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High carriage and possible hidden spread of multidrug-resistant *Salmonella* among asymptomatic workers in Yulin, China

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Food workers have frequent contact with unprocessed foods, but their carriage of *Salmonella* and potential influence on public health have not been comprehensively assessed. We investigated *Salmonella* carriage among food workers compared with non-food workers based on occupational health screening of 260,315 asymptomatic workers over an 8-year surveillance period in Yulin, China. We confirmed that healthy carriers serve as natural reservoirs for *Salmonella*, with higher carriage rates in food workers than non-food workers. The isolates from food workers also exhibited greater serovar diversity and likely higher levels of antimicrobial resistance than those from non-food workers. Factors such as meteorological, social, and hygiene factors potentially influenced the carriage rate. Genomic analysis revealed a consistent increase in antimicrobial resistance genes among *Salmonella* isolates over the study period, with the majority of these antimicrobial resistance genes located on plasmids. Additionally, we identified numerous closely related bacterial clusters, which might reflect clusters of hidden local foodborne infections. This study underscores the elevated risk posed by food workers in the persistence and dissemination of *Salmonella* as vectors/fomites. Enhanced monitoring and targeted interventions in this group may reduce the dissemination of pathogens and antimicrobial resistance genes.

Salmonella constitutes a significant global burden of foodborne disease, accounting for 93 million cases of gastroenteritis, 155,000 fatalities, and 21.2 million disability-adjusted life years annually[1,2]. It is the second leading cause of acute diarrhea in China[3] and worldwide. Asymptomatic carriers[4], individuals that harbor and shed pathogens without manifesting discernible symptoms after infection, contribute to the natural survival and transmission of *Salmonella*, especially human-specific serovars Typhi and Paratyphi A. The first recognized

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and notorious asymptomatic carrier, Typhoid Mary in the USA[5], prompted investigations into the prevalence, mechanisms[6], and health implications[7] of the asymptomatic carrier state. However, few investigations[5,8,9] have focused on the asymptomatic carriage of non-typhoid *Salmonella* (NTS), which is primarily attributed to animals[10], vegetables, and water[11]. The prevalence of asymptomatic carriage of NTS among humans and its significance in foodborne infections may have been substantially underestimated.

Asymptomatic carriers contribute to the genetic diversity of *Salmonella*, facilitating the maintenance and exchange of antimicrobial resistance genes (ARGs)[12]. Frequent in situ transmission of ARGs, primarily mediated by plasmids, has been widely reported among Enterobacteriaceae[13–15], including *Salmonella*[16]. The prevalence of ARGs in asymptomatically carried *Salmonella* is influenced by both the repeated acquisition of bacteria from food sources[17] and horizontal gene transfer from other species[18,19], providing insight into the in vivo dynamic variations of ARGs.

The prevalence of *Salmonella*, the most genomic-sequenced foodborne pathogen, in patients[20–22], food animals[23–25], and food[26–28] has been extensively investigated. However, there is limited research on the genetics and transmission of *Salmonella* in asymptomatic carriers[9,29,30], particularly among food workers (FWs) who could potentially contaminate food during preparation. This resulted in a significant gap in our understanding of its natural presence. Here, we conducted the most comprehensive investigation to date on asymptomatic *Salmonella* carriage among 260,315 workers in Yulin, China, from 2013 to 2020. The individuals were classified as FWs or non-food workers (nFWs), reflecting their exposure to unprocessed foods. We identified a significantly higher *Salmonella* carriage rate among FWs. Furthermore, we documented annual fluctuations in antimicrobial resistance (AMR) in *Salmonella*, particularly those of clinical importance. Our findings underscore the role of asymptomatic carriers in the genetic diversity of *Salmonella* and the potential implications for the transmission of foodborne diseases, highlighting the need for effective control targeting both the bacteria and ARGs.

Results

Salmonella prevalence and the correlation with environmental conditions and economic/health factors

A total of 4964 non-duplicate *Salmonella* isolates were collected from 260,315 asymptomatic workers in Yulin, China, during 2013–2020, with an overall detection rate of 1.91% (1.38–2.32%) (Supplementary Table 1). The *Salmonella* carriage rate was significantly higher in FWs at 2.03% (4392/215,982), compared to nFWs at 1.29% (572/44,333) over the 8-year period ($p < 0.001$, Table 1 and Supplementary Table 2). This difference might be attributed to FW's increased exposure to potentially contaminated foods.

Table 1 | Characteristics of the sample population and the *Salmonella* positivity rate in each group

Factors	Variable	Number ($N = 260,315$)	No. of isolates ($n = 4964$) (%)	Degrees of freedom	*P*
Sex	Male	93,235	1840 (1.97)	1	0.064
	Female	167,080	3124 (1.87)		
Age	18-29	94,012	1736 (1.84)	3	0.084
	30-39	74,086	1396 (1.88)		
	40-49	58,264	1133 (1.94)		
	50-65	33,953	699 (2.06)		
Occupation	FW	215,982	4392 (2.03)	1	<0.001*
	nFW	44,333	572 (1.29)		

FW food workers, *nFW* non-food workers.
*Significant difference (chi-squared test, two-sided). *P*-values are expressed as $p < 0.001$ due to their extremely small values.

Intriguingly, while *Salmonella* carriage rates were similar between male and female FWs, women in the nFW group, exhibited twice the carriage rate of men (1.45% vs. 0.74%), possibly due to their more frequent engagement in home cooking. Although nFWs displayed no significant age-related pattern, FWs showed a slight decrease in *Salmonella* carriage with age, from 2.14% in the 18–29 age group to 1.98% in the 50–65 age group. This trend might be attributed to the work which frequently exposed to raw food, such as slaughtering and chopping raw meat, being undertaken by junior FWs. Additionally, *Salmonella* carriage rates in nFWs decreased with time from a peak of 2.36% in 2014 to 0.84% in 2020 (Fig. 1a), possibly associated with an increased urbanization rate ($r = -0.738$, $p = 0.037$) and the number of public toilets ($r = -0.922$, $p = 0.001$) (Table 2, Fig. 1b). In contrast, FW carriage rates remain relatively stable (Fig. 1a), mirroring consistent *Salmonella* contamination levels in foods[27]. Taken together, these findings highlight an association between food contact and asymptomatic *Salmonella* carriage.

Seasonal variations in *Salmonella* carriage were evident. Both groups had the lowest detection rates (0.4–0.5%) in February, but *Salmonella* carriage in FWs increased more sharply than in nFWs, both peaking in September at 3.2% and 2.2%, respectively. These patterns aligned with the annual variations in acute diarrhea cases caused by NTS in southern China[3]. This close association may partially be attributed to temperature fluctuations in Yulin, which correlated with carriage rates in both FWs ($r = 0.71$, $p < 0.001$) and nFWs ($r = 0.473$, $p < 0.001$) (Table 2 and Fig. 1c). Even stronger correlations were found between average monthly temperatures and *Salmonella* carriage rates one month later (Table 2 and Fig. 1d), suggesting additional factors influencing seasonal variations.

Asymptomatic carriers maintain *Salmonella* diversity

From the 4964 asymptomatically carried *Salmonella* strains, 116 serovars were identified, including 32% core serovars (37/116) isolated over ≥6–8 years, constituting 92% and 93.83% of the carriage of *Salmonella* strains in the FW and nFW groups, respectively (Fig. 2a, Supplementary Table 1, and Supplementary information p1). The remaining accessory serovars were isolated less frequently, with 50 serovars detected only in one year. Serovar frequencies fluctuated over time (Supplementary Fig. 1a), with eight core serovars increasing and five decreasing substantially over the 8 years (Supplementary Fig. 1b, $r \geq 0.3$ and $p < 0.05$). Notably, all five decreasing serovars (Agona, Albany, Hadar, Infantis, and Kottbus) were frequently associated with poultry, whereas many increasing serovars (Rissen, London, and Goldcoast) were associated with swine.

