communications medicine

A Nature Portfolio journal

Article

https://doi.org/10.1038/s43856-025-00950-3

Genomic insights into antibiotic-resistant non-typhoidal *Salmonella* isolates from outpatients in Minhang District in Shanghai

Panpan Lv[1,7], Yuhang Pei[2,7], Yue Jiang[1], Qiang Wang[1], Yue Liu[3], Mengqi Qu[2], Xuebin Xu[3] ✉, Mingliang Chen[1] ✉ & Yanan Wang[2,4,5,6] ✉

Abstract

Background *Salmonella enterica* (*S. enterica*) causes tens of thousands of cases of diarrheal disease worldwide each year. However, our understanding of the genome and transmission dynamics of *S. enterica* in Minhang District in Shanghai, China is still insufficient. This study is aimed to better understand the population structure, antibiotic resistance patterns, and evolution dynamics of local strains.

Methods We sequenced 458 *S. enterica* strains from outpatients at Minhang District Central Hospital in Shanghai, China, from 2012 to 2021. Bioinformatics analyses on antibiotic resistance genes, virulence factors, mobile genetic elements, pathogenic islands, and phylogenetic relationships were performed.

Results Here we show that two dominant serovars are *S*. Enteritidis and *S*. Typhimurium isolated from outpatients in Minhang District in Shanghai, China. A total of 40 serovars and 53 sequence types (STs) are identified, two *S*. Montevideo strains isolated in 2013 belong to a newly identified ST10844, which is firstly identified in Minhang District in Shanghai, China. More than half of the isolates show resistance to fluoroquinolones and beta-lactams. Notably, 259 (56.6%) of the 458 isolates exhibit a multidrug-resistant pattern. Third-generation cephalosporin resistance gene $bla_{\text{CTX-M-55}}$ is identified in 15 (3.3%) isolates, and fluoroquinolone resistance gene *qnrS1* is identified in 42 (9.2%) isolates, both of which are strongly correlated with IS*26*. Mutations of T57S in ParC and D87Y in GyrA are observed in 149 (32.5%) and 133 (29.0%) isolates, respectively. In addition, phylogenetic analysis confirms the presence of outbreaks caused by *S*. Enteritidis and *S*. Typhimurium, respectively.

Conclusions These results suggest local expansion and evolution in *Salmonella* occurred in Shanghai, China, and the underlying emergence of the undefined multidrug-resistant clone. Our findings enlarge the knowledge of local epidemics of *Salmonella*, especially *S*. Enteritidis and *S*. Typhimurium in Shanghai, and provide a piece of useful baseline information for future whole-genome sequencing surveillance.

Plain language summary

Salmonella is a bacterium that can cause diarrhea in infected people. Non-typhoidal *Salmonella* is a combination of the types that do not cause typhoid, which is a particular type of bacterial infection. We looked at non-typhoidal *Salmonella* infections occurring in the Minhang District of Shanghai, China. We analyzed the types of *Salmonella* from fecal samples across over a 10-year period and identified the two most common types of non-typhoidal *Salmonella* responsible for infections. We found that the genetic sequences of the bacteria changed and spread within the local area over time. This study provides information that could help support future monitoring of *Salmonella* spread. This could help track development of bacteria that do not respond well to treatment and enable the development of better infection control strategies.

Salmonella enterica (*S. enterica*) is a major foodborne pathogen worldwide and non-typhoidal *Salmonella* (NTS) is one of the four pivotal causes of global diarrheal diseases listed by the World Health Organization (WHO)[1]. Invasive NTS (iNTS) infection can cause critical pathological damage in the sterile area outside the intestine in infants, elderly people, and immunocompromised populations[2]. Typhoidal serovars including *S*. Typhi and *S*. Paratyphi, are human-restricted and mainly cause enteric fever, abdominal pain, and hepatosplenomegaly in children[3]. The arisen of salmonellosis

A full list of affiliations appears at the end of the paper. ✉e-mail: xuxuebin@scdc.sh.cn; cmlbright@126.com; wangyanan1001@henau.edu.cn

poses an obvious burden on public health, especially in low-income countries with relatively poor medical and health conditions[4,5]. In 2019, 215,000, 182,000, and 23,300 fatal cases worldwide were estimated to be caused by NTS, *S.* Typhi, and *S.* Paratyphi, respectively[6]. In addition, the detection rate of NTS has shown an upward trend during non-pharmaceutical interventions in the coronavirus disease 2019 epidemic[7,8].

The commonly used antibiotics for the clinical treatment of *Salmonella* infection are still third-generation cephalosporins (3GCs) and fluoroquinolones. However, the effectiveness of these drugs is being compromised by the emergence of antimicrobial resistance (AMR)[9,10]. Fluoroquinolone and third- and fourth-generation cephalosporin-resistant *Salmonella* isolates have emerged in multiple regions abroad, and even cases of multidrug-resistant (MDR) are common[10–14]. It is worth noting that fluoroquinolone-resistant *S.* Typhi and NTS have been listed as high-priority pathogens in the 2024 WHO bacterial priority pathogens list[15,16]. It is estimated that 1.27 million deaths were attributed to AMR in 2019[17]. AMR will cause ~10 million deaths annually after 2050 if control measures are not taken[18]. Moreover, the World Bank predicted in 2017 uncontrolled AMR would lead to a 3.8% decrease in global gross domestic product by 2050[19]. Hence, urgent exploration of the population structure of AMR *Salmonella* is needed to develop better response strategies.

The occurrence of *Salmonella* infection is usually associated with the consumption of insufficiently processed livestock and poultry products and global travel[20–23]. Shanghai is an international city located on the east coast of China, with a high population density, frequent trade, and domestic and foreign travel. In China, *S.* Typhimurium and *S.* Enteritidis of human origin have been reported to be ranked as the two most common serovars[13]. The emergence of *S.* Typhimurium with rough colony further posed obstacles to the treatment of salmonellosis[24], but the prevalence of the uncharacterized rough *S.* Typhimurium lineage in Shanghai is still unclear. Given that there were few systematic studies on the genomic epidemiological characteristics of *Salmonella* from human origin in the Minhang district, Shanghai, we conducted a 10-year genomic study on *Salmonella* in the Minhang district, focusing on the study of AMR characteristics and evolution dynamic. Our study revealed that the two dominant serovars were *S.* Enteritidis and *S.* Typhimurium, multidrug resistance existed in over half of the isolates, local expansion and evolution in *Salmonella* occurred in Shanghai, there was an underlying emergence of the undefined multidrug-resistant clone, and outbreaks caused by *S.* Enteritidis and *S.* Typhimurium respectively were present. In general, these findings hold promise for providing valuable information for the prevention and control of *Salmonella* on the levels of local and nationwide.

Methods

Ethics statement

This study was approved by Ethics Committee of Minhang Hospital, Fudan University (No. 2024-037-01K), and informed consent was obtained from all participants.

Strain collection and confirmation

From May 2012 to December 2021, we collected fresh fecal samples from 15,317 suspected diarrhea outpatients in the disease surveillance system of Minhang District Central Hospital in Shanghai, which is a comprehensive hospital that receives approximately one million patients annually. Detailed records are kept for each fecal sample, including identification number, source, patient name, gender, age, sample type, and separation time. After the sample collection, the broth medium was selected with an inoculation ring for colony enrichment, and then the xylose lysine desoxycholate (XLD) agar plate was inoculated for isolation and culture. We continue to use matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) technology for *Salmonella* identification. Identification of serovar was performed using O- and H-antigen slide agglutination method according to Kauffmann White protocol.

Antimicrobial susceptibility testing (AST)

According to the guidelines of the 2023 Clinical and Laboratory Standards Institute (CLSI)[25], minimum inhibitory concentration (MIC) values and susceptibility were determined on 25 clinical antimicrobial agents using the broth microdilution method, including ampicillin, ampicillin/sulbactam, aztreonam, cefazolin, cefotaxime, cefoxitin, cefepime, cefuroxime, cefotaxime/avibactam, ceftazidime, imipenem, ertapenem, meropenem, ciprofloxacin, norfloxacin, nalidixic acid, levofloxacin, azithromycin, amikacin, gentamicin, streptomycin, tetracycline, chloramphenicol, sulfamethoxazole/trimethoprim, and polymyxin E. *Escherichia coli* ATCC 25922 was selected as a quality control strain.

