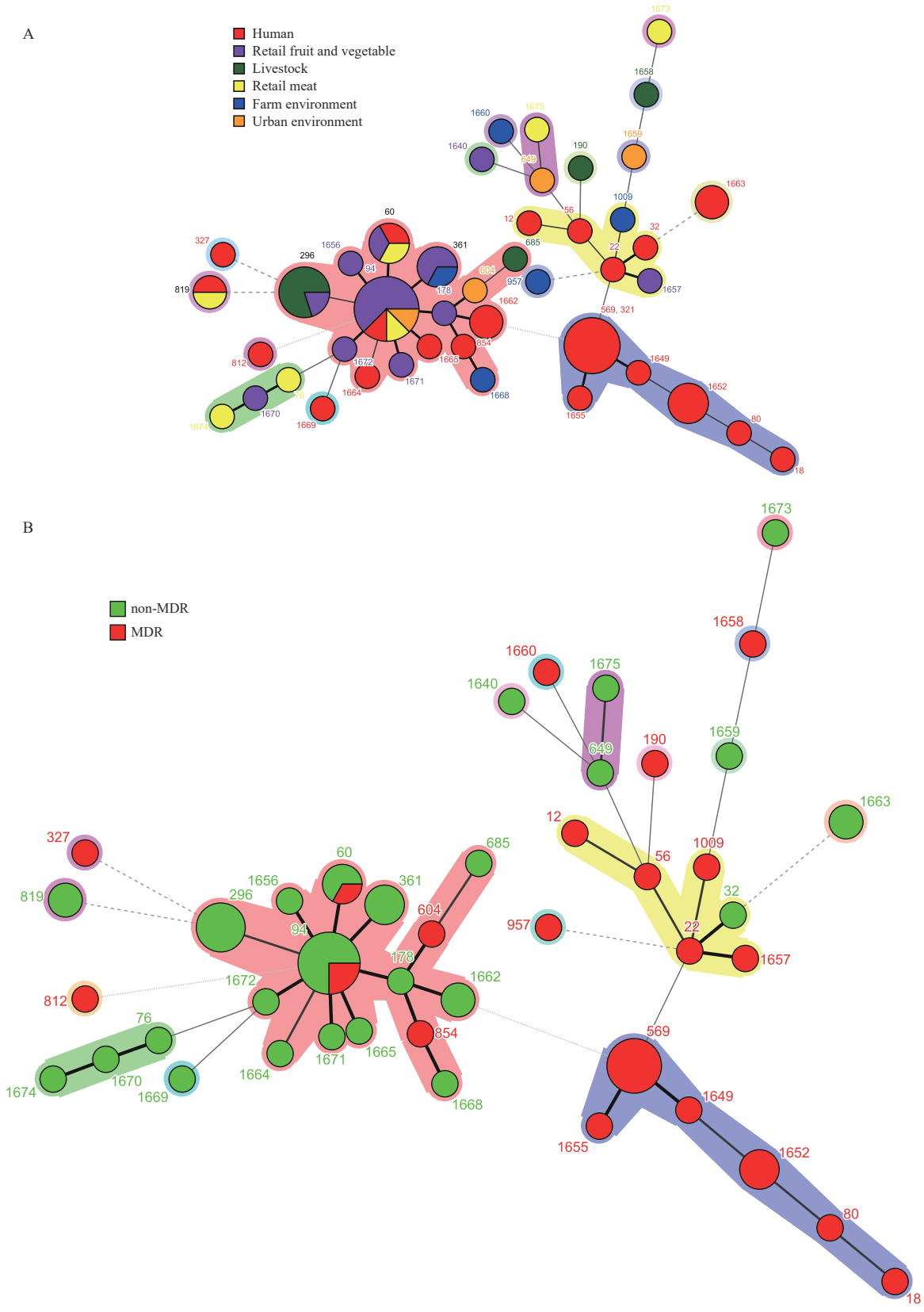

FIGURE 5. Minimum spanning tree of (A) *E. faecium* STs, (B) multidrug-resistant isolate distribution as determined by multilocus sequence typing.
Abbreviation: ST=sequence type; CC=clonal complex.

