



Original Article

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Characterization of antimicrobial resistance of *Escherichia coli* on Zhongyangshan Island, Zhejiang Province, China under the concept of "One Health"

Lin Wang^{1#}, Wanlu Guo^{1,2#}, Yubin Gao¹, Ge Zhao¹, Na Lv³, Junhui Liu¹, Sijun Zhao¹, Cuiping Song¹, Zhina Qu^{1✉}, Junwei Wang^{1✉}

¹China Animal Health and Epidemiology Center, Qingdao 266032, China

²College of Animal Science and Technology, Ningxia University, Yinchuan 750021, China

³Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China

ABSTRACT

Objective: This study aimed to investigate antimicrobial resistance (AMR) in *Escherichia (E.) coli*, a recognized indicator bacterium for drug resistance, across animal, environmental and human compartments on Zhongyangshan Island under natural conditions.

Methods: A total of 51 *E. coli* strains were isolated and identified from animal, environmental, and human samples on Zhongyangshan Island between 2022 and 2023. The drug resistance status of *E. coli* was analyzed using antimicrobial susceptibility testing, whole-genome sequencing analysis, multilocus sequence typing and pulsed-field gel electrophoresis.

Results: Antimicrobial susceptibility testing revealed that the resistance rate of *E. coli* across the “animal-environment-human” interface on Zhongyangshan Island was ≤50%. Overall, AMR levels were low, with isolates from human sources exhibiting higher resistance to seven antibiotics and a higher prevalence of multidrug resistance compared to those from animal sources, while environmental isolates showed the lowest resistance levels. Of the 51 “animal-environment-human” *E. coli* strains, 31.37% (16/51) harbored five or more different antimicrobial resistance genes. Additionally, 78 transposons were detected, including 72 insertion sequences (IS), 3 compound transposons (Tn), and 3 other types of transposons, along with 18 plasmid replicon sequence types associated with AMR. Multilocus sequence typing and pulsed-field gel electrophoresis-based evolutionary analyses indicated the *E. coli* strains from the “animal-environment-human” continuum exhibited diverse sequence types and limited genetic relatedness. Further analysis of broiler-derived *E. coli* from Zhongyangshan Island showed significantly lower resistance rates to 12 out of 14 tested antibiotics and a lower overall multidrug resistance rate (46.67%), compared with isolates from intensive broiler farms in Zhejiang Province, except for amoxicillin/clavulanic acid and meropenem. whole-genome sequencing analysis confirmed that these strains carried fewer AMR genes.

Conclusions: *E. coli*, serving as an indicator organism for AMR within the “animal-environment-human” system on Zhongyangshan Island, exhibits low AMR prevalence and limited clonal relatedness.

These findings provide a valuable baseline for investigating the ecological mechanisms underlying antimicrobial resistance emergence and transmission under the “One Health” framework.

KEYWORDS: One Health; *Escherichia coli*; Antimicrobial resistance; Zhongyangshan Island; Broilers

1. Introduction

As a comprehensive global health framework, “One Health” underscores the interconnectedness and synergistic relationships among human, animal and environmental health.

Significance

In this study, Zhongyangshan Island was utilized as a comprehensive research site for the “One Health” approach for the first time, facilitating an investigation into the interactions among drug-resistant bacteria and AMR genes across the animal-environment-human interface. This work has generated foundational data on antimicrobial resistance in Zhongyangshan Island, providing novel insights and scientific evidence to support the understanding of transmission dynamics and ecological factors that drive the spread of drug-resistant bacteria and AMR genes within the island’s ecosystem under the “One Health” framework.

[#]The authors contributed equally.

[✉]To whom correspondence may be addressed. E-mail: yffs2000@sina.com; 641117207@qq.com

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It addresses health-related challenges across these domains through systematic, interdisciplinary cooperation[1].

In recent years, antimicrobial resistance (AMR) has emerged as a critical global threat impacting public health, animal health, and ecosystem health. According to reports, AMR was directly responsible for 1.27 million deaths in 2019 and contributed indirectly to 4.95 million deaths worldwide. Notably, drug-resistant infections caused by six major pathogens—including *Escherichia (E.) coli*—accounted for 929 000 fatalities[2]. Projections suggest that by 2050, AMR could result in approximately 39 million annual deaths globally, accompanied by economic losses exceeding trillions of dollars[3]. In alignment with the “One Health” approach, the World Health Organization (WHO), the Food and Agriculture Organization of the United Nations (FAO), the World Organisation for Animal Health (WOAH) and the United Nations Environment Programme (UNEP) have jointly developed the “One Health Joint Plan of Action”[4], prioritizing the initiative “Curbing the silent pandemic of antimicrobial resistance” as one of six key focus areas. This collaborative effort aims to enhance the health of humans, animals and ecosystems through integrated, cross-sectoral cooperation and knowledge exchange. The “One Health” framework thus provides a structured and holistic foundation for the collaborative advancement of animal health, food safety and ecological sustainability.

With the ongoing advancement of intensive, large-scale and professional livestock and poultry farming practices, AMR arising from antibiotic usage has garnered increasing attention. Guided by the “One Health” framework, a growing number of researchers have initiated studies to investigate AMR across human, animal, and environmental interfaces. Zhai *et al.*[5] identified flies as potential vectors for the dissemination of NDM-producing carbapenem-resistant *Enterobacteriaceae* (CRE) among broiler farms, surrounding environments, and occupationally related personnel, suggesting their role in facilitating the spread of resistant pathogens. Li *et al.*[6] reported that in Xinyang, Henan Province, China, most farmers practice high-density grazing of meat geese in forest, riverine, or pond ecosystems, leading to horizontal transfer of drug-resistant *E. coli* genes within local environments and potentially increasing disease risks among nearby communities. Ma *et al.*[7] demonstrated that animal feces represent a critical pathway for AMR transmission; untreated manure used as fertilizer might introduce drug-resistant bacteria into soil, enabling their circulation across animals, humans, and environment. His team[8] revealed that CRE had established a continuous transmission network between broiler farms and adjacent environments, with *bla*_{NDM}-carrying plasmids and prevalent bacterial clones exhibiting annual dynamic evolution. Their findings highlight these farms as a central hub for CRE dissemination to humans through a “clone-plasmid” co-transmission mechanism. Collectively, these findings indicate that the transmission pathways of drug-resistant bacteria are becoming increasingly complex, posing

significant challenges to effective prevention and control measures and threatening both public health and ecological systems. The phenomenon of cross-species transmission of AMR pathogens now constitutes a major global public health challenge.

