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Epidemiological trends in serotypes distribution and antimicrobial resistance in *Salmonella* from humans in Taiwan, 2004-2022

Ying-Shu Liao[1], Tsai-Ling Lauderdale[2], Jui-Hsien Chang[1], Shiu-Yun Liang[1], Chi-Sen Tsao[1], Hsiao Lun Wei[1], You-Wun Wang[1], Ru-Hsiou Teng[1], Yu-Ping Hong[1], Bo-Han Chen[1], Chien-Shun Chiou[1,*]

[1] *Center for Diagnostics and Vaccine Development, Centres for Disease Control, Taichung, Taiwan*
[2] *National Institute of Infectious Diseases and Vaccinology, National Health Research Institutes, Miaoli, Taiwan*

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ABSTRACT

Objectives: *Salmonella*, a zoonotic pathogen, significantly impacts global human health. Understanding its serotype distribution and antimicrobial resistance is crucial for effective control measures and medical interventions.
Methods: We collected *Salmonella* isolates and demographic data from Taiwanese hospitals between 2004 and 2022, analyzing their serotypes and antimicrobial susceptibility.
Results: Among 40,595 isolates, salmonellosis predominated in children aged 0-4 (61.2%) years and among males (55.2%). Males also showed higher rates of extraintestinal infections (18.1% vs 16.0%, P <0.001), particularly, in the ≥65 years age group (52.4%). The top five serovars were *S.* Enteritidis (32.8%), *S.* Typhimurium (21.7%), *S.* Newport (6.2%), *S.* Stanley (4.7%), and *S.* Anatum (4.0%). Notably, *S.* Enteritidis prevalence increased from 23.9% (2004-2005) to 43.6% (2021-2022). Antimicrobial resistance was high, with a 51.6% multidrug resistance (MDR) rate. Disturbingly, MDR rates exceeded 90% in serovars Albany, Schwarzengrund, Choleraesuis, and Goldcoast. Resistance to key therapeutic agents, azithromycin, cefotaxime, and ciprofloxacin, exhibited concerning upward trends, and the surge in cefotaxime and ciprofloxacin resistance was closely linked to the emergence and spread of MDR *S.* Anatum and *S.* Goldcoast clones.
Conclusions: Prioritizing control measures against *S.* Enteritidis and closely monitoring the prevalence and spread of MDR clones are imperative to mitigate *Salmonella* infections in Taiwan.

Introduction

Salmonella, a zoonotic pathogen, is a major cause of human gastroenteritis. This genus comprises two species, *Salmonella enterica* and *S. bongori*, with over 2600 serotypes [1]. *Salmonella* serovars exhibit various host specificities, with narrow or broad host ranges. The human-restricted serovars, *S.* Typhi and *S.* Paratyphi A, B, and C, caused an estimated 14.3 million cases of typhoid and paratyphoid fever worldwide in 2017 [2]. On the other hand, nontyphoidal *Salmonella* (NTS) is estimated to cause 93.8 million illnesses annually, with 80.3 million being foodborne, resulting in 155,000 deaths [3]. NTS is a leading cause of foodborne illnesses in the United States and the second most prevalent cause of zoonotic diseases in Europe [4,5].

In Taiwan, typhoid and paratyphoid fever are infrequent, with low incidence rates at 0.147 (0.013-0.346) per 100,000 for typhoid fever and 0.036 (0.000-0.084) per 100,000 for paratyphoid fever from 1993 to 2022 (data sourced from the Taiwan National Infectious Disease Statistics System, Taiwan CDC: https://nidss.cdc.gov.tw/en/Home/Index). NTS disease is not notifiable; however, previous studies highlight its significance in foodborne diseases and community-acquired bacteremia in infants [6].

Salmonella serotyping is crucial for monitoring epidemiological trends, tracing contamination sources, and understanding antimicrobial resistance (AMR) and virulence. Despite some published data on *Salmonella* serotypes in Taiwan [7,8], there is a notable absence of long-term monitoring data. Thus, there is an urgent need for comprehensive serotyping data to understand the dynamics of serotype distribution and relationships among *Salmonella* serovars and AMR.

AMR in *Salmonella* poses a global public health threat and significantly contributes to mortality [9]. The levels of AMR in *Salmonella* isolates vary across strains, clones, serovars, geographic locations, and host sources [7,8,10–12]. In Taiwan, there has been a notable increase in multidrug resistance, especially to extended-spectrum cephalosporins (ESCs) and fluoroquinolones since 2002 [8]. The emergence and spread of multidrug resistance (MDR) clonal strains with resistance to ESCs have been observed in Taiwan [13–15]. Studies have additionally demonstrated a significantly higher prevalence of azithromycin resistance in NTS isolates from Taiwan than those from the United States

* Corresponding author: Tel: +886 4 24750452; Fax: +886 4 24750474.
E-mail address: nipmcsc@cdc.gov.tw (C.-S. Chiou).

