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Antimicrobial resistance in *Escherichia coli* and *Staphylococcus aureus* at human-animal interfaces on Chongming Island, Shanghai: A One Health perspective

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ABSTRACT

Antimicrobial resistance (AMR) is a significant concern within the One Health framework due to its ability to spread across multiple interfaces. Phenotypic data remains the primary type for AMR surveillance, but exploring association across multiple interfaces poses certain challenges. In this study, AMR phenotypic data of clinical and food animal *E. coli* and *S. aureus* from Chongming Island over the past five years were analyzed to determine key characteristics of AMR and explore its association at the human-animal interface.

The clinical *E. coli* isolates showed significant resistance to penicillins (83.92 %), cephems (63.05 %), fluoroquinolones (62.21 %), and tetracyclines (57.77 %), while *S. aureus* exhibited high resistance to penicillinase-labile penicillins (90.89 %), macrolides (51.51 %), penicillinase-stable penicillins (43.96 %), and lincosamides (43.55 %). Extended-spectrum β-lactamase (ESBL)-producing *E. coli* isolates accounted for 53.26 % (1398/2526), while methicillin-resistant *Staphylococcus aureus* (MRSA) prevalence was 43.81 % (435/993). Notably, there has been an increase in the proportion of *E. coli* isolates resistant to 8 to 12 antimicrobial classes, and in the proportion of *S. aureus* isolates resistant to 5 to 9 classes. Certain multi-drug resistance (MDR) phenotypes were first identified in food animal isolates and later emerged in clinical settings. Meanwhile, several MDR phenotypes were shared between the two interfaces, with 44 identified in *E. coli* and 12 in *S. aureus*. Further co-occurrence analysis in *E. coli* and *S. aureus* identified several co-occurrence phenotypic pairs or clusters, potentially mediated by a single plasmid or multiple plasmids within a bacterium, indicating potential associations at the human-animal interface.

To summarize, a heightened prevalence of MDR in clinical *E. coli* and *S. aureus* has been observed, with some MDR profiles appearing in food animals before emerging in clinical settings. The co-occurrence of phenotypic pairs or clusters underscores the potential for AMR association and transmission between humans and food animals. Within the One Health framework, integrating genomic data into AMR monitoring is a crucial next step.

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1. Introduction

Antimicrobial resistance (AMR) is a critical global public health issue, leading to increased illness, mortality, and significant economic burdens worldwide [1–3]. According to the Global Research on Antimicrobial Resistance (GRAM) Project, there were approximately 4.95 million bacterial drug-resistant infections worldwide in 2019, resulting in 1.27 million deaths directly attributed to AMR [4]. Even worse, the COVID-19 pandemic has further exacerbated this burden [1]. As the world's largest producer and consumer of antimicrobials, China faces an even more severe AMR crisis [5,6]. Recent studies also predicted an increasing trend in antimicrobial use over the next decade, particularly in food animals and in Asia [7,8], highlighting that the Asian continent is the main battlefront in addressing AMR.

Escherichia coli (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) are widely recognized as representative Gram-negative (G-) and Gram-positive (G+) bacterial pathogens, respectively. *E. coli* plays a dual role in the gastrointestinal tracts of humans and animals, typically existing as a harmless commensal organism but capable of causing severe, life-threatening infections [9]. *S. aureus* is not only a pathogen that can cause infections in hospital and community settings [10], but it is also a leading cause of bovine mastitis, resulting in substantial economic losses in the agricultural sector [11]. In recent years, the spread of extended-spectrum β-lactamase (ESBL)-producing *E. coli* and methicillin-resistant *Staphylococcus aureus* (MRSA) has raised increasing global concern [12–15]. Notably, ESBL-producing *E. coli* and MRSA often exhibit resistant to multiple classes of antimicrobial drugs [16,17]. Specific resistance phenotypes or genes, such as the streptothricin-resistant phenotype [18], the methicillin-resistant phenotype [14], the *bla*VIM-1 gene (found in porcine *E. coli*) [19], and the plasmid-mediated *mcr*-1 gene [20], were initially discovered in these two pathogens. Consequently, *E. coli* and *S. aureus* are frequently used as bioindicators in AMR surveillance programs.

AMR is a One Health issue as microbiota (mainly bacteria) play a vital role in linking various interfaces [21,22]. Antimicrobial-resistant bacteria (ARB) can spread to humans through various direct and indirect pathways, including the food supply, direct contact, and contaminated waterways and soils. Conversely, human waste containing ARBs from homes, hospitals, and factories contaminates rivers, waterways, and soils, turning these environmental carriers into "hot spots" for ARBs [23]. Vectors like rodents, insects, and birds can also contribute to the spread of ARBs [24]. Moreover, the movement of humans and animals between farms, as well as the transfer of patients carrying ARBs between community and hospital settings, further contributes to the spread [24,25]. Plasmids play a key role in this process, serving as vectors that facilitate the transfer of antimicrobial resistance genes (ARGs) among bacteria within and across different microbiomes, even among genetically diverse species or genera [26].

Chongming Island is unique among the districts of Shanghai due to its distinct separation, making it an ideal location for conducting One Health research and activities. Despite ongoing urbanization, the island has preserved much of its original ecological system [27]. The availability of medical facilities, especially larger hospitals, is relatively limited compared to the Shanghai metropolitan area. At the same time, Chongming Island plays a crucial role in Shanghai's breeding and agricultural industries, supplying a significant portion of the city's food [28]. This combination of factors makes Chongming Island particularly suitable for investigating the potential relationship between human and animal interfaces based on AMR phenotypic data. In this study, we explored the characteristics of clinical interfaces using phenotypic data from two bacterial species, *E. coli* and *S. aureus*, obtained from a prominent hospital on Chongming Island. We then examined the association of AMR at the human-animal interface by incorporating phenotypic data from food animal isolates. Our goal is to provide additional recommendations for AMR control and contribute to establishing a One Health surveillance network for AMR on Chongming Island.

2. Materials and methods

2.1. Study design

A dataset comprising phenotypic data of *E. coli* and *S. aureus* isolates from a nosodochium on Chongming Island, spanning 2018 to 2022, was used to explore the clinical AMR characteristics. The study also aimed to identify potential associations between AMR in clinical isolates and those from food animals on nearby farms. This effort seeks to establish a One Health surveillance framework for AMR, providing valuable insights for its control.

2.2. Sample collection and identification

Clinical samples were collected from a prominent nosodochium on Chongming Island and subjected to bacterial culture and isolation. The dataset recorded only the bacterial isolates with unique codes, without any additional patient information. Sample collection and culture adhered to standard hospital protocols. Briefly, patient swabs were placed in Luria Bertani (LB) broth (ThermoFisher, Waltham, USA) and incubated overnight at 37 °C and 200 rpm. The swab cultures were then streaked onto *E. coli* coliform chromogenic media or *Staphylococcus* chromogenic prepared plate medium (Hopebio Biotech, Qingdao, China) and incubated at 37 °C for 16 h. Colonies exhibiting similar morphological characteristics were selected and verified using the Auto ms1000, an automated microbial mass spectrometry detection system based on matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) (Autobio Company, Zhengzhou, China). The confirmed isolates were preserved at −80 °C using commercial magnetic bead strain preservation tubes (Pro-Lab Microbank, Canada).

The collection and identification of food animal-derived isolates followed a procedure similar to the one described above, with a few notable distinctions: (1) Samples for *E. coli* was collected from the rectum or cloaca of asymptomatic animals, while samples for *S. aureus* were isolated from raw milk. (2) After initial culturing in colorimetric medium, a second culture was performed using nutrient agar medium. (3) Pure colonies were identified as *E. coli* or *S. aureus* using a microbial biochemical identification system (VITEK 2 Compact, Biomerieux, France).

2.3. Testing for antimicrobial susceptibility

The antimicrobial susceptibility test (AST) for all isolates was conducted by determining the minimum inhibitory concentration (MIC) values of various antimicrobials, following the microbroth dilution protocol recommended by the Clinical & Laboratory Standards Institute (CLSI M100, 32nd Edition, United States). The AST for clinical *E. coli* included 29 antimicrobial agents from 13 distinct classes, while the AST for clinical *S. aureus* included 16 agents from 12 classes. Supplementary material: Appendix A: Additional Tables: Table A.1. In 2020, cefoxitin and aztreonam were added to the clinical *E. coli* AST panel. In 2021, additional agents including amoxicillin/clavulanic acid, ceftriaxone, ertapenem, tobramycin, tetracycline, moxifloxacin, chloramphenicol, nitrofurantoin, and colistin were incorporated. The clinical *S. aureus* AST panel was also updated in 2021 with the inclusion of tigecycline, ciprofloxacin, moxifloxacin, and chloramphenicol. Quality control was ensured using *E. coli* ATCC 25922 and *S. aureus* ATCC 25904 strains. Additionally, the AST for food animal *E. coli* isolates included 14 agents from 10 antimicrobial classes, and the AST for food animal *S. aureus* isolates comprised 18 agents from 14 antimicrobial classes. Supplementary material: Appendix A: Additional Tables: Table A.2.

The results were interpreted using the breakpoints recommended by CLSI for the antimicrobial agents. In cases where CLSI breakpoints were unavailable, alternative breakpoints from the European Committee on

Antimicrobial Susceptibility Testing (EUCAST), or the Shanghai Local Standard of Technical Specification for Antimicrobial Resistance Surveillance in Livestock and Poultry Breeding (DB31/T 1160–2019) were used. Based on these breakpoints, isolates were classified as either susceptible or non-susceptible (including both intermediate and resistant categories).

2.4. The characteristics of the resistance of clinical E. coli and S. aureus isolates

The resistance rates of all antimicrobial classes, resistance prevalence of all agents, and rates of multidrug resistance (MDR) were calculated using the "dplyr" and "comparegroups" packages in R 4.3.2 (Lucent Technologies, Jasmine Mountain, USA). Then, the Mann-Kendall trend tests were performed to test the linear trend over time using the "trend" package in R 4.3.2. The resistance profiles for each *E. coli* and *S. aureus* isolate were analyzed to explore their characteristics.

The AMR carriage, also referred to as antibiogram length, was defined as the total number of antimicrobial classes to which an isolate was phenotypically resistant. This metric was then used to evaluate MDR dynamics changes over the five-year period, employing generalized linear mixed models (GLMMs) implemented with the "lme4" package in R 4.3.2.

2.5. The association at the human-animal interface in Chongming Island

To explore the association at the human-animal interface, the correlation coefficient of the resistance rates for each antimicrobial classes and MDR rates between the clinical and food animal isolates were calculated using the "psych" package and the results were visualized by heatmap using "pheatmap" package in R 4.3.2. The Venn Diagram was employed to display the shared resistance profiles between the clinical and food animal isolates. To further investigate the potential association, this study employed pairwise co-occurrence analysis to infer whether two or more AMR phenotypes are mediated by the same plasmid or by multiple plasmids within a single bacterium [29,30]. The threshold values for the correlation coefficient and *P*-value were set at 0.5 and 0.05, respectively. Subsequently, the co-occurring phenotypes were analyzed to explore their distribution across clinical and food animal interfaces, thereby indicating potential associations between the two interfaces. The co-occurrence analysis was performed using the "polycor" package and visualized with the "corrplot" package in R 4.3.2.