limits treatment options. The *E. faecium* CC94 clade, a traditional community lineage, was detected in diarrheal feces from patients, suggesting that community isolates may be spreading into hospitals. Interestingly, the hospital CC94 isolates, ST60 and ST94, were MDR, whereas community isolates with the same ST were not. This suggests that hospital environments, with their greater use of antibacterials and disinfectants, may promote MDR formation in hospital-adapted isolates compared to community-acquired isolates. However, sampling sources were imbalanced across regions. No inpatient samples were collected in Beijing Municipality and Hubei Province due to ethical concerns, affecting the completeness of the One Health approach in this study.

In conclusion, our results suggest that effective precautionary measures should be taken to prevent the occurrence of MDR enterococci in the food chain.

Conflicts of interest: No conflicts of interest.

Acknowledgments: Tania Dottorini, Michelle Baker, and Necati Esener of the University of Nottingham for helping with data analysis and visualization.

Funding: Supported by the National Natural Science Foundation of China (32172314 and 22193064).

doi: 10.46234/ccdcw2024.246

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Submitted: October 07, 2024; Accepted: November 09, 2024

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SUPPLEMENTARY MATERIALS

Sample Collection and Transportation

To isolate bacteria, 500 g of fresh pig feces were collected from pig litter floors using sterilized spoons. Nasal swab samples were collected from the same pig litter using cotton-tip swabs (108C.USE, Copan Diagnostics, Italy). From each pig litter, one or two fecal or nasal swab samples were collected, depending on stocking density.

Pig barn floor samples, wall samples, and hospital floor samples were collected from 10 cm × 10 cm areas using sponge swabs (SS100NB, Hygiena International, Watford, UK). Two to three floor or wall samples were collected from each pig barn. From one hospital, two to three floor samples were collected with a minimum distance of 10 m between sampling sites. Soil samples were collected at depths of 2–5 cm from various locations (orchards, crop fields, vegetable fields, forest land, pig farms, fish farms, and urban areas) using sterilized buckets. Each area was represented by one to two soil samples. Liquid samples (≥100 mL each) of pig farm sewage, chicken farm sewage, fish farm water, and lake water were collected using pipettes. Two to three sewage or water samples were collected from each farm. Feed samples (500 g each) were collected from troughs using sterilized buckets, with two samples collected per pig barn.

Retail raw or cooked meat, fruit, and vegetable samples (≥200 g each) were randomly collected from major supermarkets, free-trade markets, food stores, and convenience shops using sterilized tongs.

Fecal samples (2 g each) were collected using sterilized sampling spoons from healthy or diarrheal hospitalized children (<7 years old), adults, and animal farm workers. Nasal swab samples were also collected from pig farm workers using cotton-tip swabs.

All samples were collected using aseptic techniques, stored in secure containers at 4 °C during transportation to the laboratory, and analyzed within 24 h.

Enterococcus Detection and Identification

Samples were homogenized as follows: 25 g (or mL) of pig feces, soil, feed, sewage, water, retail meat, vegetables, or fruits were homogenized with 225 mL of sterile buffered peptone water (BPW; Luqiao Inc., Beijing, China) for 1 min in a stomacher bag (Luqiao Inc.). Human or pig nasal swab samples and human fecal samples (1 g) were vigorously vortexed with 9 mL BPW for 1 min in test tubes. Floor or wall swab samples were homogenized with 10 mL BPW for 1 min in stomacher bags.

