Infection and Drug Resistance

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ORIGINAL RESEARCH

Clinical Characteristics, Serotypes and Antimicrobial Resistance of Invasive *Salmonella* Infections in HIV-Infected Patients in Hangzhou, China, 2012–2023

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Purpose: Developing countries, invasive *Salmonella* infections can cause considerable morbidity and mortality. There is a relative lack of data on coinfection with *Salmonella* in HIV-infected patients in Hangzhou, China.

Patients and Methods: In this study, we manually collected case data of patients aged >18 years with HIV combined with invasive *Salmonella* infections admitted to Xixi Hospital in Hangzhou from January 2012 to August 2023 by logging into the Hospital Information System, and identified 26 strains of invasive *Salmonella* using a fully automated microbiological identification system and mass spectrometer. Serotypes were determined using *Salmonella* diagnostic sera based on the White-Kauffmann-Le Minor scheme. Drug sensitivity tests were performed using the automated instrumental method of the MIC method.

Results: A total of 26 HIV-infected patients with invasive *Salmonella* coinfections were identified over 11 years; Twenty-five of the 26 patients (96.2%) were males, with a mean age of 33.5 years (26.75, 46.75). The most common type of infection was bloodstream infection (92.3%). One patient also had concomitant meningitis and osteoarthritis, followed by pneumonia (7.7%). The presence of multiple bacterial infections or even multiple opportunistic pathogens was clearly established in 7 (26.9%) patients. Three (11.6%) patients were automatically discharged from the hospital with deterioration of their condition, and one (3.8%) patient died. *Salmonella enteritidis* was the most common serotype in 6 patients (23.2%), and *Salmonella Dublin* was the most common serotype in 6 patients (23.2%). Drug sensitivity results revealed multidrug resistance in a total of 8 (30.8%) patients.

Conclusion: The clinical presentation of invasive *Salmonella* infection in HIV patients is nonspecific and easily masked by other mixed infections. A $CD4^+$ count <100 cells/μL and comorbid intestinal lesions may be important susceptibility factors. *Salmonella* has a high rate of resistance to common antibiotics, and the risk of multidrug resistance should not be ignored.

Keywords: *Salmonella*, invasiveness, HIV, serotype, antibiotic resistance

Introduction

Salmonella is the most complex genus in the Enterobacterales family, with a wide range of serotypes; more than 2600 serotypes have been isolated globally[1] and it is a widely spread zoonotic pathogen that spreads mainly through oral infection of contaminated food and water sources.[2] Human *Salmonella* infections are divided into typhoid fever, also known as enteric fever, caused by *Salmonella typhi* and *Salmonella paratyphi*, and a series of clinical syndromes caused by many nontyphoidal *Salmonella* serotypes (NTSs).[3] Most patients present with acute gastroenteritis with a self-limiting course.[4] A small percentage of patients develop extraintestinal disseminated infections manifesting as bacteraemia with secondary migratory lesions (eg, bone marrow, joints, heart valves, arteries, etc.) with high morbidity and mortality rates.[5,6] HIV-infected patients are at high risk for the development of invasive *Salmonella* infections and for severe

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adverse outcomes due to the presence of immunodeficiency.[7,8] Even in the era of combination antiretroviral therapy, nontyphoidal *Salmonella* remains one of the most important pathogens causing bloodstream infections in HIV-infected patients.[9]

Globally, there are approximately 10.9 million cases of enteric fever, resulting in approximately 116,800 deaths annually.[10] Invasive nontyphoidal *Salmonella* infections account for approximately 3.4 million cases and more than 680,000 deaths annually.[11,12] Looking further down the road, *Salmonella typhimurium* and *Salmonella enteritidis*, in particular, are increasingly becoming important public health threats in middle- and lower-income countries in Asia and Africa.[13] China has a population of 1.4 billion, of which approximately 70–80% of bacterial food poisoning is caused by *Salmonella*.[14] One study analyzed clinical data on *Salmonella* infections in 137 hospitals in Zhejiang Province, China, between 2018 and 2020 and reported that invasive *Salmonella* infections occurred in 20.9% of 6,111 patients in Zhejiang Province, of which bloodstream infections accounted for 52.8%.[15] Thus, the prevalence of invasive *Salmonella* infections in China is not low; however, very little data from epidemiological investigations of HIV patients with coinfections of invasive *Salmonella* infections in China are available.

The issue of drug resistance in *Salmonella*, especially nontyphoidal *Salmonella*, has also become another major global concern. Multidrug-resistant *Salmonella typhi* is considered endemic in many developing countries, such as Southeast Asia,[16] and drug resistance in nontyphoidal *Salmonella* is also high. Multidrug resistance has been reported in the literature in ≥80% of 784 NTS isolates.[17] The situation in China is not encouraging either, with a total of 178 iNTS isolates in one study, and 53.4% of the isolates showed multidrug resistance.[18] However, there are very few data on the drug resistance profile of invasive *Salmonella* infections in Chinese HIV patients.

We therefore retrospectively analyzed data on HIV-infected patients coinfected with invasive *Salmonella* infections at a hospital in Hangzhou, Zhejiang Province, China, analyzing the prevalence of serotypes, patient clinical presentations, outcomes, and *Salmonella* resistance profiles in the region to provide a baseline for the assessment of this serious disease in the Hangzhou area of China.

Materials and Methods

Study Design and Patient Population

We retrospectively investigated all *Salmonella* infection patients admitted and hospitalized from January 2012 to August 2023 at Xixi Hospital, Hangzhou, China. A total of 29 cases of *Salmonella* infection in HIV patients were retrieved, among which 3 cases with positive stool culture only were considered noninvasive infection cases and were excluded. A total of 26 nonduplicate patients were ultimately included. Clinical information, including age, sex, season of onset, underlying disease, symptoms, laboratory results, treatment, outcome, bacterial antimicrobial susceptibility and serotypes, was collected. Meteorological information was obtained from the China Meteorological Administration. Details are shown in the flow chart (Figure 1).

This study was performed at Xixi Hospital in Hangzhou, Zhejiang Province, China, which is a region located on the southeast coast of China with a subtropical monsoon climate. The Centre is the Hangzhou Public Health Clinical Centre, one of the key national tertiary teaching hospitals specialising in infectious diseases, and undertakes the clinical diagnosis and treatment of AIDS cases assigned by the Government.