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and European countries [16]. Although rare, carbapenem resistance has been identified in this pathogen in Taiwan [17,18].

This article presents comprehensive long-term surveillance findings on the demographics, epidemiological trends, and AMR patterns of *Salmonella* serovars from human salmonellosis cases from 2004 to 2022.

Materials and methods

Isolates collection, identification, and serotyping

Bacterial isolates were obtained from hospitals across the country as part of the PulseNet Taiwan disease surveillance project, which was initiated in 2004. *Salmonella* isolates were obtained from specimens provided by outpatients and inpatients seeking medical care at these hospitals. Identification and serogrouping of the isolates were conducted within the hospital facilities. The collection of isolates was temporarily halted in 2020 but resumed in 2021. The identification of bacterial isolates as *Salmonella* was confirmed using the MALDI Biotyper (Bruker Corp). For isolates collected in the initial 3 years (2004-2006), serotypes were determined using the conventional phase reversal and slide agglutination method, with antisera acquired from S&A Reagents Lab (Bangkok, Thailand). As for the isolates collected from 2007 to 2022, serotypes were ascertained through the Pulse Field Gel Electrophoresis pattern comparison approach [19]. In total, 40,595 isolates were obtained between 2004 and 2019 and in 2021-2022 and their serotypes were determined.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was conducted on isolates obtained from three periods: 2004-2010, 2013-2019, and 2021-2022. Notably, no antimicrobial susceptibility testing was performed for isolates from 2011 and 2012, and there were no isolates collected in 2020. The microbroth dilution method was used for testing, using custom-made 96-well Sensititre minimum inhibitory concentration (MIC) panels (TREK Diagnostic Systems LTD., West Sussex, UK). Initially, the MIC panel included 12 antimicrobials, namely, ampicillin, cefotaxime, ceftriaxone, nalidixic acid, ciprofloxacin, gentamicin, chloramphenicol, streptomycin, sulfamethoxazole, cotrimoxazole (trimethoprim-sulfamethoxazole), trimethoprim, and tetracycline. However, this MIC panel underwent several modifications over time. Ceftazidime and imipenem were added in 2005 and, in 2010, cefoxitin, colistin, and ertapenem were introduced, whereas ceftriaxone and trimethoprim were removed. Subsequently, in 2016, imipenem was excluded, and azithromycin was incorporated in 2017, followed by the removal of cefoxitin and ertapenem in 2019. The testing procedures adhered strictly to the manufacturer's instructions, and the interpretation of MIC results followed the guidelines outlined by the Clinical and Laboratory Standards Institute 33rd edition (2023). Although the Clinical and Laboratory Standards Institute interpretive criteria were applied for most antimicrobials, an MIC value of ≥32 μg/ml was used to indicate streptomycin resistance.

Statistical analysis

The disparity between two sets of categorical data was analyzed using the chi-square test with a 2 × 2 contingency table. Significant differences were indicated by * (P <0.05 or P <0.01) or ** (P <0.001).

Results

Demographics

Of the total cases (n = 40,595), 90.3% (n = 36,668) included gender data, with 44.8% females and 55.2% males. Among cases with available age data (n = 35,657), the 0-4 years age group constituted the highest proportion of cases (61.2%), and the 15-24 years age group had the lowest proportion (2.7%) (Figure 1). Extraintestinal infections accounted for 16.8% (5068 of 30,107) of cases, including 12.5% from blood, 1.7% from urine, and 2.6% from other sterile sites. The 5-14 years age group had the lowest extraintestinal infection rate, which increased with age. Notably, over half (52.4%) of the isolates in the ≥65 years age group were from extraintestinal infections. Males showed a significantly higher rate of extraintestinal infection than females (18.1% vs 16.0%, P <0.001). However, in the younger age groups (0-4, 5-14, and 15-24 years), females had higher extraintestinal infection rates.