Enterococcus species isolation and identification followed previously described protocols (*1*). Briefly, approximately 1 mL of diluted sample was added to 9 mL *enterococcus* broth (Difco/BD, Sparks, MD, USA) and incubated at 37 °C for 24 h to enumerate presumptive enterococci. A loopful of each solution was streaked onto mEI agar (Luqiao Inc.) and incubated at 42 °C for 40–48 h. Typical *Enterococcus* species colonies were screened and characterized by their tolerance to 6.5% (w/v) NaCl. Tolerant isolates were confirmed by PCR using *Enterococcus* species-specific primers Ent1 (5'-TAC TGA CAA ACC ATT CAT GAT G-3') and Ent2 (5'-AAT TCG TCA CCA ACG CGA AC-3') (*2*). PCR conditions were: pre-incubation at 94 °C for 5 min, followed by 30 cycles of denaturation (94 °C, 30 s), annealing (55 °C, 30 s), and elongation (72 °C, 30 s), with a final extension at 72 °C for 5 min. Species-level identification was performed using a Bruker MALDI Biotyper (Germany) and 16S rRNA gene sequencing followed by GenBank alignment as previously described (*3*).

SUPPLEMENTARY TABLE S1. Prevalence of *enterococci* in different animals, foods, environment, and human sources from four PLADs of China

Sample origin	Beijing Municipality		Hubei Province		Henan Province		Jilin Province		All	
	Number of samples	Percent enterococci, %	Number of samples	Percent enterococci, %	Number of samples	Percent enterococci, %	Number of samples	Percent enterococci, %	Number of samples	Percent enterococci, %*
Human										
Diarrheal adult feces	–	–	–	–	11	81.82	–	–	11	81.82
Diarrheal child feces	–	–	–	–	1	100.00	38	78.95	39	79.49

Continued

Sample origin	Beijing Municipality		Hubei Province		Henan Province		Jilin Province		All	
	Number of samples	Percent enterococci, %	Number of samples	Percent enterococci, %	Number of samples	Percent enterococci, %	Number of samples	Percent enterococci, %	Number of samples	Percent enterococci, %*
Rural adult feces	–	–	–	–	8	100.00	–	–	8	100.00
Rural child feces	–	–	–	–	2	100.00	–	–	2	100.00
Urban adult feces	–	–	–	–	8	37.50	–	–	8	37.50
Urban child feces	–	–	–	–	4	75.00	–	–	4	75.00
Pig farm worker feces	–	–	1	0.00	3	100.00	–	–	4	75.00
Pig farm worker nasal swab	2	0.00	1	100.00	–	–	2	50.00	5	40.00
Fish farm worker feces	–	–	–	–	6	66.67	–	–	6	66.67
Total	2	0.00	2	50.00	43	76.74	40	77.50	87	74.71[A-E]
Livestock										
Pig nasal swab	34	67.65	24	95.83	–	–	33	72.73	91	76.92
Pig feces	44	70.45	53	96.23	–	–	36	88.89	133	85.71
Total	78	69.23	77	96.10	–	–	69	81.16	224	82.14[B, C, D]
Retail Meat										
Beef	–	–	–	–	7	57.14	7	100.00	14	78.57[a-f]
Chicken	–	–	–	–	7	100.00	–	–	7	100.00[b-f]
Cooked meat	–	–	–	–	25	48.00	–	–	25	48.00[c, d, f]
Fish meat	–	–	–	–	7	28.57	–	–	7	28.57[d, f]
Pork	1	100.00	–	–	5	60.00	25	96.00	31	90.32[e]
Shrimp meat	–	–	–	–	7	28.57	–	–	7	28.57[f]
Total	1	100.00	–	–	58	51.72	32	96.88	91	68.13[C, D, E]
Retail Fruit and Vegetable										
Fruit	–	–	–	–	7	0.00	33	60.61	40	50.00[a]
Salad	–	–	–	–	23	82.61	–	–	23	82.61[b]
Vegetable	–	–	–	–	7	42.86	30	96.67	37	86.49[b]
Total	–	–	–	–	37	59.46	63	77.78	100	71.00[D, E]
Farm Environment										
Orchard soil	–	–	–	–	2	50.00	–	–	2	50.00
Crop soil	–	–	–	–	2	0.00	–	–	2	0.00
Vegetable soil	–	–	–	–	2	0.00	–	–	2	0.00
Forest soil	–	–	–	–	2	0.00	–	–	2	0.00
Pig barn floor	21	85.71	8	75.00	–	–	20	95.00	49	87.76[†]
Pig barn wall	24	66.67	4	100.00	–	–	14	78.57	42	73.81
Pig farm sewage	6	66.67	2	100.00	3	100.00	6	83.33	17	82.35