Inclusion Criteria

1. Patients >18 years old;
2. Patients infected with HIV(confirmed to be positive by a primary HIV screening test in the laboratory department and HIV antibody testing in the CDC laboratory);
3. Meets the definition of invasive *Salmonella* infection: symptoms consistent with invasive bacterial infection and positive cultures or metagenomic next-generation sequencing of *Salmonella* enterica species isolated from normally sterile body sites such as blood, cerebrospinal fluid (CSF), and bronchoalveolar lavage fluid (BALF).[19]

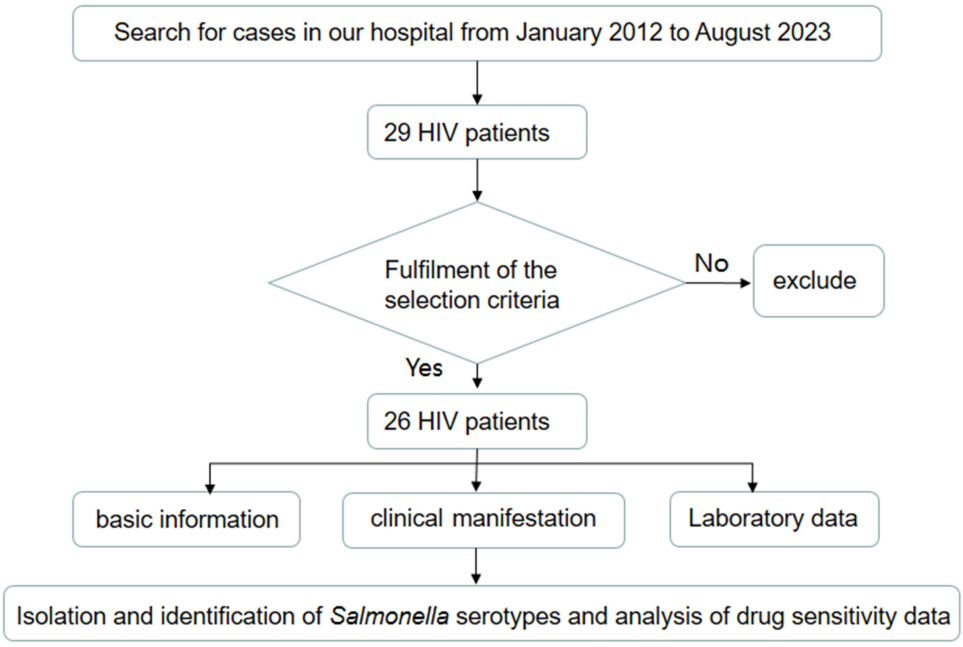

Figure 1 Flow chart of the study design.

Culture and Identification

The patient's venous blood was collected by bilateral puncture with 2 vials per side (aerobic vials(BACT/ALERT FA Plus, BioMérieux, France)+anaerobic vials(BACT/ALERT FN Plus, BioMérieux, France)) of 8–10 mL of blood per vial, or 2–3 mL of the patient's cerebrospinal fluid was withdrawn from the bedside, inoculated directly into microbial culture vials(BACT/ALERT PF Plus, BioMérieux, France), and sent to the laboratory within 2 hours. The laboratory staff placed the culture bottles in a fully automated bacterial mycobacterial culture detection instrument (BACT/ALERT 3D, BioMérieux, France) and incubated them for 5 days. The blood culture bottles were removed immediately after the blood culture was reported to be positive, and the alarm time and growth curve were recorded immediately after smear microscopy was performed by transferring the blood plate. Alternatively, under strict aseptic operation, the BALF was collected, and the precipitate was cultured and inoculated on a blood plate. After both were cultured for 18–24 hours, the typical colonies on the plate were used for the preliminary identification of *Salmonella* with a fully automated microbial mass spectrometry detection system (VITEK MS, BioMérieux, France), and Isolates identified as the genus *Salmonella* were further investigated to detect the somatic (O) antigen and flagellar (H) antigen by slide agglutination with commercial antisera (Lanzhou Biological Products Research Institute Limited Liability Company, Lanzhou, China), and the serotype was determined according to the White-Kauffmann-Le Minor scheme. OLYMPUS CX21 microscopy was employed.

Antimicrobial Susceptibility Tests

The antimicrobial susceptibility testing in this study was done using the automated instrumental method of the MIC method. *Salmonella* was added to a bacterial suspension(prepared as 3.0mL 0.45% NaCL solution + 145μL 0.5–0.63 McF units)and put into an automatic bacterial analyzer(VITEK 2 COMPACT, BioMérieux, France) for detection of the relative minimum inhibitory concentration (MIC) values of different antibiotics for bacteria, and the susceptible (S), intermediate (I), and resistant (R) values for different antibiotics were obtained according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. Antimicrobial susceptibility testing was performed using standard strains (A TCC700323, A TCC700327, A TCC29213 and A TCC25922) for microbiological quality control.

Antimicrobial sensitivity card using VITEK 2 AST-GN13, antibiotic discs include amikacin, ampicillin, ampicillin-sulbactam, aztreonam, cefazolin, cefepime, cefotetan, ceftazidime, ceftriaxone, ciprofloxacin, ertapenem, gentamicin,

imipenem, levofloxacin, furantoin, piperacillin-tazobactam, tobramycin, cotrimoxazole. Notably, *Salmonella* can exhibit activity in vitro against first- and second-generation cephalosporins, cephamycin, and aminoglycosides, but clinical treatment is not effective.[20] Therefore, the above categories of drugs were not included in the analysis.

Definitions

Multidrug resistance (MDR) was defined as resistance to three or more antimicrobial drugs, such as aminopenicillins (ampicillin), beta-lactam combinations (ampicillin-sulbactam, piperacillin-tazobactam), cephalosporins (ceftriaxone, ceftazidime, cefepime), monocyclic beta-lactams (aminoglycoside), carbapenems (imipenem), dihydrofolate reductase inhibitors (cotrimoxazole) and fluoroquinolones (ciprofloxacin, levofloxacin). Isolates with intermediate resistance were considered insensitive.

Community-acquired episodes were defined as episodes of illness prior to and within 48 hours of admission to the hospital. Otherwise, the episode was defined as a hospital episode.