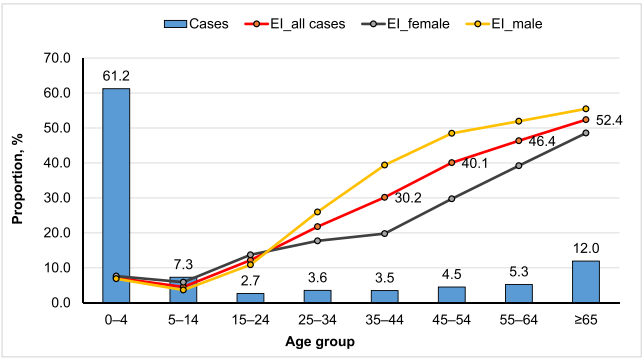

Figure 1. Distribution of salmonellosis cases (N = 35,657) across age groups and the proportions of EI in all, female, and male cases. EI, extraintestinal infections.

Distribution of Salmonella serovars

Among 40,595 isolates, 126 serotypes were identified. The top 10 serovars constituted 82.3% and the top 35 serovars accounted for 97.4% of isolates. *S.* Enteritidis and *S.* Typhimurium were the two most prevalent serovars, accounting for 32.8% and 21.7% of isolates, respectively (Supplementary Table S1). Comparing 2004-2013 with 2014-2022, among the top 20 serovars, notable increases in isolation rates occurred in *S.* Enteritidis, *S.* Anatum, *S.* Agona, and *S.* Livingstone, whereas declines were observed for 14 serovars, particularly, *S.* Stanley, *S.* Albany, *S.* Schwarzengrund, and *S.* Choleraesuis. *S.* Anatum suddenly increased in isolation rate in 2014-2015 and peaked in 2016-2019 but declined in 2021-2022. Among the top 21-35 serovars, 11 showed increased isolation rates. *S.* Goldcoast and *S.* Brancaster were rarely or not isolated in 2004-2015. Notably, *S.* Infantis, *S.* Cerro, *S.* Rissen, *S.* Havana, and *S.* Kentucky each had the highest isolation rate in 2021-2022.

Salmonella serovar distribution by age, gender, and extraintestinal Infections

Across three age groups, 68.6% of isolates were identified in the 0-14 years age group, 19.5% in the 15-64 years age group, and 12.0% in the age group over 65 (Supplementary Table S2). Among the top 35 serovars, *S.* Choleraesuis, *S.* Give, *S.* Brancaster, *S.* Typhi, and *S.* Kentucky had a distribution below 40% in the 0-14 years age group. Regarding gender, five serovars (*S.* Newport, *S.* Stanley, *S.* Anatum, *S.* Brancaster, and *S.* Typhi) significantly exceeded 44.8% in females, whereas *S.* Choleraesuis had a lower proportion (32.7%) in females. Extraintestinal infection rates were significantly higher in eight serovars than serovar Typhimurium. Noteworthy, *S.* Choleraesuis and *S.* Typhi emerged as the most invasive, with approximately 86% of isolates from extraintestinal sites. Seven serovars had significantly lower extraintestinal infection rates than *S.* Typhimurium. In addition, notable extraintestinal infection rates were observed in five less prevalent serovars, including *S.* Javiana (36.0%), *S.* IIIa 18:z4,z23:- (88.9%), *S.* Dublin (79.2%), *S.* Oranienburg (58.8%), and *S.* Paratyphi A (89.5%).

Table 1
Antimicrobial resistance rates for *Salmonella* isolates recovered from 2004-2010, 2013-2019, and 2021-2022.

Antimicrobial	All		2004-2010		2013-2019[a]		2021-2022[b]	
	N	R, %	N	R, %	N	R, %	N	R, %
Azithromycin	4641	4.5	0	0	3015	3.8	1626	5.9**
Ampicillin	25,899	42.3	16,525	38.2	7748	52.3**	1626	36.8**
Cefoxitin	9400	10.8	2193	4.4	7207	12.7**	0	0
Cefotaxime	25,899	6.6	16,525	3.1	7748	12.9**	1626	12.1
Ceftazidime	23,399	7.3	14,025	2.9	7748	14.0**	1626	13.7
Ertapenem	9384	<0.1	2177	0	7207	<0.1	0	0
Meropenem	1626	0	0	0	0	0	1626	0
Nalidixic acid	25,899	20.9	16,525	24.4	7748	15.5**	1626	10.3**
Ciprofloxacin	25,889	4.0	16,525	3.3	7739	4.8**	1625	8.4**
Ciprofloxacin[NS]	25,889	26.3	16,525	26.7	7739	25.6	1625	24.6
Gentamicin	25,899	6.7	16,525	5.5	7748	9.4**	1626	6.0**
Chloramphenicol	25,899	35.5	16,525	37.8	7748	32.2**	1626	27.6**
Streptomycin	23,731	36.2	16,518	35.7	7213	37.2*	0	0
Trimethoprim	15,975	26.3	14,349	26.1	0	0	1626	28.1
Sulfamethoxazole	25,897	52.8	16,525	53.9	7747	52.3*	1625	43.1**
Cotrimoxazole	23,727	28.7	16,520	26.0	7207	34.7**	0	0
Tetracycline	25,898	55.3	16,525	57.5	7747	54.7**	1626	35.9**
Colistin	9373	26.6	0	0	7748	24.7	1625	35.4**
Tigecycline	1626	9.6	0	0	0	0	1626	9.6

