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ORIGINAL RESEARCH

Analyzing *Shigella* in Wuhan: Serotypes, Antimicrobial Resistance, and Public Health Implications

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Objective: This study aims to delineate the epidemiological characteristics and antibiotic resistance of *Shigella flexneri* isolates from Wuhan, focusing on serotype distribution, resistance patterns, and genetic diversity.

Methods: Our study analyzed 40*Shigella flexneri* isolates collected from 2011 to 2022 in Wuhan, assessing their serotype distribution and resistance to multiple antibiotics. We conducted resistance gene detection and genetic diversity analysis using polymerase chain reaction and pulsed-field gel electrophoresis (PFGE), respectively.

Results: The study revealed significant clustering of *S. flexneri* in the Jianghan and Dongxihu districts, with serotype 2a predominating. Isolates exhibited high resistance to ampicillin, tetracycline, and trimethoprim-sulfamethoxazole, with an overall multidrug resistance (MDR) rate of 67.5%. Serotypes 1b and 2b were fully sensitive, contrasting with higher resistance in serotypes 2a and 4a to fluoroquinolones. Resistance mechanisms included *blaOXA* and *blaTEM* for ampicillin, *blaCTX-M* for cephalosporins, *tetB* for tetracycline, and *dfrA1* and *sul2* for trimethoprim-sulfamethoxazole. All 12 quinolone-resistant isolates exhibited mutations in *gyrA* (*S83L, D87N, D87G), parC* (*S80I*), and *parE* (*458A*), and novel mutations were identified in *gyrA* (*H221Y, A221V, A221E, I222N*), *parC* (*D197A*), and *parE* (*G408D*). Pulsed-field gel electrophoresis (PFGE) analysis highlighted extensive genetic diversity with dominant groups P1 and P4, and with notable regional and temporal distribution patterns. Distinct PFGE types exhibited unique antimicrobial resistance profiles, with P1 and P4 showing high rates of multidrug resistance, while P5 and P3 displayed lower resistance levels. A notable evolutionary adaptation was observed in a clone from 2016 (P4-1), which by 2017 (P4-2) had acquired aminoglycoside resistance.

Conclusion: The study underscores the significant regional specificity and genetic diversity of *S. flexneri* in Wuhan, which poses challenges for treatment due to high antibiotic resistance and MDR prevalence. Findings stress the need for enhanced surveillance and tailored public health strategies to manage shigellosis effectively.

Keywords: *Shigella flexneri*, epidemiology, resistance profile, multidrug resistance, pulsed-field gel electrophoresis

Introduction

Shigella species, which are facultative anaerobic bacilli within the Enterobacteriaceae family, are prominent global causes of diarrheal illnesses and represent a considerable public health challenge.[1] Notably, *Shigella* species require an extremely low infectious dose—as few as 10 to 100 organisms—to cause disease, making them highly transmissible and capable of causing large outbreaks, especially in areas with poor sanitation and hygiene.[2,3] *Shigella* is categorized as both a waterborne and foodborne pathogen.[4] Humans and primates serve as its primary reservoirs and hosts. These bacteria

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are present in the feces of infected humans or primates and can spread through vehicles like contaminated food or water, leading to infections and diseases in humans.[5] Numerous outbreaks have been linked to the consumption of such contaminated sources. Outbreaks related to *Shigella* are particularly common with foods that are manually processed, minimally heated, or consumed raw.[6] Foods such as groundbeef, oysters, potato salads, bean dips, raw vegetables, and fish have been identified as common sources of contamination.[7] Furthermore, *Shigella* can also spread from person to person via the fecal-oral route during foodborne and waterborne outbreaks.[8]

The *Shigella* genus is classified into four serogroups: *S. dysenteriae, S. flexneri, S. boydii*, and *S. sonnei*, based on detailed biochemical and serological analysis. *S. sonnei* is mainly found in industrialized countries, while *S. flexneri* is more prevalent in China.[9] Interestingly, even though pathogenic strains might share the same serotype, they can have different genetic backgrounds. Conversely, strains of the same serotype from different regions might share similar genetic profiles.[10] Based on the national surveillance data from 2014, the annual *shigellosis*-related morbidity rate was 11.24 cases per 100,000 people in China.[11] Currently, over 20 different serotypes of *S. flexneri* have been identified, including 1a, 1b, 1c, 1d, 2a, 2b, 2v, 3a, 3b, 4a, 4av, 4b, 5a, 5b, X, Xv, Y, Yv, 6, and 7b.[12,13] In various developing regions, *S. flexneri* serotype 1b is most frequently encountered, followed by serotype 2a.[14]

Although *Shigella* is often self-limiting, World Health Organization (WHO) guidelines advocate the use of antimicrobials to reduce the infiltration of epithelial cells, the duration and severity of diarrhea, the carrier stage of the disease, and the incidence of death.[15] However, the treatment of shigellosis is increasingly complicated by the prevalence of multidrug-resistant (MDR) strains that show resistance to commonly prescribed antimicrobials such as ciprofloxacin, third-generation cephalosporins, and ceftriaxone.[16] Recent estimates suggest that AMR contributes to approximately 700,000 global fatalities annually.[17] Substantial regional differences in multidrug-resistant *Shigella* globally underscore the importance of understanding its genetic diversity to accurately track epidemiological changes and devise suitable treatment strategies.