Statistical Analysis

The data were entered into Microsoft Excel and analyzed by SPSS version 22.0 software. Continuous variables that were not normally distributed are described as medians with interquartile ranges (IQRs) and were compared using the Kruskal–Wallis *H*-test. A $p<0.05$ was considered to indicate a statistically significant difference. The correlation between the number of cases and the mean monthly rainfall was tested using Spearman's rank correlation coefficient.

Results

Sociodemographic and Clinical Characteristics

A total of 26 patients with invasive *Salmonella* infection and HIV were identified during the 11-year study period (1 in 2012, 2 in 2013, 3 in 2014, 4 in 2015, 2 in 2016, 0 in 2017,4 in 2018, 1 in 2019, 2 in 2020, 1 in 2021, 4 in 2022, and 2 in 2023) (Figure 2).

Twenty-five (96.2%) of the 26 HIV patients with invasive *Salmonella* infection were male, and one was female (3.8%), with a mean age of 33.5 years (26.75, 46.75). Twenty-three of the patients(88.5%) presented before the initiation of highly active antiretroviral therapy. Highly active antiretroviral therapy used in the remaining three patients before the onset of the disease were tenofovir, lamivudine and efavirenz Two cases occurred in January, one in February, two in March, two in April, two in June, three in July, two in August, two in September, five in October, two in November, and three in December (Figure 3). Although the number of cases of invasive *Salmonella* infections occurring in the summer

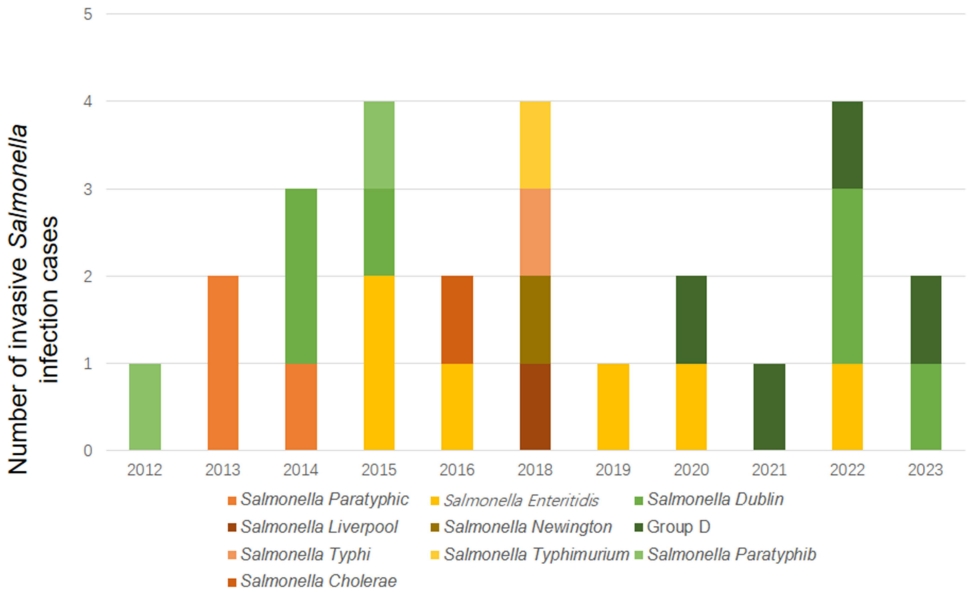

Figure 2 Distribution of the 26 invasive Salmonella cases by year of admission and serovar. The bar chart shows the number of invasive Salmonella cases diagnosed in Hangzhou in each year from 2012 to 2023, according to serovar.

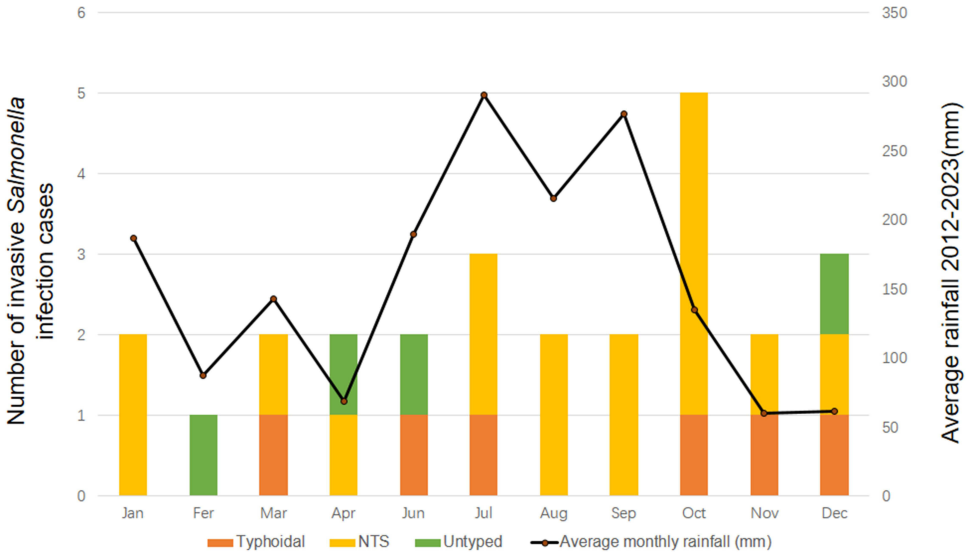

Figure 3 Distribution of the 26 invasive Salmonella cases and average rainfall by month. The bar chart shows the distribution of the 26 invasive Salmonella cases according to the month of admission. The average monthly rainfall over the 11-year period (January 2012–August 2023) in Hangzhou is shown in the line graph.

and fall seasons was greater, with a total of 16 cases (61.5%), there was no significant correlation between the number of cases and the average monthly rainfall (rs=0.069, P=0.841).

Symptoms were recorded in a total of 25 out of 26 patients, with 12 having diarrhea (48%), 2 having nausea (8%), 2 having vomiting (8%), and 2 having abdominal pain (8%). Abnormalities in routine stool test results at admission were present in 7 (26.9%) out of 26 patients; erythrocytes and pus cells were present in 3 (11.5%) patients; fecal occult blood was present in 4 (15.4%) patients; and a normal fecal result was observed in 13 (50%) patients. Additionally, 6 (23.1%) patients did not undergo a routine fecal test.