Salmonella from healthy carriers showed high genetic diversity. The two most abundant serovars (Typhimurium and Enteritidis) were responsible for 37.6% and 27.2% of strains from acute diarrhea[31] and animal sources[27], respectively, compared to only 14.7% of strains from asymptomatic carriage in our study. This was evidenced by the greater Shannon diversity index (SDI) of 2.9 for *Salmonella* serovars in healthy carriers compared to 1.9 for those from acute diarrhea cases[31] and 2.4 for animal sources[27]. Notably, Enteritidis, the 4th and 2nd serovar from animal sources and acute diarrhea cases, ranked 12th among asymptomatic carriage, accounting for merely 2.16% of isolates (Supplementary Fig. 2a and 2b, Supplementary information p1). This underscores the distinct role of asymptomatic carriers in preserving *Salmonella* genetic diversity.

A total of 113 serovars were identified in strains from the FW group, approximately twice as many as the 61 serovars from the nFW group (Supplementary Table 1 and Supplementary Fig. 2c). Among these, 64 serovars were exclusively associated with FWs, compared to only three for nFWs (Supplementary Fig. 2c). The

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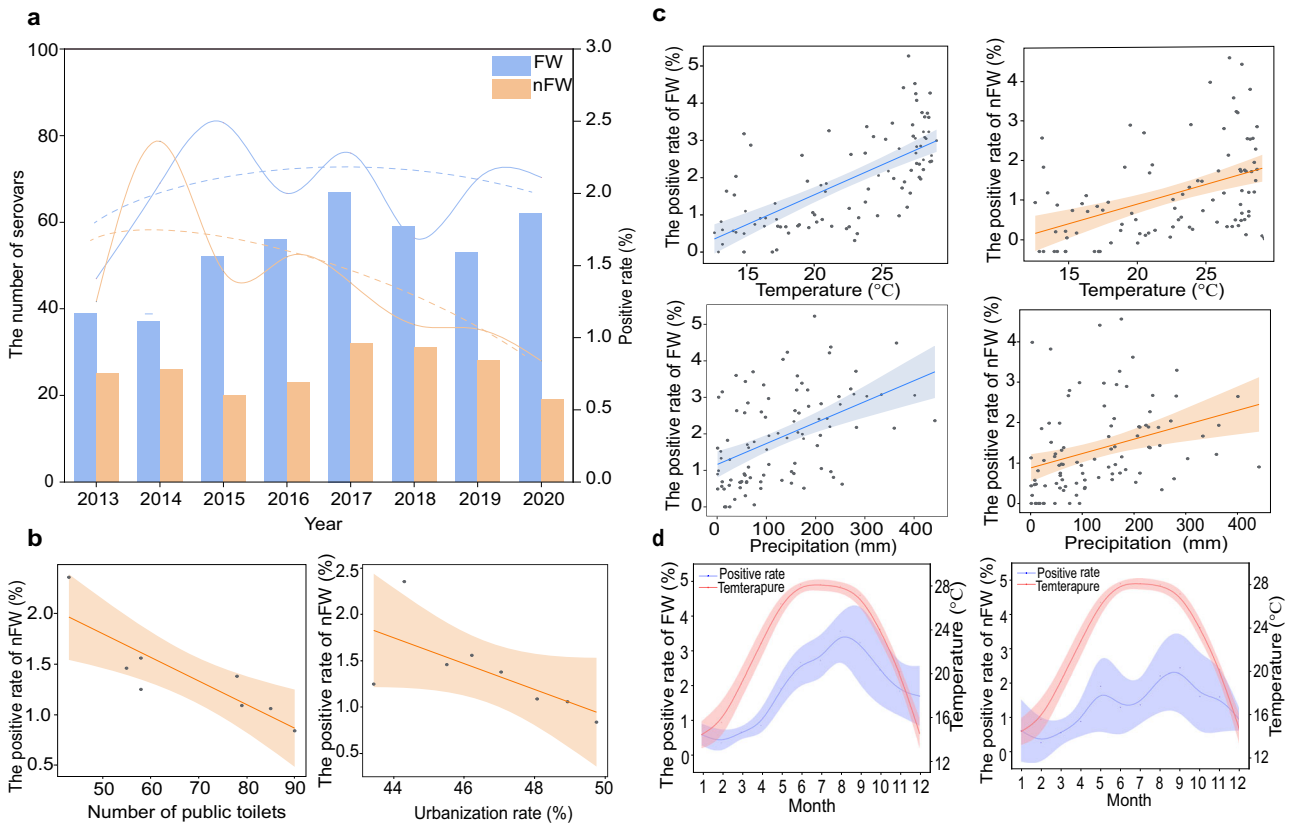

Fig. 1 | Correlation between the *Salmonella* positive detection rates in the two occupational groups, the environmental conditions, and economic/health factors. a Positive detection rates and the number of serovars present in the two occupational groups per year (FW: food workers, nFW: non-food workers). The dashed lines represent the trend in the *Salmonella* positivity rates in the two occupational groups. **b** Spearman's rank correlation analysis (two-sided) was used to analyze the annual correlations between the *Salmonella* positivity rate in the nFW group ($n = 8$) and economic/health factors (i.e., the number of public toilets and the urbanization rate) ($n = 8$) from 2013 to 2020. The points in the graph represent the *Salmonella* positivity rate in the nFW group corresponding to different numbers of public toilets or urbanization rates. The line demonstrates a negative correlation trend between the two variables, while the error bands indicate the 95% confidence interval. The number of public toilets and the urbanization rate were negatively correlated with the *Salmonella* positivity rate in the nFW group ($r = -0.922$, $p = 0.001$ and $r = -0.738$, $p = 0.037$, respectively), while no such correlation was observed in the FW group ($r = 0.048$, $p = 0.91$ and $r = 0.31$, $p = 0.456$, respectively). Multiple comparison adjustments were not applied to the *P* values. **c** Spearman's rank correlation analysis (two-sided) was used to analyze the monthly correlations between the *Salmonella* positivity rate (in the FW and nFW groups) ($n = 96$, respectively) and temperature ($n = 96$) and precipitation ($n = 96$) from 2013 to 2020. The points in the graph represent the *Salmonella* positivity rate in the FW group or nFW group corresponding to temperature and precipitation. The line demonstrates a positive correlation trend between the two variables, while the error bands indicate the 95% confidence interval. Temperature and precipitation are positively correlated with the *Salmonella* positivity rate in the FW group ($r = 0.71$, $p < 0.001$ and $r = 0.49$, $p < 0.001$, respectively). Temperature and precipitation are positively correlated with the *Salmonella* positivity rate in the nFW group ($r = 0.473$, $p < 0.001$ and $r = 0.452$, $p < 0.001$, respectively). Multiple comparison adjustments were not applied to the *p* values. **d** Error band plots of monthly temperature and *Salmonella* positivity rates in the two occupational groups over 8 years. The average positive rate (blue)/temperature (red) values per month and their respective 95% confidence bands were estimated. After adjusting the temperature by a one-month lag, the correlation between the adjusted temperature and the positivity rate in both groups increased (Spearman's rank correlation, two-sided. FW: $r = 0.724$, $p < 0.001$ and nFW: $r = 0.537$, $p < 0.001$). Multiple comparison adjustments were not applied to the *p* values.

greater number of serovars in FWs was not solely due to a higher number of isolates. The SDI of *Salmonella* from FWs remained stable at around 3.13 over 8 years, while the SDI of nFW strains decreased from 3.08 in 2014 to 2.64 in 2020. For 11 serovars (Typhimurium, Corvallis, Agona, Derby, Rissen, London, Kentucky, Weltevreden, Senftenberg, Anatum, and Albany), their positivity rates in the FW group were significantly higher than that of the nFW group ($p < 0.05$). However, the serovar rankings did not differ significantly between groups ($p = 0.557$).