Continued

Sample origin	Beijing Municipality		Hubei Province		Henan Province		Jilin Province		All	
	Number of samples	Percent enterococci, %	Number of samples	Percent enterococci, %	Number of samples	Percent enterococci, %	Number of samples	Percent enterococci, %	Number of samples	Percent enterococci, %*
Fish farm water	–	–	–	–	6	0.00	–	–	6	0.00
Pig farm soil	8	25.00	2	50.00	1	100.00	3	33.33	14	35.71†
Pig feed	8	37.50	–	–	–	–	–	–	8	37.50
Fish farm soil	–	–	–	–	1	100.00	–	–	1	100.00
Total	67	64.18	16	81.25	19	31.58	43	75.00	145	67.59E
Urban Environment										
Lake water	–	–	–	–	6	0.00	9	11.11	15	6.67
Urban soil	–	–	–	–	–	–	27	22.22	27	14.81
Hospital floor	–	–	–	–	5	20.00	–	–	5	20.00
Total	–	–	–	–	11	9.09	36	19.44	47	17.02F
All total	148	66.22	95	92.63	168	54.76	283	74.20	694	70.03

Note: "–" means no data.
* "A–F" indicates the statistical difference between sample categories. "a–f" and "ab" indicate the statistical difference within Retail Meat and Retail Fruit and Vegetable categories, respectively.
† indicates the statistical difference between the enterococcal contamination of pig barn floor and pig farm soil samples.

SUPPLEMENTARY TABLE S2. Species distribution and sample origin of enterococci isolates from four PLADs of China.

Sample origin	*E. faecalis**,†	*E. faecium*	*E. casseliflavus*	*E. hirae*	*E. gallinarum*	*E. durans*	*E. mundtii*	*E. thailandicus*	Total
Human									
Diarrheal adult feces	8 (88.89%)	1 (11.11%)	–	–	–	–	–	–	9
Diarrheal child feces	12 (38.71%)	17 (54.84%)	–	–	1 (3.23%)	1 (3.23%)	–	–	31
Rural child feces	–	2 (100.00%)	–	–	–	–	–	–	2
Rural adult feces	1 (12.50%)	6 (75.00%)	–	1 (12.50%)	–	–	–	–	8
Urban child feces	3 (100.00%)	–	–	–	–	–	–	–	3
Urban adult feces	1 (33.33%)	2 (66.67%)	–	–	–	–	–	–	3
Pig farm worker feces	3 (100%)	–	–	–	–	–	–	–	3
Fish farm worker feces	2 (50.00%)	2 (50.00%)	–	–	–	–	–	–	4
Pig farm worker nasal swab	2 (100%)	–	–	–	–	–	–	–	2
Total	32A (49.23%)	30 (46.15%)	–	1 (1.54%)	1 (1.54%)	1 (1.54%)	–	–	65
Livestock									
Pig nasal swab	63 (90.00%)	5 (7.14%)	1 (1.43%)	–	1 (1.43%)	–	–	–	70
Pig feces	108 (94.74%)	2 (1.75%)	–	4 (3.51%)	–	–	–	–	114
Total	171B (92.93%)	7 (3.80%)	1 (0.54%)	4 (2.17%)	1 (0.54%)	–	–	–	184
Retail meat									
Beef	9 (81.82%)	–	1 (9.09%)	–	–	–	–	1 (9.09%)	11
Chicken	7 (100.00%)	–	–	–	–	–	–	–	7
Cooked meat	7 (58.33%)	3 (25.00%)	2 (16.67%)	–	–	–	–	–	12
Fish meat	2 (100%)	–	–	–	–	–	–	–	2
Pork	24 (85.71%)	3 (10.71%)	–	1 (3.57%)	–	–	–	–	28
Shrimp meat	1 (50.00%)	1 (50.00%)	–	–	–	–	–	–	2