Eleven of the 26 patients (42.3%) were admitted to the hospital with respiratory symptoms as the main complaint, and 16 (61.5%) had lung lesions on admission computed tomography (CT); 10 of those patients had diffuse infectious lesions in both lungs and were considered to have clinical complications combined with Pneumocystis carinii pneumonia. Cavitary lesions were present in 2 patients on admission CT; in 1 patient, BALF NGS found the *Salmonella enteritidis* 601 sequence, and in the other patient, BALF NGS revealed a novel cryptococcal complex group 3656 sequence.

Eight of the 26 patients (30.8%) had combined intestinal lesions, including one with incomplete intestinal obstruction, three with perianal acrocyanosis, one with perianal abscess, one with adenoma of the transverse colon and duodenal bulbous ulcer, and one with a giant ulcer at the ileocecal end. The pathology was consistent with cytomegalovirus infection, which was later confirmed by perforation of the ulcer of the intestinal wall with peritonitis, and the patient was referred for surgery. One patient suffered from immunologic thrombocytopenic purpura with gastrointestinal bleeding manifested by black stools on admission to the hospital. Two patients (7.7%) had mesenteric lymphadenopathy; CT of one patient suggested multiple necrotic enlarged lymph nodes in the posterior peritoneum and mesenteric hiatus, and CT of one patient suggested multiple enlarged and necrotic lymph nodes in the posterior peritoneum and root of the mesentery. Patients were clinically diagnosed with tuberculosis in the abdominal lymph nodes, and diagnostic anti-tuberculosis treatment was effective.

Among the 26 patients, there were 11 cases of comorbid chronic underlying diseases, including 4 cases of comorbid chronic viral hepatitis B, 1 case of comorbid chronic viral hepatitis C, 1 case of comorbid chronic viral hepatitis B and chronic viral hepatitis C, 1 case of comorbid immune thrombocytopenic purpura, 1 case of comorbid Kaposi's sarcoma at final diagnosis, 1 case of comorbid type 2 diabetes mellitus, 1 case of comorbid hypertension and type 2 diabetes mellitus, and 1 case of combined bilateral cerebral infarct sequela, hypertension, and type 2 diabetes mellitus.

Twenty-three of the 26 patients had community-acquired infections, and three patients had nosocomial infections. The time from admission to diagnosis of invasive *Salmonella* infection in the three patients who developed nosocomial infections ranged from 12 to 78 days.

A total of 7 patients (26.9%) were identified as having multiple bacterial infections or even multiple opportunistic pathogen infections by culture. Cryptococcus neoformans was found in one cerebrospinal fluid culture, C. neoformans was found in two blood cultures, Cyanobacterium marneffei was found in one blood culture, C. marneffei was found in one sputum culture, Bacillus antacidus was found in one blood culture, and B. antacidus and C. marneffei were found in another blood culture.

All 26 patients were treated with parenteral antibiotics for a mean duration of 21 days (13.75, 27.25). The final outcome of treatment was assessed on the basis of the discharge record and categorised as (1) improvement: including recovery or discharge with symptomatic improvement and is able to take care of himself/herself;(2) deterioration: including death or hopeless discharge. Of these, 22 (84.6%) were discharged with improvement, 4 deteriorated, including 3 (11.6%) who were automatically discharged because of the hopelessness of treatment for deterioration, and 1 (3.8%) died. The main cause of death in these patients was respiratory failure. Detailed clinical characteristics are shown in Table 1.

Isolation and Identification of Salmonella Serotypes

Twenty-four of the patients provided blood samples (92.3%), and the mean time to report positive blood cultures was 6 days (4,7.25%). In 1 patient, in addition to a positive blood culture, a cerebrospinal fluid culture revealed Salmonella group D; 3×10^2 copies/mL of *Salmonella* were found after arthritic fluid puncture using GenSeizer™-based

Table 1 Presentation of HIV with Invasive Salmonella Infections by Serovar in the 26 Whose Isolates Were Serotyped

	All Serovars	Typhoidal *Salmonella*	Non-Typhoidal *Salmonella*	Untyped	*P*
No. of patients	26	6	16	4	
Mean age, years,median(IQR)	33.5(26.75,46.75)	28(22,34)	39.5(29.25,48.25)	47(29,70.25)	0.137
Male gender (%)	25(96.15)	6(100)	15(93.75)	4(100)	/
Presenting symptoms					
Fever(%)	25(96.15)	5(83.33)	16(100)	4(100)	/
Diarrhea(%)	12(46.15)	2(33.33)	8(50)	0(0)	/
Nausea(%)	2(7.69)	0(0)	2(12.5)	0(0)	/
Vomit(%)	2(7.69)	0(0)	2(12.5)	0(0)	/
Abdominal pain(%)	2(7.69)	0(0)	2(12.5)	0(0)	/
Investigations					
WBC,*10^9cells/L,median(IQR)	5.28(2.5,6.89)	6.07(3.55,8.04)	3.95(2.42,6.29)	7.58(3.56,9.83)	0.27
Neut,*10^9cells/L,median (IQR)	3.67(1.81,5.61)	4.5(2.43,5.79)	3.36(1.53,5.13)	5.89(2.8,9.45)	0.386
Eosi,*10^9cells/L,median (IQR)	0.01(0,0.06)	0(0,0.02)	0.04(0,0.07)	0.01(0,0.21)	0.144
Platelet,*10^9/L, median (IQR)	181.5(126.75,253.75)	204(136.25,270.25)	156.5(106.25,211.5)	236(86.25,295.75)	0.558
Creactiveprotein.mg/L,median(IQR)	38.16(17.25,91.12)	72(23.75,137.25)	28(16,62.85)	73.24(34.69,145.32)	0.203
Procalcitonin,ng/mL,median(IQR)	0.18(0.1,0.45)	0.16(0.1,0.27)	0.17(0.1,0.35)	5.6(0.83,37.49)	0.305
Hb,g/L, median(IQR)	102.5(93.75,116)	116(87,118.75)	101(88.5,111.75)	101(97,111.75)	0.428
Sodium,mmol/L, median (IQR)	134(130.8,136)	134.5(128.8,136)	133.5(131.18,136.2)	134.4(129.65,136.3)	0.969
Duration of ART					
ART native,n(%)	23(88.46)	5(88.33)	14(87.5)	4(100)	/
≤6 months,n(%)	2(7.69)	1(16.67)	1(6.25)	0(0)	/
>6 months,n(%)	1(3.85)	0(0)	1(6.25)	0(0)	/
HIV RNA<100IU/mL,n(%)	10(38.46)	5(83.33)	4(25)	1(25)	/
CD4+Tcellcount(cells/ul),media(IQR)	20(6,44)	61(20.5,98.25)	16(6,41.25)	12.5(2.25,28.75)	0.232
Patient outcomes					/
Improved, n(%)	22(84.62)	5(83.33)	14(87.5)	3(75)	
Deteriorated, n(%)	4(15.38)	1(16.67)	2(12.5)	1(25)	