Located in the central sea area of Xiangshan Bay, Ningbo, Zhongyangshan Island features a unique geographical setting characterized by minimal human disturbance, a rich migratory bird population, and a stable maritime climate—conditions that collectively establish an ideal platform for “One Health” research[9]. In this study, *E. coli* was selected as an indicator organism for AMR, and whole-genome sequencing, antimicrobial susceptibility testing and phylogenetic analysis were systematically employed to compare resistance gene profiles in *E. coli* isolated from broilers raised under natural free-range conditions on Zhongyangshan Island versus intensive farming systems in Zhejiang Province. The findings provide robust theoretical and technical foundations for advancing Zhongyangshan Island as a comprehensive “One Health” research platform, enabling integrated surveillance of pathogen transmission across animal, human, and environmental interfaces.

2. Methods

2.1. Sample collection and bacterial isolates

From February 2022 to September 2023, samples were collected at three time points (February 2022, August 2022, and September 2023). A total of 51 *E. coli* strains were isolated from samples collected across the “animal-environment-human” interface on Zhongyangshan Island, comprising 22 strains from animals, 21 from environmental sources, and 8 from humans (Table 1). Zhongyangshan Island is located in Xiangshan Bay, Qiangjiao Town, Ninghai County, Ningbo City, Zhejiang Province, China, and is one of the islands in the Qiangjiao Archipelago, with geographic coordinates of 121°34.9' E, 29°29.7' N. Additionally, 50 *E. coli* strains of broiler origin from farms in Zhejiang Province, China (isolated from cecal swab samples of growing broiler chickens collected from broiler farms in Zhejiang Province in May 2022), along with the quality control strain ATCC 25922 and the molecular quality standard reference strain H9812, were provided by China Animal Health and Epidemiology Center.

Samples were inoculated on MacConkey Agar and incubated at 37 °C for 16–18 h for isolation and purification. Based on morphological observation, smooth, moist, pink, and round single colonies were selected as presumptive *E. coli* for preliminary screening. Single colonies were then transferred to Tryptone Soya Agar and incubated at 37 °C for 18 h. The isolates were subsequently identified by MALDI-TOF mass spectrometry.

Table 1. Source and distribution of *E. coli* strains.

Sample source			Total			Sample type
			Number of samples	Positive detection	Positive rate	
Zhongyangshan Island	Environment	Soil	60	20	33.33%	Soil
		Water	47	1	2.13%	Reservoir Freshwater
		Subtotal	107	21	19.63%	
	Animal	Broilers	16	15	93.75%	Fecal Swabs
		Cattle	4	2	50.00%	Fecal Swabs
		Dogs	4	1	25.00%	Fecal Swabs
		Oysters	7	4	57.14%	Inner Shell Swabs
		Subtotal	31	22	70.97%	
	Human	Oral cavity	10	3	30.00%	Oral Swabs
		Feces	15	5	33.33%	Fecal Swabs
		Subtotal	25	8	32.00%	
	Total		163	51	31.29%	
Broiler farms in Zhejiang province	Animal	Grower chickens		50		Cecal swabs

2.2. Antimicrobial susceptibility testing

A total of 101 *E. coli* isolates (51 from Zhongyangshan Island and 50 from broiler farms in Zhejiang Province) were selected for antimicrobial susceptibility testing. The minimum inhibitory concentration (MIC) of *E. coli* against 16 antimicrobial agents representing 10 categories was determined using the broth microdilution method in 96-well microplates. ATCC 25922 was used as the quality control strain. Interpretation of results into susceptibility categories—susceptible (S), intermediate (I), and resistant (R)—was performed according to the Clinical and Laboratory Standards Institute (CLSI) guidelines[10]. Isolates exhibiting resistance to three or more antimicrobial categories were defined as multidrug-resistant (MDR).

The 16 antimicrobial agents were as follows: Penicillins: ampicillin (AMP), amoxicillin/clavulanic acid (A/C); Aminoglycosides: gentamicin (GEM), spectinomycin (SPT), apramycin (APR); Tetracyclines: tetracycline (TET); Chloramphenicols: florfenicol (FFC); Sulfonamides: sulfisoxazole (SF), trimethoprim-sulfamethoxazole (SXT); Cephalosporins: ceftiofur (CEF), ceftazidime (CAZ); Fluoroquinolones: enrofloxacin (ENR), ofloxacin (OFL); Carbapenems: meropenem (MEM); Polypeptide antibiotics: colistin (CL); Quinoxalines: mequindox (MEQ).

2.3. Whole-genome sequencing and analysis

A total of 56 *E. coli* strains (51 from Zhongyangshan Island, including 15 from chickens, and 5 randomly selected from broiler farms in Zhejiang Province) in the logarithmic growth phase were transferred to 50 mL centrifuge tubes, centrifuged at 4 °C at 4000 rpm for 5 min, and the supernatant was discarded. The bacterial cell pellets were frozen at -80 °C for 1 h and then transported on dry ice to BGI Genomics Co., Ltd. (Wuhan, China) for whole-genome sequencing (WGS) and assembly.

AMR genes were identified using the ResFinder database (<https://genepi.food.dtu.dk/resfinder>) provided by the Center for Genomic Epidemiology (CGE). Core-genome phylogenetic trees were

constructed from WGS data using BioNumerics software v7.6 and visually refined using tvBOT[11]. The reference sequences of *E. coli* ATCC 25922 were retrieved from NCBI and examined to assess inter-strain SNP differences. Mobile genetic elements (MGEs), including transposons and plasmids, were analyzed using the MobileElementFinder (<https://cge.food.dtu.dk/services/MobileElementFinder/>), with graphical outputs optimized in ChiPlot. Seven canonical *E. coli* housekeeping genes (*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, and *recA*)[12] were used as the targets for multilocus sequence typing (MLST), and ST types were assigned by querying the MLST database (<https://cge.food.dtu.dk/services/MLST/>).

