

Complex dynamic patterns of antimicrobial resistance of *Escherichia coli* isolated from poultry and swine over 10 years in Chongming Island, Shanghai

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Research Article

Keywords: Antimicrobial resistance (AMR), *Escherichia coli*, food animals, longitudinal trend analysis, Chongming Island, Shanghai

Posted Date: June 17th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1748690/v1>

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Abstract

Background:

Antimicrobial resistance (AMR) is one of the most serious threats to animal and public health. Here, we conducted a dynamic surveillance of *E. coli* on Chongming Island in Shanghai during 2009-2021 to reveal Chongming's specific epidemic AMR characteristics and trends.

Methods:

Rectal (cloaca) swabs from four poultry and nine swine farms were collected for strain culture and isolation. The routine micro-broth dilution method was used to test antimicrobial susceptibility of *E. coli* isolates against 9 antibiotic classes including 15 antimicrobials. Utilizing generalized linear mixed models (GLMMs) and co-occurrence analyses, we further explored the multidrug-resistant (MDR) combinations and dynamic patterns of *E. coli* over 10 years in two food animals.

Results:

853 MDR isolates were found among 945 collected *E. coli* isolates, 337 from poultry and 608 from swine. Both animal isolates exhibited high resistance rates (>70%) to tetracyclines, phenicols, sulfonamides, β -lactams, and aminoglycosides (only in swine). The resistance to the four antibiotic classes listed above (excluding sulfonamides) was significantly greater in swine isolates than in poultry isolates, whereas fluoroquinolones resistance was reversed. Resistance to polymyxins decreased similarly in swine (42.4% in 2009 to 0% in 2021) and poultry isolates (16.5% to 0%). However, resistance to the other seven antibiotic classes (excluding carbapenems) declined dramatically only in swine isolates, particularly fluoroquinolones (from 80.5% to 14.4%). Surprisingly, except for fluoroquinolone resistance, which showed a modest decline in poultry isolates, the resistances to the other seven antibiotic classes showed markedly divergent patterns. Using Poisson GLMMs, despite the highly diverse MDR profiles, the most prevalent MDR combinations were aminoglycosides-tetracyclines-sulfonamides in poultry and aminoglycosides-phenicols-fluoroquinolones in swine. In addition, the beta-lactams-cephalosporins and aminoglycosides-polymyxins patterns in both animals exhibited strong coefficients, indicating an enhanced synergistic risk.

Conclusion:

Our study uncovered vastly distinct AMR patterns and dynamic tendencies of poultry and swine isolates from Chongming. Due to the ongoing implementation of national anti-AMR strategies, Chongming's AMR status has improved, as indicated by the decline in MDR prevalence (particularly in swine) and low resistance to carbapenems, cephalosporins, and polymyxins. Finally, Chongming Island can use these findings to execute the One Health plan to tackle AMR.

Background

Alexander Fleming, inventor of penicillin and pioneer of the era of antibiotics, once warned that overuse of penicillin would cause resistant bacteria emerging, rendering this life-saving medication ineffective [1].

Antimicrobial resistance (AMR) has, as expected, become a global concern [2–4]. As the number of AMR bacteria has increased exponentially over the past decade, an increasing number of AMR bacteria is eluding the defenses of the last-resort antibiotics of carbapenems, tigecycline and colistin. And it is even more worrisome that some of these bacteria are multiple-drug-resistant (MDR), extensively-drug-resistant (XDR), and even pan-drug-resistant (PDR) [5–8]. AMR is estimated to kill 700,000 people worldwide annually, and by 2050, this figure will have reached 10 million [9]. Apart from the ongoing spread of AMR, the previously robust antibiotic development pipeline has dwindled to a trickle of effective molecules [10, 11]. Dr. Tedros Adhanom Ghebreyesus, the director-general of the World Health Organization (WHO), stated at the 2019 World Health Assembly: "Together with our partners, we are intensifying the battle against antimicrobial resistance, one of the most critical health concerns of our time." [12] Certainly, China is also affected by the crisis, which is not surprising given that China is the world's largest producer and consumer of antibiotics [13, 14].