Less than half (49/116) of the *Salmonella* serovars coexisted in both groups. These shared serovars had similar relative frequencies between FWs and nFWs ($r = 0.9587$) (Fig. 2b), suggesting close associations. Typhimurium and Corvallis were the most frequent serovars in both groups. Almost all serovars were more frequently isolated from FWs except for Thompson and Hadar, which were significantly more prevalent in nFWs (Fig. 2c). Both serovars were associated with poultry and showed decreasing prevalence over time[27].

Increasing antimicrobial resistances in *Salmonella* isolates

The susceptibility tests of all isolates to 29 antimicrobial agents across 12 drug classes revealed high resistance rates to tetracycline (TET) (67.8%), chloramphenicol (CHL) (52.7%), and trimethoprim-sulfamethoxazole (SXT) (39.0%). More than 10% of the isolates showed resistance to seven other agents, with an overall multidrug resistance (MDR, resistant to ≥3 classes of antimicrobials) rate of 32.98% (Supplementary Table 1). Resistance to first-line antibiotics for salmonellosis was detected, including quinolones [ciprofloxacin (CIP, 12.9%), moxifloxacin (MXF, 7.0%), levofloxacin (LVX, 5.3%), and norfloxacin (NOR, 3.8%)] and third-generation cephalosporins [ceftriaxone (CRO, 10.9%), ceftazidime (CAZ, 5.4%), and cephalosporin (SCP, 2.8%)]. Other antibiotics, such as carbapenems (EIP and MEM) had resistance rates of less than 1.2%, but displayed increasing trends. Additionally, 2.8% of the isolates were resistant to colistin (COL) (Fig. 3a). Alarmingly, 143 strains (2.9%) displayed resistance to both quinolones and third-generation cephalosporins, with 14 also resistant to carbapenems and

Table 2 | Correlation analysis of the *Salmonella* positivity rates in the two occupational groups, taking environmental conditions and economic/health factors into consideration

	Correlate analysis[a]	FW		nFW	
		r	*p*	*r*	*p*
Environmental factors	Temperature	0.71	<0.001*	0.473	<0.001*
	Precipitation	0.49	<0.001*	0.452	<0.001*
Economic/ health factors	GDP	0.476	0.233	−0.619	0.102
	Per capita GDP	0.476	0.233	−0.619	0.102
	Water consumption	−0.443	0.272	0.563	0.146
	Number of hospitals	0.204	0.629	−0.683	0.062
	Number of hospital beds	0.214	0.61	−0.69	0.058
	Number of public toilets	0.048	0.91	−0.922**	0.001*
	Urbanization rate	0.31	0.456	−0.738*	0.037*
Temperature adjustment[b]	Two-month lag	0.592	<0.001*	0.348	0.001*
	One-month lag	0.724	<0.001*	0.537	<0.001*

FW food workers, *nFW* non-food workers, *GDP* Gross Domestic Product.
*Significant difference (Spearman's rank correlation, two-sided). *P*-values are expressed as $p < 0.001$ due to their extremely small values.
[a]Spearman's rank correlation analysis was used to analyze correlations of *Salmonella* positive rate in FW and nFW groups with economic/health factors and environmental conditions, respectively.
[b]To further explore the relationship between temperature and the *Salmonella* positivity rates of the two occupational groups, correlations were analyzed between the temperature values for a given month and the *Salmonella* positivity rates for that month, one month prior, and two months prior. After adjusting the temperature with a one-month lag, the correlations between the adjusted temperatures and the positivity rates were stronger in both groups.

six exhibiting extensive drug resistance across quinolones, third-generation cephalosporins, carbapenems, and colistin (Supplementary Fig. 3 and Supplementary information p1).

Resistance frequencies varied over the 8 years, with increases noted for tetracyclines, quinolones, and carbapenems, which are extensively used in clinical settings. In contrast, resistance to the majority of drugs, including aminoglycosides, cephalosporin, and penicillin inhibitors, remained relatively stable. A decline in colistin resistance was observed after a peak in 2015–2016 (17%), possibly linked to governmental regulatory changes (http://www.moa.gov.cn/nybgb/2016/dibaqi/201712/t20171219_6102822.htm) (Fig. 3a).

Using least absolute shrinkage and selection operator (LASSO) logistic analysis, we identified four resistance phenotypes (aminoglycoside, fluoroquinolone, tetracycline, and colistin) that exhibited close serovar associations (Fig. 3b, Supplementary Fig. 4, Supplementary Table 3, and Supplementary information p1). Based on antimicrobial susceptibility, serovars were categorized into three groups: six high-resistance (≥8 antimicrobials), 25 multi-resistance (3–8 antimicrobials), and 85 low-resistance (<3 antimicrobials). All six high-resistance serovars appeared only once throughout the 8-year period, indicating external introductions. Over half (13/25) of the multi-resistance group consisted of the core serovars isolated in ≥6 of the 8 years. In contrast, accessory serovars accounted for 72% (61/85) of the low-resistance group, demonstrating their lower AMR levels (Fig. 3b and Supplementary information p2). Further subdivision of the core serovars by their yearly carriage rates revealed that the eight serovars with increased prevalence exhibited resistance to, on average, 4.93 antimicrobials. In contrast, the five serovars with decreased prevalence exhibited resistance to only 2.70 antimicrobials, emphasizing the role of AMR in natural adaptation and competition.

Furthermore, our findings revealed higher resistance in FWs compared to nFWs for all drugs, except levofloxacin and colistin. This included five antimicrobials (Fig. 3c; cefotaxime, ceftriaxone, aztreonam, chloramphenicol, and fosfomycin) with statistically significant differences ($p < 0.05$). As a result, FW strains exhibited a slightly higher MDR rate (33.4%) compared to nFW strains (29.9%), although the difference was not statistically significant. The FW group exhibited more MDR patterns, showing an increasing trend over the 8 years, while the nFW group had fewer MDR patterns with frequent changes (Supplementary Fig. 5a).

Plasmids as major drivers of AMR dissemination

We genomic sequenced 1689 strains, encompassing all MDR strains (1637) and those resistant to quinolones/third-generation cephalosporins/carbapenems/colistin. Genomic analysis predicted 162 ARGs across 11 classes (Supplementary Fig. 6a and 6b, and Supplementary information p2). MDR rates over 50% were found in 29 serovars. These serovars all exhibited open panels of ARGs, with high chances of finding new MDR patterns per additional sequenced genome by the rarefaction curves (Supplementary Fig. 7). This suggested the presence of additional genetic diversity beyond our reports.

We predicted plasmids in 1302 of the 1689 genomes using a Kraken-based tool[32] and found that the majority of ARGs were located in contigs associated with plasmids (Supplementary Fig. 8), indicating the importance of plasmids in spreading resistance. Plasmid distribution varied among serovars, with specific plasmids showing high positivity rates in certain serovars, such as IncHI1A/B plasmids in 87.8% of the Goldcoast strains and IncN plasmids in 88.2% of Braenderup and Cerro strains (Supplementary Fig. 6c).