2.6. Statistical analysis

The bar charts, pie charts, scatter plot, and heatmaps in this study were generated by package of "ggplot2" and "ggprism." in R 4.3.2. The data distribution test of resistance rates of clinical and food animal isolates was conducted before the calculation of correlation coefficient. The distribution of AMR carriage was also tested to determine whether it followed a Poisson distribution. The categorical data analysis was conducted using the chi-square test or Fisher exact test using the built-in function of R 4.3.2. The *P*-value of 0.05 was chosen as statistically significant.

3. Results

3.1. The status of AMR in clinic E. coli and S. aureus isolates

During the sampling period from 2018 to 2022, a total of 2625 clinical isolates of *E. coli* and 994 clinical isolates of *S. aureus* were collected. The annual sampling numbers for both species were presented in Supplementary material: Appendix A: Additional Tables: Table A.3.

The AST results revealed that a significant proportion of clinical *E. coli* isolates were resistant to penicillins (83.92 %), cephems (63.05 %), fluoroquinolones (62.21 %), and tetracyclines (57.77 %). However, resistance to nitrofurans (6.73 %), fosfomycins (6.40 %), carbapenems (3.73 %), and polymyxins (0.74 %) was observed in a relatively small proportion of isolates (Fig. 1A). Among the six antimicrobial classes that included multiple agents, the resistance prevalence varied significantly within the same class, except for the carbapenems and fluoroquinolones (Fig. 1C). Within the tetracycline class, tigecycline exhibited an almost negligible resistance prevalence (0.34 %), while tetracycline showed resistance in more than half of the isolates (57.77 %). Additionally, among the seven agents classified as cephems, the highest resistance prevalence was observed with the first-generation agent cefazolin (61.95 %), followed by second-generation agents (excluding cefoxitin [17.27 %], cefuroxime [55.09 %]), and third-generation agents such as cefotaxime (53.26 %), ceftazidime (34.90 %), and ceftriaxone (52.07 %). The lowest resistance prevalence was found with the fourth-generation agent cefepime (25.71 %).

The clinical isolates of *S. aureus* isolates demonstrated high resistance to penicillinase-labile penicillins (90.89 %), macrolides (51.51 %), penicillinase-stable penicillins (43.96 %), lincosamides (43.55 %). Notably, all *S. aureus* isolates were susceptible to glycopeptides and oxazolidinones (Fig. 1B). Additionally, the isolates exhibited low resistance to folate pathway antagonists (5.84 %) and ansamycins (2.72 %). Among the tetracyclines and fluoroquinolones, which each contain three agents in the clinical *S. aureus* AST, resistance rates varied except for ciprofloxacin and moxifloxacin (Fig. 1D). Furthermore, the resistance prevalence for tigecycline (3.57 %) and minocycline (7.35 %), both tetracyclines, was below 10 %.

The AMR traits within the hospital were further elaborated. In summary, there were no significant differences in resistance rates of both *E. coli* and *S. aureus* to most antimicrobial classes between male and female patients or between outpatients and inpatients. Supplementary material: Appendix A: Additional Tables: Table A.4-A.5. Notably, isolates from neonates and the neonatology department exhibited the lowest resistance rates across nearly all antimicrobial classes. Additionally, resistance rates were higher in isolates obtained from sputum, throat swabs, and urine compared to those from other sampling sites. Supplementary material: Appendix B: Additional Figures: Fig. B.1-B.5.

3.2. Trends in variation of resistance rates and prevalence

The clinical *E. coli* AMR dataset comprised 8 classes of continuous data spanning from 2018 to 2022. The linear trend test (2018–2022) over this period revealed that resistance rates for six classes—β-Lactam combination agents, cephems, carbapenems, fluoroquinolones, folate pathway antagonists, and fosfomycin—displayed significant trends. Of these, five classes showed an upward trend ($R > 0$, $P < 0.05$), while resistance to folate pathway antagonists exhibited a downward trend ($R = -0.072$, $P < 0.001$, Fig. 2A). Notably, the change in resistance rates were relatively minor in magnitude ($R < 0.2$). Furthermore, no discernible patterns in resistance prevalence were observed for the 15 agents with continuous data. Supplementary material: Appendix B: Additional Figures: Fig. B.6.

Linear trend tests were performed on resistance rates to 11 antimicrobial classes using 5-year continuous data from clinical *S. aureus* isolates. A slight downward trend was observed in resistance rates to penicillinase-labile penicillins, penicillinase-stable penicillins, fluoroquinolones, lincosamides, and aminoglycosides, with the most significant decrease noted in fluoroquinolone resistance ($R = -0.177$, $P < 0.001$, Fig. 2B). Conversely, resistance rates to folate pathway antagonists and tetracyclines exhibited a slight upward trend ($R = 0.215$ and 0.281, $P < 0.001$). No linear trends were detected in resistance rates to macrolides and ansamycins ($P = 0.213$ and 0.606). Among the tetracyclines and fluoroquinolones, which each included three agents, tetracycline, minocycline, and levofloxacin showed no significant changes in resistance over time. Supplementary material: Appendix B: Additional Figures: Fig. B.7.

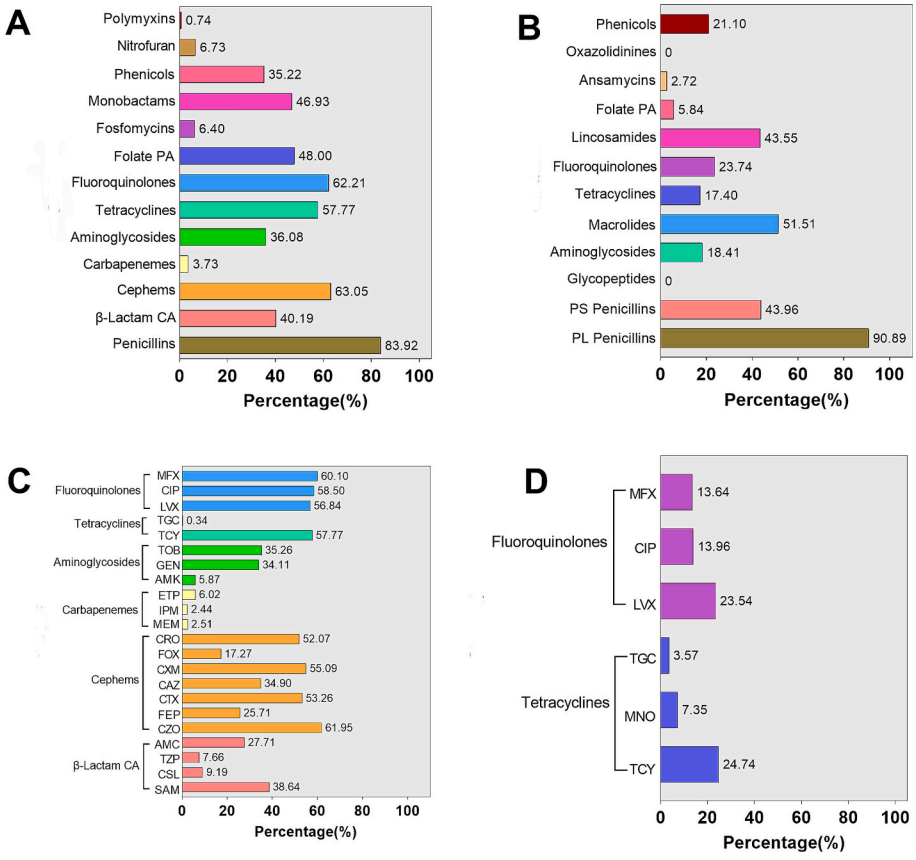

Fig. 1. Bar charts showing resistance rates of clinical *E. coli* (A, C) and *S. aureus* (B, D) isolates to all antimicrobial classes and antimicrobial agents. In antimicrobial susceptibility test (AST) of clinical *E.coli*, classes of β-Lactam combination agents, cephems, carbapenems, aminoglycosides, tetracyclines, and fluoroquinolones have two or more agents (C). In AST of *S. aureus,* classe of fluoroquinolones and tetracyclines all have three agents (D). *β-Lactam CA* β-Lactam combination agents, *Folate PA* Folate pathway antagomists, *SAM* Ampicillin/Dsulbactam, *CSL* Cefoperazone/Sulbactam, *TZP* Piperacillin/ Tazobactam, *AMC* Amoxicillin/ Clavulanic acid, *CZO* Cefazolin, *FEP* Cefepime, *CTX* Cefotaxime, *CAZ* Ceftazidime, *CXM* Cefuroxime, *FOX* Cefoxitin, *CRO* Ceftriaxone, *MEM* Meropenem, *IPM* Imipenem, *ETP* Ertapenem, *AMK* Amikacin, *GEN* Gentamicin, *TOB* Tobramycin, *TCY* Tetracycline, *TGC* Tigecycline, *LVX* Levofloxacin, *CIP* Ciprofloxacin, *MFX* Moxifloxacin; *PL Penicillins* Penicillinase-labicle Penicillins, *PS Penicillins* Penicillinase-stable Penicillins, *TCY* Tetracycline, *MNO* Minocycline, *TGC* Tigecycline, *LVX* Levofloxacin, *CIP* Ciprofloxacin, *MFX* Moxifloxacin.