2.4. Pulsed-field gel electrophoresis

Pulsed-field gel electrophoresis (PFGE) was performed following the standard protocol for *E. coli* established by the U.S. Centers for Disease Control and Prevention, as well as the method described by Gao *et al.*[13]. Strain H9812 was used as the molecular size standard, and the resulting gel images were analyzed using BioNumerics software.

2.5. Ethical statement

The study received approval from Institutional Animal Welfare Ethics and Committee of China Animal Health and Epidemiology Center (Ethical approval number: DWFL-2022-05).

3. Results

3.1. AMR analysis of *E. coli* from “animal–environment–human” interface on Zhongyangshan Island

As an indicator of AMR, 51 *E. coli* strains isolated from “animal–environment–human” samples on Zhongyangshan Island (Table 2) were subjected to antimicrobial susceptibility testing. The

results showed that the overall level of AMR in *E. coli* was low, with resistance rates $\leq 50\%$. Across all three sources, *E. coli* strains exhibited no resistance to meropenem and colistin, but displayed varying degrees of resistance to ampicillin, tetracycline, florfenicol, sulfisoxazole, and trimethoprim-sulfamethoxazole. The AMR rate and prevalence of MDR among *E. coli* from environmental sources were significantly lower than those from humans and animals (Figures 1A and 1B; Tables 2 and 3). Among these, human origin isolates showed higher resistance to seven antibiotics (ampicillin, gentamicin, tetracycline, sulfisoxazole, trimethoprim-sulfamethoxazole, enrofloxacin, ofloxacin) and a higher prevalence. The overall MDR rate of *E. coli* across the animal-environment-human continuum on Zhongyangshan Island was 29.41% (15/51). Among human isolates, the predominant resistance profiles were 3-drug resistance (25.00%, 2/8) and 4-drug resistance (25.00%, 2/8). Animal isolates exhibited 3-drug resistance (13.64%, 3/22), 4-drug resistance (9.09%, 2/22), 5-drug resistance (4.55%, 1/22), and 6-drug resistance (4.55%, 1/22). Environmental isolates were primarily 3-drug resistance (9.52%, 2/21), 4-drug resistance (4.76%, 1/21), and 5-drug resistance (4.76%, 1/21) antimicrobial categories. These findings indicated that the overall burden of AMR remained low on Zhongyangshan Island.

Analysis of AMR genes (Figure 1C) revealed that 96.08% (49/51) of *E. coli* isolates harbored the *mdf* (A) gene, which encodes an antimicrobial efflux pump. Only two isolates—Z230930 (ST515) from soil and Z230926 (ST224) from oyster—lacked this gene, and notably, these two *E. coli* strains did not carry any detectable AMR genes. A tetracycline resistance gene, *tet* (A), was present in 35.29% (18/51) of the isolates, consistent with their phenotypic tetracycline resistance. The 51 *E. coli* strains were distributed across 43 distinct branches, exhibiting a scattered distribution of STs and distant genetic relationships. Meanwhile, AMR genes were unevenly distributed among *E. coli* from different sources, with most showing no clear correlation; however, 31.37% (16/51) of isolates still carried five or more AMR genes.

Mobile Genetic Elements (MGEs) play a crucial role in the spread of bacterial AMR. To further investigate the horizontal transfer potential of AMR genes among *E. coli* isolates from Zhongyangshan Island, the distribution of AMR-associated transposons and plasmids within MGEs was analyzed.

Transposons are mobile DNA elements capable of relocating within chromosomes or plasmids and include insertion sequences (ISs), composite transposons (Tns), and other transposon types. Analysis revealed that 78 distinct transposon sequences were identified in 51 *E. coli* strains from the “animal-environment-human” interface on Zhongyangshan Island, comprising 72 ISs, 3 Tns, and 3 other types, including non-composite transposons and transposable phage Mu. All strains harbored the MITEEC1 sequence, followed by ISEc1 detected in 29 strains and ISEc31 in 21 strains (Figure 1D). Based on strain sources, both animal- and environmental-derived isolates exhibited high transposon diversity, with 52 types (176 occurrences) and 56 types (172 occurrences) identified, respectively. Among these, soil-derived *E. coli* showed the highest transposon diversity (56 types, 144 occurrences), significantly exceeding that of water-derived strains (4 types, 4 occurrences). For indicator animal-

derived strains, chicken fecal-derived *E. coli* displayed the greatest transposon diversity (42 types, 134 occurrences), followed by oyster-derived (19 types, 33 occurrences) and cattle-derived strains (7 types, 8 occurrences), whereas dog fecal-derived *E. coli* contained only one type. Human fecal-derived *E. coli* exhibited significantly higher transposon diversity (30 types, 46 occurrences) compared to oral swab-derived strains (9 types, 18 occurrences). At the strain level, 96.08% (49/51) of *E. coli* strains individually carried three or more transposon types, indicating a substantial potential for horizontal transfer of AMR genes.

Plasmids in bacteria can carry and transfer various genes, including antibiotic resistance genes, virulence factors, and genes involved in metabolic pathways. Through horizontal gene transfer, plasmids facilitate the dissemination of these genes among bacterial populations, enhancing bacterial adaptability and survival under environmental stressors. Among the 51 *E. coli* strains isolated from “animal-environment-human” sources on Zhongyangshan Island, 18 distinct plasmid replicon types associated with AMR were detected in 31 strains. Twenty strains—10 from environmental sources and 10 from animal sources—lacked detectable plasmid replicons. Of the identified replicons, IncFIB was the most prevalent (20/51, 39.22%), followed by IncFII (19/51, 37.25%). Plasmid replicons were particularly abundant in soil-derived environmental isolates and chicken fecal-derived strains (Figure 1E).

Table 2. Antimicrobial susceptibility testing results of *E. coli* from “animal-environment-human” samples on Zhongyangshan Island.