Whole-genome sequencing (WGS), quality control, and assembly

Genomic DNA was extracted from each isolate using the QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany). DNA purity was evaluated using Nanodrop (Thermo Fisher Scientific, USA) and concentration was quantified using Qubit Fluorometer 3.0 (Thermo Fisher Scientific, USA). Libraries were built using Illumina Nextera XT Kits according to the producer's standards and sequenced using the Illumina Novaseq 6000 platform (Illumina, USA). Process of raw data FASTQs using Trimomatic version 0.36 to remove adapters and low-quality regions[26]. The trimmed reads were assembled using SPAdes v.3.6.2[27].

Bioinformatics analysis

The annotation thresholds for functional genes were performed according to previous studies[13,28]. *Salmonella* In Silicon Typing Resource (SISTR) v1.1.2 and SeqSero2 v.1.3.1 were used for serovar prediction[29,30]. MLST v.2.0 was used to predict the sequence type (ST) by identifying 7 housekeeping genes[31]. With ABRicate v.1.0.1 (https://github.com/tseemann/abricate), the comprehensive antibiotic resistance database (CARD) and virulence factor database (VFDB) were called with default parameters to determine the removable and non-removable antibiotic resistance genes (ARGs) and VFs, respectively[32,33]. Plasmid identification was performed using PlasmidFinder v.2.0.1[34]. Mobile genetic elements (MGEs) such as MITEEc1, MITEs, ISs, and Tns that can lead to horizontal transfer of ARGs were identified using MobileElementFinder v.1.0.3[35]. ARGs located on chromosomes due to point mutations were identified using ResFinder v.4.1 by calling the PointFinder Database[36,37]. *Salmonella* pathogenic islands (SPIs) were confirmed using SPIFinder v.2.0[38].

Phylogenetic analysis

We selected 179 *S.* Enteritidis and 106 *S.* Typhimurium and its monophasic variant *S.* I 1,4,[5],12:i:- genomes for phylogenetic analysis. *S.* Enteritidis str. P125109 and *S.* Typhimurium str. LT2 were selected as reference genomes. Snippy v.4.6.0 was used to map the genome sequences of *S.* Enteritidis, *S.* Typhimurium, and *S.* I 1,4,[5],12:i:- to the reference genome, respectively[39], and to identify the single-nucleotide polymorphisms (SNPs). SNPs were extracted using SNP-sites[40]. A total of 1479 SNPs were identified in *S.* Enteritidis and 2878 SNPs in *S.* Typhimurium to construct the maximum likelihood phylogenetic tree using RAxML v.8.2.12 after recombinant regions were removed using Gubbins v.2.4.1[41,42].

Statistical analyses and reproducibility

Histogram, dot, line, and pie charts were generated using GraphPad Prism v.9.5.0, and multiple comparison tests were performed for MIC. When $P < 0.05$, there was a statistically significant difference.

Visualization

The minimum spanning tree (MST)-based core-genome multilocus sequence typing (cgMLST) was conducted using GrapeTree[43]. Heatmaps were generated using ImageGP (https://www.bic.ac.cn/BIC/#/). The co-occurrence network diagram was made using Gephi v.0.10.1[44]. iTOL v.6.9.1 was used for visualization and annotation of phylogenetic trees[45]. All the software used in this study has been listed in the "Methods" section.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Results

Dataset

A total of 458 *Salmonella* isolates were isolated from outpatients in the Minhang District of Shanghai, China, from 2012 to 2021 (Supplementary Fig. 1a). The emergence of these isolates was mostly concentrated in June to October (Supplementary Fig. 1b). The isolates were mainly from young people aged 21–40 years (50.44%) (Supplementary Fig. 1c), and the ratio of females (54.80%) to males (45.20%) was 1.2:1 (Supplementary Fig. 1d). A total of 40 *Salmonella* serovars were identified using both traditional serology and WGS prediction. *S.* Enteritidis was predominant (39.08%) (Supplementary Fig. 1e), accounting for more than 30% each year on average (Supplementary Fig. 1f) and most from ages 21 to 40 years (Supplementary Fig 2), and was followed by *S.* Typhimurium (15.07%, 69/458), *S.* I 1,4,[5],12:i:- (8.08%, 37/458), *S.* London (4.37%, 20/458), *S.* Thompson (3.71%, 17/458) and *S.* Infantis (3.49%, 16/458) (Supplementary Fig. 1e).

A total of 53 STs were identified. ST11 (38.65%) and ST19 (12.88%) account for 50%, followed by ST34 (9.83%, 45/458), ST155 (4.37%, 20/458), and ST26 (3.71%, 17/458) (Supplementary Fig. 1g). Similar temporal changes were observed between dominant serovars and STs (Supplementary Fig. 1f, h). Furthermore, five STs were detected in 2012, and at least two previously undefined STs were detected each year thereafter (Supplementary Table 1). The vast majority (98.88%) of *S.* Enteritidis belong to ST11, 85.51% of *S.* Typhimurium belong to ST19, and all *S.* I 1,4,[5],12:i:- belong to ST34. The MST based on cgMLST displayed the temporal distribution of different STs (Supplementary Fig. 3).

AMR characteristics of *Salmonella* in Minhang District in Shanghai, China

AST results indicated that streptomycin (62.66%) showed the highest AMR rate, followed by nalidixic acid (55.24%), ampicillin (53.93%), ampicillin/sulbactam (42.58%), tetracycline (34.28%), polymyxin E (28.38%), sulfamethoxazole (17.25%), imipenem (14.19%), chloramphenicol (13.54%), and cefazolin (12.88%) (Supplementary Table 2). Interestingly, 458 isolates were 100% sensitive to cefotaxime/avibactam (Supplementary Table 2). Among the classes of antibiotics, aminoglycoside had the highest resistance level (64.19%), followed by beta-lactam (61.35%), fluoroquinolone (59.17%), tetracycline (34.28%), polymyxin E (28.38%), sulfonamide (17.25%), chloramphenicol (13.54%) and macrolide (4.15%) (Fig. 1a). Differences in the distribution of AMR rates of different classes of antibiotic in different serovars was observed (Fig. 1b). Compared to other serovars, *S.* Enteritidis showed higher AMR rate to nalidixic acid, *S.* I 1,4,[5],12:i:- showed higher AMR rate to cefepime and *S.* Thompson showed higher AMR rate to cefotaxime, ceftazidime, norfloxacin, and levofloxacin (Fig. 1c).