[a] Statistical comparison of resistance rates for isolates between 2004-2010 and 2013-2019
[b] Statistical comparison of resistance rates for isolates between 2013-2019 and 2021-2022;
* , chi-square P <0.05;
** , P <0.001. N, number of isolates tested; R, resistance rate; NS, nonsusceptible.

Trends of AMR in Salmonella isolates

Among all isolates, over 50% displayed resistance to sulfamethoxazole and tetracycline, whereas 20-42.3% exhibited resistance to seven antimicrobials (ampicillin, nalidixic acid, chloramphenicol, streptomycin, trimethoprim, cotrimoxazole, and colistin). In addition, 4.5% were resistant to azithromycin, 6.6-10.8% to ESCs (cefotaxime and/or ceftazidime) and cefoxitin, 4.0% to ciprofloxacin, and 9.6% to tigecycline (Table 1). Although ciprofloxacin resistance was observed in only 4.0% of isolates, 26.3% exhibited nonsusceptibility to ciprofloxacin. One isolate (*S.* Infantis) in 2013 demonstrated resistance to carbapenems. Comparing 2004-2010 with 2013-2019, higher resistance rates were observed for eight antimicrobials and lower rates for four. In contrast, 2021-2022 isolates showed higher resistance for azithromycin, ciprofloxacin, and colistin and lower rates for six antimicrobials (ampicillin, nalidixic acid, gentamicin, chloramphenicol, sulfamethoxazole, and tetracycline). Notably, azithromycin resistance increased from 3.8% to 5.9%, and ciprofloxacin resistance surged from 4.8% to 8.4%, whereas ESC resistance slightly decreased.

Trends of susceptibility to eight antimicrobials for all isolates are shown in Supplementary Figure S1. Resistance to ampicillin, sulfamethoxazole, and tetracycline resistance peaked in 2013 and then declined. Chloramphenicol and nalidixic acid resistance decreased since 2004, with a higher level of chloramphenicol resistance observed around 2017. Cefotaxime resistance peaked in 2017-2019 but has substantially declined since 2021. Ciprofloxacin resistance increased in 2018, peaked in 2021, and significantly declined in 2022. Gentamicin resistance was higher between 2013 and 2018 but has gradually declined since 2019.

MDR in Salmonella serovars

Among the top 22 serovars, *S.* Typhimurium, *S.* Stanley, *S.* Anatum, *S.* Albany, *S.* Schwarzengrund, *S.* Choleraesuis, and *S.* Goldcoast were highly resistant, with a strikingly high MDR rate ranging from 80.4% to 98.6% (Table 2). Five serovars (*S.* Agona, *S.* Albany, *S.* Weltevreden, *S.* Mbandaka, and *S.* Goldcoast) showed notably higher resistance to azithromycin, and another five (*S.* Typhimurium, *S.* Anatum, *S.* Choleraesuis, *S.* Infantis, and *S.* Goldcoast) had a significantly elevated cefotaxime resistance. Four serovars (*S.* Typhimurium, *S.* Schwarzengrund, *S.* Choleraesuis, and *S.* Goldcoast) exhibited significantly higher ciprofloxacin resistance. *S.* Enteritidis displayed an exceptionally high colistin resistance rate of 72.4%; however, excluding it reduced the colistin resistance rate for all other 125 serovars to 2.6%.

Serovars and their contribution to cefotaxime and ciprofloxacin resistance

From 2004 to 2022, *S.* Typhimurium, *S.* Anatum, *S.* Enteritidis, *S.* Goldcoast, and *S.* Agona were key contributors to the cefotaxime resistance (Figure 2a). A significant increase in cefotaxime resistance from 2016 was strongly correlated with *S.* Anatum, *S.* Typhimurium, and *S.* Goldcoast. Despite having a relatively low cefotaxime resistance rate (2.2%), *S.* Enteritidis ranked as the third contributor owing to its significant number of isolates. *S.* Agona made a notable contribution to cefotaxime resistance in 2019-2021, with a specific rate of 20.3% for the period. For ciprofloxacin resistance (Figure 2b), *S.* Typhimurium, *S.* Choleraesuis, *S.* Goldcoast, *S.* Schwarzengrund, and *S.* Albany were major contributors. *S.* Choleraesuis and *S.* Goldcoast had remarkably high ciprofloxacin resistance rates at 82.9% and 84.4%, respectively (Table 2). Although *S.* Choleraesuis was the primary contributor in 2004-2005, its contribution declined over time. Notably, *S.* Goldcoast became the predominant contributor in 2018-2019.