Continued

Sample origin	*E. faecalis*[*,†]	*E. faecium*	*E. casseliflavus*	*E. hirae*	*E. gallinarum*	*E. durans*	*E. mundtii*	*E. thailandicus*	Total
Total	50[B, C, D] (80.65%)	7 (11.29%)	3 (4.84%)	1 (1.61%)	–	–	–	1 (1.61%)	62
Retail fruit and vegetable									
Fruit	6 (30.00%)	3 (15.00%)	9 (45.00%)	1 (5.00%)	1 (5.00%)	–	–	–	20
Salad	3 (15.79%)	11 (57.89%)	2 (10.53%)	–	1 (5.26%)	2 (10.53%)	–	–	19
Vegetable	24 (75.00%)	2 (6.25%)	4 (12.50%)	–	1 (3.13%)	–	1 (3.13%)	–	32
Total	33[A, D] (46.48%)	16 (22.54%)	15 (21.13%)	1 (1.41%)	3 (4.23%)	2 (2.82%)	1 (1.41%)	–	71
Farm environment									
Orchard soil	–	1 (100.00%)	–	–	–	–	–	–	1
Pig barn floor	32 (74.42%)	1 (2.33%)	1 (2.33%)	8 (18.60%)	–	1 (2.33%)	–	–	43
Pig barn wall	20 (64.52%)	3 (9.68%)	2 (6.45%)	3 (9.68%)	2 (6.45%)	–	–	1 (3.23%)	31
Pig farm sewage	12 (85.71%)	–	1 (7.14%)	–	1 (7.14%)	–	–	–	14
Pig farm soil	3 (60.00%)	–	2 (40.00%)	–	–	–	–	–	5
Pig feed	3 (100.00%)	–	–	–	–	–	–	–	3
Fish farm soil	–	–	–	–	–	–	1 (100%)	–	1
Total	70[B, C] (71.43%)	5 (5.10%)	6 (6.12%)	11 (11.22%)	3 (3.06%)	1 (1.02%)	1 (1.02%)	1 (1.02%)	98
Urban environment									
Lake water	–	1 (100.00%)	–	–	–	–	–	–	1
Urban soil	2 (33.33%)	2 (33.33%)	1 (16.67%)	–	–	–	1 (16.67%)	–	6
Hospital floor	–	1 (100.00%)	–	–	–	–	–	–	1
Total	2[C, D] (25.00%)	4 (50.00%)	1 (12.50%)	–	–	–	1 (12.50%)	–	8
Total	358 (73.36%)	69 (14.14%)	26 (5.33%)	18 (3.69%)	8 (1.64%)	4 (0.82%)	3 (0.61%)	2 (0.41%)	488

Note: "–" means negative.

* Percentage data means the proportion of *Enterococcus* species in this sample origin category.

† "A–D" indicates the statistical difference within the constitution of *E. faecalis* and *E. faecium* between different categories.

SUPPLEMENTARY TABLE S3. Distribution of MICs and resistance among 488 *enterococcal* strains isolated in the food chain from four PLADs of China.