Antibiotics are a primary driver of AMR bacteria; as antibiotic use increases, so does the incidence of AMR [15]. Approximately 73% of all antibiotics sold worldwide are used on food animals, particularly poultry and swine produced in intensive animal production systems [16, 17]. Compared to humans, low (sub-therapeutic) amounts of antibiotics in the feed of food animals are used largely for growth promotion and mass prevention. Admittedly, this low-dose, prolonged antibiotic use exacerbates the onset and spread of AMR, despite the widespread acceptance of antibiotics in animal husbandry in more than half of the world's countries [18–20]. Therefore, food animals of poultry and swine are implicated as AMR reservoirs [21]. It has been proven that China and India have the highest levels of AMR in their animal populations [22]. Importantly, AMR bacteria in food animals might be transmitted directly to farm workers, as well as indirectly to a larger population through contaminated food, water, and soil, posing a threat to public health [23, 24].

Among the various microorganisms, *Escherichia coli* (*E. coli*) occupies a unique position due to its capacity to serve as a harmless commensal in the digestive tract of humans and animals, but also to cause serious infections which may result in life-threatening or economic losses [25]. In a 2019 comprehensive study of global AMR bacterial burden, *E. coli* was identified as one of the major pathogens accounting for 929,000 deaths attributable to AMR and 3.57 million deaths associated with AMR [26]. In particular, the ability of extended-spectrum beta-lactamase (ESBL)-producing *E. coli* to hydrolyze third-generation cephalosporins poses a significant threat to global health [27]. *E. coli* is also the pathogen with the highest rate of clinical isolates in China [28]. Moreover, *E. coli* possesses a diverse array of resistance characteristics, relatively open pan-genomes, a high capacity to acquire antimicrobial resistance genes (ARGs) via horizontal gene transfer, and the ability to act as both a donor and a recipient in ARGs transmission [29]. In general, *E. coli* has been utilized as a biomarker to detect AMR in numerous medical or environmental situations, including food animal farms, hospitals, and the interfaces between wild animals, domestic animals, and human beings [30, 31]. In the meantime, AMR in *E. coli* occurs in both human and animal populations and could be transmitted to a vast number of species via environmental pathways. In the context of One Health, it is also a perfect object for multisector and interdisciplinary AMR disposal.

The majority of high-income nations have implemented AMR surveillance in animals for more than a decade; however, monitoring data on AMR are scarce in low- and middle-income countries (LMICs) [32]. Given the

highest AMR burden are in LMICs, robust measures based on evidence data of surveillance are crucial, and data collection is the first substantial step [26]. Shanghai, as a representative of China's megacities, is one of the first provinces to conduct AMR surveillance on animals in 2008, and the surveillance area covered Chongming Island in the second year [33]. Chongming Island has a direct impact on Shanghai's food security as one major supplier of meat, eggs, and milk. Food and environmental security are likely to be of the utmost importance for the establishment of the Chongming global ecological island. Concurrently, for the establishment of the Chongming global ecological island, food and environmental security are expected to be of paramount importance. Concurrently, the One Health concept was developed to address health concerns and contribute to the achievement of the global ecological island goals. In this study, we investigate the surveillance data of AMR in poultry and swine since 2009 to determine the present profiles and trends of *E. coli* AMR in Chongming Island's food animals. Prior to initiating the One Health project to reduce the prevalence of AMR on Chongming Island, this study will give a comprehensive understanding of AMR in food animals and serve as a reference for baseline data. This is, to the best of our knowledge, the first regional longitudinal investigation of AMR in food animals over a 10-year period, particularly in LMICs.

Methods

Sample collection and identification of *E. coli* isolates

Chongming Island, located near the estuary of the Yangtze River, was selected as the sampling site for targeted poultry and swine farms. The sampling period began in 2009, and lasted until 2021. No strains were collected in 2010, 2011 and 2012 (only in poultry). At least one poultry or swine farm was chosen annually to collect swabs of fresh excrement or rectal (cloaca) swabs of swine and poultry. All samples originated from four poultry and nine swine farms, with poultry farm P4 being only sampling farm since 2014 and one swine farm (S5) with a 5-year sampling period. Figure 1 depicted the distribution of sampling farms and times.

Individual sample was collected following the routine protocols outlined below. In brief, a sterilized cotton swab was inserted into the pig anus or poultry cloaca for 1.5-2.0 cm and rotated 2–3 times. After adding 10 ml of sterile transport medium, the feces-stained swab was stored at enclosed and homiothermic (0–4 °C) units for shipment within 24 hours. Then the swabs were inoculated at 36°C for 18–24 hours with *E. coli* coliform chromogenic media (Hopebio biotech company, Qingdao, China). One small, purified blue/purple spherical colony was chosen from each plate and sub-cultured for an additional 18–24 hours on chromogenic media. The pure colony was then identified as *E. coli* using a microbial biochemical identification system (VITEK 2 Compact, Biomerieux, France). The confirmed isolates were stored at -80°C.