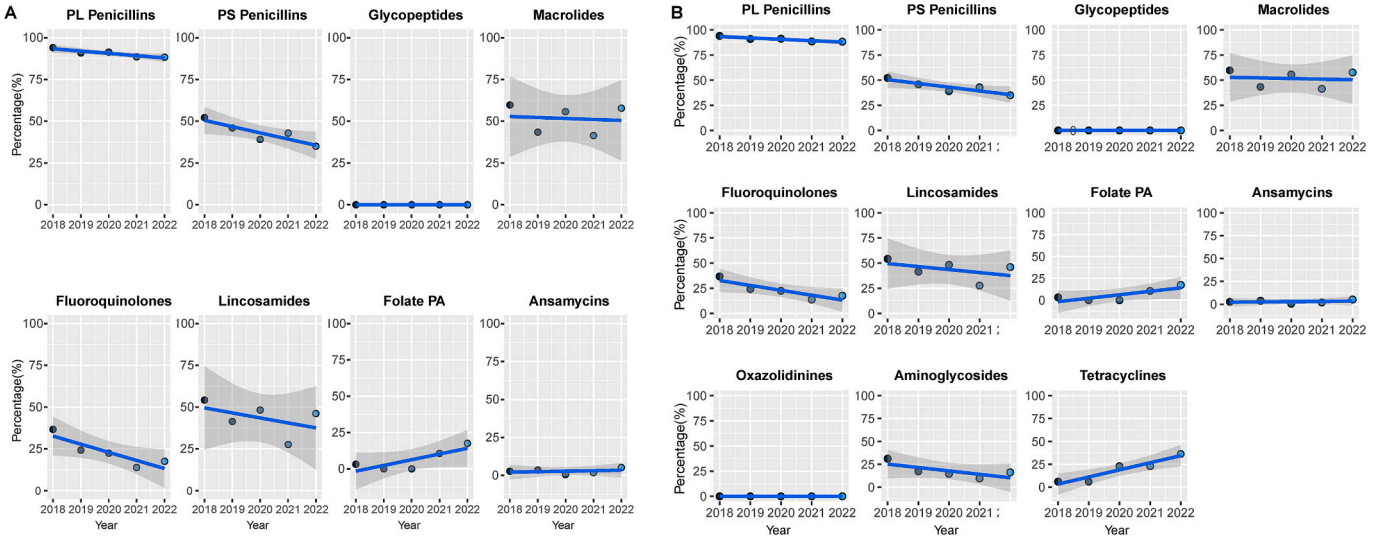

Fig. 2. Trends of the resistance rates of *E. coli* (A) and *S. aureus* (B) isolates to various antimicrobial classes from 2018 to 2022. Only complete data from these 5 years will be displayed. The trends of resistance rates were fitted by linear models in R. The gray shadow represents the 95 % confidence interval. *β-Lactam CA* β-Lactam combination agents; *Folate PA* Folate pathway antagonists; *PL Penicillins* Penicillinase-labile Penicillins; *PS Penicillins* Penicillinase-stable Penicillins.

3.3. The status of extended-spectrum beta-lactamases (ESBL) positive E. coli and methicillin-resistant S. aureus (MRSA)

The ESBL-positive isolates account for 53.26 % of all tested clinical *E. coli* isolates (Fig. 3A). The linear trend test indicated no significant change in prevalence over the five years ($P = 0.136$, Fig. 3B). The prevalence of MRSA was 43.81 % (Fig. 3C), with a slight downward trend observed in the linear trend test ($R = -0.106$, $P < 0.001$, Fig. 3D).

The characteristics of ESBL-positive *E. coli* and MRSA were further analyzed in relation to gender, age, hospitalization status, and hospital department. There was a significant difference in the prevalence of ESBL-positive *E. coli* between male and female patients (61.02 %/48.13 %, $\chi^2 = 41.91$, $P < 0.001$), while there was no difference for MRSA (44.35 %/42.97 %, $\chi^2 = 0.185$, $P = 0.667$). The prevalence of ESBL-positive *E. coli* (53.77 %/48.39 %, $\chi^2 = 2.609$, $P = 0.106$) and MRSA (44.02 %/28.57 %, $\chi^2 = 1.339$, $P = 0.247$) did not differ significantly between inpatients and outpatients. Among the four age groups, geriatric patients had the highest prevalence of both ESBL-positive *E. coli* (56.14 %) and MRSA (51.17 %), whereas neonates had the lowest prevalence (21.62 %/13.64 %). Supplementary material: Appendix B: Additional Figures: Fig. B.8. Similarly, the Department of Neonatology had the lowest prevalence of ESBL-positive *E. coli* (20.59 %) and MRSA (14.29 %).

3.4. The status and dynamics of MDR in clinical E. coli and S. aureus isolates

Of the 2625 clinical *E. coli* isolates tested, 1877 (71.50 %) were MDR isolates, showing resistance to three or more antimicrobial classes (Fig. 4A). None of the isolates were resistant to all 13 antimicrobial classes, and only one isolate was resistant to 12 classes. An upward trend in the proportion of MDR isolates was observed from 2018 to 2022, particularly among those resistant to 9 to 12 classes (Fig. 4B-C). Notably, a significant number of isolates resistant to 8 and 9 classes have been detected since 2021, with the emergence of isolates resistant to 10 and 11 classes beginning that same year. Additionally, isolates resistant to 12 classes were first detected in 2022.

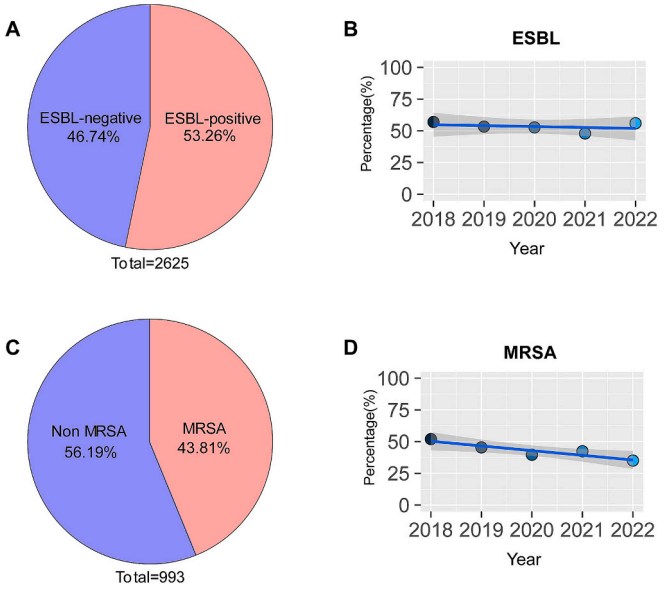

Fig. 3. The prevalence and trends of clinical ESBL-positive *E. coli* and MRSA from 2018 to 2022. (A) the prevalence of ESBL-positive *E. coli*; (B) the prevalence trends of ESBL-positive *E. coli* from 2018 to 2022. The trends of resistance rates were fitted by linear models in R. The gray shadow represents the 95 % confidence interval; (C) the prevalence of MRSA; (D) the prevalence trends of ESBL-*E. coli* from 2018 to 2022. The trends of resistance rates were fitted by linear models in R. The gray shadow represents the 95 % confidence interval.

The proportion of MDR *S. aureus* isolates was 50.80 % (505/994), with none showing resistance to all 12 antimicrobial classes (Fig. 4D). There was no significant trend in the proportion of MDR *S. aureus* over the past five years ($P = 0.885$), the highest proportion of MDR isolates was recorded in 2022 (61.69 %, Fig. 4E-F). Additionally, isolates resistant to 8 and 9 antimicrobial classes were exclusively identified in 2021 and 2022.

A significant difference was observed in the proportion of MDR *E. coli* isolates between male and female patients (75.29 % vs. 68.94 %, $\chi^2 = 12.409$, $P < 0.001$), while no significant difference was found in MDR *S. aureus* isolates between genders (50.50 % vs. 51.28 %, $\chi^2 = 0.057$, $P = 0.811$). For isolates from inpatients and outpatients, the proportion of MDR *S. aureus* was significantly higher in inpatients compared to outpatients (51.22 % vs. 21.43 %, $\chi^2 = 4.903$, $P = 0.027$), whereas the proportion in *E. coli* isolates showed no significant difference (71.48 % vs. 71.37 %, $P = 0.972$). Among the four age groups, neonates had the lowest MDR rates, while geriatric patients had the highest for both *E. coli* and *S. aureus*. Across hospital departments, the MDR rate of *E. coli* was particularly concerning, with rates exceeding 60 % in all departments except Neonatology. Similarly, in the MDR rate of *S. aureus*, the Neonatology department had the lowest rate among the eight departments analyzed. Supplementary material: Appendix B: Additional Figures: Fig. B.9.

The Poisson GLMMs were used to investigate the variation of AMR carriage (the total number of antimicrobial classes to which an isolate was phenotypically resistant) over the five years. The AMR carriages of *E. coli* in 2020, 2021, and 2022 were found to be higher compared to 2018 (odds ratio [OR] = 1.09, 1.31, and 1.59, $P < 0.05$), suggesting a deteriorating trend of *E. coli* MDR (Table 1). In contrast, the AMR carriages in *S. aureus* isolates in 2019, 2020, and 2021 were lower than that of 2018 (OR = 0.83, 0.91, 0.78, $P = 0.003$, 0.147, 0.001), suggesting a decline in the MDR situation of *S. aureus*. However, the slight increase in AMR carriage observed in 2022 (OR = 1.06, $P = 0.354$) indicates that the issue of MDR should not be overlooked.

3.5. The characteristic of AMR profile identified from E. coli and S. aureus

A total of 294 AMR profiles were identified among the clinical *E. coli* isolates. The profiles with six antimicrobial classes (antibiogram lengths 6) had the highest number of unique profiles (51 types), followed by antibiogram lengths 5 (47 types) and antibiogram lengths 4 (43 types, Fig. 5A). Notably, there was a significant increase in the diversity of AMR profiles, with profiles of antibiogram lengths ranging from 7 to 12 appearing predominantly in 2020, 2021, and 2022 (Fig. 5B). Interestingly, each antibiogram length had at least one dominant AMR profile as the most common phenotype, simultaneously providing the focus on AMR monitoring and control (Table 2). Supplementary material: Appendix A: Additional Tables: Table A.6.

Among the 119 AMR profiles of clinical *S. aureus* isolates, both antibiogram length 3 and 4 exhibited 22 different profiles, followed by antibiogram length 6 with 19 types and length 5 with 18 types (Fig. 5C). The AMR profiles in 2022 (68 types) and 2021 (54 types) were significantly higher compared to those in 2018 (33 types), 2019 (27 types), and 2020 (29 types) (Fig. 5D). Additionally, there was an increase in the proportion of antibiogram length exceeding 3 in 2021 (68.52 %, 37/54) and 2022 (83.83 %, 57/68). Each antibiogram length of *S. aureus* also had a dominant AMR profile (Table 2). Supplementary material: Appendix A: Additional Tables: Table A.7.