Abbreviations: WBC, white blood cell count; Neut, neutrophil count; Eosi, eosinophil count; Hb, Haemoglobin; ART, antiretroviral treatment.

metagenomic next-generation sequencing (mNGS) results. In addition, 2 patients provided alveolar lavage samples (7.7%), and the time to report positivity in the alveolar lavage fluid was 3 days in both patients.

Of the 26 serotyped isolates, 1 (3.8%) was *Salmonella typhi*, 5 (19.2%) were *Salmonella paratyphi*, and 16 (61.6%) were NTS, with 6 (23.2%) being *Salmonella enteritidis* and 6 (23.2%) being *Salmonella Dublin*; these were the most common serotypes. Another 4 strains (15.4%) had unspecified serotypes (Table 2).

Antimicrobial Resistance Profiles

Drug sensitivity was detected for 24 of the 26 strains. Of these, 6 were TS, 15 were NTS and 3 were untyped. The in vitro resistance rates of the 6 TS isolates to ampicillin, ampicillin-sulbactam, and cotrimoxazole were 50% (3/6), 33.3% (2/6), and 50% (3/6), respectively. Overall, 16.7% (1/6) of the isolates were sensitive to ciprofloxacin, and 16.7% (1/6) were affected by ampicillin-sulbactam. All the strains were susceptible to ceftriaxone, cefepime, aztreonam, imipenem, levofloxacin, and piperacillin-tazobactam.

The in vitro resistance rates of 15 NTS isolates to ampicillin, ampicillin-sulbactam, aztreonam and ceftriaxone were 86.7% (13/15), 78.6% (11/14), 6.7% (1/15), and 6.7% (1/15), respectively. Additionally, 6.7% (1/15) of the isolates were sensitive to cefepime, 26.7% (4/15) were sensitive to levofloxacin, and 7.1% (1/14) were sensitive to ampicillin sulbactam. All the strains were susceptible to imipenem, ciprofloxacin, cotrimoxazole, and piperacillin-tazobactam. One patient had no drug sensitivity to ampicillin-sulbactam.

In vitro resistance to ampicillin and ampicillin-sulbactam was 100% (3/3) in all 3 untyped isolates. Overall, 66.7% (2/3) of the isolates were susceptible to levofloxacin. The isolates were sensitive to ceftriaxone, cefepime, aztreonam, imipenem, cotrimoxazole, and piperacillin-tazobactam; one patient had no drug sensitivity to ciprofloxacin; and two patients were sensitive to ciprofloxacin (Table 3, Figure 4).

Among the MDR strains detected in 8 cases, 2 were *Salmonella paratyphi C*, 1 of which was resistant to ampicillin, ampicillin-sulbactam, and cotrimoxazole and was susceptible to ciprofloxacin; 1 was resistant to ampicillin and cotrimoxazole and was susceptible to ampicillin-sulbactam; 3 were *Salmonella Dublin*, 1 of which was resistant to ampicillin, ampicillin-sulbactam, ceftriaxone, and aztreonam and was susceptible to levofloxacin and cefepime; 2 were

Table 2 Serogroups, Serotypes and Distribution of 26 Cases of Invasive Salmonella Infections in HIV Patients

Group(No.)	Serogroup	Serotype	No.	Percent
Typhoidal (6)	B	paratyphib	2	7.7
	C1	paratyphic	3	11.5
	D	typhi	1	3.8
NTS(16)	B	typhimurium	1	3.8
	C1	cholerae	1	3.8
	D	dublin	6	23.2
	D	enteritidis	6	23.2
	D	Liverpool	1	3.8
	E2	newington	1	3.8
Untyped (4)	D	Group D[#]	4	15.4
Total			26	100

Notes: [#]*Salmonella* Groups D could not be identified as NTS or typhoid, so they belonged to the untyped group.
Abbreviation: NTS, nontyphoidal *Salmonella*.

Table 3 Study of Salmonella Serotype Resistance in 24 HIV Patients with Invasive Salmonella Infection

All Serotypes	Antimicrobial Susceptibility, n (%)										
	Pattern	AMP	SAM[a]	CRO	FEP	ATM	IPM	CIP[b]	LEV	SXT	TZP
Typhoidal (n=6)	S	3	3	6	6	6	6	5	6	3	6
	I	0	1	0	0	0	0	1	0	0	0
	R	3	2	0	0	0	0	0	0	3	0
NTS (n=15)	S	2	2	14	14	14	15	14	11	15	15
	I	0	1	0	1	0	0	0	4	0	0
	R	13	11	1	0	1	0	0	0	0	0
Untyped (n=3)	S	0	0	3	3	3	3	2	1	3	3
	I	0	0	0	0	0	0	0	2	0	0
	R	3	3	0	0	0	0	0	0	0	0
Total (n=24)	S	5	5	23	23	23	24	21	18	21	24
	I	0	2	0	1	0	0	1	6	0	0
	R	19	16	1	0	1	0	0	0	3	0

Notes: [a]There was 1 case without ampicillin-sulbactam;[b]There was 1 case without ciprofloxacin in the NTS and 1 case without ciprofloxacin in Untyped.
Abbreviations:R, resistant; S, sensitive; I, intermediate; AMP, ampicillin; SAM, ampicillin-sulbactam; CRO, ceftriaxone; FEP, cefepime; ATM, aztreonam; IPM, imipenem; CIP, ciprofloxacin; LEV, levofloxacin; SXT, trimethoprim-sulfamethoxazole; TZP, Piperacillin-tazobactam.

resistant to both ampicillin and ampicillin-sulbactam and susceptible to levofloxacin; and 1 was *Salmonella enteritidis* and resistant to ampicillin and ampicillin-sulbactam and susceptible to levofloxacin. Two *Salmonella* group D strains were susceptible to ampicillin, ampicillin-sulbactam, and levofloxacin.