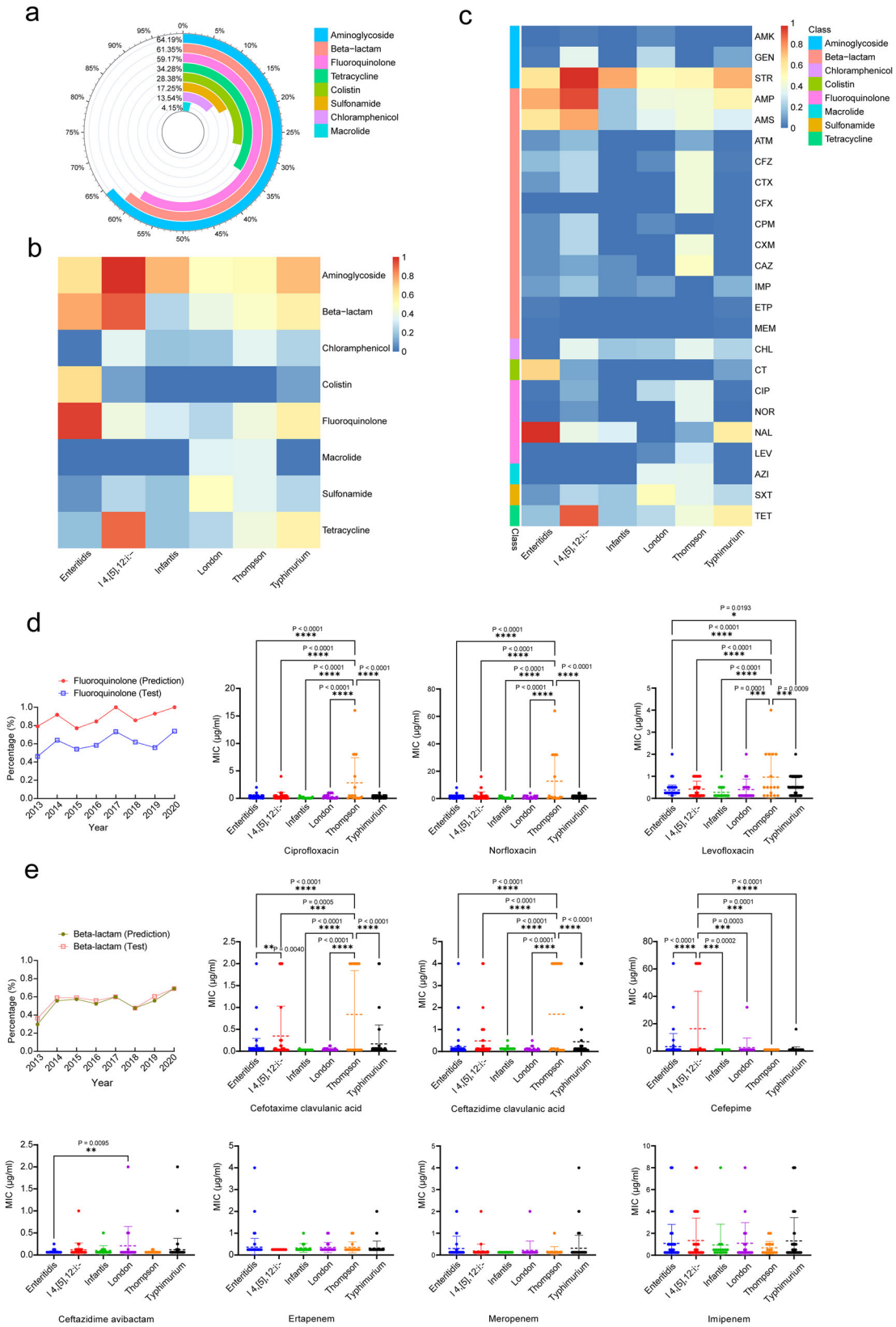
From the perspective of the time dimension, the resistance rate of fluoroquinolones did not show an obvious increase trend, while beta-lactams showed an obvious increase trend, and after 2014, the resistance rates of both were above 40% (Fig. 1d, e). Since 2018, the MICs of beta-lactam agents meropenem, imipenem, and ceftazidime clavulanic acid showed a downward trend, while the MICs of fluoroquinolone agents norfloxacin, ciprofloxacin, and levofloxacin did not change significantly from year to year (Supplementary Fig. 4a, b). An obvious fact was observed that ciprofloxacin, norfloxacin, levofloxacin, cefotaxime/clavulanic acid, and ceftazidime/clavulanic acid had significantly higher MICs against *S.* Thompson than other serovars (Fig. 1d, e). The MICs of cefepime to *S.* I 1,4,[5],12:i:- isolates also exhibited higher levels than other serovars (Fig. 1e). On the other hand, the MICs of polymyxin E showed a downward trend after 2017 and was significantly higher in *S.* Enteritidis compared to other serovars. The MICs of azithromycin showed a downward trend after 2018 and were significantly higher in *S.* London and *S.* Thompson compared to other serovars (Supplementary Fig. 5). It's worth noting that a total of 259 (56.55%) isolates showed MDR patterns based on the results of AST, with the highest showed resistance to 7 classes of antibiotic agents (Supplementary Table 3). Among MDR isolates, the most common (18.92%) MDR pattern was resistant to four drugs: aminoglycoside, beta-lactam, fluoroquinolone, and polymyxin E. Among the 6 dominant serovars and STs, *S.* I 1,4,[5],12:i:- and ST34 had the highest MDR rate, at 91.89% and 93.33%, respectively.

The draft genomes of 458 *S. enterica* were examined to elucidate the AMR profiles. The results were not exactly the same as AST (Supplementary Table 4). Aminoglycosides and fluoroquinolones had the highest resistance rates, at 100% and 86.46% (Supplementary Fig. 6a), respectively, and both predicted results were higher than AST. This may be due to the presence of unexpressed antibiotic resistance genes (ARGs) in the results of WGS analysis. For beta-lactams (51.75%), tetracyclines (33.62%), and macrolides (3.71%), the predicted results of these three showed similar to AST. In addition, the resistance rate of sulfonamides displayed an increasing trend, and was above 40% after 2014 (Supplementary Fig. 6b). The AMR rates of beta-lactams, sulfonamides, and tetracyclines in *S.* I 1,4,[5],12:i:- displayed higher levels, while the AMR rates of rifampicin and trimethoprim in *S.* London displayed higher levels (Supplementary Fig. 6c).

Distribution and dynamics of ARGs in Minhang District in Shanghai, China

Among the predicted 61 ARGs, the top 20 are *AAC(6′)-Iy* (64.63%), $bla_{\text{TEM-1}}$ (43.45%), *APH(3″)-Ib* (40.61%), *APH(6)-Id* (40.61%), *sul2* (40.61%), *AAC(6′)-Iaa* (34.93%), *tet(A)* (23.58%), *floR* (11.35%), *sul1* (9.83%), *qnrS1* (9.17%), *tet(B)* (8.95%), *dfrA12* (8.52%), *sul3* (7.42%), *ANT(3″)-IIa* (7.21%), *cmlA1* (6.55%), *aadA2* (5.90%), *arr-3* (5.68%), *APH(3′)-Ia* (5.46%), and *AAC(3)-IV* (4.15%) (Supplementary Table 5). For fluoroquinolones, a total of 82.53% of isolates acquired chromosome mutations in the *gyrA* (D87Y, D87G, D87N, S83F, and S83Y), *gyrB* (E466D) and *parC* (T57S and S80I) (Supplementary Table 6) and 9 plasmid-mediated quinolone resistance (PMQR) genes were detected, including *qnrS1* (9.17%), *oqxA* (2.18%), *oqxB* (2.18%), *qnrB17* (1.75%), *qepA2* (1.31%), *qnrB4* (1.09%), *qnrB5* (0.66%), *qnrS2* (0.44%), and *qnrA1* (0.22%) (Supplementary Table 5). For beta-lactams, 10 ARGs were screened, including $bla_{\text{TEM-1}}$ (43.45%), $bla_{\text{CTX-M-55}}$ (3.28%), $bla_{\text{OXA-1}}$ (2.40%), $bla_{\text{CMY-59}}$ (1.97%), $bla_{\text{TEM-141}}$ (1.75%), $bla_{\text{CTX-M-14}}$ (1.31%), $bla_{\text{DHA-1}}$ (1.09%), $bla_{\text{CARB-3}}$ (0.66%), $bla_{\text{CTX-M-9}}$ (0.66%), and $bla_{\text{OXA-10}}$ (0.66%) (Supplementary Table 5). For fosfomycin-RGs, *fosA7* and *fosA3* were detected in 14 and 7 isolates, respectively. For macrolides, 17 isolates were resistant to azithromycin, of which 2 had chromosome mutations in AcrB (R717L) and 15 had plasmid-mediated resistance gene *mph*(A) (Supplementary Tables 5 and 6). We also identified *qacH* gene that was associated with resistance to disinfecting agents and antiseptics in 33 isolates. Importantly, based on WGS analysis, more than 50% (58.95%) of the isolates carried at least 3 ARG types belonging to different classes of antibiotics, and exhibiting MDR (Fig. 2a). Of particular concern is the 100% MDR rate observed in *S.* I 1,4,[5],12:i:- and the MDR rate of *S.* Enteritidis also up to about 75% (Fig. 2b). Equally concerned, among 6 dominant STs, the MDR rata of ST34 also reached 100%. More importantly, we have noticed that the MDR rate is increasing year by year (Fig. 2c).