Testing for antimicrobial susceptibility

A total of 15 types of antibiotics belonging to 9 classes, namely beta-lactam (ampicillin and amoxicillin-clavulanic acid), cephalosporins (ceftiofur and ceftazidime), carbapenems (meropenem), aminoglycosides (spectinomycin and gentamicin), tetracyclines (doxycycline and tetracycline), phenicols (florfenicol), sulfonamides (sulfaisoxazole and sulfamethoxazole), fluoroquinolones (enrofloxacin and ofloxacin), and polymyxins (colistin) in China, were included in the antimicrobial susceptibility testing (AST). Among of them, ceftiofur, florfenicol, and enrofloxacin were used for livestock and fowl specially, ofloxacin and colistin have

been banned from using in food animals in China. The minimum inhibitory concentration (MIC) values of 15 antibiotics were determined using the microbroth dilution protocol (CLSI M100, 28th edition) recommended by the Clinical & Laboratory Standards Institute (CLSI), and the results were interpreted using the CLSI recommend breakpoint for each antibiotic. In addition, three types of antibiotics namely ceftazidime (2016), spectinomycin (2012) and meropenem (2016) were added to the surveillance program, and doxycycline was employed only in 2012–2017. *E. coli* ATCC25922 was selected as a quality control organism for all antimicrobial susceptibility testing.

Statistical analysis

The differences between poultry and swine in the phenotypic resistance rates for antibiotic classes and the prevalence of resistant to antibiotics were determined using Chi-square testing, and *P*-values of 0.05 were considered statistically significant. The Chi-square tests were conducted using IBM SPSS Statistics 22 and the graphics were created using GraphPad Prism 9 and OriginLab Origin 2021. GraphPad Prism 9 was used to present the dynamic trends of phenotypic resistance rates and the resistance prevalence of antibiotics.

To define the MDR patterns, the overall resistance profile was calculated by combining the resistance phenotype to each individual class. Then the antibiogram length (also called "AMR carriage") was defined as the total number of antibiotic classes to which an isolate was phenotypically resistant [30]. Subsequently, the antibiogram length was chosen as dependent variable to test whether it differed between the two hosts and the different years by utilizing generalized linear mixed models (GLMMs), and the analysis was implemented in the "lme4" R package. In the meantime, the antibiogram length profiles from two to seven of poultry and swine were visualized as stacked histogram generated by GraphPad Prism 9. To investigate the co-occurrence of AMR phenotypes, a pairwise co-occurrence matrix of the 9 classes of antibiotic resistance phenotypes (absence and presence) was constructed. The "polycor" package in R was adopted, and the "corrplot" package was utilized to visualize the matrix. To detect a correlation between two phenotypes, a *P*-value of 0.05 was chosen as statistically significant.

Results

Sampling information of *E. coli* isolates

Over a sampling period of more than a decade (2009 to 2021, excluding 2010 and 2011) on Chongming Island, a total of 945 *E. coli* isolates were collected, comprising 337 poultry isolates and 608 swine isolates, from four poultry farms (Coded P1-P4) and nine swine farms (S1-S9). The number of isolates collected varies annually. There were the maximum isolates obtained in 2009, with 103 chicken and 118 swine strains. However, the minimum number was obtained for poultry isolates in 2019, with ten isolates, and for swine isolates in 2016, with 29 isolates. Among all poultry isolates, 243 breeding-hen isolates (P1, P4) and 56 layer-hen isolates (P2) were identified. In swine, 60 isolates from weaned pigs were obtained from S8 (5 isolates), S5 (35 isolates), and S7 (20 isolates), while 265 isolates from growing pigs were collected from S8 (25), S5 (73), S6 (58), S7 (65), and S9 (44) farms. The distribution of farms, the sampling period, and the quantity of *E. coli* isolates collected were depicted in Fig. 1 and Table S1 (Supplementary files).

The status of AMR in poultry and swine *E. coli* isolates

The AST results revealed that both poultry and swine isolates were more than 70% resistance rates of phenicols, tetracycline, sulfonamides, and beta-lactam, with the highest 86.1% for tetracycline in poultry and 92.6% for florfenicol in swine. The resistance rate of aminoglycosides in swine isolates was also more than 70%. Only one swine isolate displayed resistance phenotype in 2019. The resistance rates of swine isolates for beta-lactams, aminoglycosides, tetracyclines, phenicols and polymyxins were significantly higher than those of poultry isolates ($P < 0.01$), whereas the resistance rates of poultry isolates for fluoroquinolones were significantly higher than those of swine isolates. There were no differences in resistance rates of sulfonamides and cephalosporins between the two hosts (Fig. 2A, Table 1). In addition, the resistance rates of breeding-hen, layer-hen, weaned pigs and growing pigs were shown Fig S1(A, B) and Table S2.