Approximately 86.7% of quinolone-resistant strains possessed genes or mutations associated with quinolone resistance. Plasmid-mediated quinolone resistance (PMQR) genes were prevalent, with *qnrS* (56.03%), *oqxA* (46.10%), and *aac(6')-Ib-cr* (45.74%) being the most common. Mutations in *gyrA*, *parC*, or *parE* genes were identified in 224 strains, 146 of which also carried PMQR genes. Similarly, 74.7% of detected third-generation cephalosporin resistance was attributed to seven ARGs, predominated by bla_{TEM} (50.12%), $bla_{CTX\text{-}M}$ (43.2%), and bla_{OXA} (16.47%) (Supplementary Tables 4–6, Supplementary information p2).

Only 13.9% (11/79) of carbapenem-resistant strains were predicted to encode a carbapenemase (Supplementary Tables 4 and 7), and only 36 of 132 colistin-resistant strains were predicted to carry the *mcr*−1 or *mcr*-9 genes (Fig. 3d). Furthermore, only one of nine tigecycline-resistant strains harbored the *tet(X4)* gene and the plasmid-mediated efflux pump *tmexCD-toprJ* was not found. These findings indicated the presence of potentially new drug-resistance mechanisms in *Salmonella* that are yet to be explored. Associations between ARGs and plasmid replicon types were also identified (Supplementary Fig. 9 and Supplementary Table 8), highlighting the role of plasmid in the spread of ARGs.

Long-term persistence of HC5s among asymptomatic workers

All genomes were uploaded into EnteroBase[33] for core genome multilocus sequence typing (cgMLST) and subsequent hierarchical clustering (HierCC) of the obtained sequence types (STs). The majority (75%, 22/29) of the MDR serovars ($n \geq 5$) were each associated with one HC900 cluster, namely a single-linkage cluster with branch lengths of ≤900 allelic differences. The exceptions were serovars Derby, Kentucky, Kottbus, Stanley, Goldcoast, and Newport, each separating into two HC900s, and Cerro, which fell into three HC900s. Furthermore, we approximated genetically closely related strain groups as HC5 clusters, a threshold that has been widely used in disease outbreak investigations[34,35]. Nearly 94% (218/232) of the 232 identified HC5 clusters were each associated with no more than three isolates in any month (Supplementary Fig. 10). The remaining 14 HC5 clusters had four or more isolates in at least one month, suggesting them as potentially Surge HC5s (SuHC5s).

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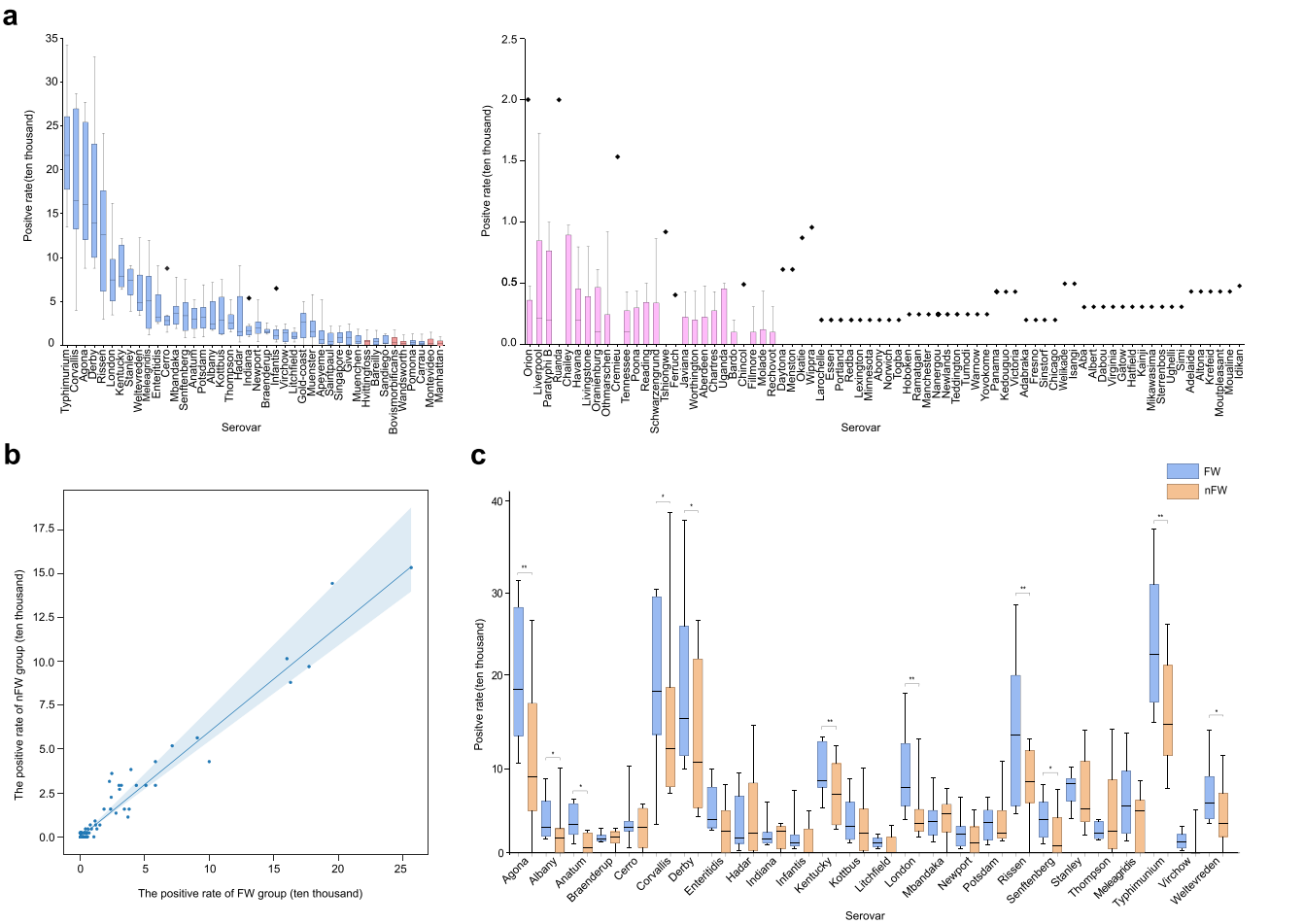

Fig. 2 | The *Salmonella* serovar positivity rates. The serovar of each strain obtained in this study was identified, and biological replicates were performed. **a** Positivity rate of the *Salmonella* serovars ($n = 116$) detected in this study. Data are shown as the mean ± s.e.m. Boxplot elements are median; the bounds of box, upper and lower quartiles; the whiskers, 1.5× interquartile range. The rhombuses represent outliers beyond $x \pm 3\sigma$ in each serovar. The serovars are ordered according to their positivity rate and the number of years in which they appeared. Blue bars represent the serovars that were present for 6–8 years, red bars represent serovars that were present for 5 years, and pink bars represent serovars that were present for fewer than 4 years. **b** Spearman's rank correlation (two-sided) was used to analyze the correlation between the *Salmonella* positivity rate in the FW group and nFW group ($n = 49$, $r = 0.9587$). The points in the graph represent the *Salmonella* positivity rate in the FW group corresponding to that in the nFW group. The line demonstrates a positive correlation trend between the two variables, while the error bands indicate the 95% confidence interval. **c** Comparison of the *Salmonella* ($n = 26$) positivity rates in the two occupational groups. Fisher's exact test was used if N was less than 20 and one expected cell was less than or equal to five. Data are shown as the mean ± s.e.m. Boxplot elements are median; the bounds of box, upper and lower quartiles, and the whiskers, 1.5× interquartile range. The positivity rates in the FW group were higher than in the nFW group for 11 of the 26 serovars that were detected in each of the 8 years (chi-squared test, two-sided, $^{*}0.01 < p < 0.05$, $^{**}p < 0.01$) (FW food workers, nFW non-food workers). Multiple comparison adjustments were not applied to the *P* values. Source data are provided as a Source Data file.