3.6. The AMR association between clinical and food animal isolates

A total of 44 *E. coli* AMR profiles were shared between clinical and food animal sources (Fig. 6A). Among these shared profiles, 7 were found in clinical isolates during 2018, 2019, and 2020, comprising four

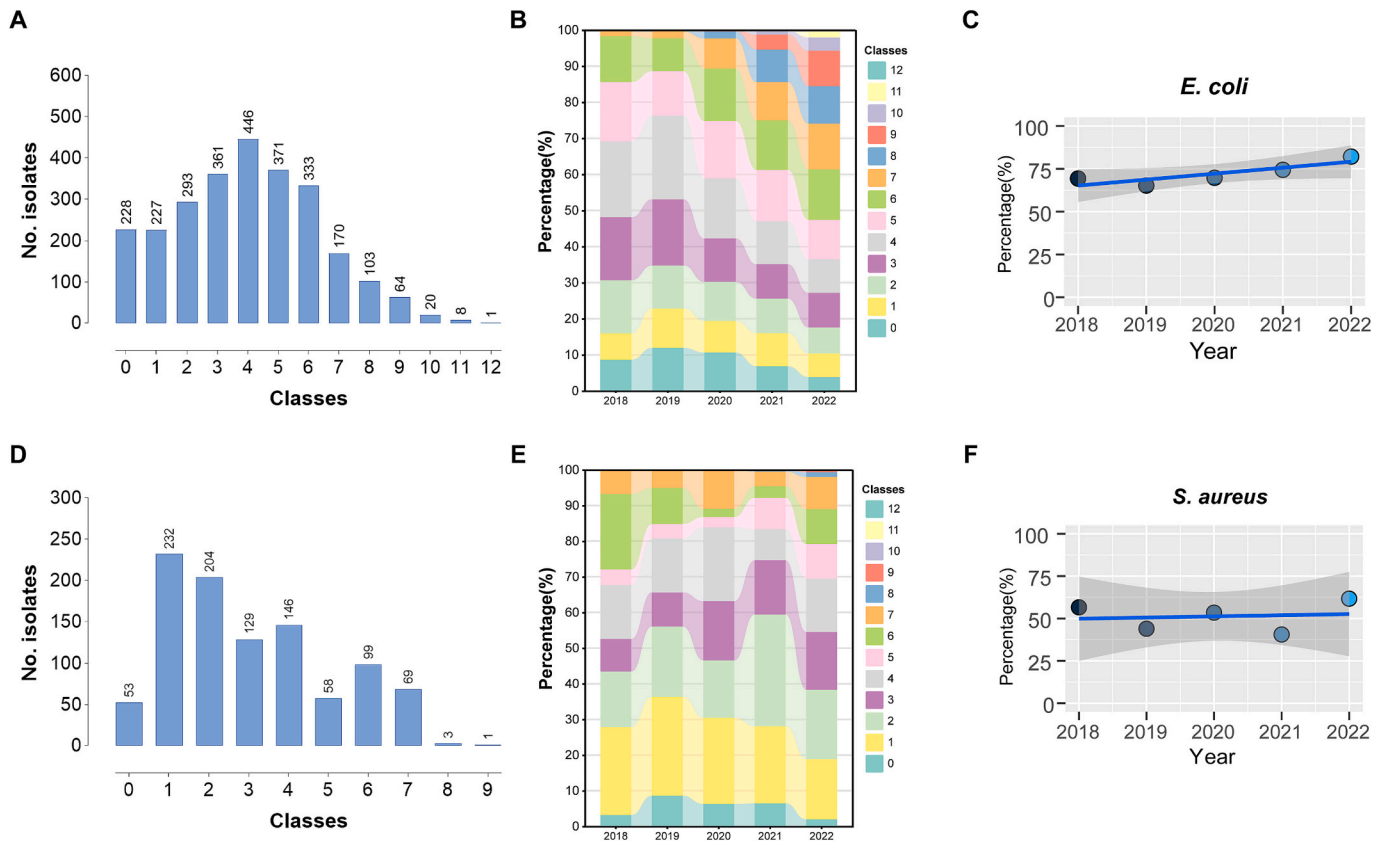

Fig. 4. The status and trends of MDR *E. coli* (A-C) and *S. aureus* (D—F) isolates from 2018 to 2022. (A, D) Number of isolates in each antimicrobial class; (B, E) The percentage of isolates in each antimicrobial class annually; (C, F) The proportion trends of MDR isolates. The trends of resistance rates were fitted by linear models in R. The gray shadow represents the 95 % confidence interval.

Table 1
Results of a Poisson generalized linear mixed model examining the likelihood of antibiogram length within different years.

Year	No. Isolates	Estimate	OR	SE	95 % *CI*	Z score	*P*-value
E. coli							
2018	590	Reference					
2019	578	−0.08987	0.91	0.03596	0.85, 0.98	−2.499	0.0124
2020	516	0.08735	1.09	0.0359	1.02, 1.17	2.433	0.015
2021	555	0.27393	1.32	0.034	1.23, 1.41	8.058	<0.001
2022	386	0.46518	1.59	0.0359	1.48, 1.70	12.958	<0.001
S. aureus							
2018	251	Reference					
2019	198	−0.18199	0.83	0.06277	0.74, 0.94	−2.899	0.003739
2020	174	−0.09362	0.91	0.06452	0.80, 1.03	−1.451	0.146799
2021	217	−0.24664	0.78	0.06183	0.69, 0.88	−3.989	<0.001
2022	154	0.05942	1.06	0.0641	0.94, 1.20	0.927	0.353978

Variable of estimate was the main output of GLMM analysis, the OR value was calculated from estimate. OR > 1 indicates the antibiogram length was higher than reference, OR < 1 indicate the antibiogram length was lower than reference. *P*-value <0.05 indicates that the comparison among sampling years has statistical significance.

profiles with an antibiogram length of 1, two with a length of 2, and one with a length of 3. In 2021, 24 new AMR profiles emerged in clinical isolates, with an additional 13 profiles observed in 2022. In contrast, *E. coli* AMR profiles from food animals were more frequently detected in the first three years (30/44), with 17 profiles in 2018, 6 in 2019, and 7 in 2020, suggest that specific AMR profiles, particularly those with an antibiogram length of three or more (MDR isolates), may have appeared earlier in *E. coli* from food animals. Supplementary material: Appendix A: Additional Tables: Table A.8.

For *S. aureus* isolates, 12 shared profiles by both interfaces were identified (Fig. 6B), consisting of 3 profiles with antibiogram length 3 and 1 profiles with length 4. Appendix A: Additional Tables: Table A.9. Interestingly, the four profiles also emerged in clinical profiles in 2021 or 2022 and were observed during the initial three years in food animal isolate profiles. This observation aligned with that of *E. coli*, which could potentially strengthen the validity of our conclusion.

The phenotypic data of *E. coli* and *S. aureus* isolates from food animals were integrated into the dataset to explore AMR associations between clinical and food animal sources. Resistance rates and the proportion of MDR isolates were calculated. Supplementary material: Appendix A: Additional Tables: Table A.10-A.11. The correlation coefficient of resistance rates was then computed to evaluate the AMR association between the clinical and food animal interfaces. The correlation coefficient heatmap for *E. coli* revealed a strong negative

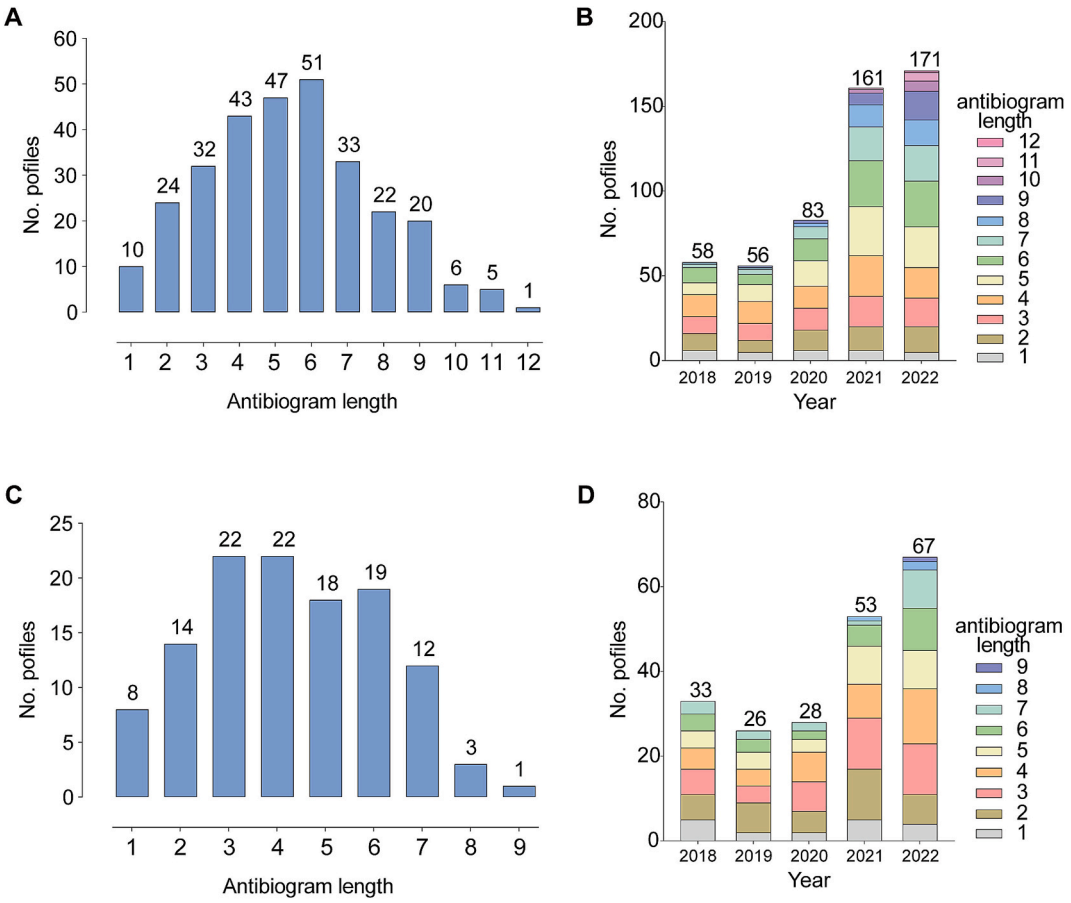

Fig. 5. The status of AMR profiles in *E. coli* and *S. aureus* at the clinical interfaces. The quantity of *E. coli* (A) and *S. aureus* (C) AMR profiles categorized by antibiogram length; the quantity of *E. coli* (B) and *S. aureus* (D) AMR profiles categorized by years and antibiogram length.

correlation in the resistance rate of cephems between the clinical and food animal isolates ($r = -1.000$, $P < 0.001$, Fig. 6C). No significant one-to-one correlations were found between other phenotypic resistance rates, including MDR rates. Similarly, the analysis of phenotype correlations between clinical *S. aureus* resistance rates and those from food animal isolates showed the same results (Fig. 6D), indicating that establishing connections at the human-animal interface based on a single AMR phenotype is highly challenging.

Co-occurrence analysis identified a distinct cluster in the clinical phenotype dataset of *E. coli*, specifically penicillins-β-lactam combination agents-cephems-monobactams (Pen-βLac-Cep-Mon) ($P < 0.01$, correlation coefficient > 0.5, Fig. 6E). Additionally, 13 pairs of co-occurring phenotypes were found in clinical *E. coli* isolates. To further explore these co-occurrences, the phenotypes were examined across both human and animal interfaces. Since the monobactams class was not included in the food animal *E. coli* AST, an alternative cluster, Pen-βLac-Cep, was analyzed. This cluster was detected in 125 AMR profiles of clinical isolates, eight profiles of food animal isolates, and six profiles shared by both interfaces. Moreover, 11 of the 13 co-occurring phenotypic pairs were identified in both human and food animal interfaces (Table 3).