Table 1

Resistant rates for various antibiotic classes and the resistance prevalence of antibiotics in *E. coli* isolates, categorized by host.

Antibiotic classes	Poultry (%) (n = 337)	Swine (%) (n = 608)	P value	Antibiotic agents	Poultry (%) (n = 337)	Swine (%) (n = 608)	P value
beta-lactam	75.7	86	< 0.001	Ampicillin	75.4	85.9	< 0.001
				Amoxicillin/clavulanic acid	48.7	42.3	NS
Cephalosporins	23.1	22.5	NS	Ceftiofur	23.1	22.5	NS
				Ceftazidime	6.3	1.9	0.012
Carbapenems	0	0.03	NS	Meropenem	0	0.03	NS
Aminoglycosides	46.3	70.4	< 0.001	Spectinomycin	46.6	65.7	< 0.001
				Gentamicin	19.2	37.2	< 0.001
Tetracyclines	86.4	91.8	0.008	Doxycycline	71.3	85.9	< 0.001
				Tetracycline	86.1	91.6	0.007
Phenicols	76.6	92.6	< 0.001	Florfenicol	76.6	92.6	< 0.001
Sulfonamides	85.2	87.2	NS	Sulfaisoxazole	82.8	85.4	NS
				Sulfamethoxazole	71.5	75.2	NS
Fluoroquinolones	52.5	43.3	0.006	Enrofloxacin	49.8	41.3	0.027
				Ofloxacin	46.3	39	0.029
Polymyxins	7.7	21.9	< 0.001	Colistin	7.7	21.9	< 0.001
NS, not significant.							

Among all isolates from both hosts, the prevalence of resistant to ampicillin, doxycycline, tetracycline, florfenicol, sulfaisoxazole and sulfamethoxazole were more than 70%, while resistance to ceftazidime, meropenem were less than 10%. In swine isolates, the highest resistance prevalence was 92.6% for florfenicol which was the most commonly used phenicols class in veterinary practice, and in poultry isolates, the highest resistance prevalence was 86.1% for tetracycline which was another the most widely used antibiotic in animal husbandry. There was a higher prevalence of resistance to three antibiotics (ceftazidime, enrofloxacin, and ofloxacin) in poultry isolates than in swine isolates, but the opposite was true for the

remaining seven antibiotics (Fig. 2B, Table 1). In addition, the resistance prevalence for antibiotics of breeding-hen, layer-hen, weaned pigs and growing pigs were shown Fig S1(C, D) and Table S2.

AMR Trends in Poultry and Swine

Two antibiotic classes, polymyxins and carbapenems, exhibited similar trends of resistance rate between poultry and swine isolates. The rate of resistance to carbapenems has remained essentially unchanged at 0.0% since it was included in the surveillance plan in 2016. In contrast, poultry isolates resistance to polymyxins decreased from 16.5% in 2009 to 0% in 2021, while swine isolates resistance rates decreased from 42.4–0%. For the other seven classes, the resistance trends in poultry and swine isolates exhibited unique patterns. Apart from beta-lactam, the resistance rates of the other six classes in swine fell significantly, with fluoroquinolones exhibiting the greatest decline from 80.5–14.4%. Moreover, the decrease ranges of classes of cephalosporins and aminoglycosides in swine were also larger (cephalosporins: from 53.4% in 2009 to 12.6% in 2021, aminoglycosides: from 73.7–52.3%). Despite the decreasing trends of tetracyclines, phenicols and sulfonamides in swine isolates, the resistance rates in 2021 were still more than 65.0%, and the decline of these three classes became clear after 2018. For poultry isolates, only fluoroquinolones showed a slight decline trend (from 68.0–50.0%), meaning that the other six classes of antibiotics had no decline trends. In 2021, resistance rates to aminoglycosides, tetracyclines, phenicols, sulfonamides, and beta-lactam were all more than 70% in poultry (Fig. 3).