Discussion

In this study, we present a comprehensive analysis of the extended 19-year epidemiological landscape of *Salmonella* serovars and AMR in isolates from human cases in Taiwan. The availability of serotyping data allowed us to examine the distribution of *Salmonella* serovars across age groups and genders, assess their invasiveness, and analyze AMR trends among different serovars. Our findings reveal substantial shifts in the prevalence of *Salmonella* serovars and AMR patterns from 2004 to 2022. Notably, the dynamic changes in AMR levels were closely linked to specific serovars or MDR clones, highlighting the intricate interplay between *Salmonella* serovars and AMR evolution over the years.

The data in Figure 1 illustrates a distribution of salmonellosis cases across age groups, which is consistent with patterns observed worldwide [20,21]. Our findings highlight an exceptionally high rate of ex-

Table 2
Antimicrobial resistance in *Salmonella* serovars, 2004–2022

Serovar	AZI	AMP	FOX	CTX	ETP	NAL	CIP	CIP^{NS}	GEN	CHL	STR	TMP	SUL	SXT	TET	TIG	COL	MDR
Enteritidis		22.8	2.8	2.2	0	9.5	0.3	10.8	0.8	4.7	23.8	6.3	34.0	16.5	33.8	4.6	72.4**	33.8
Typhimurium	5.2	75.2**	16.0**	9.1**	0	25.4**	7.2**	34.8**	14.6**	60.1**	73.7**	17.3	83.1**	19.4	82.3**	12.6	3.0	81.4
Newport/Bardo	3.3	25.8	6.0	3.2	0	18.7	1.1	21.1	4.4	24.9	19.4	19.6	26.3	19.6	57.5	1.0	2.8	27.9
Stanley	0	65.6**	4.3	3.0	0	5.1	0.5	5.9	0.6	87.2**	18.1	91.6**	88.3**	87.6**	88.3**	0.0	1.7	87.9
Anatum	1.1	85.5**	86.6**	77.4**	0	20.6	3.5	87.0**	4.6	85.0**	80.6**	79.6**	88.5**	82.1**	87.0**	9.2	2.7	88.0
Agona	9.8**	43.0	8.6	6.1	0	26.0**	2.4	27.3	7.6	44.7**	47.2**	4.1	50.8	8.1	46.0	14.8	1.9	45.5
Albany	32.2**	97.4**	4.5	2.8	0	89.9**	4.6	90.0**	6.4	97.2**	10.1	98.7**	99.2**	98.7**	97.1**	0.0	3.4	98.1
Derby	2.0	39.4	7.7	4.1	0	17.2	0	21.2	8.4	41.9**	68.8**	20.6	80.9**	16.9	79.1**	22.5*	1.2	77.2
Paratyphi B var. Java	3.6	8.6	4.5	3.2	0	2.5	0.2	3.2	0.6	8.0	5.0	0.8	12.1	3.0	6.7	3.7	1.5	8.1
Weltevreden	14.7**	8.8	5.2	2.9	0	2.8	0.6	4.5	1.5	7.7	11.7	12.2	24.8	15.6	24.8	3.2	2.3	15.3
Braenderup	3.5	27.2	3.4	2.0	0	1.5	0	5.4	1.1	4.8	4.8	27.2	25.2	24.2	31.5	3.7	2.1	24.3
Bareilly	0	4.8	6.3	2.6	0	36.1**	1.3	40.9**	0.4	5.0	4.4	0.6	5.2	3.0	4.8	0.0	1.3	5.4
Virchow	0	9.0	5.4	2.3	0	73.3**	1.8	74.8**	2.8	70.2**	5.0	85.8**	75.3**	72.0**	71.2**	0.0	1.5	71.7
Livingstone var. 14+	5.9	58.3**	4.5	2.9	0	6.3	3.4	41.3**	2.4	59.7**	25.1	51.0**	56.8	45.5**	63.1	5.6	2.0	62.1
Schwarzengrund	0	82.9**	7.2	5.7	0	95.4**	14.4**	95.4**	56.6**	77.0**	63.2**	88.0**	93.8**	86.5**	87.5**	0.0	1.4	94.9
Hadar/Istanbul	0	10.9	1.6	0.9	0	7.8	1.6	9.9	0.6	6.2	87.6**	1.0	5.9	3.8	96.6**	0.0	2.3	15.5
Mbandaka	14.7*	14.2	4.8	3.2	0	4.6	0.5	6.4	0.5	13.2	11.4	4.3	18.7	14.8	25.1	0.0	0.9	16.4
Choleraesuis	20.0	81.2**	16.7	10.6*	0	97.3**	82.9**	97.3**	59.6**	82.2**	74.8**	75.6**	98.6**	74.8**	88.4**	0.0	3.6	98.6
Potsdam	0	12.6	9.0	5.0	0	2.0	1.5	4.0	1.5	6.0	6.8	2.5	9.5	5.7	9.0	0.0	3.5	9.5
Montevideo	5.6	9.7	2.4	3.8	0	4.9	0.5	27.6	1.1	6.5	9.3	55.0**	61.6	61.0**	57.3	0.0	0	13.0
Infantis	0	13.5	1.6	12.3*	1.6	11.7	0	12.3	4.3	12.3	3.3	30.0	23.9	13.1	11.7	0.0	0	13.5
Goldcoast	45.5**	88.4	63.6**	88.4**	0	81.6**	84.4**	89.1**	82.3**	89.1**	83.0**	90.3**	89.8**	83.0**	88.4**	91.9**	3.4	90.5
Other 106 serovars	3.9	21.8	7.8	4.9	0	20.0	4.2	26.2	2.5	26.3	19.3	16.7	27.3	17.4	32.9	7.2	4.1	31.1
All serovars	4.5	42.3	10.8	6.6	<0.1	20.9	4.0	26.3	6.7	35.5	36.2	26.5	52.8	28.7	55.3	9.4	27.3	51.6