Antibiotic	Sample types or species	MIC_{50}††	MIC_{90}††	Resistance (%)§§	Number of strains with MIC (μg/mL) of*												
					0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	250	500	1000	>1000
Ampicillin	*Enterococcus* species	≤0.5	4	33 (6.76)	249** (≤0.5)				31	76	85	14	4			29 (>16)			
	Human†	4	>16	24[A] (36.92)	3				2	12	17	7	1			23			
	Food†	2	4	5[B] (3.76)	8				11	49	53	7	2			3			
	Farm§	≤0.5	2	3[B] (1.06)	237				16	14	12	0	1			2			
	Environment¶	≤0.5	4	1[B] (0.94)	77				8	8	12	0	0			1			
	E. faecalis	≤0.5	4	11[a] (3.07)	224				14	42	58	9	2			9			
	E. faecium	4	>16	20[b] (30.77)	11				4	9	23	2	2			18			
Penicillin	*Enterococcus* species	2	4	31 (6.35)	3 (≤0.06)	5	5	22	57	260	87	18				31 (>8)			
	Human	2	>8	20[A] (30.77)	1	0	0	5	7	21	10	1				20			
	Food	2	2	2[B] (1.50)	2	3	2	12	47	56	9	0				2			
	Farm	2	4	9[B] (3.19)	0	1	1	3	3	181	68	16				9			
	Environment	2	8	5[B] (4.72)	0	1	3	5	2	59	20	11				5			
	E. faecalis	2	4	9[a] (2.51)	1	2	0	8	33	232	70	3				9			
	E. faecium	2	>8	17[b] (24.64)	2	2	0	6	7	19	13	3				17			