A cluster of aminoglycosides-tetracyclines-folate pathway antagonists (Ami-Tet-Fpa) was identified in the food animal interface and further analyzed across both human and animal datasets. Supplementary material: Appendix B: Additional Figures: Fig. B.10. This cluster was found in 10 profiles of food animal isolates, 11 profiles of clinical isolates, and four profiles shared by both interfaces. Additionally, six co-occurrence pairs (three of which were identical to those in clinical isolates) were identified in food animal isolates. Most of these pairs, except for Cep-Fos and Fos-Phe, were detected in both human and food animal interfaces (Table 3). These findings suggest a potential association between human and food animal interfaces, possibly mediated by MDR phenotypes or inferred plasmid which mediated multidrug resistance.

Co-occurrence analysis of the clinical *S. aureus* phenotypic dataset identified three distinct clusters: Ami-Mac-Lin (correlation coefficient $>$ 0.75), Ami-Mac-Flu-Lin, and PSPen-Ami-Flu-Lin (correlation coefficient > 0.5, Fig. 6F). All three clusters were present in both clinical and food animal AMR profiles (24.37 %/0.29 %, 9.24 %/0.29 %, 5.04 %/0.57 %, Table 2). Additionally, three pairs of co-occurring AMR phenotypes were identified in clinical *S. aureus* isolates, with the PLPen-PSPen pair found in both clinical (34.35 %) and food animal (0.86 %) interfaces (Table 3).

The AST for *S. aureus* from food animals included three additional classes: β-lactam combination agents, cephems, and diterpenes. Consequently, although three clusters—PSPen-βLac-Cep, PSPen-βLac-Cep-Mac, and PSPen-βLac-Cep-Flu-Lin-Dit—were identified in the phenotypic dataset of *S. aureus* isolates from food animals, only PSPen-Mac (adjusted from PSPen-βLac-Cep-Mac, 31.09 %/0.29 %) and PSPen-Flu-Lin (adjusted from PSPen-βLac-Cep-Flu-Lin-Dit, 10.08 %/0.57 %) were used to assess their presence across both human and animal interfaces (Table 3). Additionally, eight co-occurrence phenotype pairs were identified in the food animal phenotypic dataset, six of which were also observed in both clinical and food animal interfaces. Supplementary material: Appendix B: Additional Figures: Fig. B.10. These findings from *S. aureus* further highlight the potential of using MDR as a tool for assessing cross-interface associations in G+ bacteria.

4. Discussion

AMR has emerged as a critical challenge to global public health and

Table 2
The proportion of predominant AMR profiles with different antibiogram length in clinical *E. coli* and *S. aureus*.

AMR profiles with different antibiogram length	Proportion of AMR profiles (%)
E. coli	
Flu	28.63 (65/227)
Pen	47.58 (108/227)
Pen-Cep	20.14 (59/293)
Pen-Flu	21.50 (63/293)
Pen-Fpa	26.62 (78/293)
Pen-Cep-Flu	19.39 (70/361)
Pen-Cep-Flu-Fpa	16.82 (75/446)
Pen-βLac-Cep-Flu	18.16 (81/446)
Pen-Cep-Ami-Flu-Fpa	16.98 (63/371)
Pen-βLac-Cep-Flu-Fpa	15.09 (56/371)
Pen-βLac-Cep-Ami-Flu-Fpa	31.53 (105/333)
Pen-βLac-Cep-Ami-Flu-Fpa-Mon	23.53 (40/170)
Pen-βLac-Cep-Ami-Tet-Flu-Fpa-Mon	28.16 (29/103)
Pen-βLac-Cep-Ami-Tet-Flu-Fpa-Mon-Phe	43.75 (28/64)
Pen-βLac-Cep-Ami-Tet-Flu-Fpa-Fos-Mon-Phe	50.00 (10/20)
Pen-βLac-Cep-Ami-Tet-Flu-Fpa-Fos-Mon-Phe-Nit	50.00 (4/8)
S. aureus	
PLPen	89.66 (208/232)
PLPen-PSPen	48.53 (99/204)
PLPen-Mac-Lin	55.81 (72/129)
PLPen-PSPen-Mac-Lin	59.59 (87/146)
PLPen-PSPen-Mac-Flu-Lin	24.14 (14/58)
PLPen-PSPen-Mac-Tet-Lin	18.97 (11/58)
PLPen-PSPen-Ami-Mac-Flu-Lin	62.63 (62/99)
PLPen-PSPen-Ami-Mac-Tet-Flu-Lin	73.91 (51/69)

Pen Penicillins, *βLac* β-Lactam combination agents, *Cep* Cephems, *Ami* Aminoglycosides, *Tet* Tetracyclines, *Flu* Fluoroquinolones, *Fpa* Folate pathway antagomists, *Fos* Fosfomycins, *Mon* Monobactams, *Phe* PheniPols, *Nit* Nitrofuran, *PLPen* Penicillinase-labicle Penicillins, *PSPen* Penicillinase-stable Penicillins, *Mac* Macrolides, *Lin* Lincosamides.

clinical treatment, affecting countries or regions at all economic levels [1–3]. AMR is a One Health issue that involves interactions between humans, animals, and the environment [21,22,31]. Understanding the associations between these interfaces, especially though the analysis common phenotypic data, is crucial and demands immediate attention. In this study, we described the AMR characteristics of clinical *E. coli* and *S. aureus* isolates and explore the association between the clinical and food animal interfaces using the phenotypic data combined with co-occurrence analysis. Certain co-occurrence phenotypic pairs and clusters may serve as indicators for measuring association across various interfaces. This study represents a pioneering effort to investigate cross-interface associations using AMR phenotypic data without relying on genomic data. In addition, compared to other large-scale AMR monitoring efforts and surveys, this study specifically focuses on a small island, offering detailed descriptions of AMR characteristics and insights into AMR associations and transmission across various interfaces within the One Health framework.

The study revealed that *E. coli* isolates exhibited significant resistance rates (over 50 %) to penicillins, cephems, fluoroquinolones, and tetracyclines, while *S. aureus* isolates showed notable resistance rates (over 40 %) to penicillins, macrolides, and lincosamides. These findings align with resistance patterns observed in Shanghai and other provinces across China [32]. The persistently high resistance rates are largely attributed to the widespread use of specific antimicrobials like penicillins and cephalosporins, commonly prescribed in China's public healthcare institutions [33]. Recent reports have highlighted the emergence of carbapenem, colistin, and tigecycline resistance among Gram-negative bacteria [20,34,35], and varying levels of vancomycin and linezolid resistance in clinical *S. aureus* isolates [36,37]. In our study, clinical *E. coli* and *S. aureus* isolates showed low resistance or remained susceptible to these drugs, indicating these drugs continued efficacy on Chongming Island. The zero-resistance rate to linezolid in *S. aureus* aligns with previous findings on other regions [38]. However, ongoing surveillance is crucial, given the potential AMR association and transmission across the One Health interfaces highlighted by our findings.

ESBL-producing bacteria emerged in the 1980s, and the high prevalence of ESBL-producing *E. coli* in the community has contributed to their spread in hospitals [39]. Recent global surveillance data showed that the prevalence of ESBL-producing *E. coli* in China remains alarmingly high at 60–70 %, significantly higher than in Europe (15 %), North America (10 %), and Southeast and East Asia (20–40 %) [40,41]. Our study also found a high prevalence of ESBL-producing *E. coli* isolates (53.26 %) on Chongming Island. Both our findings and previous research confirm that the global spread of ESBL-producing *E. coli* continues unabated.

MRSA has been a prevalent global resistant phenotype since its emergence in 1961 [14,42]. In 2022, the prevalence in Chongming was consistent with that in Shanghai at 43.8 % [32], significantly higher than the national average of 28.9 % and that of some developed countries like Germany (7.6 %), Australia (17.65 %), and Japan (35.85 %) [43]. However, it was lower than in some African countries, like Egypt (63 %) [44] and Nigeria (46 %) [45]. Although monitoring data showed a downward trend, reducing the prevalence of MRSA remains a key focus for Shanghai in its future efforts.

The high proportion of MDR *E. coli* and *S. aureus* in Chongming Island, consistents with findings from several studies in other regions [46,47]. Over the five-year period, the MDR rate of *E. coli* exhibited an increasing trend, whereas that of *S. aureus* showed no clear pattern of change. Generalized linear mixed models were employed to evaluate the MDR changes in these two bacterial species, further supporting our conclusions. A detailed analysis on the AMR profiles indicated that profiles with an antibiogram length exceeding three became more prevalent after 2020, particularly those with an antibiogram length exceeding six. This gradual accumulation of AMR and the increased diversity of AMR profiles in these bacterial species heightens the risk of transitioning to extensive-drug resistance and pan-drug resistance. A previously reported co-occurrence analysis [29,30] identified several co-occurrence phenotypic pairs and clusters in *E. coli* and *S. aureus*, suggesting potential transmission mediated by a plasmid with multiple resistance genes or by multiple plasmids within a single bacterium. To definitively validate these findings, it is crucial to employ genome sequence analysis into AMR surveillance.

Our results highlight the complexity of AMR dynamics and association across multiple interfaces [48]. Notably, certain AMR profiles emerged in food animal isolates before being detected in clinical settings, suggesting possible transmission routes from animals to humans. However, determining AMR association and transmission across multiple interfaces remains challenging with the current dataset. This emphasizes the urgent need to integrate whole genome sequencing (WGS) into AMR surveillance, a call also echoed by the WHO [49,50]. Currently, only a few countries have incorporated bacterial genome data into routine AMR surveillance. The United States pioneered this effort in 2014 through the National Antimicrobial Resistance Monitoring System (NARMS), which operates as a multi-sectoral, multidisciplinary, and multi-scenario framework within the One Health approach. [51–53]. As WGS becomes more affordable, more countries are expected to adopt this method for AMR monitoring [54].

In conclusion, our study provides a usable methodological framework for investigating associations across various interfaces using phenotypic data. We also identified several high likelihoods of co-occurrence phenotypic pairs and clusters that can serve as indicators for measuring associations at human-animal interface. However, it is imperative to rapidly integrate genomic data into AMR surveillance to fully address the monitoring requirements of the One Health framework.