Abbreviations: AMP (ampicillin), AZI (azithromycin), CHL (chloramphenicol), CIP (ciprofloxacin), CIP^{NS} (ciprofloxacin-nonsusceptible), COL (colistin), CTX (cefotaxime), ETP (ertapenem), FOX (cefoxitin), GEN (gentamicin), IMI (imipenem), NAL (nalidixic acid), STR (streptomycin), SUL (sulfamethoxazole), SXT (trimethoprim/sulfamethoxazole), TET (tetracycline), TAZ (ceftazidime), TIG (tigecycline), TMP (trimethoprim), N (no data). MDR, multi-drug resistant, is defined as resistant to three or more antimicrobial classes, including AZI, AMP/FOX/CTX/TAZ, ETP/IMI, NAL, CIP, GEN, CHL, STR, TMP/SUL/SXT, TET, TIG, and COL.

* chi-square P <0.01;

** , P <0.001, stand for a rate for a serovar significantly higher than that for all serovars.

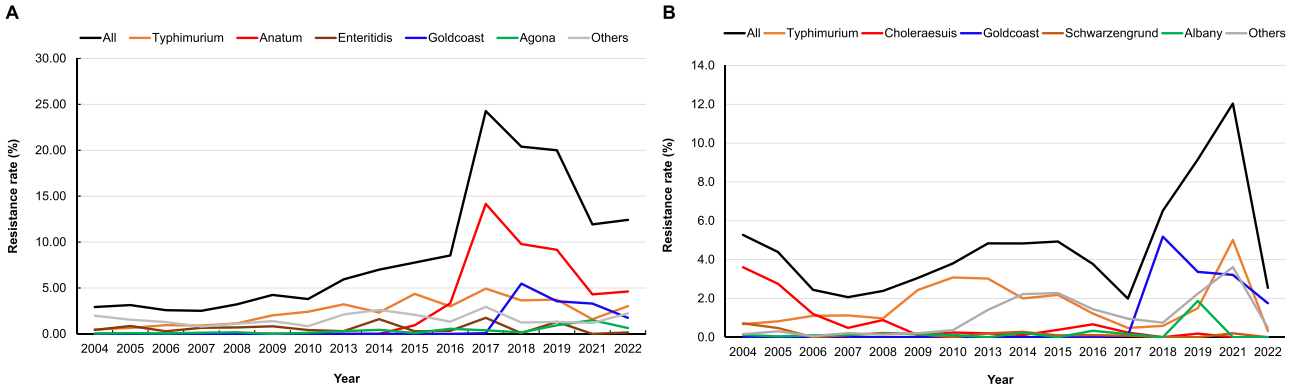

Figure 2. Contribution of *Salmonella* serovars to cefotaxime resistance (a) and ciprofloxacin resistance (b).

traintestinal infections (16.8%), which notably increases with age. Regarding sample sources, 83.2% of isolates are from feces, 12.5% from blood, 1.7% from urine, and 2.6% from other sterile sites. This differs from *Salmonella* surveillance data from the US FoodNet (1996-2006), which reports 89% of isolates from feces and only 5% from blood [21], and European surveillance data in 2021, which shows 92% of isolates from feces and only 2% from blood [20]. The variation in the ratio of invasive salmonellosis between Taiwan and other countries may not be attributed to *Salmonella* virulence but could be influenced by health care–seeking behavior, specimen sampling criteria and rates, and other factors. Nevertheless, further investigation is needed to determine the reasons behind the higher rate of invasive salmonellosis in Taiwan.