Drug	Animal (n=22)		Environment (n=21)		Human (n=8)	
	Resistant count	Resistance rate (%)	Resistant count	Resistance rate (%)	Resistant count	Resistance rate (%)
AMP	7	31.82	3	14.29	3	37.50
A/C	1	4.55	0	0.00	0	0.00
GEM	1	4.55	0	0.00	2	25.00
SPT	4	18.18	2	9.52	0	0.00
TET	10	45.45	4	19.05	4	50.00
FFC	8	36.36	4	19.05	1	12.50
SF	5	22.73	5	23.81	4	50.00
SXT	5	22.73	3	14.29	3	37.50
CEF	2	9.09	0	0.00	0	0.00
CAZ	2	9.09	0	0.00	0	0.00
ENR	1	4.55	0	0.00	1	12.50
OFL	1	4.55	0	0.00	1	12.50
MEM	0	0.00	0	0.00	0	0.00
APR	-	-	-	-	-	-
CL	0	0.00	0	0.00	0	0.00
MEQ	-	-	-	-	-	-

Note: “-” indicated that the drug has no clinical breakpoints. AMP: ampicillin; A/C: amoxicillin/clavulanic acid; GEM: gentamicin; SPT: spectinomycin; TET: tetracycline; FFC: florfenicol; SF: sulfisoxazole; SXT: trimethoprim-sulfamethoxazole; CEF: ceftiofur; CAZ: ceftazidime; ENR: enrofloxacin; OFL: ofloxacin; MEM: meropenem; APR: apramycin; CL: colistin; MEQ: mequindox.

3.2. Phylogenetic analysis of *E. coli* from “animal-environment-human” sources on Zhongyangshan Island

The 51 *E. coli* strains from Zhongyangshan Island were assigned to 29 STs, with 13 strains having no ST types assigned. The distribution

of ST types was not concentrated: three strains each belonged to ST196 and ST998, two strains each to ST10, ST2522, ST5156, ST6168 and ST6745, and one strain each to the remaining ST types. Based on sample categories from the “animal-environment-human” interface, an MLST-based minimum spanning tree was constructed (Figure 2A). The results showed that the highest ST diversity occurred in animal-derived isolates (15 STs), followed by environmental isolates (12 STs) and human-associated isolates (5 STs). Notably, *E. coli* ST5156 was detected in both environmental soil and human-derived samples, ST196 was shared between soil and chicken fecal samples, and ST998 was identified in both oyster and soil samples. Only one housekeeping gene allele difference was observed between soil-derived ST10 and either chicken-derived ST48 or human-derived ST34, as well as between oyster-derived ST224 and dog-derived ST6488, indicating close genetic relatedness. Two allele differences were found between soil-derived ST2522 and broiler-associated ST4511 and ST6422. All other ST pairs exhibited three or more allele differences across the seven housekeeping genes, demonstrating distant genetic relationships and an overall pattern of polymorphic, decentralized distribution.

Forty-two *E. coli* strains from the “animal-environment-human” interface on Zhongyangshan Island were selected for PFGE cluster analysis. Human-derived strains (Z230901 and Z230903) and oyster-derived strains (Z220849 and Z220851) exhibited 100% similarity (Figure 2B), indicating they belonged to the same homologous clone. Additionally, pairwise similarities exceeding 90% were observed between soil-derived pairs Z220312 and Z220313, Z220314 and Z220315, and human-derived strains Z220827 and Z220828, suggesting clonal relatedness among these isolates. Overall, chicken-derived strains were distributed across multiple clusters, reflecting high PFGE pattern diversity and genetic heterogeneity. Notably, some soil-derived and broiler-derived *E. coli* strains showed more than 80% similarity, and the broiler-derived strain Z230912 shared 83.3% similarity with oyster-derived strains Z220849 and Z220851, indicating close phylogenetic relationships. In contrast, *E. coli* strains from different environmental sites were more distantly related, suggesting spatial heterogeneity in strain distribution, likely due to the large geographic area of Zhongyangshan Island and resulting microbial diversity across sampling locations.

Table 3. MDR results of *E. coli* isolates from animal-environment-human interface on Zhongyangshan Island.

Sample type	3-drug resistance		4-drug resistance		5-drug resistance		6-drug resistance		MDR	
	Isolates count	Resistance rate	Isolates count	Resistance rate	Isolates count	Resistance rate	Isolates count	Resistance rate	Isolates count	Resistance rate
Animal (<i>n</i> =22)	3	13.64%	2	9.09%	1	4.55%	1	4.55%	7	31.82%
Environment (<i>n</i> =21)	2	9.52%	1	4.76%	1	4.76%	0	0.00%	4	19.05%
Human (<i>n</i> =8)	2	25.00%	2	25.00%	0	0.00%	0	0.00%	4	50.00%