Our research has several limitations: (1) Although the farms are located near the medical institutions, they operate independently and use different types of antibacterial drugs, making it challenging to establish a direct association between the two interfaces; (2) The hospital samples are collected from patients, whereas the samples from food

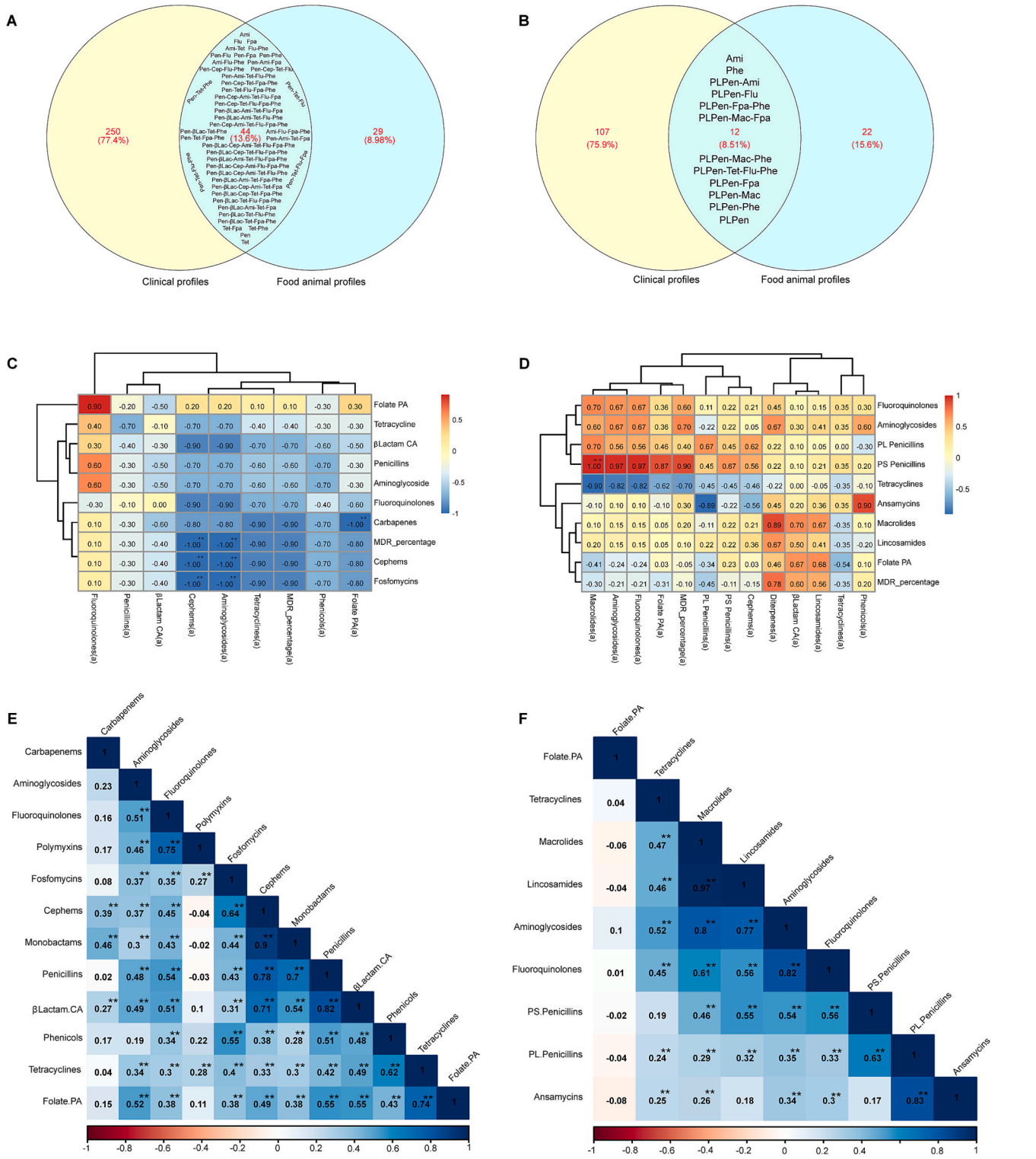

Fig. 6. The association of *E. coli* (A, C, E) and *S. aureus* (B, D, F) between the clinical and food animal interfaces. A-B The shared profiles between the two interfaces of *E. coli* (A) and *S. aureus* (B). *Pen* Penicillins, *βLac* β-Lactam combination agents, *Cep* Cephems, Ami Aminoglycosides, *Tet* Tetracyclines, *Flu* Fluoroquinolones, *Fpa* Folate pathway antagonists, *Fos* Fosfomycins, *Mon* Monobactams, *Phe* PheniPols, *Nit* Nitrofuran, *PLPen* Penicillinase-labile Penicillins, *PSPen* Penicillinase-stable Penicillins, *Gly* Glycopeptides, *Mac* Macrolides,*Lin* Lincosamides,*Ans* Ansamycins, *Oxa* Oxazolidinines. C—D The correlation of resistance rate of every antimicrobial class between the clinical *E. coli*, *S. aureus*, and that of food animals. The sign (a) indicates the classes used in food animals. *βLactam CA* β-Lactam combination agents; *Folate PA* Folate pathway antagonists; *PL Penicillins* Penicillinase-labile Penicillins; *PS Penicillins* Penicillinase-stable Penicillins. *E*-F Co-occurrence analysis of antimicrobial class in clinical *E. coli* and *S. aureus*. The numbers within the boxes reflect values for the correction coefficient (r). The legends beneath the two heat maps indicate whether the link between resistant phenotypes is positive (closer to 1; darker blue) or negative (less than 1; lighter blue) (closer to −1, dark red). $^{*}P < 0.05$, $^{**}P < 0.01$. *βLactam CA* β-Lactam combination agents; *Folate PA* Folate pathway antagonists; *PL Penicillins* Penicillinase-labile Penicillins; *PS Penicillins* Penicillinase-stable Penicillins. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3
The proportion of the co-occurrence cluster and pairwise phenotypes at the human-animal interface.

Co-occurrence AMR phenotypes	*E. coli*			Co-occurrence AMR phenotypes	*S. aureus*		
	Proportion in clinical profiles (%)	Proportion in food animal profiles (%)	Proportion in shared profiles (%)		Proportion in clinical profiles (%)	Proportion in food animal profiles (%)	Proportion in shared profiles (%)
Clinical isolates				Clinical isolates			
Pen-Flu	52.72 (155/294)	19.33 (23/119)	45.45 (20/44)	Ami-Tet	17.65 (21/119)	0.00 (0/34)	0.00 (0/12)
Pen-βLac[a]	52.38 (154/294)	18.49 (22/119)	34.09 (15/44)	PLPen-PSPen	34.45 (41/119)	0.86 (3/34)	0.00 (0/12)
Pen-Phe	32.31 (95/294)	29.41 (35/119)	50.00 (22/44)	PLPen-Ans	17.65 (21/119)	–	–
Pen-Fpa[a]	44.90 (132/294)	25.21 (30/119)	47.73 (21/44)	Ami-Mac-Lin	24.37 (29/119)	0.29 (1/34)	0.00 (0/12)
βLac-Flu	34.69 (102/294)	7.56 (9/119)	20.45 (9/44)	Ami-Mac-Flu-Lin	9.24 (11/119)	0.29 (1/34)	0.00 (0/12)
βLac-Fpa[a]	29.25 (86/294)	10.92 (13/119)	25.00 (11/44)	PSPen-Ami-Flu-Lin	5.04 (6/119)	0.57 (2/34)	0.00 (0/12)
Ami-Flu	30.27 (89/294)	13.45 (16/119)	25.00 (11/44)	Food animal isolates		0.00 (/34)	
Flu-Col	2.38 (7/294)	0.84 (1/119)	0.00 (0/44)	PSPen-Ami	15.13 (18/119)	0.57 (2/34)	0.00 (0/12)
Cep-Fos	23.47 (69/294)	–	–	PLPen-Mac	55.46 (66/119)	3.74 (13/34)	25.00 (3/12)
Fos-Phe	9.52 (28/294)	–	–	Ami-Lin	24.37 (29/119)	0.57 (2/34)	0.00 (0/12)
Tet-Phe	24.15 (71/294)	27.73 (33/119)	45.45 (20/44)	Flu-Lin	18.49 (22/119)	0.86 (3/34)	0.00 (0/12)
Ami-Fpa	26.19 (77/294)	21.01 (25/119)	27.27 (12/44)	Ami-Dit	–	0.57 (2/34)	–
Tet-Fpa	26.19 (77/294)	25.21 (30/119)	43.18 (19/44)	Lin-Dit	–	0.86 (3/34)	–
Pen-βLac-Cep-Mon[b]	42.52 (125/294)	6.72 (8/119)	13.64 (6/44)	Mac-Flu	28.57 (34/119)	1.44 (5/34)	0.00 (0/12)
Food animal isolates				Mac-Lin	51.26 (61/119)	0.57 (2/34)	0.00 (0/12)
Flu-Phe	24.49 (72/294)	18.49 (22/119)	38.64 (17/44)	PSPen-βLac-Cep	–	0.86 (3/34)	–
Pen-Cep	65.65 (193/294)	14.29 (17/119)	27.27 (12/44)	PSPen-βLac-Cep-Mac[c]	31.09 (37/119)	0.29 (1/34)	–
βLac-Ami	27.21 (80/294)	12.61 (15/119)	20.45 (9/44)	PSPen-βLac-Cep-Flu-Lin-Dit[d]	10.08 (12/119)	0.57 (2/34)	–

[a] The profiles shared by human and food animal isolates.
[b] the proportion of the profile was obtained by the adjusted profile of Pen-βLac-Cep.
[c] the adjusted profile of PSPen-Mac obtained the proportion of the profile.
[d] the proportion of the profile was obtained by the adjusted profile of PSPen-Flu-Lin. *Pen* Penicillins, *βLac* β-Lactam combination agents, *Cep* Cephems, *Ami* Aminoglycosides, *Tet* Tetracyclines, *Flu* Fluoroquinolones, *Fpa* Folate pathway antagonists, *Fos* Fosfomycins, *Mon* Monobactams, *Phe* PheniPols, *Nit* Nitrofuran, *PLPen* Penicillinase-labile Penicillins , *PSPen* Penicillinase-stable Penicillins , *Gly* Glycopeptides , *Mac* Macrolides , *Lin* Lincosamides , *Ans* Ansamycins , *Oxa* Oxazolidinines.

animals are taken from healthy animals, which may lead to bias in the drug resistance rates; (3) The number of isolates from food animals is significantly lower than that from human clinical samples, which could result in less accurate results and potentially unreliable conclusions; (4) The inference methods used in this study do not equate to empirical research, and the conclusions drawn here need further validation through genomic data.

5. Conclusion

Phenotypic data continues to be the primary form of AMR surveillance. In our study, phenotypic data of clinical *E. coli* and *S. aureus* were utilized to explore the AMR characteristics and trends on Chongming Island. Subsequently, the potential association of AMR at the human-animal interface were investigated by incorporating phenotypic data from food animals into the pooled dataset. The findings revealed a worsening situation of MDR among G+ and G- bacteria in Chongming Island's clinical scenario, characterized by an increase in the diversity of AMR profiles and longer antibiogram lengths. Some MDR profiles manifest in food animal interface before clinical settings, highlighting the potential for AMR association and transmission between humans and food animals. Meanwhile, several co-occurrence phenotypes that could serve as indicators for AMR association were identified in both human and food animal interfaces. Our findings indicate the pressing need for incorporating genomic data into AMR monitoring.