The incidence of ceftazidime resistance was low in both poultry and swine isolates. therefore, no clear trends were found. Resistance to amoxicillin/clavulanic acid, gentamicin, and doxycycline has decreased in poultry and swine isolates, and amoxicillin/clavulanic acid resistance has decreased by more than 80%. Resistance to the remaining eight antibiotics (ampicillin, ceftiofur, spectinomycin, tetracycline, enrofloxacin, ofloxacin, sulfaisoxazole, and sulamethoxazole) varied greatly between poultry and swine isolates. Other than ampicillin, seven of the other antibiotics showed declining trends in swine, with the most notable declines in ceftiofur (from 53.6–12.4%), spectinomycin (from 91.3–50.5%), enrofloxacin (from 76.3–14.4%), ofloxacin (from 78.8–11.7%), and sulfamethoxazole (from 98.3–57.7%). Comparatively, Antibiotic resistance patterns in poultry were complex, with an upward trend for tetracycline, an initially rising and then declining trend for sulfaisoxazole, a falling and rising trend for ampicillin, a slight decline trend for enrofloxacin and ofloxacin, and no discernible trends for ceftazidime, spectinomycin, or sulfamethoxazole (Fig. 4).

MDR combinations in poultry and swine isolates

Of the 945 isolates from poultry and swine tested, only five (four in 2018 and one in 2020) were susceptible to all 15 antibiotics. None of the isolates was resistant to all antibiotic classes. There were 853 multidrug-resistant isolates (with resistance to at least three different antibiotic classes, MDR), with most being resistant to four to seven antibiotic classes (Fig. 5A). MDR distribution patterns usually fluctuated annually. While non-MDR poultry isolates peaked in 2018, non-MDR swine isolates peaked in 2020. In poultry, only the isolates collected in 2021 were all MDR strains, while all swine isolates collected prior to 2017 were MDR (Fig. 5B).

The Poisson GLMMs were used to investigate the probability of AMR carriage (also called antibiogram lengths, defined as the total number of antibiotic classes to which an isolate was resistant) for the hosts and the sampling years. The antibiogram length of swine was significantly higher than that of poultry [odds ratio (OR) = 1.19, 95% confidence interval (CI): 1.12–1.27, $P < 0.001$]. Based on all isolates of poultry and swine, antibiogram lengths from 2012 to 2015 did not present significant differences with those in 2009 ($P = 0.871, 0.811, 0.130, 0.404$), while antibiogram lengths in all years after 2016 were significantly lower than that of 2009 ($OR < 1, P = 0.015$ in 2016, $P < 0.001$ from 2017 to 2021, Table 2).

Table 2
Results of a Poisson generalized linear mixed model examining the likelihood of antibiogram length within the two hosts and different years

	No. isolates	Estimate	SE	Z score	Pvalue
Hosts					
Poultry	337	Reference			
Swine	608	0.175	0.033	5.39	< 0.001
Year					
2009	221	Reference			
2012	46	0.011	0.067	0.163	0.871
2013	77	0.013	0.055	0.239	0.811
2014	75	-0.088	0.058	-1.515	0.130
2015	60	-0.052	0.062	-0.834	0.404
2016	47	-0.175	0.072	-2.421	0.015
2017	79	-0.211	0.060	-3.530	< 0.001
2018	73	-0.302	0.064	-4.731	< 0.001
2019	60	-0.265	0.067	-3.926	< 0.001
2020	76	-0.450	0.066	-6.804	< 0.001
2021	131	-0.390	0.053	-7.381	< 0.001

Seventy-five antibiotic combination profiles of poultry and swine *E. coli* isolates were found between 2009 and 2021: 17 in poultry, 19 in swine, and 39 common to both (Supplementary Table S2). Then, we evaluated the variations and tendencies of antibiogram length profiles ranging from two to seven (Fig. 6, Table S2). We defined the dominant profile as appearing almost every year and comprising the majority of isolates, and we defined profile diversity as the number of distinct profiles in certain years. Overall, we identified the evident dominant profiles in antibiogram length of four (beta-lactam-tetracyclines-phenicols-sulfonamides), antibiogram length of five (beta-lactam-tetracyclines-phenicols-sulfonamides-fluoroquinolones in poultry and beta-lactam-aminoglycosides-tetracyclines-sulfonamides-fluoroquinolones in swine), antibiogram length of

six (beta-lactam-cephalosporins-aminoglycosides-tetracyclines-phenicols-sulfonamides in poultry and beta-lactam-aminoglycosides-tetracyclines-phenicols-sulfonamides-fluoroquinolones in poultry and swine), as well as antibiogram length seven (beta-lactam-cephalosporins-aminoglycosides-tetracyclines-phenicols-sulfonamides-fluoroquinolones). None of dominant profiles were detected in antibiogram length of two (only in swine) and three (Fig. 6).