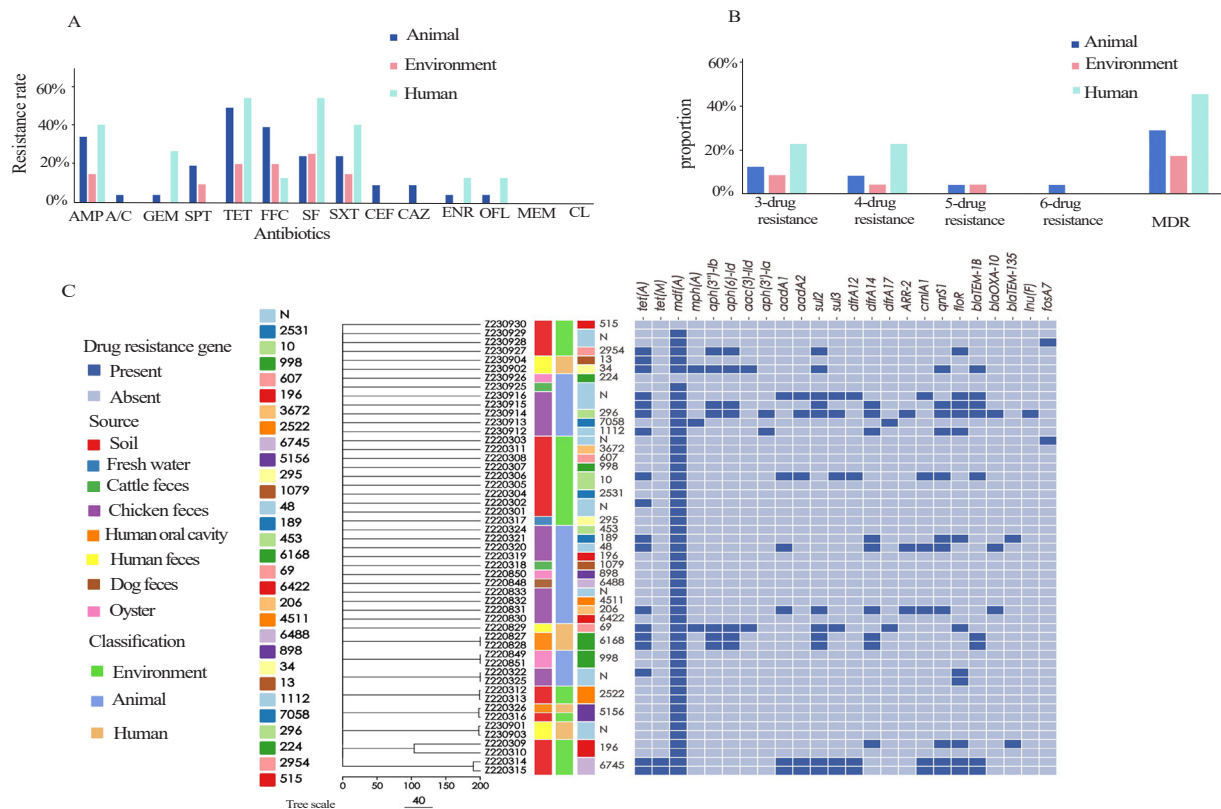


Figure 1. AMR profile of *E. coli* from “animal-environment-human” source on Zhongyangshan Island. A: AMR rates of *E. coli* isolates on Zhongyangshan Island; B: MDR rates of *E. coli* isolates on Zhongyangshan Island; C: Resistance gene of *E. coli* isolates on Zhongyangshan Island. Continued on next page.

E

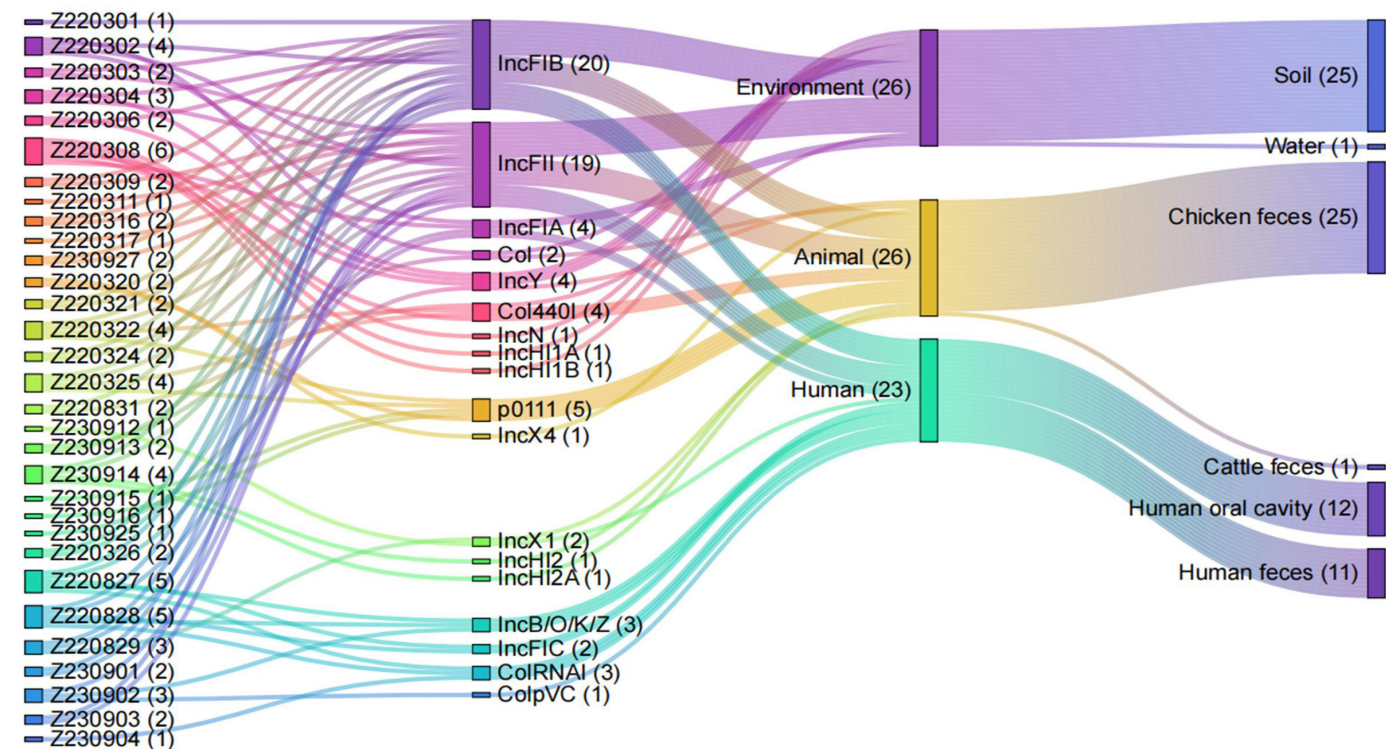


Figure 1 continued. AMR profile of *E. coli* isolates from “animal-environment-human” source on Zhongyangshan Island. D: Transposon of *E. coli* isolates on Zhongyangshan Island; E: Plasmid analysis of *E. coli* on Zhongyangshan Island.