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Ethics

The experimental protocol was established, according to the ethical guidelines of the Helsinki Declaration and was approved by the Human Ethics Committee of Chongming Hospital Affiliated to Shanghai University of Medicine and Health Sciences (CMEC-2021-KT-15). Written informed consent was obtained from individual or guardian participants. In this study, all sensitive information related to personal identity has been deleted. Animal ethical approval was granted by the Ethics Committee of Shanghai Jiao Tong University School of Medicine (A-2021-014), and obtained the consent of the farm management personnel before sampling.

CRediT authorship contribution statement

Chao Lv: Writing – original draft, Visualization, Software, Methodology, Formal analysis, Data curation. **Minjian Qian:** Writing – original draft, Methodology, Investigation. **Bingqing Sun:** Methodology, Investigation. **HuiPing Ye:** Resources, Investigation. **Min Li:** Visualization, Methodology, Formal analysis. **Nan Zhou:** Visualization, Methodology, Formal analysis. **Zile Cheng:** Visualization, Methodology, Formal analysis. **Yiwen Chen:** Visualization, Software, Methodology. **Xiaokui Guo:** Supervision, Funding acquisition. **Jun Shang:** Writing – original draft, Resources, Investigation. **Li Zhang:** Writing – review & editing, Supervision, Data curation, Conceptualization. **Yongzhang Zhu:** Writing – review & editing, Supervision, Methodology, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors of the present study declare no competing interests.

Data availability

Data will be made available upon formal request and supervision by the corresponding authors.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.onehlt.2024.100910.

References

[1] S. Rehman, A parallel and silent emerging pandemic: antimicrobial resistance (AMR) amid COVID-19 pandemic, J. Infect. Public Health 16 (2023) 611–617, https://doi.org/10.1016/j.jiph.2023.02.021.
[2] U. Theuretzbacher, Accelerating resistance, inadequate antibacterial drug pipelines and international responses, Int. J. Antimicrob. Agents 39 (2012) 295–299, https://doi.org/10.1016/j.ijantimicag.2011.12.006.
[3] WHO, Antimicrobial Resistance. https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance, 2023.
[4] Antimicrobial Resistance Collaborators, Global burden of bacterial antimicrobial resistance in 2019: A systematic analysis, Lancet 399 (2022) 629–655, https://doi.org/10.1016/S0140-6736(21)02724-0.
[5] R. Hao, R. Zhao, S. Qiu, L. Wang, H. Song, Antibiotics crisis in China, Science 348 (2015) 1100–1101, https://doi.org/10.1126/science.348.6239.1100-d.
[6] T.P. Van Boeckel, C. Brower, M. Gilbert, B.T. Grenfell, S.A. Levin, T.P. Robinson, A. Teillant, R. Laxminarayan, Global trends in antimicrobial use in food animals, Proc. Natl. Acad. Sci. USA 112 (2015) 5649–5654, https://doi.org/10.1073/pnas.1503141112.
[7] K. Tiseo, L. Huber, M. Gilbert, T.P. Robinson, T.P. Van Boeckel, Global trends in antimicrobial use in food animals from 2017 to 2030, Antibiotics (Basel). 9 (2020) 918, https://doi.org/10.3390/antibiotics9120918.
[8] R. Mulchandani, Y. Wang, M. Gilbert, T.P. Van Boeckel, Global trends in antimicrobial use in food-producing animals: 2020 to 2030, PLOS Glob. Public Health. 3 (2023) e0001305, https://doi.org/10.1371/journal.pgph.0001305.
[9] J.B. Kaper, J.P. Nataro, H.L. Mobley, Pathogenic *Escherichia coli*, Nat. Rev. Microbiol. 2 (2004) 123–140, https://doi.org/10.1038/nrmicro818.
[10] B.P. Conlon, Staphylococcus aureus chronic and relapsing infections: evidence of a role for persister cells: an investigation of persister cells, their formation and their role in *S. Aureus* disease, Bioessays 36 (2014) 991–996, https://doi.org/10.1002/bies.201400080.
[11] A.I. Molineri, C. Camussone, M.V. Zbrun, G. Suárez Archilla, M. Cristiani, V. Neder, L. Calvinho, M. Signorini, Antimicrobial resistance of *Staphylococcus aureus* isolated from bovine mastitis: Systematic review and meta-analysis, Prev. Vet. Med. 188 (2021) 105261, https://doi.org/10.1016/j.prevetmed.2021.105261.
[12] G. Peirano, J.D.D. Pitout, Extended-Spectrum β-lactamase-producing Enterobacteriaceae: update on molecular epidemiology and treatment options, Drugs 79 (2019) 1529–1541, https://doi.org/10.1007/s40265-019-01180-3.
[13] T. Ramatla, T. Mafokwane, K. Lekota, M. Monyama, G. Khasapane, N. Serage, J. Nkhebenyane, C. Bezuidenhout, O. Thekisoe, "One health" perspective on prevalence of co-existing extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella pneumoniae*: a comprehensive systematic review and meta-analysis, Ann. Clin. Microbiol. Antimicrob. 22 (2023) 88, https://doi.org/10.1186/s12941-023-00638-3.
[14] A.S. Lee, H. de Lencastre, J. Garau, J. Kluytmans, S. Malhotra-Kumar, A. Peschel, S. Harbarth, Methicillin-resistant *Staphylococcus aureus*, Nat. Rev. Dis. Primers 31 (2018) 18033, https://doi.org/10.1038/nrdp.2018.33.
[15] A. Ciapponi, A. Bardach, M.M. Sandoval, M.C. Palermo, E. Navarro, C. Espinal, R. Quirós, Systematic review and Meta-analysis of deaths attributable to antimicrobial resistance, Latin America, Emerg. Infect. Dis. 29 (2023) 2335–2344, https://doi.org/10.3201/eid2911.230753.
[16] H. Huang, P. Wan, X. Luo, Y. Lu, X. Li, W. Xiong, Z. Zeng, Tigecycline resistance-associated mutations in the MepA efflux pump in *Staphylococcus aureus*, Microbiol. Spectr. 11 (2023) e0063423, https://doi.org/10.1128/spectrum.00634-23.
[17] S.J. Dunn, C. Connor, A. McNally, The evolution and transmission of multi-drug resistant *Escherichia coli* and *Klebsiella pneumoniae*: the complexity of clones and plasmids, Curr. Opin. Microbiol. 51 (2019) 51–56, https://doi.org/10.1016/j.mib.2019.06.004.
[18] R. Hummel, H. Tschäpe, W. Witte, Spread of plasmid-mediated nourseothricin resistance due to antibiotic use in animal husbandry, J. Basic Microbiol. 26 (1986) 461–466, https://doi.org/10.1002/jobm.3620260806.
[19] J. Fischer, I. Rodríguez, S. Schmoger, A. Friese, U. Roesler, R. Helmuth, B. Guerra, *Escherichia coli* producing VIM-1 carbapenemase isolated on a pig farm, J. Antimicrob. Chemother. 67 (2012) 1793–1795, https://doi.org/10.1093/jac/dks108.
[20] Y.Y. Liu, Y. Wang, T.R. Walsh, L.X. Yi, R. Zhang, J. Spencer, Y. Doi, G. Tian, B. Dong, X. Huang, L.-F. Yu, D. Gu, H. Ren, X. Chen, L. Lv, D. He, H. Zhou, Z. Liang, J.H. Liu, J. Shen, Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study, Lancet Infect. Dis. 16 (2016) 161–168, https://doi.org/10.1016/S1473-3099(15)00424-7.
[21] T.P. Robinson, D.P. Bu, J. Carrique-Mas, E.M. Fèvre, M. Gilbert, D. Grace, S.I. Hay, J. Jiwakanon, M. Kakkar, S. Kariuki, R. Laxminarayan, J. Lubroth, U. Magnusson, P. Thi Ngoc, T.P. Van Boeckel, M.E.J. Woolhouse, Antibiotic resistance is the quintessential one health issue, Trans. R. Soc. Trop. Med. Hyg. 110 (2016) 377–380, https://doi.org/10.1093/trstmh/trw048.
[22] G. Berg, D. Rybakova, D. Fischer, T. Cernava, M.C.C. Vergès, T. Charles, X. Chen, L. Cocolin, K. Eversole, G.H. Corral, M. Kazou, L. Kinkel, L. Lange, N. Lima, A. Loy, J.A. Macklin, E. Maguin, T. Mauchline, R. McClure, B. Mitter, M. Ryan, I. Sarand, H. Smidt, B. Schelkle, H. Roume, G.S. Kiran, J. Selvin, R.S.C. de Souza, L. van Overbeek, B.K. Singh, M. Wagner, A. Walsh, A. Sessitsch, M. Schloter, Microbiome definition re-visited: old concepts and new challenges, Microbiome 8 (2020) 103, https://doi.org/10.1186/s40168-020-00875-0.
[23] S. Banerjee, M.G.A. van der Heijden, Soil microbiomes and one health, Nat. Rev. Microbiol. 21 (2023) 6–20, https://doi.org/10.1038/s41579-022-00779-w.
[24] P.M. da Costa, L. Loureiro, A.J.F. Matos, Transfer of multidrug-resistant bacteria between intermingled ecological niches: the interface between humans, animals and the environment, Int. J. Environ. Res. Public Health 10 (2013) 278–294, https://doi.org/10.3390/ijerph10010278.
[25] M.A. Argudín, A. Deplano, A. Meghraoui, M. Dodémont, A. Heinrichs, O. Denis, C. Nonhoff, S. Roisin, Bacteria from animals as a Pool of antimicrobial resistance genes, Antibiotics (Basel). 6 (2017) 12, https://doi.org/10.3390/antibiotics6020012.
[26] S. Castañeda-Barba, E.M. Top, T. Stalder, Plasmids, a molecular cornerstone of antimicrobial resistance in the one health era, Nat. Rev. Microbiol. 22 (2024) 18–32, https://doi.org/10.1038/s41579-023-00926-x.
[27] S.L. Liu, X.J. Wang, X.J. Su, Study on the construction of Chongming Ecological Island in Shanghai, Chin. Nation. Conditions Strength. 09 (2020) 56–59 (in Chinese).
[28] F. Luo, G.X. Lu, X.A. Shen, Y.X. Lin, Countermeasures and measures for developing ecological animal husbandry in Chongming Island, Shanghai J. Anim. Husbandry Vet. Med. 06 (2013) 52–53 (in Chinese).
[29] J.M. Hassell, M.J. Ward, D. Muloi, J.M. Bettridge, T.P. Robinson, S. Kariuki, A. Ogendo, J. Kiiru, T. Imboma, E.K. Kang'ethe, E.M. Öghren, N.J. Williams, M. Begon, M.E.J. Woolhouse, E.M. Fèvre, Clinically relevant antimicrobial resistance at the wildlife-livestock-human interface in Nairobi: an epidemiological study, Lancet Planet. Health 3 (2019) e259–e269, https://doi.org/10.1016/S2542-5196(19)30083-X.
[30] D. Muloi, J. Kiiru, M.J. Ward, J.M. Hassell, J.M. Bettridge, T.P. Robinson, B.A. D. van Bunnik, M. Chase-Topping, G. Robertson, A.B. Pedersen, E.M. Fèvre, M.E. J. Woolhouse, E.K. Kang'ethe, S. Kariuki, Epidemiology of antimicrobial-resistant *Escherichia coli* carriage in sympatric humans and livestock in a rapidly urbanizing city, Int. J. Antimicrob. Agents 54 (2019) 531–537, https://doi.org/10.1016/j.ijantimicag.2019.08.014.
[31] D.W. Kim, C.J. Cha, Antibiotic resistome from the one-health perspective: understanding and controlling antimicrobial resistance transmission, Exp. Mol. Med. 53 (2021) 301–309, https://doi.org/10.1038/s12276-021-00569-z.
[32] Y. Guo, L. Ding, Y. Yang, R. Han, D. Yin, S. Wu, D. Zhu, F. Hu, Multicenter antimicrobial resistance surveillance of clinical isolates from major hospitals - China, 2022, China CDC Weekly. 5 (2023) 1155–1160, https://doi.org/10.46234/ccdcw2023.217.
[33] Y. Yang, X. Geng, X. Liu, X. Wen, R. Wu, D. Cui, Z. Mao, Antibiotic use in China's public healthcare institutions during the COVID-19 pandemic: an analysis of Nationwide procurement data, 2018-2020, Front. Pharmacol. 13 (2022) 813213, https://doi.org/10.3389/fphar.2022.813213.
[34] R.F. Potter, A.W. D'Souza, G. Dantas, The rapid spread of carbapenem-resistant Enterobacteriaceae, Drug Resist. Updat. 29 (2016) 30–46, https://doi.org/10.1016/j.drup.2016.09.002.
[35] T. He, R. Wang, D. Liu, T.R. Walsh, R. Zhang, Y. Lv, Y. Ke, Q. Ji, R. Wei, Z. Liu, Y. Shen, G. Wang, L. Sun, L. Lei, Z. Lv, Y. Li, M. Pang, L. Wang, Q. Sun, Y. Fu, H. Song, Y. Hao, Z. Shen, S. Wang, G. Chen, C. Wu, J. Shen, Y. Wang, Emergence of plasmid-mediated high-level tigecycline resistance genes in animals and humans, Nat. Microbiol. 4 (2019) 1450–1456, https://doi.org/10.1038/s41564-019-0445-2.
[36] Y. Cong, S. Yang, X. Rao, Vancomycin resistant *Staphylococcus aureus* infections: a review of case updating and clinical features, J. Adv. Res. 21 (2020) 169–176, https://doi.org/10.1016/j.jare.2019.10.005.
[37] C. Crowe-McAuliffe, V. Murina, K.J. Turnbull, S. Huch, M. Kasari, H. Takada, L. Nersisyan, A. Sundsfjord, K. Hegstad, G.C. Atkinson, V. Pelechano, D.N. Wilson, V. Hauryliuk, Structural basis for PoxtA-mediated resistance to phenicol and oxazolidinone antibiotics, Nat. Commun. 13 (2022) 1860, https://doi.org/10.1038/s41467-022-29274-9.
[38] A. Shariati, M. Dadashi, Z. Chegini, A. van Belkum, M. Mirzaii, S.S. Khoramrooz, D. Darban-Sarokhalil, The global prevalence of Daptomycin, Tigecycline, Quinupristin/Dalfopristin, and linezolid-resistant Staphylococcus aureus and coagulase-negative staphylococci strains: a systematic review and meta-analysis, Antimicrob. Resist. Infect. Control 9 (2020) 56, https://doi.org/10.1186/s13756-020-00714-9.
[39] S.H. Lob, L.E. Nicolle, D.J. Hoban, K.M. Kazmierczak, R.E. Badal, D.F. Sahm, Susceptibility patterns and ESBL rates of *Escherichia coli* from urinary tract infections in Canada and the United States, SMART 2010-2014, Diagn. Microbiol.