Our serotyping data revealed a lower serovar diversity of *Salmonella* isolates in Taiwan from 2004 to 2022, with only 126 serovars identified in 40,595 isolates, and the top 35 serovars accounting for 97.4% of isolates (Supplementary Table S1). In comparison, a study in the United States recorded 687 serovars in 46,639 isolates from 1996 to 2006 [21]. Our data indicate that the prevalence of serovars changed over time, with a particularly notable increasing trend for *S.* Enteritidis (Supplementary Table S1). *S.* Enteritidis was the most common isolated serovar, accounting for 23.9% of isolates in 2004-2005 and increasing to 43.6% in 2021-2022 (Supplementary Table S1). *S.* Enteritidis has been a major contributor to human salmonellosis in the United Kingdom since the late 1980s and is the most prevalent serovar worldwide [22]. Eggs are the primary vehicle for *S.* Enteritidis infection in humans [23]. Successful intervention measures implemented to prevent contamination and growth of *S.* Enteritidis in eggs have effectively reduced the incidence of *S.* Enteritidis infection in humans in the United States and Europe [5,23]. Given that *S.* Enteritidis infections have consistently accounted for around 40% of human salmonellosis, with a rising trend, controlling this organism is essential to reducing the incidence of salmonellosis in Taiwan.

Our study highlights a significant concern regarding AMR in *Salmonella* isolates from humans in Taiwan. Notably, *S.* Typhimurium, *S.* Stanley, *S.* Anatum, *S.* Albany, *S.* Schwarzengrund, *S.* Choleraesuis, and

S. Goldcoast demonstrate an extremely high prevalence of resistance, with an MDR rate exceeding 80% (Table 2). The dynamic patterns in AMR are intricately linked to the fluctuations in major serovars. Particularly noteworthy are the escalating trends in resistance to azithromycin, ESCs (e.g. cefotaxime and ceftazidime), ciprofloxacin, and colistin. In a study by Lauderdale et al. of 798 *Salmonella* isolates from humans between 1998 and 2002, only one isolate (*S.* Schwarzengrund) exhibited resistance to cefotaxime and 2.0% of isolates were resistant to ciprofloxacin [7]. However, the rate of cefotaxime resistance has surged to 12.1%, and the rate of ciprofloxacin resistance has reached 8.4% for isolates from 2021-2022, indicating a concerning upward trajectory over time (Table 1). The rise in cefotaxime resistance is primarily attributed to *S.* Typhimurium, *S.* Anatum, and *S.* Goldcoast (Figure 2a). The emergence of MDR clones of *S.* Anatum and *S.* Goldcoast is particularly pivotal for the high level of cefotaxime resistance observed between 2016 and 2022, whereas the MDR *S.* Goldcoast clone significantly contributes to ciprofloxacin resistance between 2018 and 2022 (Figure 2b).

The significant increase in the prevalence of *S.* Anatum and *S.* Goldcoast was accompanied by the emergence of specific MDR clones [13,14]. *S.* Anatum was not common between 2004 and 2014; however, the isolation rate has abruptly increased since 2015 [13]. The increase in isolation of *S.* Anatum was accompanied by the emergence of an MDR *S.* Anatum clone in 2015 [13]. *S.* Anatum became the third most prevalent serovar in 2017 and the majority (94.1%) of the isolates belonged to the MDR clone [13]. The origin of the MDR *S.* Anatum clone is unknown; however, MDR strains of the clone have been isolated in the United States from seafood imported from Taiwan and the Philippines and travelers who returned from these two countries [24]. MDR *S.* Anatum strains typically harbor an IncC plasmid that carries 11 resistance genes, including $bla_{\text{DHA-1}}$ and *qnrB4*; thus, *S.* Anatum has become the largest contributor to cefotaxime resistance since 2016 (Figure 2a).