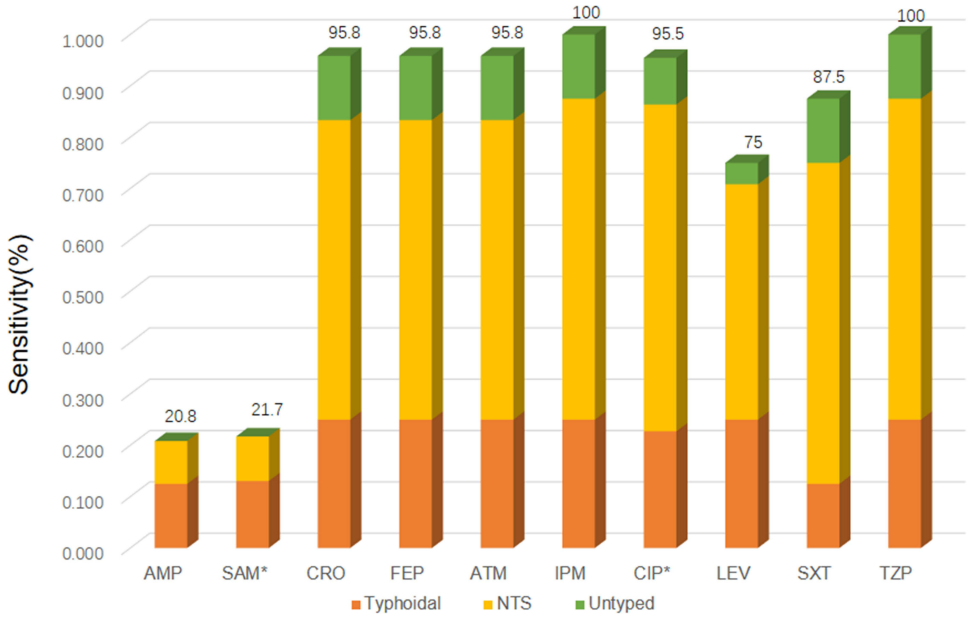

Figure 4 Antibiotic sensitivity pattern of the 24 invasive *Salmonella* cases.
Notes:*There was 1 case without ampicillin-sulbactam and 1 case without ciprofloxacin in the NTS.There was 1 case without ciprofloxacin in Untyped.
Abbreviations:AMP, ampicillin; SAM, ampicillin-sulbactam; CRO, ceftriaxone; FEP, cefepime; ATM, aztreonam; IPM, imipenem; CIP, ciprofloxacin; LEV, levofloxacin; SXT, trimethoprim-sulfamethoxazole; TZP, Piperacillin-tazobactam.

Discussion

To our knowledge, this study is the first detailed report on invasive *Salmonella* infections in HIV-infected patients in Hangzhou, China. Previously, we focused on Cryptococcus, Mycobacterium tuberculosis, nontuberculous Mycobacteria, C. marneffei and cytomegaloviruses among the types of opportunistic infections occurring in HIV patients, and the burden of *Salmonella*, a large group of gram-negative bacilli, on HIV patients may be underestimated.

Salmonellosis is usually characterized by fever, abdominal pain, diarrhea, nausea and vomiting.[5] In contrast, the current study showed that many patients did not experience gastrointestinal symptoms. This finding is similar to that of previous studies showing that the lack of typical diarrhea symptoms in many immunosuppressed patients with invasive nontyphoidal *Salmonella* infections is associated with severe cellular immune abnormalities.[21] In this study, we found that many patients even started with respiratory symptoms, and a total of 7 patients clearly presented with multiple bacterial infections or even multiple opportunistic pathogen infections through multisite sampling and examination. This finding suggested that mixed infections caused by the other pathogens in HIV-infected patients tend to mask the *Salmonella* infection itself and are easily missed. Four out of the 26 patients had poor treatment outcomes; 3 were automatically discharged due to deterioration of their condition, and 1 died. Among these four patients, blood cultures revealed both *C*. marneffei and B. antacidus in one patient, blood cultures revealed B. antacidus in another patient, and lung CT suggested diffuse infection in both lungs in one patient; these findings were considered associated with Pneumocystis jiroveci pneumonia, suggesting that the prognosis of patients with mixed infections may be worse.

Twenty-three of the 26 infections in this study occurred prior to the initiation of highly active antiretroviral therapy. $CD4^+$ T-cell counts were <200 cells/μL in all 25 patients for whom data were available, and 23 of these patients had $CD4^+$ T-cell counts <100 cells/μL, which is similar to the findings of a previous study.[22,23] The main mechanism is an imbalance in the function of monocytes and macrophages, which results in defects in effector functions such as phagocytosis, microbicidal activity and cytokine production.[24] Additionally, immune evasion fails to properly phagocytose and kill bacteria in the presence of microorganisms associated with opportunistic infections. Eight of the 26 patients had combined intestinal lesions, and the gastrointestinal tract is a site of early and severe $CD4^+$ T-cell depletion in HIV infection,[25] in particular interleukin-17-producing T cells (Th17 cells). Loss of intestinal mucosal interleukin-17 cells during HIV infection may be a key mechanism by which *Salmonella* transmission from the gut leads to invasive disease in these patients.[23] Two of the 26 patients had combined mesenteric lymphadenopathy, as demonstrated in a previous study.[26] Surgical removal of mouse mesenteric lymph nodes (MLNs) led to an increase in the number of *Salmonella typhimurium* strains reaching systemic sites early after infection. This finding suggested that MLNs are important sites of immune protection during *Salmonella* infection,[27] and highly active antiretroviral therapy has been shown to prevent intestinal infections by inhibiting viral replication and restoring immunity.[28]