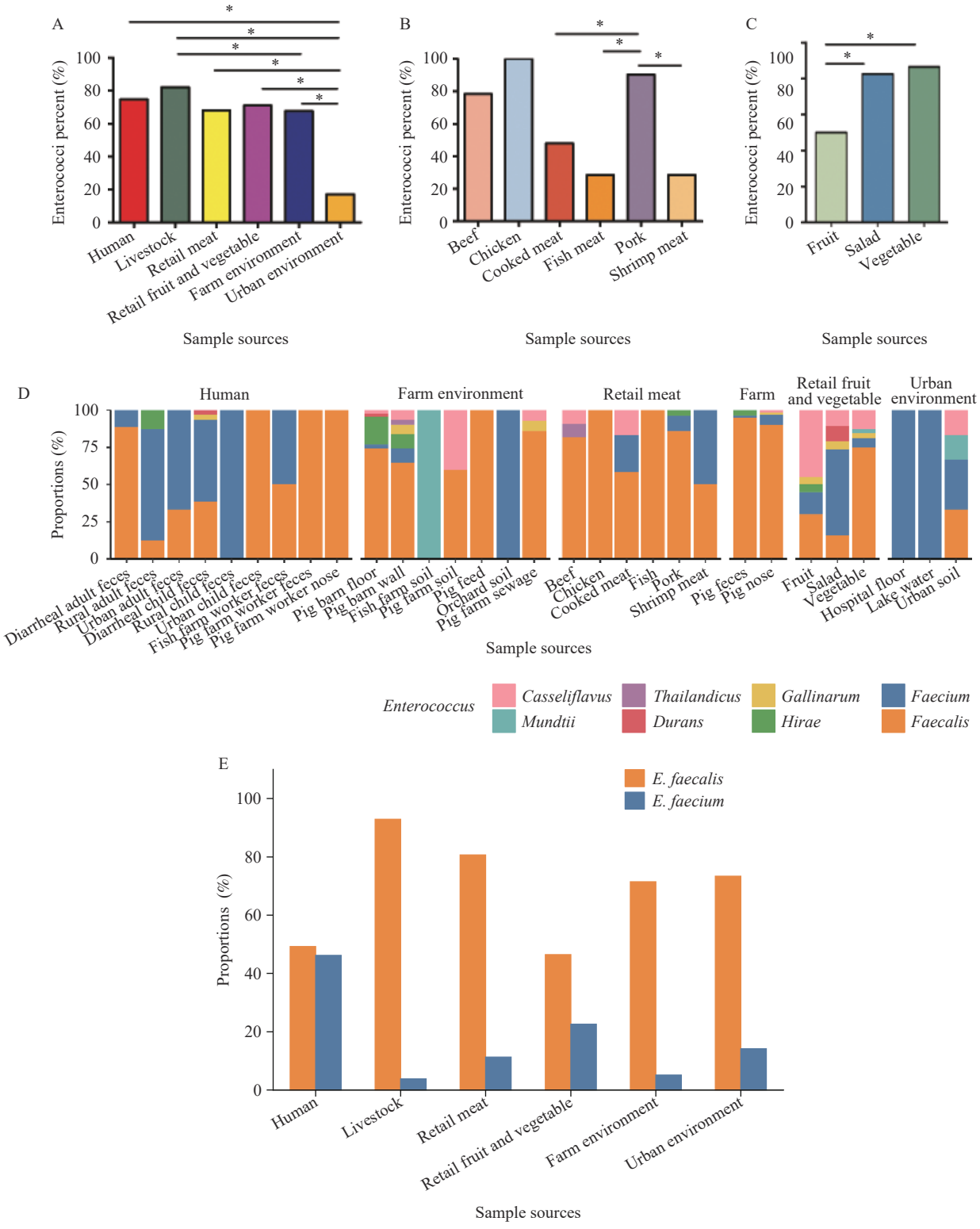
Continued

Antibiotic	Sample types or species	MIC_{50}††	MIC_{90}††	Number of strains with MIC (μg/mL) of*													
				Resistance (%)§§	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	250	500	1000	>1000
Erythromycin	*Enterococcus* species	>8	>8	368 (75.41)	15 (≤0.125)		11	26	18	22	28	24				344 (>8)			
	Human	>8	>8	41[A] (63.08)	2		4	2	4	5	7	4				37			
	Food	2	>8	46[B] (34.59)	8		7	23	14	16	19	14				32			
	Farm	>8	>8	278[C] (98.58)	2		0	0	0	0	2	6				272			
	Environment	>8	>8	97[D] (91.51)	5		0	1	0	1	2	2				95			
	E. faecalis	>8	>8	288[a] (80.45)	8		7	20	8	15	12	5				283			
	E. faecium	>8	>8	47[b] (68.12)	1		2	1	5	3	10	18				29			
Ciprofloxacin	*Enterococcus* species	2	>8	188 (38.52)	6 (≤0.125)		10	51	165	52	16	6				182 (>8)			
	Human	>8	>8	48[A] (73.85)	2		4	2	4	5	7	4				37			
	Food	1	4	14[B] (10.53)	3		2	28	73	13	3	0				11			
	Farm	>8	>8	175[A, C] (62.06)	0		5	15	61	26	10	6				159			
	Environment	2	>8	52[C] (40.06)	0		6	9	30	9	6	3				43			
	E. faecalis	4	>8	184[a] (51.40)	2		1	18	123	30	7	4				173			
	E. faecium	1	8	11[b] (15.94)	3		3	19	20	13	3	2				6			
Daptomycin	*Enterococcus* species	2	4	8 (1.64)	0 (≤0.125)		3	6	108	277	86	5				3 (>8)			
	Human	2	4	0 (0)	0		0	0	12	36	17	0				0			
	Food	2	2	1 (0.75)	0		0	2	31	95	4	1				0			
	Farm	2	4	7 (2.48)	0		3	4	62	142	64	4				3			
	Environment	2	4	6 (5.66)	0		1	2	26	54	21	0				2			
	E. faecalis	2	4	7 (1.96)	0		3	2	80	219	47	4				3			
	E. faecium	2	4	0 (0)	0		0	0	8	36	25	0				0			
Vancomycin	*Enterococcus* species	1	4	8 (1.64)	73 (≤0.5)				202	121	72	10	2	1		7 (>32)			
	Human	1	4	0 (0)	17				26	14	7	1	0	0		0			
	Food	2	4	0 (0)	19				45	33	29	7	0	0		0			
	Farm	1	4	8 (2.84)	35				129	71	35	2	2	1		7			
	Environment	1	4	4 (3.77)	20				45	18	17	1	1	0		4			
	E. faecalis	1	4	7 (1.96)	20				20	3	3	1	1	6		304			
	E. faecium	≤0.5	4	1 (1.45)	35				14	9	10	0	0	0		1			
Tetracycline	*Enterococcus* species	>32	>32	382 (78.28)	58 (≤0.5)				29	12	5	2	3	14		365 (>32)			
	Human	>32	>32	44[A] (67.69)	12				7	2	0	0	2	5		37			
	Food	32	>32	70[A] (52.63)	35				15	8	4	1	1	7		62			
	Farm	>32	>32	265[B] (93.97)	7				7	2	0	1	0	2		263			
	Environment	>32	>32	91[B] (85.85)	7				5	1	1	1	0	2		89			
	E. faecalis	>32	>32	311[a] (86.87)	20				20	3	3	1	1	6		304			
	E. faecium	2	>32	34[b] (40.58)	29				3	3	0	0	2	4		28			