Nearly 90% (752/848) of the HC5-clustered isolates were from FWs. Concordantly, 168 and seven of the 232 HC5 clusters were exclusively associated with FWs and nFWs, respectively, while the remaining 57 were found in both groups (Table 3). Notably, SuHC5s were significantly more likely to be found in both groups than the non-SuHC5s (12/14 versus 45/218, $p < 0.0001$), underscoring their wider spreading.

The majority (65%, 139/232) of the HC5s were transient, with all associated isolates reported within six months. Four of these transient HC5s were SuHC5s, including HC5_424918 in Typhimurium (five isolates in November 2017), HC5_425122 in Goldcoast (four isolates in May 2019), HC5_425076 (four isolates in April 2019) and HC5_425352 (five isolates in December 2019) in London, each forming an independent branch in the cgMLST trees (Supplementary Fig. 11). There were also 40 (17%) long-persisting HC5s with time spanning of ≥24 months, including eight SuHC5s, a significantly higher proportion than in the transient HC5s ($p = 0.0008$). Additionally, four HC5s lasted for ≥6 years. Among them, HC5_4181 in Agona likely reflected repetitive introduction from abroad followed by long-term regional transmission (Supplementary Fig. 12), and HC5_285619 (Typhimurium) and HC5_350459 (Albany) have also been isolated from Canada and Guangdong, China, respectively, leaving HC5_424364 (London) the only cluster without prior reports (Supplementary Fig. 11).

Discussion

Asymptomatic carriage of *Salmonella*, particularly those of antibiotic resistance, poses a substantial yet often overlooked threat to public health. This 8-year, laboratory-based surveillance investigates the prevalence, antibiotic resistance, and genetic characteristics of *Salmonella* among adult carriers, including both FWs and nFWs.

Healthy carriages of pathogens like *S. enterica* have been widely documented[36,37]. Despite low frequency (1–2%) compared to patients exhibiting symptoms, the role of healthy carriers in the dissemination of infectious diseases remains critical due to the large population base they represent. Our research reveals a vast, previously overlooked, natural reservoir of *Salmonella*. FWs exhibited significantly higher frequencies of *Salmonella* with more complex serovars than nFWs,

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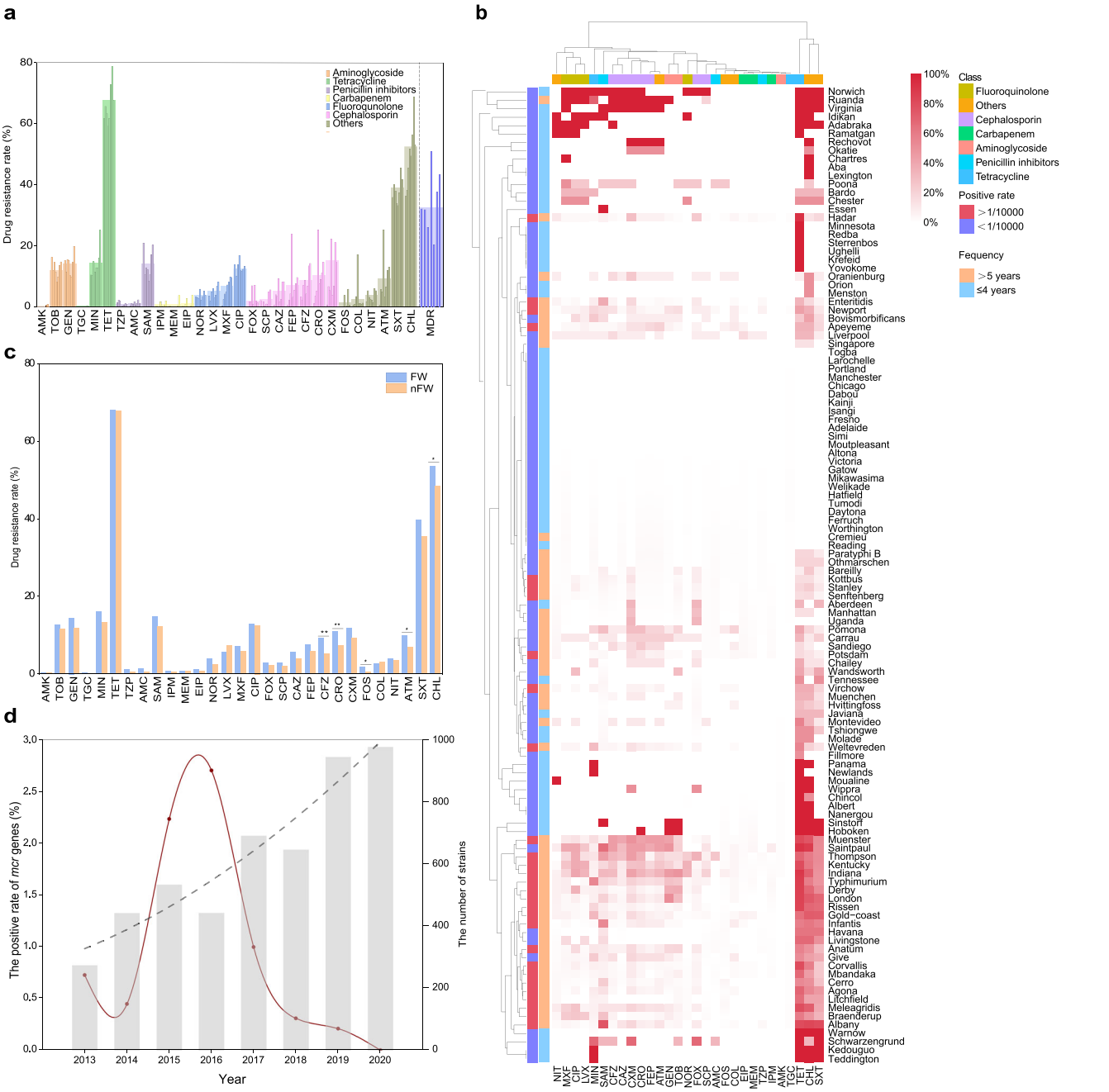