The highest diversity in poultry happened in 2009 with 27 distinct profiles and the lowest occurred in 2019 with 7 profiles, whereas the most diversity in swine occurred in 2021 with 31 profiles and the least in 2018 with 7 profiles (Table S2). When analyzed by antibiogram lengths, the highest diversity in poultry mainly centralized in the first few years of surveillance, such as the highest diversity of antibiogram length of three occurred in 2014 with 6 distinct profiles. Conversely, the greatest diversity of antibiogram length profiles in swine was primarily dispersed in 2021 (except for antibiogram lengths of six and seven in 2009). Furthermore, the number of isolates in each profile was not uniform; poultry and swine isolates were the most numerous in one or two profiles in every year (Fig. 6).

Co-occurrence analysis revealed two obvious clusters for beta-lactam-phenicols-fluoroquinolones in poultry and aminoglycosides-tetracyclines-sulfamides in pigs ($P < 0.05$, correlation coefficient > 0.5). The correction coefficient of two pairs of AMR phenotypes, beta-lactam-cephalosporins, and aminoglycosides-polymyxins, had correction coefficients of more than 0.5 in poultry, indicating a greater likelihood of co-occurrence. Two pairs of phenotypes (tetracyclines-polymyxins and cephalosporins-tetracyclines) exhibited a negative connection; nevertheless, the correlation is weak ($r < 0.5$, Fig. 7A). Furthermore, swine isolates had a higher number of co-occurrence pairs (9 pairs) with correlation coefficients more than 0.5, with the highest pair being sulfamides-polymyxins (0.88, $P < 0.01$). It was also noteworthy to see that fluoroquinolones had a higher likelihood of co-occurrence with polymyxins (0.66, $P < 0.01$), while polymyxin resistance was not the predominant resistance trait in swine (Fig. 7B).

Discussion

In this work, we offer a comprehensive report on the surveillance of isolates from poultry and swine farms on Chongming Island, which is located near the estuary of the Yangtze River in Shanghai, China. This is a longitudinal study with a sample period of more than ten years, as opposed to the cross-sectional studies previously undertaken on food animal farms in various regions of China. As far as we are aware, this work complements shorter-term dynamic surveillance studies.

In general, the prevalence of *E. coli* AMR in Chongming Island's food animals is alarming. Both poultry and swine *E. coli* isolates are highly resistant to tetracyclines, phenicols, sulfamates, beta-lactam ($> 70\%$), and aminoglycosides, fluoroquinolones ($> 40\%$). According to past reports on antibiotic usage in China, the top three antibiotics used in food animals are tetracyclines (45.90%), beta-lactam (10.87%), and macrolides (9.72%), and this high level of resistance may be attributable to the quantity of antibiotics usage [16, 22]. Given the early application of tetracyclines (1960s), sulfonamides (1960s), and beta-lactam (1970s), the use time may also be a key factor affecting resistance [34, 35]. In addition, swine isolates are more resistant to most antibiotic classes than poultry isolates (except for cephalosporins and fluoroquinolones), especially in beta-lactam, aminoglycosides, tetracyclines, phenicols, and polymyxins ($P < 0.001$); possibly because of

antibiotic usage in swine is significantly higher than in poultry in China (52.2% Vs 19.6%) [36]. However, antibiotic consumption data was not collected from each farm in this study, and we assume that the antibiotic dosage in Chongming Island is the same as that in other regions based on east China is the region with the most quantity of antibiotic usage [36]. Interestingly, according to Zhang's research of antibiotic usage in China, the resistance prevalence differences of three antibiotics (amoxicillin/clavulanic acid, ofloxacin, and enrofloxacin) between swine and poultry do not support the phenomenon that high usage is associated with high AMR occurrence [36].