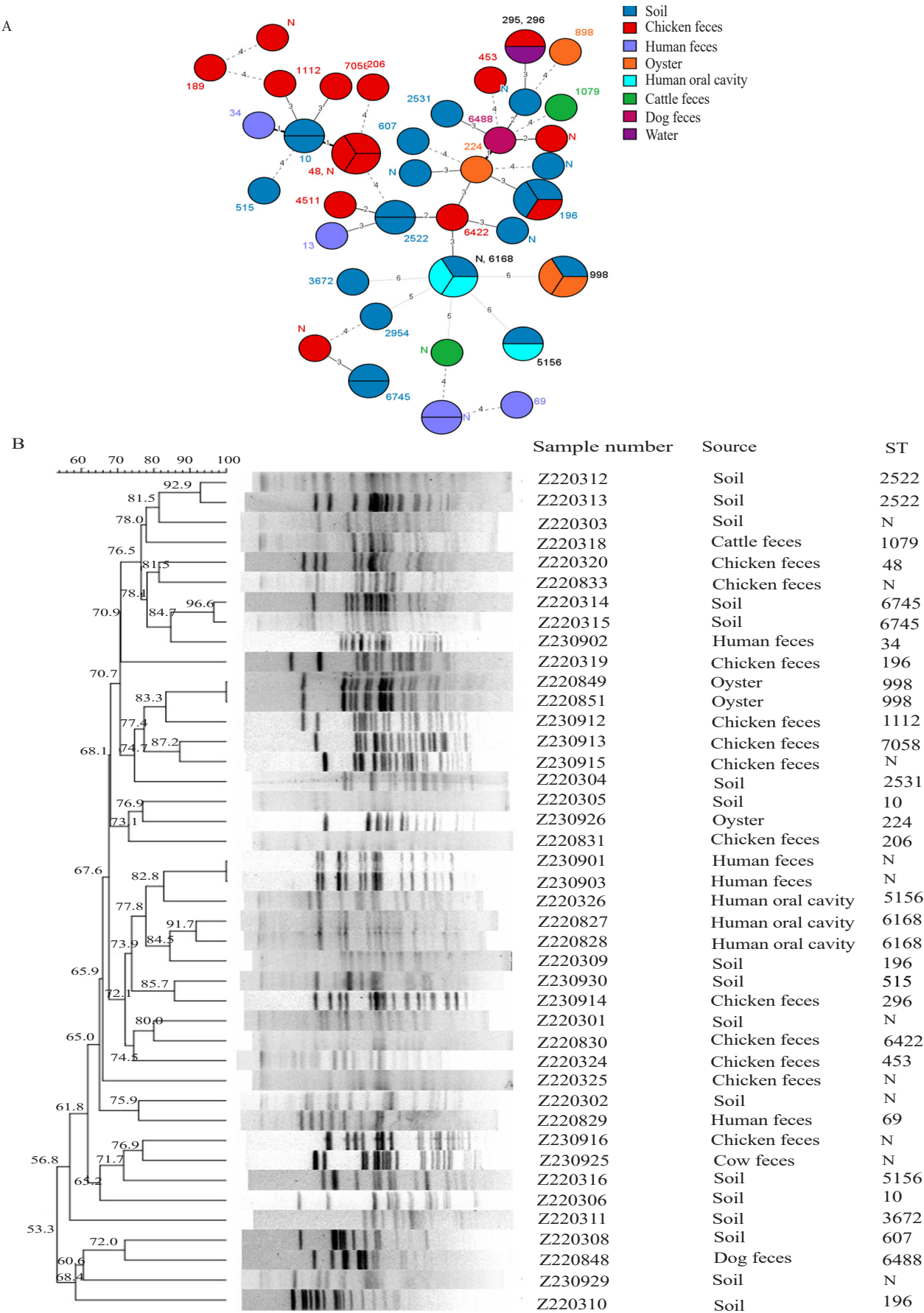


Figure 2. Phylogenetic analysis of *E. coli* isolates from “animal-environment-human” on Zhongyangshan Island. A: Minimum spanning tree (MST) based on multilocus sequence typing (MLST) of *E. coli* isolates from “animal-environment-human” source; B: Pulsed-field gel electrophoresis cluster analysis of different sources of *E. coli* isolates from Zhongyangshan Island.