We then selected dominant ARGs from each class of antibiotics for the temporal variation study. It can be surveyed that the proportion of some ARGs shows an increasing trend, such as *APH(3″)-Ib*, *APH(6)-Id*, $bla_{\text{TEM-1}}$, *sul2*, *tet(A)*, and *gyrA* p.D87Y (Fig. 3a). The distribution of ARGs also varies among different serovars. *AAC(6′)-Iaa* was only present in *S.* I 1,4,[5],12:i:-, *S.* London, and *S.* Typhimurium, while *AAC(6′)-Iy* was observed in *S.* Enteritidis, *S.* Infantis, and *S.* Thompson, exclusivity. Compared with other serovars, $bla_{\text{CTX-M-55}}$, *sul2*, and *tet(B)* were more common in *S.* I 1,4,[5],12:i:-, *qnrS1* was more common in *S.* Tompson, *arr-3* and *dfrA27* were more common in *S.* London and *gyrA* p.D87Y was more common in *S.* Enteritidis (Fig. 3b). Moreover, *fosA3*, $bla_{\text{OXA-1}}$, and *catB3* were mainly found in *S.* I 1,4,[5],12:i:-, *fosA7* were mainly identified in *S.* Derby, *cmlA1*



were mainly detected in *S.* Typhimurium, and *mph(A)* were mainly observed in *S.* Tompson and *S.* London (Supplementary Fig. 7).

In order to explore the correlation between ARGs, we constructed the ARGs co-occurrence network, which was selected from the dominant ARGs in each class of antibiotic (Fig. 4a). For *AAC(6′)-Iy*, the weight values between *AAC(6′)-Iy* and $bla_{\text{TEM-1}}$, *sul2*, and *APH(3″)-Ib* are the highest, at 260, 242, and 236, respectively, indicating the strongest correlation. For $bla_{\text{CTX-M-55}}$, the weight values between $bla_{\text{CTX-M-55}}$ and $bla_{\text{TEM-141}}$, *qnrS1*, $bla_{\text{TEM-1}}$, *floR*, *sul2*, and *tet(A)* are the highest, at 16, 14, 12, 12, 12, and 12, respectively, indicating the strongest correlation. For *mph(A)*, the weight

Fig. 1 | Phenotypic distribution of different antibiotics in clinical testing. **a** Overall AMR rate clinical testing. $n = 458$. **b** Distribution characteristics of AMR in dominant serovars of different classes. **c** Distribution characteristics of AMR in dominant serovars of different antibiotics. **d** Distribution characteristics of fluoroquinolone resistance. **e** Distribution characteristics of beta-lactam resistance. The abbreviations for different antibiotics are as follows: AMP Ampicillin, AMS Ampicillin/Sulbactam, ATM Aztreonam, CFZ Cefazolin, CTX Cefotaxime, CFX Cefoxitin, CPM Cefepime, CXM Cefuroxime, CZA Cefotaxime/Avibactam, CAZ Ceftazidime, IMP Imipenem, ETP Ertapenem, MEM Meropenem, CIP Ciprofloxacin, NOR Norfloxacin, NAL Nalidixic acid, LEV Levofloxacin, AZI Azithromycin, AMK Amikacin, GEN Gentamicin, STR Streptomycin, TET Tetracycline, CHL Chloramphenicol, SXL Sulfamethoxazole, CT Polymyxin E. The error bar represents the standard deviation. The "*" represents P values. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$. No significant differences were not shown. Data are presented as mean values with SD. d-e, *S.* Enteritidis, $n = 179$; *S.* I 4,[5],12:i:-, $n = 37$; *S.* Infantis, $n = 16$; *S.* London, $n = 20$; *S.* Thompson, $n = 17$; and *S.* Typhimurium, $n = 69$. d and e, 2012, $n = 22$; 2013, $n = 91$; 2014, $n = 61$; 2015, $n = 61$; 2016, $n = 84$; 2017, $n = 15$; 2018, $n = 21$; 2019, $n = 43$; 2020, $n = 42$; and 2021, $n = 18$.

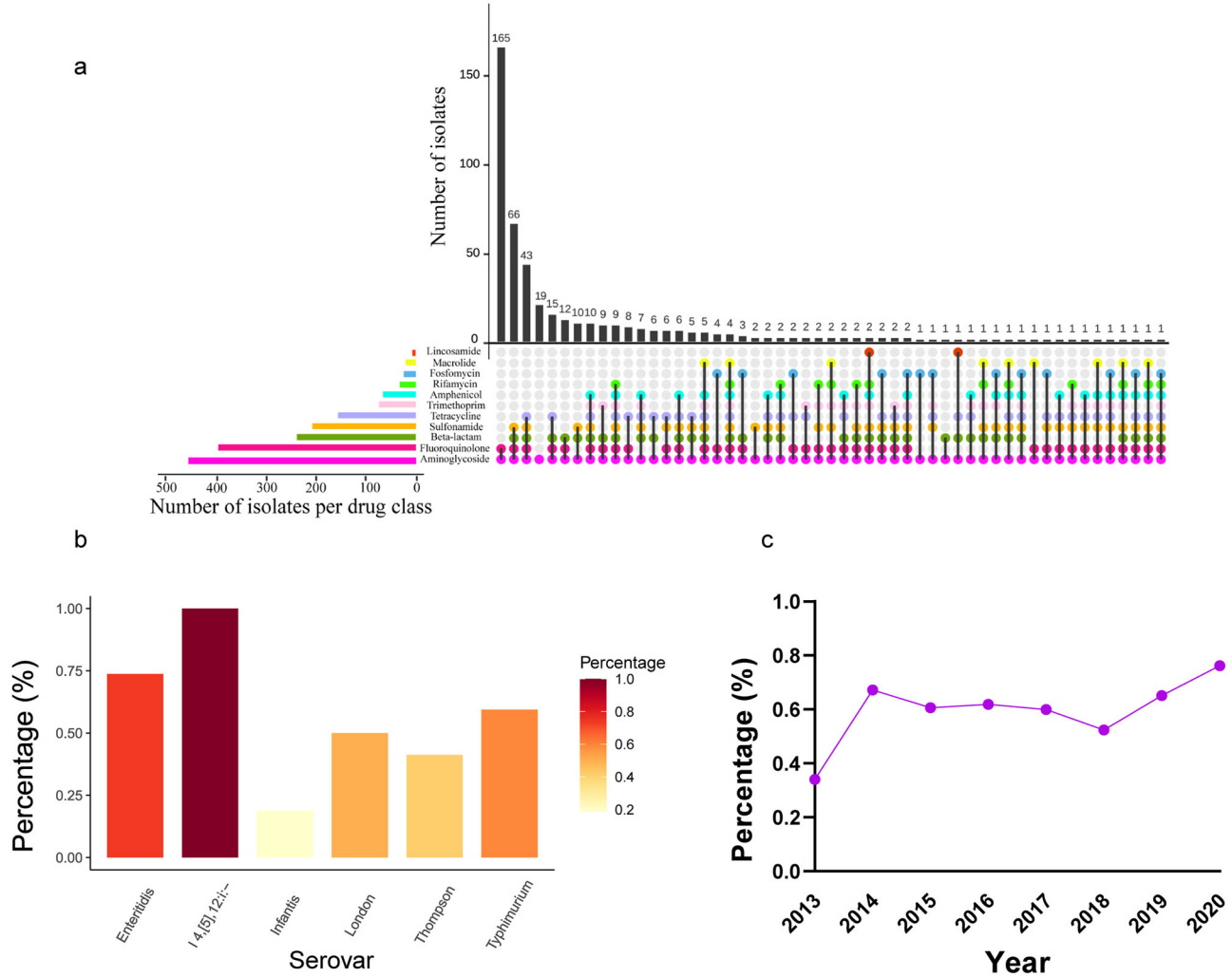


Fig. 2 | Distribution characteristics of multidrug resistance (MDR) predicted. **a** UpSet plot showing the diversity of antibiotic resistance profiles for 458 isolates. **b** Distribution characteristics of MDR in different serovars. *S.* Enteritidis, $n = 179$; *S.* I 4,[5],12:i:-, $n = 37$; *S.* Infantis, $n = 16$; *S.* London, $n = 20$; *S.* Thompson, $n = 17$; *S.* Typhimurium, $n = 69$. **c** Temporal changes of MDR isolates. 2013, $n = 91$; 2014, $n = 61$; 2015, $n = 61$; 2016, $n = 84$; 2017, $n = 15$; 2018, $n = 21$; 2019, $n = 43$; 2020, $n = 42$.

values between *mph(A)* and *sul1*, *arr-3*, *sul2*, and *tet(A)* are the highest, at 30, 20, 20, and 20, respectively, indicating the strongest correlation. The coexistence of ARGs belonging to different antibiotic classes also explains, to some extent, the emergence of MDR.