The MDR *S.* Goldcoast clone emerged as a major contributor to cefotaxime and ciprofloxacin resistance after it became prevalent (Figures 2a and b). *S.* Goldcoast was not detected in Taiwan until 2014; however, an MDR clone surfaced in 2017 and became prevalent in 2018 [14]. MDR *S.* Goldcoast strains carry an IncHI2-IncHI2A megaplasmid usually housing over 10 resistance genes, including $bla_{\text{CTX–M-55}}$ and *qnrS13*, along with an efflux pump activator known as *ramAp* [14,25]. This plasmid-borne *ramA* can activate the expression of efflux pumps, such as *acrAB-tolC*, elevating resistance to various antimicrobials, including azithromycin and ciprofloxacin [25]. The IncHI2-IncHI2A plasmid in the MDR *S.* Goldcoast clone may be highly mobile, as evidenced by its presence in *S.* Agona. In some *S.* Agona strains, all resistance genes have been integrated into the chromosomes, likely through an IS26-mediated transposition [17]. MDR *S.* Goldcoast strains with carbapenem resistance were identified in a nosocomial salmonellosis outbreak at a hospital in central Taiwan in 2020-2021, where carbapenem resistance was developed through the acquisition of a $bla_{\text{OXA-48}}$-carrying IncL plasmid by the strains [18].

Despite the low prevalence of *S.* Infantis between 2004 and 2022, its significance is underscored by the emergence of a $bla_{\text{CTX–M–65}}$–carrying *S.* Infantis clone in Taiwan in 2021. The MDR *S.* Infantis clone, previously identified in South and North America and some European countries [26], typically harbored nine resistance genes, of which $bla_{\text{CTX–M–65}}$ and four other genes had moved from a pESI-like megaplasmid into the chromosomes [15]. Chickens are suspected to be the primary source of this clone because an investigation revealed 68.1% (258/379) of *Salmonella* isolates recovered from chicken meat being *S.* Infantis and all belonging to the MDR clone [15]. The diverse Pulse Field Gel Electrophoresis genotypes among the isolates from retail chicken meat suggest the evolution and proliferation of $bla_{\text{CTX–M–65}}$–carrying *S.* Infantis strains on chicken farms. Although human cases caused by the MDR *S.* Infantis clone remain limited, the isolation rate increased in 2022. Therefore, careful monitoring of the emergence of the MDR *S.* Infantis clone is warranted because chicken is the most consumed meat in Taiwan.

S. Choleraesuis, a swine-adapted serovar, typically causes severe manifestations, such as pneumonia and septicemia, in pigs but infrequently leads to infections in humans. Nevertheless, it has been a prominent serovar associated with human salmonellosis in Thailand and Taiwan [27,28]. Isolates of *S.* Choleraesuis from human and swine sources have displayed high rates of resistance to multiple antimicrobials, particularly, cefotaxime and ciprofloxacin [8,29]. Our findings reveal that *S.* Choleraesuis ranked as the seventh most common serovar in 2004-2005; however, its prevalence has significantly decreased over the years (Supplementary Table S1). Notably, in 2022, no *S.* Choleraesuis was identified among 1488 *Salmonella* isolates recovered. A study by Su et al. suggests that the decline in *S.* Choleraesuis infections in humans may be attributed to various control measures implemented on farms and changes in agricultural practices [30].

In conclusion, *Salmonella* isolates obtained from human salmonellosis cases in Taiwan between 2004 and 2022 reveal a concerning upward trend in AMR. Notably, there is a troubling increase in resistance to azithromycin, cefotaxime, and ciprofloxacin. In recent years, the emergence and spread of MDR clones of *S.* Anatum, *S.* Goldcoast, and *S.* Infantis, as well as *S.* Typhimurium, imposes a potential risk of compromising the effectiveness of essential therapeutic drugs. Urgent and continuous monitoring of *Salmonella* resistance, especially MDR clones, is critical for effective control and public health protection in Taiwan.

Declarations of competing interest

The authors have no competing interests to declare.

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Ethical approval

The collection of bacterial isolates was executed through a series of projects, all of which obtained ethical approval from the institutional review board of the Centers for Disease Control, Ministry of Health and Welfare. These projects were registered under the institutional review board numbers 101010, 102004, 104113, and 10711.

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Author contributions

YS, JH, SY, CT, HL, YW, RH, YP, and BH performed microbiological laboratory work and collected and analyzed the data. TL and CS drafted the original manuscript. All authors contributed to the study design and data interpretation and revised the manuscript for intellectual content. All authors have read and approved the final version.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this manuscript, the authors used Grammarly and ChatGPT 3.5 to check and edit the text to improve the readability of the content. After using this tool/service, the authors reviewed and edited the content as needed and took full responsibility for the content of the publication.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijregi.2024.100372.

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