Since 2013, the Chinese government has adopted a series of policies to control, minimize, and prohibit animal antibiotic use [33, 37, 38]. From the AMR trend in swine, six of nine antibiotic classes and twelve of fifteen antibiotics have a downward trend, whereas the majority of the AMR for antibiotics in poultry (excluding classes of fluoroquinolones and polymyxins, and antibiotic agents of amoxicillin/clavulanic acid, gentamicin, doxycycline, sulfisoxazole, and colistin) have no obvious downward trend. We deduced that the explanation for poultry's low sensitivity to policy is its smaller size and lower disease resistance compared to swine [39, 40]. Another reason may be that the policy directly influences the prevalence of AMR by altering antibiotic usage, and the amount of antibiotics used in swine is far larger than in poultry. In addition, the sampling size in poultry may be insufficient, leading to fluctuation between surveillance years. In 2015 and 2016, the Chinese government prohibited the use of ofloxacin as an antibacterial agent in food animals and the use of colistin as an animal growth-promoting feed ingredient [41, 42]. The persistent decline of the AMR trend in poultry and swine demonstrates the undeniable efficacy of these two policies [43]. The prevalence of resistance to colistin has decreased to 0% in 2017 (0% in 2016 for poultry), but the incidence of resistance to ofloxacin is still over 10% (an upward trend in poultry from 2019). The high rate of resistance in past years, the difficulties of regulating farm rather than feed enterprises, and enrofloxacin's synergistic effect in AMR ($r = 0.88$ in poultry and $r = 0.90$ in swine; data not shown) all contribute to the higher incidence of resistant to ofloxacin.

Another characteristic of the AMR problem is the emergency and rapid development of MDR [44, 45], and this study also reveals a high prevalence of MDR (90.3%). AMR carriage was defined to represent the features of MDR *E. coli* isolates. The higher AMR carriage in swine was consistent with the higher prevalence of AMR in this species. The dramatic drop in AMR carriage since 2016 in two hosts is also persuasive evidence of the positive impact of antibiotic policies that restrict or prohibit their use in food animals. Whole-genome sequence (WGS) undertaken on 31 Swiss farms revealed that clonal transmission from animal to animal or via contaminated stable surfaces, rather than the introduction of new strains, was the predominant mode of transmission [46]. Several dominant profiles (which appear in multiple years and have a preponderance of isolates number) are identified in analysis of trends of AMR carriage profiles ranging from two to seven in our study, and we infer that they may always exist in the farm in the absence of all-in and all-out management and good hygiene. In addition, some resistance phenotypes in poultry and pigs may be synergistic [30]. As demonstrated by this study, β -lactam-phenicols-fluoroquinolones in poultry and aminoglycosides-tetracyclines-sulfamides in swine are evident co-occurrence phenotype clusters ($r > 0.5$, $P < 0.01$), indicating potential circulating conjugative MDR plasmids within the *E. coli* population of the two hosts, particularly the cluster of aminoglycosides-tetracyclines-sulfonamides with the reason that antibiotic resistance genes

(ARGs) conferring resistance phenotypes to tetracyclines and sulfonamides are typically attributed to mobile genetic elements [47, 48].

One of the five objectives of the Global Action Plan on Antimicrobial Resistance issued by WHO, FAO, and OIE, is to strengthen the knowledge and evidence basis through surveillance [49]. AMR Surveillance in animals is inadequately documented in LMICs, particularly data on continuous, systematic longitudinal surveillance, although point prevalence surveys are important surveillance measures [32]. However, local prevalence surveys are imperfect substitutes for surveillance networks and may not reveal the dynamics of AMR within farms, even in the regions, or national level. Several investigations on the dynamics of extended-spectrum cephalosporin-resistant *E. coli* (ESC-R-Ec) in European pig farms concluded that longitudinal surveillance is necessary since the presence of ESC-R-Ec fluctuates throughout a pig's lifetime [50, 51]. Certainly, the pattern of AMR differs globally, with different bacteria and bacteria–drug combinations predominating in different places and resulting in a variety of surveillance strategies. Moreover, as the world's largest producer of poultry and swine as well as the greatest producer and consumer of antibiotics, understanding the current status of AMR in food animals in China is crucial for worldwide AMR surveillance [16, 32]. According to Daniel et al systematic review, eastern China and India will benefit the most from future surveillance initiatives [52]. Consequently, this 10-year longitudinal surveillance of AMR in food animals on Chongming Island is part of China's animal AMR surveillance network and a supplement to the absence of animal AMR surveillance in LMICs.

Due to its relative isolation and flourishing animal husbandry, Chongming Island is a good environment for AMR surveillance based on empirical evidence. In the process of constructing the global ecological island, the potential threat to environmental health, food security, and public health posed by AMR in *E. coli* generated from food animals must be addressed. One Health is regarded as the most effective strategy for coordinating many sectors of animals, public health, and the environment to combat health risks, such as zoonosis, AMR, and food security, and so on [53–55]. Hence, Chongming Island will be regarded as the ideal regions for implementing One Health to combat AMR. In addition, our research demonstrates that swine can serve as an indicator animal for evaluating One Health strategies. Moreover, there are two major limitations to our research. The lack of critical data from animal farms, such as the number of animals present, antibiotics used, and disease reports, as well as the low number of animal samples, is the primary limitation of our study. Secondly, the study lacks genomic sequence data. Combining genomic ARGs and mobile genetic elements with phenotypic data will offer more convincing information for deciding how to combat AMR.