Fig. 3 | Antibiotic resistance rates of *Salmonella* strains carried by asymptomatic workers. a Total antibiotic resistance rate of 29 antibiotics and multidrug-resistance (MDR) rates over the 8 years. The background color in the bar chart indicates the total antibiotic resistance rate of each antimicrobial agent. **b** Heatmap of the antibiotic resistance rates for 116 serovars and 29 antibiotic agents [including amikacin (AMK), gentamicin (GEN), tobramycin (TOB), tetracycline (TET), minocycline (MIN), tigecycline (TGC), amoxicillin-clavulanate (AMC), ampicillin-sulbactam (SAM), piperacillin-tazobactam (TZP), ertapenem (EIP), imipenem (IPM), meropenem (MEM), ciprofloxacin (CIP), levofloxacin (LVX), moxifloxacin (MXF), norfloxacin (NOR), cefazolin (CFZ), cefepime (FEP), cefoperazone-sulbactam (SCP), cefoxitin (FOX), ceftazidime (CAZ), ceftriaxone (CRO), cefuroxime (CXM), chloramphenicol (CHL), aztreonam (ATM), colistin (COL), fosfomycin w/G6P (FOS), nitrofurantoin (NIT), and trimethoprim-sulfamethoxazole (SXT)]. **c** The chi-square test was used to compare the antibiotic resistance rates of 29 antibiotics between the two occupational groups. For five antibiotics, the resistance rates were higher in the FW group than in the nFW group (two-sided, $*0.01 < p < 0.05$, $**p < 0.01$) (FW food workers, nFW non-food workers). Multiple comparison adjustments were not applied to the *P* values. Source data are provided as a Source Data file. **d** Trends in the *mcr* gene detection rate among *Salmonella* strains isolated from asymptomatic workers. The bar graph shows the number of *Salmonella* strains obtained from asymptomatic workers each year, the gray dashed line indicates the trend in the number of *Salmonella* strains collected per year, and the red curve indicates the *mcr* gene positivity rate in the *Salmonella* strains. After the colistin ban was implemented in April 2017, the annual *mcr* gene positivity rate decreased from 1% in 2017 to 0.001% in 2020.

likely due to occupational exposure to contaminated food. This finding aligns with previous research emphasizing the role of food contact in the transmission of *Salmonella*[38]. The increased likelihood of contamination in FWs, who handle and process raw food, highlights the need for targeted interventions in this occupational group to prevent the spread of *Salmonella*. Conversely, nFWs showed lower and decreasing carriage rates over time, reflecting increased economic/health status in Yulin.

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Table 3 | Prevalence of HC5 clusters determined based on HierCC (Hierarchical Clustering of CgMLST) for different serovars

Serovar	Number of strains (FW, nFW)	HC5				FW	nFW	FW&nFW	Same year	Different year
		Strains (≥2) (%)	Clusters	Strains (/cluster)	Clusters (/100)	Clusters (strains)	Clusters (strains)	Clusters (strains)	Clusters (strains)	Clusters (strains)
Typhimurium	270 (240, 30)	100 (37)	34	2.9	34	25 (67)	1 (2)	8 (31)	21 (47)	13 (53)
Derby	198 (172, 26)	70 (35.3)	25	2.8	35.7	18 (52)	1 (2)	6 (16)	18 (43)	7 (27)
London	147 (138, 9)	94 (63.9)	32	2.9	34	27 (75)	0 (0)	5 (19)	21 (60)	11 (34)
Agona	116 (104, 12)	95 (81.9)	15	6.3	15.8	10 (20)	0 (0)	5 (75)	11 (30)	4 (65)
Kentucky	112 (103, 9)	63 (56.3)	21	3	33.3	17 (50)	1 (2)	3 (11)	10 (27)	11 (36)
Corvallis	99 (81, 18)	75 (75.8)	14	5.4	18.7	9 (22)	2 (4)	3 (49)	10 (22)	4 (53)
Rissen	97 (89, 8)	36 (37.1)	12	3	33.3	10 (25)	0 (0)	2 (11)	10 (24)	2 (12)
Albany	75 (69, 6)	61 (81.3)	12	5.1	19.7	8 (32)	0 (0)	4 (29)	5 (12)	7 (49)
Meleagridis	72 (65, 7)	58 (80.6)	7	8.3	12	4 (15)	0 (0)	3 (43)	2 (9)	5 (49)
Thompson	47 (38, 9)	23 (48.9)	5	4.6	21.7	2 (6)	0 (0)	3 (17)	2 (4)	3 (19)
Gold-coast	42 (36, 6)	27 (64.3)	7	3.9	25.9	5 (16)	0 (0)	2 (11)	3 (9)	4 (18)
Indiana	38 (35, 3)	22 (57.9)	8	2.75	36.4	5 (0)	0 (0)	3 (12)	2 (9)	6 (13)
Muenster	37 (34, 3)	22 (59.4)	3	7.3	13.6	2 (5)	0 (0)	1 (17)	2 (5)	1 (17)
Cerro	33 (30, 3)	19 (57.6)	7	2.7	36.8	5 (11)	0 (0)	2 (8)	4 (10)	3 (9)
Infantis	22 (19, 3)	13 (59.1)	5	2.6	38.5	2 (4)	0 (0)	3 (9)	1 (2)	4 (11)
Stanley	21 (18, 3)	4 (19)	2	2	50	1 (2)	0 (0)	1 (2)	2 (4)	0 (0)
Mbandaka	20 (17, 3)	12 (60)	2	6	16.7	0 (0)	0 (0)	2 (12)	1 (3)	1 (9)
Enteritidis	20 (16, 4)	5 (25)	2	2.5	40	1 (2)	1 (3)	0 (0)	1 (2)	1 (3)
Weltevreden	20 (19, 1)	8 (40)	4	2	50	4 (8)	0 (0)	0 (0)	4 (8)	0 (0)
Anatum	19 (16, 3)	10 (52.6)	3	3.3	30	2 (4)	0 (0)	1 (6)	2 (4)	1 (6)
Braenderup	16 (14, 2)	5 (31.3)	2	2.5	40	2 (5)	0 (0)	0 (0)	2 (5)	0 (0)
Saintpaul	14 (14, 0)	5 (35.7)	2	2.5	40	2 (5)	0 (0)	0 (0)	2 (5)	0 (0)
Newport	13 (8, 3)	6 (46.1)	2	3	33.3	1 (4)	1 (2)	0 (0)	2 (6)	0 (0)
Hadar	12 (11, 1)	4 (33.3)	2	2	50	2 (4)	0 (0)	0 (0)	2 (4)	0 (0)
Potsdam	10 (9, 1)	0 (0)	0	0	0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Apeyeme	7 (7, 0)	5 (71.4)	2	2.5	40	2 (5)	0 (0)	0 (0)	2 (5)	0 (0)
Kottbus	7 (7, 0)	0 (0)	0	0	0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Hvittingfoss	5 (5, 0)	2 (40)	1	2	50	1 (2)	0 (0)	0 (0)	1 (2)	0 (0)
Senftenberg	5 (5, 0)	4 (80)	1	4	25	1 (4)	0 (0)	0 (0)	1 (4)	0 (0)

Based on the sequenced genomes of the multidrug-resistant strains and the quinolones/third-generation cephalosporins/carbapenem/colistin antibiotic-resistant strains, HC5 analyses were performed for each of the 29 serovars (containing five or more strains in each serovar) to determine the genomic clusters of the strains.
FW food workers, *nFW* non-food workers.

Seasonal variations in *Salmonella* carriage rates correlated with temperature fluctuations, emphasizing the complex interplay between environmental conditions and bacterial prevalence. The observed seasonal patterns aligned with the annual patterns in acute diarrhea cases caused by NTS in southern China[3], suggesting a possible link between *Salmonella* carriage and the regional epidemiology of diarrheal diseases. The findings are also consistent with previously identified associations between the frequencies of many foodborne pathogens like *Escherichia coli* O157 and *Campylobacter* in food animals and the climate condition, particularly latitudinal gradients in the northern hemisphere[36,37]. Notably, the average monthly temperatures correlated more strongly with *Salmonella* carriage rates one month later, likely reflecting a time delay in the enrichment of *Salmonella* in natural repositories, such as animals, before transmission to humans. Additionally, precipitation (Supplementary Fig. 13), especially the dual-peaked monsoon rainfall in Yulin[39], likely contributed to the dual *Salmonella* peaks observed in May and September, particularly among nFWs. This underscores the importance of considering both the short-term and delayed environmental effects on the epidemiology of *Salmonella*.