Characteristics of MGEs and VFs

Among 458 *Salmonella* genomes, the plasmid replicons detection rate was 83.41% (382/458), with 34 plasmid replicons (Supplementary Table 7). The dominant plasmid replicons are IncFIB(S) ($n = 224$) and IncFII(S) ($n = 224$), followed by IncX1 ($n = 128$), Col(pHAD28) ($n = 56$), Col440I ($n = 43$), ColRNAI ($n = 35$), IncQ1 ($n = 32$), IncHI2A ($n = 26$), IncHI2 ($n = 26$), IncC ($n = 14$), IncI1-I(Alpha) ($n = 11$), Col156 ($n = 10$). Over time, IncFIB(S), IncFII(S), IncX1, and Col440I showed an increasing trend (Supplementary Fig. 8a). In terms of serovar distribution, IncFIB(S), IncFII(S), and IncX1 were mainly distributed in *S.* Enteritidis, followed by *S.* Typhimurium. Col(pHAD28) was mainly distributed in *S.* London. IncC was mainly distributed in *S.* Thompson. IncHI2, IncHI2A, and IncQ1 were mainly distributed in *S.* I 1,4,[5],12:i:- (Supplementary Fig. 8b).

For other MGEs, a total of 3089 MGEs were identified, belonging to 82 types, divided into 4 classes (Supplementary Table 8). The majority of MGEs are insertion sequences (ISs, $n = 2007$), followed by MITEEc1 ($n = 925$), unit transposons (Tns, $n = 156$), and miniature inverted repeats transposable elements (MITESen1, $n = 1$). The number of isolates carrying IS*26*, IS*Ecl10*, IS*Kpn2*, IS*Sen7*, and Tn2 presented a growing trend (Supplementary

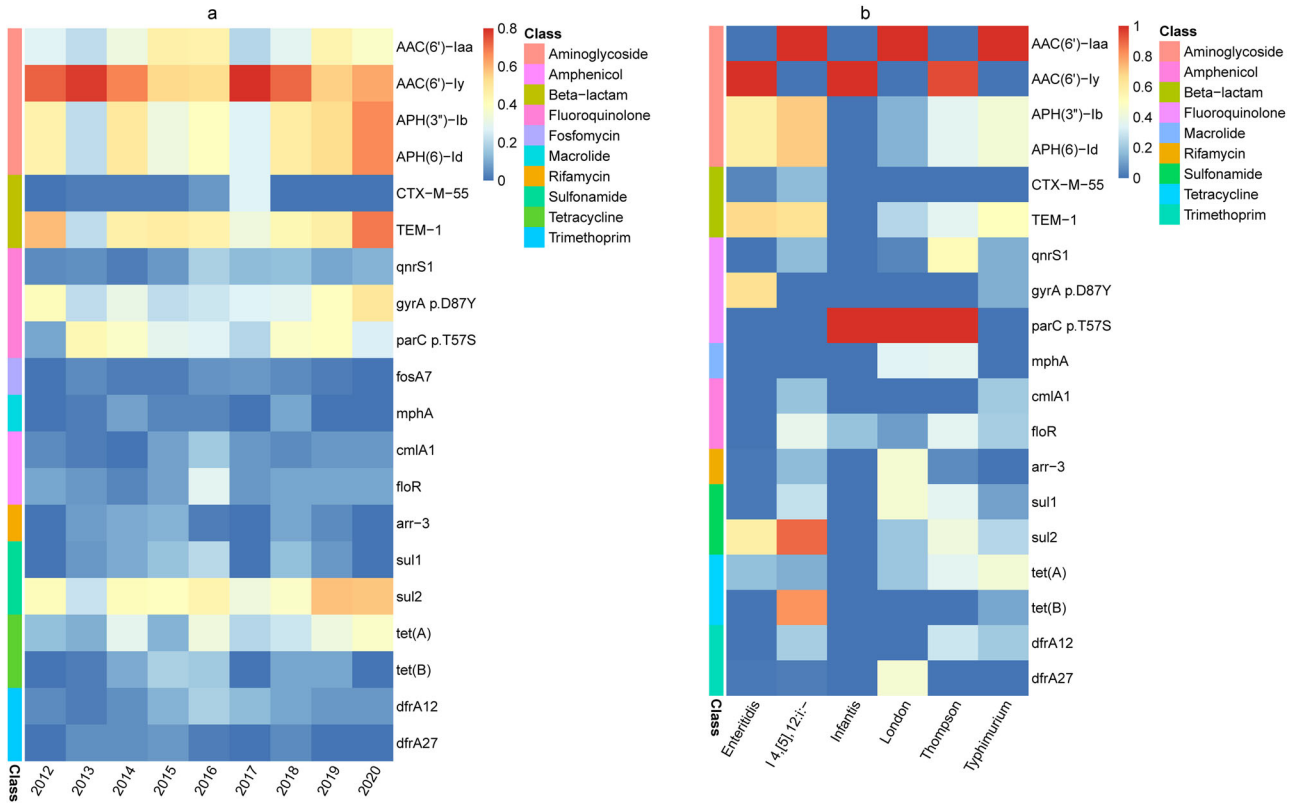


Fig. 3 | Distribution characteristics of antibiotic resistance genes (ARGs). **a** Temporal changes. 2012, $n = 22$; 2013, $n = 91$; 2014, $n = 61$; 2015, $n = 61$; 2016, $n = 84$; 2017, $n = 15$; 2018, $n = 21$; 2019, $n = 43$; 2020, $n = 42$. **b** Serovars distribution. ARGs are selected from dominant genotypes in different antibiotic classes. *S.* Enteritidis, $n = 179$; *S.* I 4,[5],12:i:-, $n = 37$; *S.* Infantis, $n = 16$; *S.* London, $n = 20$; *S.* Thompson, $n = 17$; *S.* Typhimurium, $n = 69$.

Fig. 9a). There are also differences in the distribution of MGEs among serovars (Supplementary Fig. 9b). IS*Ech12* was principally detected in *S.* Infantis. IS*Ecl10* and IS*Sen7* were principally detected in *S.* Enteritidis and *S.* Typhimurium, and IS*Kpn2* and Tn2 were principally detected in *S.* Enteritidis. IS*Sty2* was principally detected in *S.* Tompson and *S.* London.

In order to explore the correlation between ARGs and MGEs, correlation networks were constructed (Fig. 4). We found that several ARGs are closely associated with MGEs. The weight values between IS*26* and bla_{OXA-1}, *qnrS1*, *fosA3*, and *floR* are higher than other MGEs, with weight values of 9, 29, 5, and 34, respectively, indicating that IS*26* is more conducive to the dissemination of the four ARGs compared to other MGEs (Fig. 4b–e). The correlation between plasmids and ARGs was also discrepant. For beta-lactam resistance genes, the weight values between bla_{OXA-1} and IncHI2, bla_{CTM-55} and IncFIB(S), and bla_{OXA-1} and IncHI2A are higher than others, with weight values of 9, 8, and 8, respectively (Fig. 4f). For fluoroquinolone resistance genes, the weight values between *qnrS1* and Col4401, Col(pHAD28) and IncC are higher than others, with weight values of 16, 12, and 11, respectively (Fig. 4g). For fosfomycin resistance genes, the weight values between *fosA3* and IncHI2 and IncHI2A are higher than others, with weight values of both 4 (Fig. 4h). For amphenicol resistance genes, the weight values between *floR* and IncHI2A and IncHI2 are higher than others, with weight values of 20 and 19, respectively (Fig. 4i).