Conclusion

Our study focused on Chongming Island's AMR *E. coli* isolates from food-producing animals during 2009–2021 revealed an alarming scenario with > 90% MDR phenotypes and high resistance to phenicols, tetracycline, sulfonamides, beta-lactam, aminoglycosides, and fluoroquinolones. The AMR trend patterns of poultry and swine isolates are distinct: the resistance phenotypes to the majority of antibiotic classes and the prevalence of resistance to the majority of antibiotics decreased in swine isolates, while poultry isolates exhibited less pronounced downward trends. AMR carriage in swine isolates was much higher than in poultry,

and distinct MDR clusters were discovered, as determined by GLMMs and co-occurrence analyses. Given food animals as the primary source of animal protein for humans, the One Health approach should be implemented to coordinate the public health and veterinary sectors in order to combat the AMR threat. In addition, this surveillance work on food animals is an important contribution to the food animal surveillance network in LMICs.

Declarations

Acknowledgements

The authors would like to thank all participants for participating in this study.

Funding

This study was supported by the National Natural Science Foundation of China (Grant No. 32170141), China Medical Board (no. 20-365) and Shanghai Jiao Tong University Integrated Innovation Fund (no. 2020-01).

Authors' contributions

YZZ and SXH conceived and designed experiments. CL, JS, and WGZ performed the whole data integration and analysis, ML, CYG and NZ collected all the AMR datasets from multiple databases. CL and YZZ wrote the first draft of the manuscript. XNZ, XKG, and SXH improved this research and edited the manuscript. All authors approved the submitted version.

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Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

Competing interests

The authors declare that there are no competing interests.

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Figures

Figure 1

Distribution of sampling farms in Chongming Island and the number of *E. coli* isolates collected from 2009 to 2021. The brown, sky blue, and pink zones denoted municipalities with both poultry and swine farms, swine farms and poultry farms. The gray regions had no sample sites. The bar graph in the lower-left corner displayed the number of isolates gathered for each year.

Figure 2

Radar charts showing percentages of *E. coli* isolates resistant to nine antibiotic classes and fifty antibiotics. Asterisks indicate statistically significant variations in the resistance phenotype or prevalence of specific

antibiotics between swine and poultry, * $P<0.05$, ** $P<0.01$. AMP- ampicillin, AMC-amoxicillin/clavulanic acid, TIO-ceftiofur, CAZ-ceftazidime, MEM-meropenem, SPT-spectinomycin, GEN-gentamicin, DOX-doxycycline, TCY-tetracycline, FLR -florfenicol, SUL-sulfaisoxazole, SXT-sulfamethoxazole, ENR-enrofloxacin, OFX-ofloxacin, COL-colistin.

Figure 3

Trends in the proportion of *E. coli* isolates resistant to various antibiotic classes from 2009 to 2021. Only one antibiotic was selected for each of the classes of phenicols, polymyxins and carbapenems.

Figure 4

Trends in the prevalence of antibiotic-resistant *E. coli* isolates from 2009 to 2021. AMC-amoxicillin/clavulanic acid, AMP-ampicillin, TIO-ceftiofur, CAZ-ceftazidime, SPT-spectinomycin, GEN-gentamicin, DOX-doxycycline, TCY-tetracycline, ENR-enrofloxacin, OFX-ofloxacin, SUL-sulfaisoxazole, SXT-sulfamethoxazole. The trends of meropenem, florfenicol and colistin were same as the classes of carbapenems, phenicols and polymyxins.

Figure 5

Number of antibiotic classes (A) and percentage of antibiotic classes annually (B).

Figure 6

Variations and trends in antibiogram length from two and seven. Blac-beta-lactam, ceph-cephalosporins, carb-carbapenems, amin-aminoglycosides, tetr-tetracyclines, phen-phenicols, sulf-sulfonamides, fluo-fluoroquinolones, and poly-polymyxins. Antibiotic classes are not ranked in any particular order across all profiles.

Figure 7

Correlations between antimicrobial resistance characteristics in *E. coli* isolates from poultry (A) and swine (B). The numbers within the boxes reflect values for the correction coefficient (r). The legends beneath the two heat maps indicate whether the link between resistance phenotypes is positive (closer to 1; darker blue) or negative (less than 1; lighter blue) (closer to -1, dark red). * $P<0.05$, ** $P<0.01$.

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