Compared to those previously reported among acute diarrhea cases and animal sources, our study observed substantially higher serovar diversity among asymptomatic carriers, underscoring the unique role of the carriers in preserving *Salmonella* genetic diversity (Supplementary information p2–3). Significant differences in *Salmonella* serovar rankings were observed between asymptomatic workers and those with salmonellosis ($p < 0.001$). Healthy carriers exhibited notably lower frequencies of Enteritidis, the predominant poultry-associated serovar[40], indicating its lower capacity for colonizing in humans. In contrast, Typhimurium and its variants, predominant in acute diarrhea cases, had high asymptomatic carriage rates. This indicates that some of the *Salmonella* isolates from patients, notably those of Typhimurium, might not be the direct causative agent of their acute diarrhea, but a hitchhiker. Furthermore, some *Salmonella* serovars might colonize humans without causing acute diarrhea, resulting in greater serovar diversity in our investigation than those from patients. However, these asymptomatically carried *Salmonella* might result in illness during periods of weakened immunity, demanding continuous monitoring.

We identified 37 core serovars consistently isolated over the study period, highlighting their persistent presence in both FWs and nFWs. A selective decline in serovars associated with poultry was observed, accompanied by an increase in those linked to swine. This is not attributable to the swine production or dietary changes, as the relative consumption of swine over chicken decreased from 1.82× in 2013 to 0.75× in 2020, and the Pork/Poultry production ratio stabilized around

1.71 between 2013 and 2019 (http://tjj.gxzf.gov.cn/tjsj/tjnj/). Detailed analyses indicated that such variations were possibly associated with the greater levels of AMR in the swine-related serovars, many of which are increasingly concerning because of their MDR and rising association with acute infections in China[27,41–43].

High levels of antimicrobial resistance, especially >30% of MDR, were observed in *Salmonella*. Alarmingly, high resistance rates to commonly used antimicrobials, including quinolones and third-generation cephalosporins, indicated decreasing effectiveness of standard treatment regimens. The observed increase in resistance to tetracyclines, fluoroquinolones, and carbapenems further highlighted challenges in clinical management and the need for continuous surveillance (Supplementary information p3–4). Meanwhile, colistin resistance declined rapidly in 2017, particularly among FWs, likely reflecting the effective policy interventions banning the associated veterinary antibiotics (http://www.moa.gov.cn/nybgb/2016/dibaqi/201712/t20171219_6102822.htm), suggesting that FWs may act as an indicator of the outcome of veterinary antibiotic bans. However, resistance to fluoroquinolone, despite bans on four agents, only slightly decreased, possibly due to continued high usage of other agents in the same class in livestock[44] (Supplementary Fig. 5b and Supplementary information p3).

Notably, we identified genes/mutations responsible for only 11–27% of resistances to carbapenem, tigecycline, and colistin. The plasmid-mediated efflux pump *tmexCD-toprJ*, associated with increased tigecycline resistance in various clinical gram-negative bacteria[45], was not found in our strains, indicating the presence of other unclear resistance mechanisms. In particular, our analyses revealed a time-dependent increase in the genetic diversities of both ARGs and their plasmid vectors, demanding further investigations.

The *Salmonella* carried by FWs and nFWs is genetically similar, as indicated by their analogous serovar distributions and the presence of many highly clonal HC5 clusters. FWs, due to their higher rates of *Salmonella* carriage and frequent contact with unprocessed foods, likely contributed to the dissemination of *Salmonella* through food contamination during their operations, similar to the historical case of "Typhoid Mary"[5]. Alternatively, both FWs and nFWs might have been exposed to the same contamination sources, such as raw meats or animals[46,47]. Over 16% (38) of the 232 HC5 clusters had their initial isolates from nFWs, a value consistent with our random permutation tests, indicating no significant transmission trends. Nonetheless, phylogenetic analysis revealed that nFWs isolates clustered with the FW isolates in 57 HC5 clusters, with the first strains of 42 clusters isolated from FWs and 15 from nFWs. This is likely due to sampling bias, but contaminated cooking staff remains a significant risk factor for salmonellosis. Restaurants and other food establishments are common sites for *Salmonella* outbreaks and sporadic cases[38]. Inadequate practices by cooking staff, such as improper holding temperatures, inadequate cooking, contaminated equipment, sourcing food from unsafe origins, and poor personal hygiene, have been identified as major risk factors in the FDA Model Food Code[38]. Overall, FWs can act as vectors or fomites for transmission, or their establishments may serve as waystations for the pathogen from farm to consumer.

Regular screening of FWs and nFWs, as part of government regulation, provides baseline data on the time-dependent dynamics of human-associated *Salmonella* and can potentially detect their cryptic local dissemination and population expansion. We identified 14 SuHC5s that were isolated ≥4 isolates during peak months, a criterion similar to disease outbreaks, except those outbreaks involve symptomatic patients. The serovars associated with these SuHC5s were also frequently linked to acute diarrhea in China. Additionally, HC5 clusters varied in their isolation spans. Most HC5 clusters were transient and disappeared within six months of first isolation, while 40 HC5 clusters persisted for over two years during the study period, often overlapping with SuHC5s. Some of these HC5 clusters, such as HC_4181 in Agona, likely resulted from repeated importations, while many others, not reported in public databases, likely represented local endemicity in Yulin, especially London HC5_424364.

This study has some limitations. The nFW cohort reflected only a small subset of the community, only the workers who contact with the public were included in nFW group. The absence of detailed exposure data and the lack of sampling from local diarrhea patients or contaminated food sources limit the broader interpretation of our findings. Additionally, the retrospective design limited the examination of epidemiological associations within the HC5 clusters.

In conclusion, we present a comprehensive analysis of *Salmonella* prevalence, serovar distribution, AMR, and potential transmission dynamics among asymptomatic carriers in Yulin, China. Our findings reveal a complex interplay between environmental conditions, occupational exposure, and genetic diversity in shaping *Salmonella* epidemiology. These findings emphasize the need for targeted interventions, continuous surveillance, and a One Health approach to address the multifaceted challenges posed by *Salmonella*. Future research will explore specific mechanisms of transmission, sources of contamination, and the impact of interventions on *Salmonella* dynamics in both food-related and non-food occupational settings.

Methods

Samples and *Salmonella* strains

In this study, participants were asymptomatic workers, including both FWs and nFWs. An FW was defined as an individual who worked in food and/or beverage production or operation. An nFW was defined as an individual who worked in a public setting, such as in leisure, entertainment, beauty salons, cosmetics shops, or shopping malls, with direct contact with customers. The nFW group belongs to a public-facing occupation, just like the FW group, except that they do not process raw food materials during working hours. Therefore, nFWs were used as the control group in this study.

From January 2013 to December 2020, 260,315 workers (aged 18–65) were sampled in Yulin during their occupational health examinations, following the laws and regulations. Individuals with diarrhea and those who had taken antibiotics in the week prior to the physical examination were excluded (Supplementary Table 1). Fecal samples were cultured for *Salmonella* spp. as described previously[48]. Strains were stored at −80 °C. The basic characteristics of age, sex, and occupation were recorded, but their names and national identity numbers were encrypted due to privacy protection regulations, preventing the determination of the precise number of individuals included more than once. The individuals who carried *Salmonella* were treated with antibiotics until the clearance of *Salmonella* (the test becomes negative). As a result, while the individuals might have been sampled multiple times, the associated *Salmonella* isolates were unique. The serovars, resistance phenotypes, and genotypes of the *Salmonella* isolates were analyzed. The study was approved by the Ethics Committee of the National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention (approval number: ICDC-2022001), which agreed to waive informed consent and allowed secondary analysis of strains and data for the following reasons: (1) We can only access anonymous records. (2) We have further protected personal privacy by providing the results of the FW and nFW groups rather than disclosing personal information.