A total of 129 VFs were detected and each strain carried a large number of virulence genes (ranging from 94 to 117). Most of the VFs were present in all isolates (Supplementary Table 9). We randomly selected 45 types for analysis (Supplementary Table 10). Most of them belong to *Salmonella* Pathogenic Islands (SPIs)-encoded type III protein secretion system (T3SS) or fimbriae-related genes. No significant changes were observed in VFs (Supplementary Fig. 10a). However, differences were observed in different serovars (Supplementary Fig. 10b). The *astA*, *cdtB*, *faeCDE*, and *gyvA* genes were mainly found in *S.* London and the *pefAD* and *rck* genes were mainly found in *S.* Enteritidis and *S.* Typhimurium. The *entA*, *lpfB*, *pipB2*, and *steB* genes were observed to be more prevalent in people over 50 years old (Supplementary Fig. 10c).

A total of 15 SPIs were detected, one of which was "not named". Among them, the detection rates of SPI-1, SPI-2, SPI-3, and SPI-9 were 100%. No significant changes were observed in SPIs (Supplementary Fig. 11a). The distribution of SPIs varied in serovars (Supplementary Fig. 11b). SPI-4 existed in *S.* Typhimurium, *S.* I 1,4,[5],12:i:-, and *S.* London. SPI-10 was only existed in *S.* Enteritidis. We also detected SGI-1, which existed in *S.* Infantis and *S.* London. SPI-8 was more common in people under 50 years old, and SPI-4, SPI-10, SPI-13, and SPI-14 were more common in people over 50 years old (Supplementary Fig. 11c).

Phylogenetic analysis confirms the presence of outbreaks caused by *S.* Typhimurium and *S.* Enteritidis isolates

We selected 106 *S.* Typhimurium (and monophasic, called *S.* I 1,4,[5],12:i:-) and 179 *S.* Enteritidis for phylogenetic analysis, respectively. *S.* Typhimurium had two evolutionary branches, with ST19 and ST34 being the dominant clones (Fig. 5a). ST34 carried more ARGs than ST19 and the MGEs carried by both also differ. IncFIB(S) and IncFII(S) were only detected in ST19, while IncHI2 and most of IncQ1 and IncHI2A were detected in ST34. In our early passive surveillance, we recorded 12 suspect outbreak events of *S.* Typhimurium. According to the previous report[46], an outbreak of foodborne disease is defined as the occurrence of two or more cases of similar diseases caused by ingestion of ordinary food. In addition, isolates that cause diseases need to meet the requirement of having 2-32 SNP differences[47]. Finally, we confirmed 8 outbreaks caused by *S.* Typhimurium. In addition, for *S.* Typhimurium and *S.* I 1,4,[5],12:i:-, 19 isolates with rough colonies and 87 with smooth colonies were confirmed. Compared with the smooth *S.* Typhimurium, all rough *S.* Typhimurium belonged to ST34, carried IncHI2 and IncHI2A, and showed a higher ARGs coverage rate, such as $bla_{CTX-M-55}$, *dfrA12*, and *floR*. Notably, three outbreaks of rough *S.* Typhimurium isolates were observed, and the strains in the seventh outbreak all carried

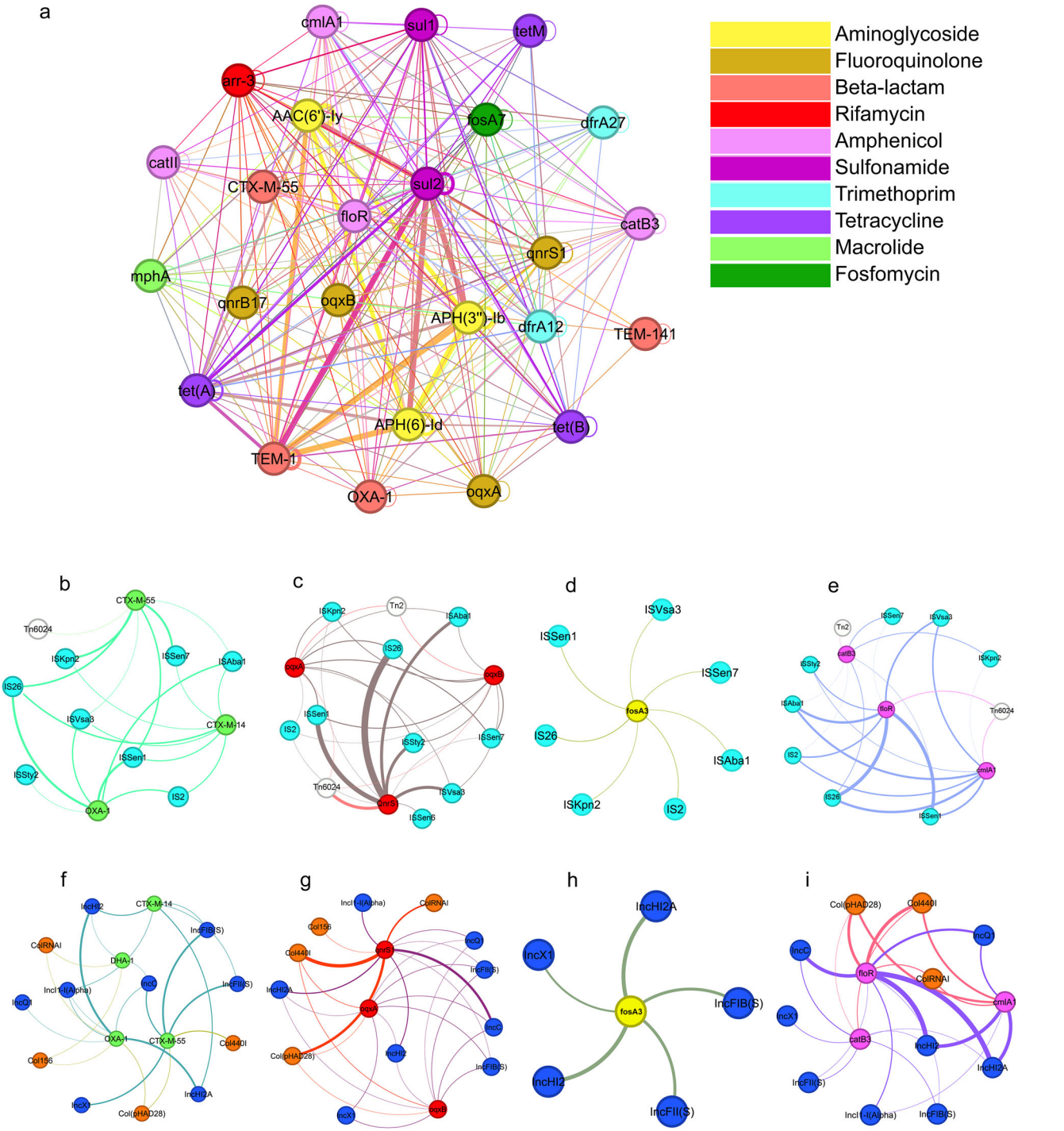


Fig. 4 | Co-occurrence networks of ARGs, plasmids, and MGEs. a Co-occurrence network of 25 ARGs which are selected from dominant genotypes in different antibiotics classes. *AAC(6′)-Iy*, $n = 296$; bla_{TEM-1}, $n = 199$; *APH(3″)-Ib*, $n = 186$; *APH(6)-Id*, $n = 186$; *sul2*, $n = 186$; *tet(A)*, $n = 108$; *floR*, $n = 52$; *sul1*, $n = 45$; *qnrS1*, $n = 42$; *tet(B)*, $n = 41$; *dfrA12*, $n = 39$; *cmlA1*, $n = 30$; *arr-3*, $n = 26$; $bla_{CTX-M-55}$, $n = 15$; *mphA*, $n = 15$; *dfrA27*, $n = 15$; *fosA7*, $n = 14$; *tetM*, $n = 14$; *catB3*, $n = 11$; *catII*, $n = 11$; bla_{OXA-1}, $n = 11$; *oqxA*, $n = 10$; *oqxB*, $n = 10$; $bla_{TEM-141}$, $n = 8$; *qnrB17*, $n = 8$. **b–i** Co-occurrence network of beta-lactam-RGs, fluoroquinolone-RGs, fosfomycin-RG, and amphenicol-RGs and mobile genetic elements (MGEs) (**b–e** indicate insertion sequence and **f–i** indicate plasmids). The edge widths represent connection weights. The higher the weight value is, the more co-occurrence events it indicates. **b–e** Tn*6024*, $n = 26$; IS*26*, $n = 216$; ISKpn*2*, $n = 207$; Tn*2*, $n = 115$; ISSen6, $n = 23$; MITEEc1, $n = 458$; ISAba*1*, $n = 29$; ISSty*2*, $n = 52$; IS*1133*, $n = 23$; ISSen*1*, $n = 169$; cn_5129_ISVsa3, $n = 22$; IS*2*, $n = 23$; ISSen*7*, $n = 229$; ISE*c110*, $n = 293$; IS*1006*, $n = 32$; ISKpn*72*, $n = 44$; IS*6100*, $n = 23$; ISSen*9*, $n = 63$; and ISVsa*3*, $n = 42$. **f–i** IncFIB(S), $n = 224$; IncI1-I(Alpha), $n = 11$; Col440I, $n = 43$; IncC, $n = 14$; IncHI2A, $n = 26$; IncX1, $n = 128$; Col(pHAD28), $n = 56$; IncHI2, $n = 26$; IncQ1, $n = 32$; CoIRNAI, $n = 35$; Col156, $n = 10$, and IncFII(S), $n = 224$.