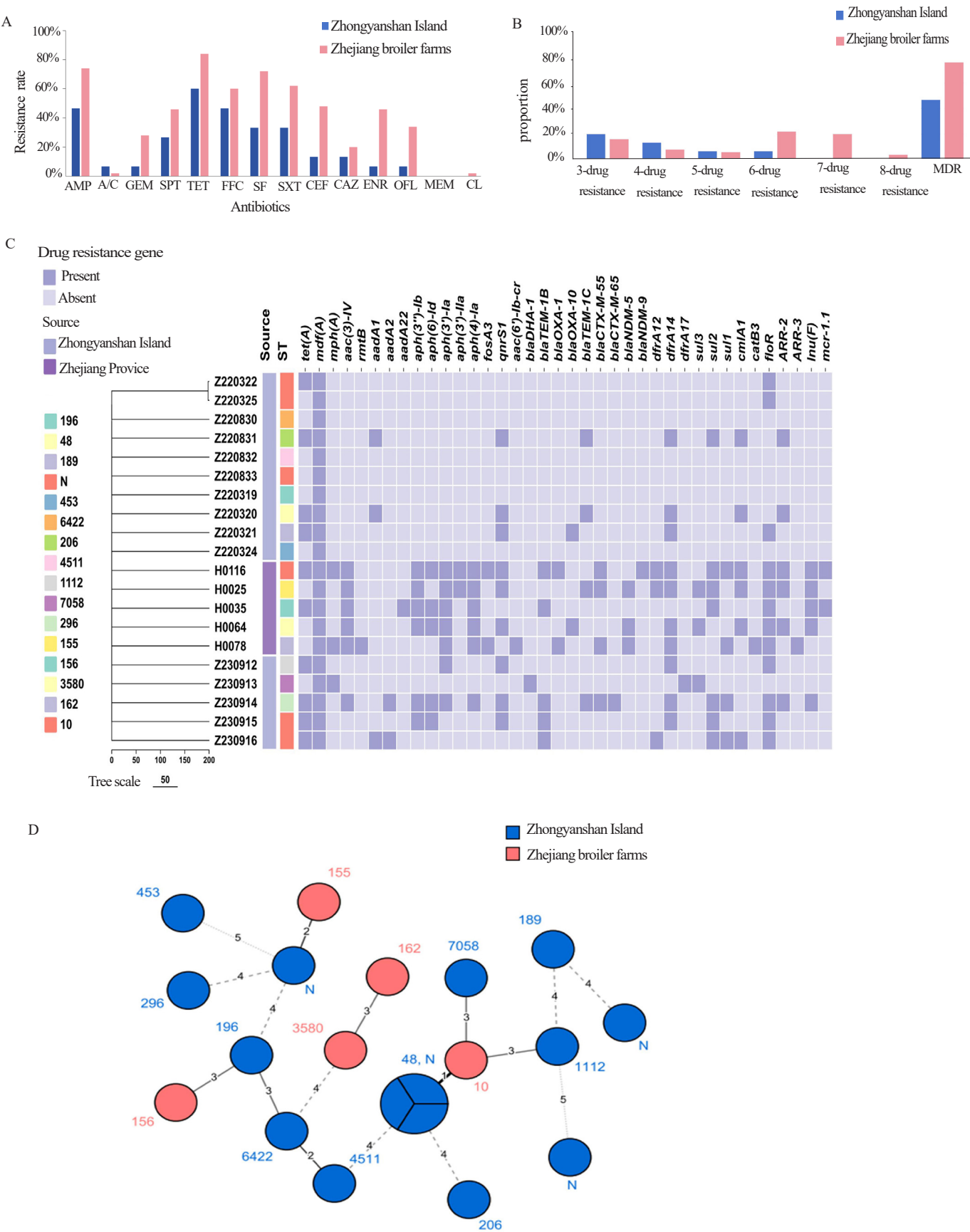


Figure 3. Antimicrobial susceptibility testing analysis of broiler-derived *E. coli* isolates from different regions. A: Comparative analysis of AMR in broiler-derived *E. coli* isolates between Zhongyanshan Island and Zhejiang broiler farms; B: Comparative analysis of MDR in broiler-derived *E. coli* isolates between Zhongyanshan Island and Zhejiang broiler farms; C: Comparative analysis of AMR genes in broiler-derived *E. coli* isolates between Zhongyanshan Island and Zhejiang broiler farms; D: Minimum spanning tree (MST) based on multilocus sequence typing (MLST) of broiler-derived *E. coli* isolates from Zhongyanshan Island and Zhejiang broiler farms.

3.3. Comparative analysis of broiler-derived *E. coli* between Zhongyangshan Island and Zhejiang broiler farms

Antimicrobial susceptibility testing against 14 antibiotics was performed on 15 broiler-derived *E. coli* strains from indicator animals on Zhongyangshan Island and 50 strains from intensive broiler farms in Zhejiang Province (same province). As shown in Figure 3A and Table 4, among the 14 tested antibiotics, *E. coli* isolates from broilers on Zhongyangshan Island were resistant to 12 antibiotics, whereas those from Zhejiang broiler farms exhibited resistance to 13 antibiotics. Notably, except for amoxicillin/clavulanic acid and meropenem, the resistance rates of *E. coli* from Zhongyangshan Island broilers to the remaining 12 antibiotics were significantly lower than those observed in *E. coli* from Zhejiang farm broilers. MDR analysis revealed that the highest resistance level among Zhongyangshan Island isolates was to 6-drug resistance (6.67%, 1/15), while isolates from Zhejiang broiler farms showed resistance to up to 8-drug resistance (4.00%, 2/50). Moreover, the overall MDR rate of *E. coli* from broilers on Zhongyangshan Island (46.67%) was significantly lower than that of *E. coli* from Zhejiang farm broilers (76.00%) (Figure 3B and Table 5).

WGS was performed on 15 broiler-derived *E. coli* strains from Zhongyangshan Island and 5 selected *E. coli* strains from Zhejiang farm broilers randomly to analyze their AMR genes. As shown in Figure 3C, the number of AMR genes carried by *E. coli* strains from Zhejiang farm broilers—all of which harbored more than 10 resistance genes—was significantly higher than that in Zhongyangshan Island isolates, among which only one strain carried more than 10 resistance genes. All strains carried *mdf* (A), an antimicrobial efflux pump gene, while $\geq 50\%$ of the strains carried *tet* (A) (tetracycline resistance), *dfrA14* (trimethoprim resistance), and *floR* (florfenicol resistance), consistent with the observed AMR phenotypes. Eleven AMR genes, including *aac*(6')-IIb-*cr* (aminoglycoside resistance), *bla*_{NDM-5} and *bla*_{NDM-9} (β -lactam resistance) and *mcr*-1.1 (polymyxin resistance), were detected exclusively in *E. coli* from Zhejiang broiler farms. Conversely, five AMR genes, such as *aadA1* and *aadA2* (aminoglycoside resistance) and *bla*_{CTX-M-65} (β -lactam resistance), were unique to *E. coli* strains from broilers on Zhongyangshan Island. Phylogenetic analysis using MLST-based MST of *E. coli* from broilers on Zhongyangshan Island and Zhejiang broiler farms (Figure 3D) revealed a dispersed, multi-type distribution between the two groups, indicating a distant genetic relationship.

Table 4. AMR profiles of broiler-derived *E. coli* isolates from Zhongyangshan Island and Zhejiang broiler farms.

Drug	Zhongyangshan Island (n=15)		Zhejiang broiler farms (n=50)	
	Resistant count	Resistance rate	Resistant count	Resistance rate
AMP	7	46.67%	37	74.00%
A/C	1	6.67%	1	2.00%
GEM	1	6.67%	14	28.00%
SPT	4	26.67%	23	46.00%
TET	9	60.00%	42	84.00%
FFC	7	46.67%	30	60.00%
SF	5	33.33%	36	72.00%
SXT	5	33.33%	31	62.00%
CEF	2	13.33%	24	48.00%
CAZ	2	13.33%	10	20.00%
ENR	1	6.67%	23	46.00%
OFL	1	6.67%	17	34.00%
MEM	0	0.00%	0	0.00%
CL	0	0.00%	1	2.00%

Note: AMP: ampicillin; A/C: amoxicillin/clavulanic acid; GEM: gentamicin; SPT: spectinomycin; TET: tetracycline; FFC: florfenicol; SF: sulfisoxazole; SXT: trimethoprim-sulfamethoxazole; CEF: ceftiofur; CAZ: ceftazidime; ENR: enrofloxacin; OFL: ofloxacin; MEM: meropenem; CL: colistin.

4. Discussion

As a major antimicrobial-resistant indicator bacterium[14], the antimicrobial susceptibility of *E. coli* could reflect the overall antimicrobial resistance level on Zhongyangshan Island. In this study, the evolution of *E. coli* from the “animal-environment-human” interface on Zhongyangshan Island was analyzed through a “One Health” perspective. The results revealed that *E. coli* isolates across the “animal-environment-human” continuum exhibited a dispersed distribution of ST types, and certain soil-derived *E. coli* strains showed close genetic relatedness to those from chicken feces, oysters, and human oral swabs. This might be associated with the higher abundance of *E. coli* in soil samples compared to other types.