Serovar identification

The 4964 strains were serovard by slide agglutination using commercial antiserum in accordance with a protocol from the Statens Serum Institut (Danish) and were determined according to the White–Kauffmann–Le Minor scheme[49].

Antimicrobial susceptibility testing

All 4964 *Salmonella* isolates were analyzed for susceptibility to Phoenix NMIC413 (Becton, Dickinson Co., Franklin Lakes, NJ, USA), which includes 29 antimicrobial agents [amikacin (AMK), gentamicin (GEN), tobramycin (TOB), TET, minocycline (MIN), tigecycline (TGC), amoxicillin-clavulanate (AMC), ampicillin-sulbactam (SAM), piperacillin-tazobactam (TZP), EIP, imipenem (IPM), MEM, CIP, LVX, MXF, NOR, cefazolin (CFZ), cefepime (FEP), SCP, cefoxitin (FOX), CAZ, CRO, cefuroxime (CXM), CHL, aztreonam (ATM), COL, fosfomycin w/ G6P (FOS), nitrofurantoin (NIT), and SXT]. The drug resistance of these isolates to antibiotics was identified according to the minimum inhibitory concentration with reference to the Clinical and Laboratory Standards Institute criteria M100, 30st Edition[50]. *Escherichia coli* ATCC 25922 was used as a quality control strain during the antimicrobial susceptibility tests.

Genome sequencing analysis

The genomic DNAs of MDR *Salmonella* and quinolone/third-generation cephalosporins/carbapenem/colistin antibiotic-resistant strains were extracted using the Wizard Genomic DNA Purification kit (Promega, Madison, USA). The DNA concentrations were quantified using a Qubit 4™ fluorometer with the Qubit dsDNA HS Assay kit (Thermo Fisher Scientific, MA, USA). Libraries were constructed using the MGIEasy FS DNA Library Prep Set (MGI, China) and were paired-end sequenced on the MGISEQ-200RS and MGISEQ-2000RS sequencing platforms (MGI). Raw reads were processed using fastp, which filtered low-quality bases ($Q < 30$) and kept only reads with >50 bp in size. The remaining high-quality reads were assembled using SPAdes v3.13.2, with additional options of '−careful -k 33,55,77'. ARGs and the replicon types of plasmids were screened using ResFinder v4.1.0 and PlasmidFinder.

Plasmid analyses

Kraken was used to predict and assign categories for contigs and identify their origin (i.e., plasmid or chromosomal)[32].

Phylogenetic analysis of genomic sequences

All genomes were uploaded into EnteroBase (http://enterobase.warwick.ac.uk) for cgMLST genotyping and subsequent HierCC clusterings. The HC900 and HC5 clusters were retrieved. Additionally, the minimum spanning trees (MSTs) were built and visualized using GrapeTree[51].

Environmental conditions and economic/health factors

Several environmental conditions and economic/health factors were explored as drivers of dynamic changes in the *Salmonella* positivity rate[52–54]. The environmental conditions were the mean temperature (°C) and mean precipitation (mm). The daily records of these data for Yulin were downloaded from the China Meteorological Data Service Center (http://data.cma.cn). We generated approximately 1 km × 1 km gridded mean temperature (°C) and precipitation (mm) datasets from 2012 to 2020 from the acquired records using ANUSPLIN-SPLINA version 4.36. We then aggregated the gridded datasets to the city-month level using QGIS version 3.34. The economic and health factor data from 2013 to 2020 were obtained from the Guangxi Zhuang Autonomous Region Bureau of Statistics (http://tjj.gxzf.gov.cn). The data included the gross domestic product (GDP), per capita GDP, water consumption, number of hospitals, number of hospital beds, number of public toilets, and the urbanization rate.

Consumption data

The consumption data for swine and poultry in Yulin, Guangxi Zhuang Autonomous Region, were calculated by multiplying the urban and rural populations with the respective annual per capita consumption data, which were sourced from the Guangxi Statistical Yearbooks spanning the years 2014 to 2023 (http://tjj.gxzf.gov.cn/tjsj/tjnj/).

Statistical analysis

All statistical analyses were performed using R version 4.0.4 with two-sided tests. The chi-square test was used to explore whether sex, age, and occupational factors affected the *Salmonella* carriage rate among the study population. Owing to differences in the sex and age distribution between the two occupational populations, the crude positivity rates could not be compared directly. To eliminate the influence of different age and sex distributions between the two groups, it was necessary to conduct standardized data processing for the FW and nFW groups. First, the FW group was taken as the standard population, and the expected number of positives in the standard population was calculated. Next, the standardized positivity rate was calculated, and the standardized rates were compared by a chi-square test. The chi-square test was also used to compare the annual number of serovars, the annual serovar positivity rate, and the MDR-*Salmonella* positivity rate between the two occupational groups. Fisher's exact test was used if N was <20 and one expected cell was less than or equal to five. The rank−sum test was used to compare the differences in the distribution of *Salmonella* serovars isolated from asymptomatic workers and patients with diarrhea from previous studies. We searched a study on *Salmonella* involving 36,822 patients with diarrhea in China from 2014 to 2021[31], a time period similar to our study. Spearman's rank correlation analysis was used to examine the correlations between the *Salmonella* positivity rates (in the FW and nFW groups) with environmental conditions and economic/health factors. Correlation analysis was also used to further explore the relationships between temperature and the *Salmonella* positivity rates in the two occupational groups. Spearman's rank correlation analysis was conducted between the temperature values for a given month and the *Salmonella* positivity rate for that month, one month prior, and two months prior. In addition, Spearman's rank correlation was used to analyze the association between *Salmonella* serovars isolated from FWs and nFWs, as well as the association between ARGs and plasmid replicon types. A *t*-test was used to compare the number of ARGs in plasmids and chromosomes. P values < 0.05 were considered statistically significant. We used the LASSO to construct a comprehensive model for predicting the phenotype of 12 drugs. The model encompassed 122 variables, including year, sex, age, occupation, urbanization rate, number of public toilets, and various serovars. Lambda parameter optimization was executed through 10-fold cross-validation. To address data imbalance, we employed multiple rounds of under-sampling, with the frequency determined by the ratio of sample numbers between negative and positive. We used the R "glmnet" package (4.1-8) to perform the LASSO regression analysis.

Reporting summary

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

Data availability

All assembled genome sequences were deposited in the NCBI database under BioProject accession numbers PRJNA1088298, PRJNA1088321, PRJNA1088386, PRJNA1088617, PRJNA1088619. The research data generated in this study have been deposited in the figshare repository (https://doi.org/10.6084/m9.figshare.26490733). The source data generated in this study are provided in the Supplementary Information. Source data are provided with this paper.

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Author contributions

X.L. and M.W. prepared the manuscript. M.L. and J.X. reviewed the manuscript. F.D., D.J., C.Z., T.M., B.P., M.Y., Z.Z., and Y.W. (Yongning Wu) provided all technical assistance. M.L. and Y.W. (Yannong Wu) collected samples and isolated bacteria. M.L., M.W., Y.P., and L.Y. carried out the experiments. M.W., Z.L., W.Z., and Y.Z. performed the data analysis. B.K. and X.L. designed the study and led the writing of the paper. All authors had full access to all of the data in the study, had read and approved the final manuscript, and had final responsibility for the decision to submit for publication.

Competing interests

The authors declare no competing interests.

Additional information

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