Continued

Antibiotic	Sample types or species	MIC_{50}††	MIC_{90}††	Number of strains with MIC (μg/mL) of*												
				Resistance (%)§§	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	250	500	1000	>1000
Chloramphenicol	*Enterococcus* species	32	>64	285 (58.40)		2 (≤1)			4	105	80	12	75	52		158 (>64)			
	Human	8	32	7[A] (10.77)		2			1	24	29	2	3	2		2			
	Food	4	64	20[A] (15.04)		0			1	73	38	1	2	7		11			
	Farm	>64	>64	256[B] (90.78)		0			0	4	13	9	69	43		144			
	Environment	64	>64	88[B] (83.02)		0			2	7	5	4	26	20		42			
	E. faecalis	64	>64	257[a] (71.79)		1			0	52	42	6	67	44		146			
	E. faecium	4	32	10[b] (14.49)		1			1	36	20	1	4	2		4			
High-level gentamicin	*Enterococcus* species	≤250	>500	234 (47.95)							254 (≤250)						15	219 (>500)	
	Human	≤250	>500	22[A] (33.85)							43						4	18	
	Food	≤250	500	14[B] (10.53)							119						2	12	
	Farm	>500	>500	195[C] (69.15)							87						9	186	
	Environment	>500	>500	63[C] (59.43)							43						3	60	
	E. faecalis	>500	>500	213[a] (59.50)							145						11	202	
	E. faecium	≤250	>500	15[b] (21.74)							54						2	13	
High-level streptomycin	*Enterococcus* species	1000	>1000	265 (54.30)							223 (≤500)							41	224 (>1000)
	Human	≤500	>1000	22[A] (33.85)							43							5	17
	Food	≤500	>1000	37[A] (27.81)							96							13	24
	Farm	>1000	>1000	203[B] (71.99)							79							21	182
	Environment	>1000	>1000	68[B] (64.15)							38							10	58
	E. faecalis	>1000	>1000	233[a] (65.08)							125							25	208
	E. faecium	≤500	>1000	19[b] (27.54)							50							8	11

* Determined according to CLSI recommendations; *E. faecalis* ATCC™29212 was used as a control bacterium for these experiments. Dotted and solid bars indicate the breakpoints for intermediary and complete resistance, respectively.
† Meant enterococcal strains isolated from human or food samples, respectively.
§ Meant enterococcal strains isolated from food-producing animals and farm environments.
¶ Meant enterococcal strains isolated from farm and urban environment samples.
** Meant the number of strains.
†† The MIC50 value (the MIC required to inhibit 50% of cells),and the MIC90 value (the MIC required to inhibit 90% of cells).
§§ "A–D" indicates the statistical difference of AMR among Human, Food, Farm, and Environment categories. "a, b" indicates the statistical difference of AMR between *E. faecalis* and *E. faecium*.



SUPPLEMENTARY FIGURE S1. Summary of enterococci detection rate through the food chain. (A) Enterococci detection rate in various sample sources; (B) retail meat; (C) retail fruits and vegetables; (D) *Enterococcus* species distribution in various sample sources; (E) detection rates of *E. faecalis* and *E. faecium* in various sample sources.
* Indicates a statistically significant difference between sample sources.

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