$bla_{CTX-M-14}$. It can be also observed that all rough *S.* Typhimurium isolates carried IncHI2 and IncHI2A, which may lead to the emergence of multiple resistance genes.

For *S.* Enteritidis, the phylogenetic tree showed two branches, and most (98.9%, 177/179) of isolates belonged to ST11 (Fig. 5b). Eighteen outbreaks occurred from 2012 to 2020, including one caused by ST183 and 16 by ST11.

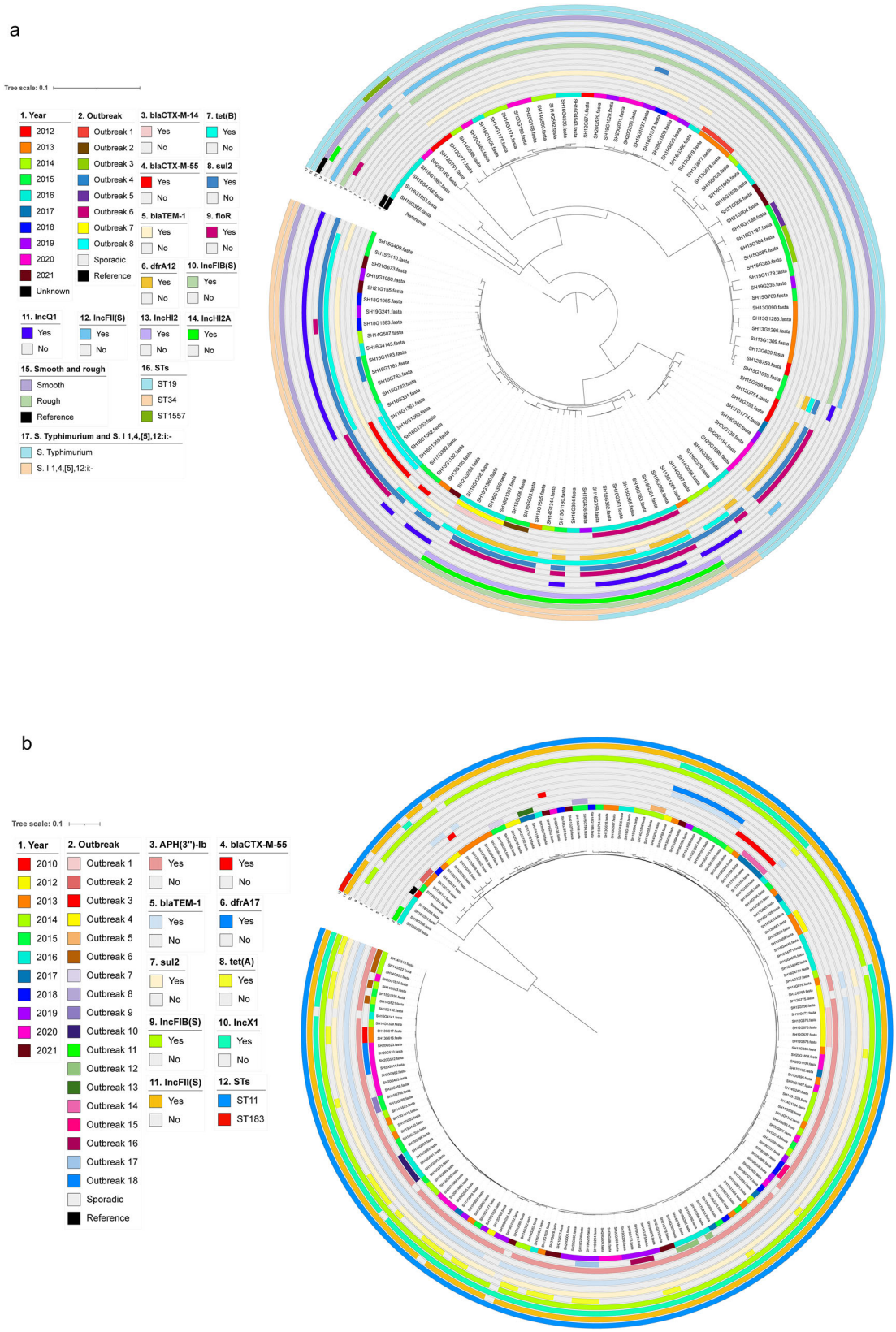


Fig. 5 | Phylogenetic analysis of *S.* Typhimurium and *S.* Enteritidis. a Phylogenetic analysis of *S.* Typhimurium and its monophasic variant *S.* I 1,4,[5],12:i:-. The circle represents annotations 1–17 in sequence from the inside out. $n = 106$. **b** Phylogenetic analysis of *S.* Enteritidis. The circle represents annotations 1–12 in sequence from the inside out. $n = 179$.

Only IncFII(S) was detected in ST183, while more IncFII(S), IncFIB(S), and IncX1 were detected in ST11. No ARGs were detected in ST183 isolates. Eight isolates of ST11 carried $bla_{CTX\text{-}M\text{-}55}$. The gene *dfrA17* was not observed in the outbreak strains in ST11. *APH(3″)-Ib*, $bla_{TEM\text{-}1}$, *sul2*, and *tet(A)* were more prevalent in ST11 isolates. In addition, the strains that emerged in the thirteenth outbreak all carried $bla_{CTX\text{-}M\text{-}55}$.

Discussion

In this study, we analyzed 458 *Salmonella* genomes of human origin from Minhang District in Shanghai, China, elucidating the epidemiological, AMR, and virulence characteristics, and evolutionary dynamics, and providing valuable information for future WGS surveillance of *Salmonella*. WGS has been widely used for public health and international surveillance of AMR, especially bacteria that can cause gastrointestinal diseases[28,48–53].

In the present study, the diarrhea outpatients caused by *Salmonella* were mainly found in young adults aged 21–40 years (~50%), and more common in male patients (1.2:1). This feature showed some difference in the distribution of age and gender of NTS-causing salmonellosis in other countries. In Denmark during 2013-2022, patients aged 15–84 years accounted for 76.1% of the total NTS cases, nearly equal in male and female[54]. In Armenia from 2016 to 2021, over 60% of NTS infections were found in the young age group of under 18 years old[55]. These deviations may reflect unique local epidemiological or environmental factors, such as regional dietary habits and centralized industrialization scale of pork and poultry products[20,21], healthcare access to foodborne infections, or diagnostic practices towards NTS infections[56].

International trade plays a crucial role in the transmission of *Salmonella*[21,22]. Shanghai is a city with frequent domestic and foreign trade, which leads to a wide variety of serovars of *Salmonella*. A total of 40 serovars were identified, with *S.* Enteritidis (39.08%) having the highest detection rate, followed by *S.* Typhimurium (15.07%) and *S.* I 1,4,[5],12:i:- (8.80%). A total of 17 isolates of *S.* Thompson were identified, and previous studies reported a close relationship between *S.* Thompson and seafood[13,57–59].