In our study, *Salmonella enteritidis* and *Salmonella Dublin* were the most common serotypes, and both serotypes together caused 46.4% of the invasive infections. Previous studies have confirmed that *Salmonella enteritidis* and *Salmonella typhimurium* are the most common serotypes globally, while *Salmonella Dublin* is considered the most invasive serotype.[16,29] There appear to be regional differences in the prevalence of *Salmonella* serotypes, and differences in the distribution of serotypes in a region may be related to the presence of local animal hosts or food sources.[30]

Published reports of invasive nontyphoidal *Salmonella* in Africa suggest that the disease is highly seasonal.[31] This is because the ideal temperature range that favours the growth of *Salmonella* is 35–43°C.[32] In summer, a hot and humid climate is conducive to the reproduction of microorganisms, and food is more susceptible to bacterial contamination. Hangzhou is located on the southeast coast of China and has a subtropical monsoon climate, with the summer and fall seasons from June-November. The results of this study showed that 16 cases occurred in summer and fall, which was greater than the 10 cases that occurred in winter and spring. A study in China revealed that the most common causes of outbreaks of *Salmonella* infections in Zhejiang Province, China, were cross-contamination, inappropriate storage temperatures, and failure to adequately heat food.[33] In conclusion, *Salmonella* is a group of foodborne pathogens that are transmitted primarily via the fecal–oral route, and exposure to water and food contaminated with animal feces, as well as temperature and humidity, is strongly associated with infection with this pathogen.

Antibiotic-resistant *Salmonella* strains have emerged globally as a result of widespread antibiotic use.[1] However, few studies in China have reported the resistance rates of HIV-infected patients to *Salmonella* infection. In the present study,

the resistance rate of *Salmonella* isolates to ampicillin and ampicillin/sulbactam was high, similar to the findings of previous studies in China.[34] Eight patients with community-acquired infections even developed infections with MDR bacteria, which undoubtedly greatly increased the difficulty of diagnosis and treatment. Thus, resistance to commonly used antimicrobial agents for the treatment of *Salmonella* bacteremia is a major concern in developing countries with limited health care facilities. Monitoring antimicrobial resistance in *Salmonella* is important for physicians in order to develop effective treatment plans to minimize complications, and our study also provides valuable information on antibiotic resistance in HIV-infected patients with *Salmonella* coinfections in China.

Nonetheless, our study has several limitations. First, this was a retrospective study based on data from a single hospital, and our study was limited by this, its small sample size and potential selection bias. We were unable to calculate the incidence of invasive *Salmonella* infections among HIV-infected patients under ambulatory conditions in Hangzhou. This implies that further large-scale, multicenter studies are necessary. Second, not all the isolates were obtained. Therefore, we suggest that additional molecular typing methods for *Salmonella* be encouraged in future studies and can be used to study the mechanism of drug resistance. However, our study adds to the limited literature on invasive *Salmonella* infections in HIV-infected patients in China and emphasizes the need for continuous and improved surveillance of these important cases.

In summary, the clinical presentation of invasive *Salmonella* infections in HIV patients is nonspecific, and a $CD4^+$ count <100 cells/μL and comorbid intestinal pathology may be important susceptibility factors. Therefore, it is particularly important to actively search for pathogens by culturing or even NGS in suspected patients during treatment.

Conclusion

Salmonella infection is an important but underappreciated burden on HIV-infected patients, and the clinical presentation is easily masked by other mixed infections. *Salmonella enteritidis* and *Salmonella Dublin* were the predominant serotypes identified in this study. Antibiotic resistance is also another easily overlooked problem in Chinese patients with HIV infected with invasive *Salmonella* and warrants further research and surveillance.

Ethics Statement

This study was approved by the Ethics Committee of Hangzhou Xixi Hospital (HangXiMedLunReview 2024 Study No. 007) and was conducted in accordance with the principles of the Declaration of Helsinki. Due to the retrospective nature of this study, the requirement for written informed consent was waived. The data was anonymized or maintained with confidentiality.

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Disclosure

The authors report no conflicts of interest in this work.

References

1. Ibrahim GM, Morin PM. Salmonella serotyping using whole genome sequencing. *Front Microbiol*. 2018;9:2993. doi:10.3389/fmicb.2018.02993
2. Christenson JC. Salmonella Infections. *Pediatr Rev*. 2013;34(9):375–383. doi:10.1542/pir.34.9.375
3. Feasey NA, Dougan G, Kingsley RA, et al. Invasive non-typhoidal Salmonella disease: an emerging and neglected tropical disease in Africa. *Lancet*. 2012;379(9835):2489–2499. doi:10.1016/S0140-6736(11)61752-2
4. Baker S, Uche IV, MacLennan CA, et al. A systematic review of the incidence, risk factors and case fatality rates of Invasive Nontyphoidal Salmonella (iNTS) disease in Africa (1966 to 2014). *PLoS Negl Trop Dis*. 2017;11(1):e0005118. doi:10.1371/journal.pntd.0005118
5. Cohen JI, Bartlett JA, Corey GR. Extra-intestinal manifestations of Salmonella infections. *Medicine*. 1987;66(5):349–388. doi:10.1097/00005792-198709000-00003
6. Majowicz SE, Musto J, Scallan E, et al. The global burden of nontyphoidal Salmonella gastroenteritis. *Clin Infect Dis*. 2010;50(6):882–889. doi:10.1086/650733
7. Crump JA, Sjölund-Karlsson M, Gordon MA, et al. Epidemiology, clinical presentation, laboratory diagnosis, antimicrobial resistance, and antimicrobial management of invasive Salmonella infections. *Clin Microbiol Rev*. 2015;28(4):901–937. doi:10.1128/CMR.00002-15
8. Stanaway JD, Parisi A, Sarkar K, et al. The global burden of non-typhoidal Salmonella invasive disease: a systematic analysis for the global burden of disease study 2017. *Lancet Infect Dis*. 2019;19(12):1312–1324. doi:10.1016/S1473-3099(19)30418-9