The results showed that human-derived *E. coli* isolates from Zhongyangshan Island exhibited the highest overall AMR prevalence, which might be associated with the irregular human activities and frequent landings on the island. Resistance levels in animal-derived isolates were intermediate, while environmental-derived *E. coli* demonstrated the lowest AMR rates. However, the overall AMR rate among *E. coli* isolates from Zhongyangshan Island was still significantly lower than that observed in broiler isolates from intensive farms in Guangdong[15] and Zhejiang provinces[16].

Table 5. MDR results of broiler-derived *E. coli* isolates from Zhongyangshan Island and Zhejiang broiler farms.

Sample Type	3-drug Resistance		4-drug Resistance		5-drug Resistance		6-drug Resistance		7-drug Resistance		8-drug Resistance		MDR	
	Isolates count	Resistance rate	Isolates count	Resistance rate	Isolates count	Resistance rate	Isolates count	Resistance rate	Isolates count	Resistance rate	Isolates count	Resistance rate	Isolates count	Resistance rate
Zhongyangshan Island	3	20.00%	2	13.33%	1	6.67%	1	6.67%	0	0.00%	0	0.00%	7	46.67%
Zhejiang broiler farms	8	16.00%	4	8.00%	3	6.00%	11	22.00%	10	20.00%	2	4.00%	38	76.00%

At the same time, except for amoxicillin/clavulanic acid (6.67% resistance), the resistance rates of broiler-derived *E. coli* from Zhongyangshan Island were lower than those of *E. coli* isolated from broiler farms in Zhejiang Province in our laboratory studies, as well as lower than the resistance rates reported for broiler-derived *E. coli* from domestic farms in most published papers. For example, Dai *et al.*[17] isolated *E. coli* strains carrying the *mer-1* gene from broiler farms in Fujian Province, China, which exhibited resistance approaching 90% to tetracycline and doxycycline, and over 70% resistance to aztreonam, ceftazidime and ampicillin. Similarly, Lv *et al.*[18] identified 108 *E. coli* strains from thirty broiler farms in Henan Province, China, revealing resistance rates exceeding 90% to ampicillin, amoxicillin and clindamycin. Among these, 57 pathogenic *E. coli* strains were resistant to 15–19 different antibiotics. In comparison with other “One Health” study sites, it was found that, except for ceftazidime (13.33%), broiler-derived *E. coli* from Zhongyangshan Island showed lower resistance to the tested antibiotics than *E. coli* from healthy broilers on Chongming Island, Shanghai, China[19]. This indicated that the overall AMR level in broiler-derived *E. coli* in the Zhongyangshan Island region remained low.

Analysis of AMR genes revealed that *E. coli* from broilers on Zhongyangshan Island carried a relatively diverse set of resistance genes, and the carriage rates of *tet* (A), *aadA*, *sul1*, *sul2*, *bla*_{CTX-M}, and others were significantly lower than those observed in *E. coli* isolates from multiple broiler farms in Shandong Province across the entire breeding cycle[20]. Compared with *E. coli* from Zhejiang broiler farms, strains from Zhongyangshan Island did not carry 11 AMR genes, including *aac*(6′)-*Ib*-*cr*, *bla*_{NDM}, *mer-1.1*. Although these isolates harbored multiple AMR genes, as well as transposons and plasmids capable of mediating horizontal gene transfer, the overall risk of AMR transmission remained low. This is likely due to the absence of anthropogenic antibiotic use on Zhongyangshan Island, suggesting that AMR levels in *E. coli* from animals, the environment, and humans were maintained at a natural baseline. In contrast to the “One Health” research sites on Chongming Island in Shanghai, China, and the demonstration zone in Hainan Province, both of which are significantly impacted by anthropogenic activities and can be regarded as urban interfaces[19], Zhongyangshan Island remains in a relatively pristine condition. This makes it an ideal setting for investigating the genetic evolution patterns and ecological dissemination mechanisms of antimicrobial-resistant bacteria and resistance genes across the “animal-environment-human” continuum. However, this study only monitored *E. coli* resistance on Zhongyangshan Island during 2022–2023, and long-term follow-up research is still needed to assess the potential influence of drug-resistant bacteria and resistance genes carried by wild birds on the AMR profile of *E. coli* in this region.

5. Conclusions

This study provides a novel perspective and theoretical foundation for further research on the traceability and ecological mechanisms of antimicrobial-resistant bacteria and resistance genes under the “One Health” framework in Zhongyangshan Island. Leveraging the unique

geographical setting of Zhongyangshan Island, this study applied the “One Health” concept for the first time to investigate the distribution characteristics and transmission pathways of *E. coli* AMR genes and MGEs, revealing the resistance profile of *E. coli* through a multi-perspective analysis across the “animal-environment-human” interface. The AMR levels in *E. coli* from the “animal-environment-human” system on Zhongyangshan Island were significantly lower than those observed in intensive broiler farming systems, with an overall low resistance rate. WGS revealed that resistance genes were dispersed among 29 ST-type strains, without forming a dominant clonal lineage, indicating limited clonal expansion.

In this study, Zhongyangshan Island was utilized as a comprehensive research site for the “One Health” approach for the first time, facilitating an investigation into the interactions among drug-resistant bacteria and AMR genes across the “animal-environment-human” interface. This work has generated foundational data on AMR on Zhongyangshan Island, providing novel insights and scientific evidence to support the understanding of transmission dynamics and ecological factors that drive the spread of drug-resistant bacteria and AMR genes within the island’s ecosystem under the “One Health” framework. Additionally, future research should focus on long-term systematic and dynamic monitoring to elucidate AMR gene transmission pathways across the “animal-environment-human” interface, and to establish targeted AMR prediction models, thereby contributing to safeguarding public health.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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Data availability statement

The data supporting the findings of this study are available from the corresponding author upon request.

Authors' contributions

Wang L and Guo WL contributed to the conceptualization and design of the study, performed data curation, investigation, methodology development, formal analysis, and visualization, and drafted and revised the manuscript. Gao YB, Zhao G, and Lv N participated in the investigation, data curation, methodology, and formal analysis. Liu JH provided resources, participated in project administration, and acquired funding. Zhao SJ and Song CP contributed to study supervision, project administration, and validation, with Song CP also involved in funding acquisition. Qu ZN and Wang JW conceived and supervised the study, were responsible for project administration and resource coordination, acquired funding, and critically reviewed and edited the manuscript. All authors read and approved the final manuscript.

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