The harm of *Salmonella* to humans and animals mainly stems from its virulence, which can inject toxic proteins into host cells through the T3SS encoded by SPI to exert pathogenic effects[60]. We found that some T3SS-related genes such as *invA*, *invB*, and *invC* were present in all isolates (Supplementary Table 9). In addition, we speculated that during the process of strain variation, AMR may increase and virulence may decrease, as certain ARGs in *S.* I 1,4,[5],12:i:- have a higher proportion than *S.* Typhimurium, while virulence factors have a significantly lower proportion (Supplementary Fig. 6c and Supplementary Fig. 10b).

The emergence of *S.* I 1,4,[5],12:i:- ST34 has always been related to MDR, which initially showed resistance to ampicillin, chloramphenicol, gentamicin, and tetracycline[61]. It is of concern that the number of MDR ST34 strains has rapidly increased since 2017, and the antibiotic resistance spectrum has recently added third-generation cephalosporins and fluoroquinolone[62,63]. In our previous research, MDR *S.* I 1,4,[5],12:i:- ST34 has been reported to be the dominant clone in China, which has attracted public attention and reflection on addressing this issue[28]. In this study, MDR ST34 showed a high occurrence rate in both clinical AST and WGS analysis results, at 93.33% and 100%, respectively, involving resistance to cefotaxime, cefepime, ceftazidime, and ciprofloxacin. The extremely high resistance rate of *Salmonella* ST34 in the region puts forward higher requirements for the improvement of disease surveillance systems and the rational use of antibiotics, as the non-standard use of antibiotics is the fundamental reason for the exacerbation of bacterial resistance.

3GC-resistant and carbapenem-resistant *Enterobacterales* and fluoroquinolone-resistant *S.* Typhi and NTS have been listed as critical- and high-priority pathogens in the 2024 WHO BPPL, respectively[15,16]. Our results confirmed an increasing trend in the proportion of fluoroquinolone-resistant *Salmonella* with an annual proportion of over 30%. A total of 9 ARG types and 8 mutation types conferring fluoroquinolone resistance were detected in the 458 isolates. Notably, antibiotic susceptibility testing results showed that there were 65 isolates that had poor sensitivity to imipenem and meropenem. However, our WGS analysis did not detect the imipenem-resistant genes. This fact led us to suspect the emergence of uncharacterized genes conferring resistance to carbapenems.

Azithromycin has been recommended as the first choice for the treatment of *Salmonella*-infected patients when fluoroquinolone and beta-lactam failed to produce satisfactory results[64]. However, azithromycin-resistant *Salmonella* has gradually surfaced around the world due to the extensive use of azithromycin[13,28,65–68]. Similarly, in our analysis, seventeen (3.7%) *Salmonella* isolates were found to be resistant to azithromycin, of which fifteen carried azithromycin-RG *mph(A)*, and two harbored the mutation R717L in AcrB.

Fosfomycin typically inhibits the synthesis of peptidoglycan in bacterial cell walls to exert antibacterial effects and is commonly used to treat infections of AMR *Enterobacteriaceae*[69,70]. The fosfomycin resistance genes especially *fosA3* and *fosA7* were widespread in *Salmonella* spp. strains[10,13,28,71,72]. We found that 7 and 14 isolates harbored *fosA3* and *fosA7*, respectively, which were dispersed across 5 serovars.

Outbreaks of salmonellosis linked with food or animals have been reported worldwide, for example in Europe, America, and Oceania[73–75]. In China in 2020, among bacterial pathogens, *Salmonella* was most closely associated with outbreaks and illnesses[76]. During our previous surveillance period, a total of 25 suspect outbreaks were recorded. These outbreak events were eventually confirmed by genome-wide analysis, with 17 in *S.* Enteritidis and 8 in *S.* Typhimurium. A previous study reported the discovery of a novel rough *S.* Typhimurium lineage in China, which showed better environmental adaptability as well as greater AMR[24]. Notably, in our study, three outbreaks caused by rough *S.* Typhimurium were observed. However, rough *S.* Typhimurium lineage were identified in Equus caballus in the USA in October 1992, frozen squid in India on 26 July 2001, and frozen eel fish in Vietnam on 4 June 2007 in recent reports[10].

WGS analysis is crucial for monitoring antibiotic-resistant bacteria and the spread of ARGs within bacteria and in the environment, and plays a key role in identifying ARGs[10,13,28,36,77–80]. However, the determination of bacterial resistance still needs to be validated through clinical trials, as WGS analysis may detect certain ARGs, the expression of which may require complex conditions, and these mechanisms need to be further studied in the future. There are some limitations in this study. Firstly, in some years, the sample size is relatively small, such as in 2017, which leads to some discontinuity in the research results in the time dimension. Secondly, our samples came from non-children hospitals, which results in a small sample size for children under 14 years old and cannot reflect the prevalence of *Salmonella* infection in the pediatric population well. Finally, although we conducted a correlation analysis between ARGs and MGEs, we did not conduct an in-depth genetic environment analysis, and the genetic environment and transmission patterns of important ARGs need to be further explored.

In summary, we conducted a retrospective study on 458 *Salmonella* isolates of human origin in Minhang District in Shanghai, China over the past 10 years, revealing the population structure, antibiotic resistance patterns, and evolution dynamics. Two *S.* Montevideo strains isolated in August 2013 belonged to ST10844 were firstly detected in Minhang District in Shanghai, earlier than its first assigned in the EnteroBase in October 2023, indicated that it has been already spread in Shanghai for a long time. In the rampant era of bacterial diseases and AMR, our findings provide valuable information for future prevention and control.

Data availability

The assembled 458 genomes used in this study have been submitted to the NCBI project number PRJNA1154820. Source data for the figures in the manuscript are available as Supplementary Data 1 and 2. The numerical data plotted (source data) in the graphs in Figs. 1–5 and Supplementary Figs. 1–11 are included in Supplementary Data 1 and 2, respectively. All other data are available from the corresponding author upon reasonable request.

Received: 11 September 2024; Accepted: 3 June 2025;
Published online: 12 June 2025

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Acknowledgements

This study was supported in part by grants from the National Key Research and Development Program of China (2023YFC2307101), the National Natural Science Foundation of China (82272381 and 82472322), the Young TopNotch Talents Foundation of Henan Agricultural University (30501278), Training Program for Outstanding Young Medical and Pharmaceutical Talents of Minhang District Health System (No. mwyjyx08), and Project for Young scientist of the Joint Funds of Science and Technology Research and Development Plan of Henan Province, China (235200810058). We also thank the Veterinary Big Data and Bioinformatics Center, Henan Agricultural University, for their support and help.

Author contributions

X.B.X., Y.N.W., and M.L.C. conceived and designed the project. P.P.L., M.L.C., Y.L., and Q.W. collected hospital isolates and epidemiological data,

extracted DNA, and organized the sequencing. Y.H.P., Y.N.W., X.B.X., Y.L., M.L.C., P.P.L., M.Q.Q., and Y.J. analyzed data. Y.H.P., P.P.L., M.L.C., Y.N.W., and X.B.X. drafted the manuscript. All authors have read and approved the submitted version.

Competing interests
The authors declare no competing interests.

Additional information
Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s43856-025-00950-3.

Correspondence and requests for materials should be addressed to Xuebin Xu, Mingliang Chen or Yanan Wang.

Peer review information *Communications Medicine* thanks Sadia Isfat Ara Rahman and the other, anonymous, reviewer(s) for their contribution to the peer review of this work. [Peer review reports are available].

[1]Research and Translational Laboratory of Acute Injury and Secondary Infection, and Department of Laboratory Medicine, Minhang Hospital, Fudan University, Shanghai, China. [2]International Joint Research Center of National Animal Immunology, College of Veterinary Medicine, Henan Agricultural University, Zhengzhou, Henan, China. [3]Department of Microbiology, Shanghai Municipal Center for Disease Control and Prevention, Shanghai, China. [4]CAS Key Laboratory of Pathogen Microbiology and Immunology, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China. [5]Longhu Laboratory, Zhengzhou, Henan, China. [6]Henan Academy of Innovations in Medical Science, Zhengzhou, Henan, China. [7]These authors contributed equally: Panpan Lv, Yuhang Pei.
✉e-mail: xuxuebin@scdc.sh.cn; cmlbright@126.com; wangyanan1001@henau.edu.cn