Infect. Dis. 85 (2016) 459–465, https://doi.org/10.1016/j.diagmicrobio.2016.04.022.
[40] Y. Chong, S. Shimoda, N. Shimono, Current epidemiology, genetic evolution and clinical impact of extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae*, Infect. Genet. Evol. 61 (2018) 185–188, https://doi.org/10.1016/j.meegid.2018.04.005.
[41] M. Azam, V. Kumar, K. Siddiqui, A.T. Jan, J.S.M. Sabir, I.A. Rather, S. Rehman, Q. M.R. Haq, Pharmaceutical disposal facilitates the mobilization of resistance determinants among microbiota of polluted environment, Saudi Pharm. J. 28 (2020) 1626–1634, https://doi.org/10.1016/j.jsps.2020.10.009.
[42] K.R. Alkharsah, S. Rehman, A. Alnimr, A. Diab, A. Hawwari, S. Tokajian, Molecular typing of mrsa isolates by spa and PFGE, J. King Saud Univer.-Sci. 31 (2019) 999–1004, https://doi.org/10.1016/j.jksus.2018.07.018.
[43] M. Rödenbeck, O. Ayobami, T. Eckmanns, M.W. Pletz, J. Bleidorn, R. Markwart, Clinical epidemiology and case fatality due to antimicrobial resistance in Germany: a systematic review and meta-analysis, 1 January 2010 to 31 December 2021, Euro Surveill. 28 (2023) 2200672, https://doi.org/10.2807/1560-7917.ES.2023.28.20.2200672.
[44] A. Azzam, H. Khaled, M. Mosa, N. Refaey, M. AlSaifi, S. Elsisi, F.K. Elagezy, M. Mohsen, Epidemiology of clinically isolated methicillin-resistant *Staphylococcus aureus* (MRSA) and its susceptibility to linezolid and vancomycin in Egypt: a systematic review with meta-analysis, BMC Infect. Dis. 23 (2023) 263, https://doi.org/10.1186/s12879-023-08202-2.
[45] C.K. Ezeh, C.N. Eze, M.E.U. Dibua, S.C. Emencheta, A meta-analysis on the prevalence of resistance of *Staphylococcus aureus* to different antibiotics in Nigeria, Antimicrob. Resist. Infect. Control 12 (2023) 40, https://doi.org/10.1186/s13756-023-01243-x.
[46] S.J. Dunn, C. Connor, A. McNally, The evolution and transmission of multi-drug resistant *Escherichia coli* and *Klebsiella pneumoniae*: the complexity of clones and plasmids, Curr. Opin. Microbiol. 51 (2019) 51–56, https://doi.org/10.1016/j.mib.2019.06.004.
[47] L.M. Assis, M. Nedeljković, A. Dessen, New strategies for targeting and treatment of multi-drug resistant *Staphylococcus aureus*, Drug Resist. Updat. 31 (2017) 1–14, https://doi.org/10.1016/j.drup.2017.03.001.
[48] D.M. Muloi, E. Jauneikaite, M.F. Anjum, S.Y. Essack, D.A. Singleton, M.R. Kasudi, M.J. Wade, B. Egyir, J.G. Nunn, J.T. Midega, S.J. Peacock, N.A. Feasey, K.S. Baker, R.N. Zadoks, SEDRIC genomics surveillance working group, exploiting genomics for antimicrobial resistance surveillance at one health interfaces, lancet, Microbe 4 (2023) e1056–e1062, https://doi.org/10.1016/S2666-5247(23)00284-7.
[49] E. Jauneikaite, K.S. Baker, J.G. Nunn, J.T. Midega, L.Y. Hsu, S.R. Singh, A. L. Halpin, K.L. Hopkins, J.R. Price, P. Srikantiah, B. Egyir, I.N. Okeke, K.E. Holt, S. J. Peacock, N.A. Feasey, SEDRIC genomics surveillance working group, genomics for antimicrobial resistance surveillance to support infection prevention and control in health-care facilities, Lancet Microb. 4 (2023) e1040–e1046, https://doi.org/10.1016/S2666-5247(23)00282-3.
[50] K.S. Baker, E. Jauneikaite, J.G. Nunn, J.T. Midega, R. Atun, K.E. Holt, K. Walia, B. P. Howden, H. Tate, I.N. Okeke, A. Carattoli, L.Y. Hsu, K.L. Hopkins, D.M. Muloi, N. E. Wheeler, D.M. Aanensen, L.C.E. Mason, J. Rodgus, R.S. Hendriksen, S.Y. Essack, B. Egyir, A.L. Halpin, D.R. MacCannell, J. Campos, P. Srikantiah, N.A. Feasey, S. J. Peacock, SEDRIC genomics surveillance working group, evidence review and recommendations for the implementation of genomics for antimicrobial resistance surveillance: reports from an international expert group, lancet, Microbe 4 (2023) e1035–e1039, https://doi.org/10.1016/S2666-5247(23)00281-1.
[51] USDA, FSIS NARMS Multi-Year Report – 2014-2019 | Food Safety and Inspection Service. http://www.fsis.usda.gov/news-events/publications/fsis-narms-multi-year-report-2014-2019, 2023.
[52] B. Wu, F. Yan, C.W. Fu, Comparison of bacterial resistance surveillance and governance between China and the United States, China Health Rses. 24 (2021) 615–618, https://doi.org/10.13688/j.cnki.chr.2021.210244. [in Chinese].
[53] H. Pan, X.L. Li, W. Fang, M. Yue, Analysis of significant human and foodborne pathogens and their resistance to antimicrobials in the USA in the past two decades: implications for surveillance and control of antimicrobial resistance in China, J. Zhejiang University (Agricult. & Life Sci.). 44 (2018) 237–246 (in Chinese).
[54] S. Argimón, M.A.L. Masim, J.M. Gayeta, M.L. Lagrada, P.K.V. Macaranas, V. Cohen, M.T. Limas, H.O. Espiritu, J.C. Palarca, J. Chilam, M.C. Jamoralin, A.S. Villamin, J. B. Borlasa, A.M. Olorosa, L.F.T. Hernandez, K.D. Boehme, B. Jeffrey, K. Abudahab, C.M. Hufano, S.B. Sia, J. Stelling, M.T.G. Holden, D.M. Aanensen, C.C. Carlos, Integrating whole-genome sequencing within the National Antimicrobial Resistance Surveillance Program in the Philippines, Nat. Commun. 11 (2020) 2719, https://doi.org/10.1038/s41467-020-16322-5.