9. Taramasso L, Tatarelli P, Di Biagio A. Bloodstream infections in HIV-infected patients. *Virulence*. 2016;7(3):320–328. doi:10.1080/21505594.2016.1158359
10. Carey ME, Dyson ZA, Danielle JI, et al. Global diversity and antimicrobial resistance of typhoid fever pathogens: insights from a meta- analysis of 13,000 Salmonella T yphi genomes. *Elife*. 2023;12(12):e85867. doi:10.7554/eLife.85867
11. Ao TT, Feasey NA, Gordon MA, et al. Global burden of invasive nontyphoidal Salmonella disease, 20101. *Emerg Infect Dis*. 2015;21(6):941–949. doi:10.3201/eid2106.140999
12. Kariuki S, Gordon MA, Feasey N, et al. Antimicrobial resistance and management of invasive Salmonella disease. *Vaccine*. 2015;33:C21–C29. doi:10.1016/j.vaccine.2015.03.102
13. Kariuki S, Owusu-Dabo E. Research on invasive nontyphoidal Salmonella disease and developments towards better understanding of epidemiology, management, and control strategies. *Clin Infect Dis*. 2020;71(Supplement_2):S127–S129. doi:10.1093/cid/ciaa315
14. Zhan Z, Kuang D, Liao M, et al. Antimicrobial susceptibility and molecular typing of Salmonella senftenberg isolated from humans and other sources in Shanghai, China, 2005 to 2011. *J Food Prot*. 2017;80(1):146–150. doi:10.4315/0362-028X.JFP-16-255
15. Hu Y, Wang J, Chen S, et al. Non-typhoidal Salmonella invasive infections in China. *The Lancet. Infectious Diseases*. 2022;22(7):939. doi:10.1016/S1473-3099(22)00347-4
16. Mughini-Gras L, Pijnacker R, Duijster J, et al. Changing epidemiology of invasive non-typhoid Salmonella infection: a nationwide population-based registry study. *Clin Microbiol Infect*. 2020;26(7):941.e949–941.e914. doi:10.1016/j.cmi.2019.11.015
17. Kalonji LM, Post A, Phoba M-F, et al. InvasiveSalmonellaInfections at multiple surveillance sites in the democratic Republic of the Congo, 2011–2014. *Clin Infect Dis*. 2015;61(suppl 4):S346–S353. doi:10.1093/cid/civ713
18. Zhan Z, Xu X, Gu Z, et al. Molecular epidemiology and antimicrobial resistance of invasive non-typhoidal Salmonella in China, 2007–2016. *Infect Drug Resist*. 2019;12(12):2885–2897. doi:10.2147/IDR.S210961
19. Crump JA, Heyderman RS. A perspective on invasive Salmonella disease in Africa. *Clin Infect Dis*. 2015;61(suppl 4):S235–S240. doi:10.1093/cid/civ709
20. Takkar VP, Kumar R, Khurana S, et al. Comparison of ciprofloxacin versus cephelexin and gentamicin in the treatment of multi-drug resistant typhoid fever. *Indian Pediatr*. 1994;31(2):200–201.
21. Brown M, Eykyn SJ. Non-typhoidal Salmonella bacteraemia without gastroenteritis: a marker of underlying immunosuppression. review of casesat St. Thomas' Hospital 1970–1999. *J Infect*. 2000;41(3):256–259. doi:10.1053/jinf.2000.0750
22. Kankwatira AM, Mwafulirwa GAK, Gordon MA. Non-typhoidal Sal-monella bacteraemia-an under-recognized feature of AIDS in African adults. *Trop Doct*. 2004;34(4):198–200. doi:10.1177/004947550403400404
23. Gordon MA, Banda HT, Gondwe M, et al. Non-typhoidal Salmonella bacteraemia among HIV-infected Malawian adults: high mortality and frequent recrudescence. *AIDS*. 2002;16(12):1633–1641. doi:10.1097/00002030-200208160-00009
24. Cacemiro MC, Espíndola MS, Galvão-Lima LJ, et al. Immune response against Salmonella enteritidis is unsettled by HIV infection. *Adv Exp Med Biol*. 2018;1057:29–39.
25. Brenchley JM, Douek DC. HIV infection and the gastrointestinal immune system. *Mucosal Immunol*. 2008;1(1):23–30. doi:10.1038/mi.2007.1
26. Voedisch S, Koenecke C, David S, et al. Mesenteric lymph nodes confine dendritic cell-mediated dissemination ofSalmonella entericaSerovar typhimurium and limit systemic disease in mice. *Infect Immun*. 2009;77(8):3170–3180. doi:10.1128/IAI.00272-09
27. Griffin AJ, McSorley SJ. Development of protective immunity to Salmonella, a mucosal pathogen with a systemic agenda. *Mucosal Immunol*. 2011;4(4):371–382. doi:10.1038/mi.2011.2
28. Galanis E, DMALF W, Patrick ME, et al. Web-based surveillance and global Salmonella distribution, 2000–2002. *Emerg Infectious Dis*. 2006;12(3):381–388. doi:10.3201/eid1205.050854
29. Balasubramanian R, Im J, Lee J-S, et al. The global burden and epidemiology of invasive non-typhoidal Salmonella infections. *Hum Vaccin Immunother*. 2019;15(6):1421–1426. doi:10.1080/21645515.2018.1504717
30. Yue M, Liu D, Li X, et al. Epidemiology, serotype and resistance of Salmonella isolates from a Children's Hospital in Hangzhou, Zhejiang, China, 2006–2021. *Infection and Drug Resistance*. 2022;15:4735–4748. doi:10.2147/IDR.S374658
31. Gordon MA, Graham SM, Walsh AL, et al. Epidemics of invasive Salmonella enterica serovar enteritidis and S. entericaSerovar typhimurium infection associated with multidrug resistance among adults and children in Malawi. *Clin Infect Dis*. 2008;46(7):963–969. doi:10.1086/529146
32. Milazzo A, Giles LC, Zhang Y, et al. Heatwaves differentially affect risk of Salmonella serotypes. *J Infect*. 2016;73(3):231–240. doi:10.1016/j.jinf.2016.04.034
33. Sun L, Zhang H, Chen J, et al. Epidemiology of foodborne disease outbreaks caused by nontyphoidal Salmonella in Zhejiang Province, China, 2010–2019. *Foodborne Pathog Dis*. 2021;18(12):880–886. doi:10.1089/fpd.2021.0006
34. Ran L, Wu S, Gao Y, et al. Laboratory-based surveillance of nontyphoidal Salmonella infections in China. *Foodborne Pathog Dis*. 2011;8(8):921–927. doi:10.1089/fpd.2010.0827