The antimicrobial resistance pattern differs from place to place and even in the same place in two separate regions,[18] and antibiotic resistance patterns of *Shigella* in different regions of China have not been adequately monitored and systematically analyzed.[19,20] This rise of antibiotic-resistant isolates has become a serious concern, highlighting the urgent need for research to gain deeper insights into the epidemiology, antibiotic resistance patterns, and genetic characteristics of *Shigella* across different regions. Comprehensive and systematic studies are essential to bridge these knowledge gaps and enhance our understanding of this key pathogen.

In reality, research into the epidemiological characteristics of *Shigella* is primarily concentrated across major cities and between major urban regions in China.[9,21] However, there is a noticeable deficiency in studies focusing on associations within the inner areas of major cities. Moreover, a significant lack of data exists regarding the prevalence, serotypes, and antimicrobial resistance (AMR) patterns of *Shigella* strains isolated from the various administrative districts of Wuhan. Consequently, this study has been designed to investigate the epidemiological characteristics of shigellosis and its AMR patterns in this specific region.

Materials and Methods

Bacterial Isolates and Identification

From 2011 to 2022, a total of 40 non-duplicate *Shigella flexneri* isolates were recovered from stool specimens collected at sentinel hospitals across nine administrative districts in Wuhan. These isolates represent all available and successfully preserved strains obtained during routine clinical microbiological testing over the 11-year study period. Although the sample size is limited, the isolates span multiple years, serotypes, and geographic regions, and thus provide a meaningful snapshot of the regional epidemiology of *S. flexneri*. These isolates were used for descriptive analyses of serotype distribution, antimicrobial resistance, and clonal diversity in this region. The isolation and identification procedures and methods are strictly carried out in accordance with relevant operating protocols.[22] Specifically, fresh fecal samples (10 g/mL) were inoculated into bottles containing 100 mL of *Shigella* broth supplemented with novobiocin (2 mg/L) (Sigma-Aldrich). After homogenization for 5 minutes at 260 rpm, the samples were incubated at 37°C for 24 hours. Following incubation, the enriched samples were streaked onto MacConkey and *Salmonella*–

Shigella (SS) agar plates (Becton Dickinson) and incubated at 37°C for 20 hours. Colonies suspected to be *Shigella* were identified as convex, colorless, light pink on MacConkey agar and red/colorless on SS agar plates. These colonies were then subcultured onto commercial GN (Gram-negative) plates (Biomérieux) and subjected to biochemical tests using VITEK2 Compact (Biomérieux) for species identification. Authenticated *Shigella* isolates were preserved at −80°C until further use.

Serotyping

To determine the serotype of *Shigella* isolates, fresh cultures are mixed with four types of polyvalent *Shigella* antisera. A positive serotyping reaction is indicated by visible agglutination particles forming within minutes. The isolates are classified into groups A, B, C, or D, with further subtypes within these groups. For instance, group B includes subtypes such as 2a, 2b, 3a, and 1b.[23]

Antimicrobial Susceptibility Testing

The antimicrobial susceptibility of all *Shigella* isolates was assessed by determining the minimum inhibitory concentrations (MICs) of 12 common antimicrobial agents. These antibiotics include Ampicillin (AMP), Piperacillin (PIP), Ceftazidime (CAZ), Ceftriaxone (CRO), Cefepime (FEP), Tetracycline (TET), Minocycline (MNO), Ciprofloxacin (CIP), Levofloxacin (LVX), Trimethoprim-sulfamethoxazole (SXT), Gentamicin (GEN), and Tobramycin (TOB). This was done using a commercially available 96-well microtiter plate (GN4F plate, Thermo Fisher Scientific) pre-encapsulated with antibiotics in twofold serial dilutions. A Shigella bacterial suspension of 0.5 McFarland standard was prepared and dispensed into the GN4F plates, followed by incubation at 37°C for 24 hours. MIC concentrations were then determined, and the results were categorized as resistant or susceptible based on the MIC breakpoints recommended by the Clinical and Laboratory Standards Institute.[24] *Escherichia coli* ATCC 25922 was used as a control strain.

Polymerase Chain Reaction for Antimicrobial Genes

Genomic DNA from each isolate was extracted and purified using the commercial Bacteria DNA Kit (TIAN-GEN Biotech). Polymerase Chain Reaction (PCR) assays were conducted according to previously described protocols to screen for various antimicrobial genes,[9,25] including β-lactamase genes, tetracycline-resistance genes, sulfonamide-and trimethoprim-resistance genes, aminoglycoside resistance genes, plasmid-mediated quinolone resistance (PMQR) genes, and quinolone resistance-determining regions (QRDR). The specific gene names and primers used in the study are summarized in Supplementary Table S1. PCR amplification was performed on the T100 thermal cycler (Bio-Rad Laboratories). Agarose gel electrophoresis was used to visualize the PCR products, which were stained with EcoDye™ DNA Staining Solution (BIOFACT). PCR products of QRDR, including *gyrA, gyrB, parC* and *parE* were sent to Sangon Biotech (Shanghai, China) for nucleotide sequencing after being purified. The results were analyzed by the Basic Local Alignment Search Tool (BLAST) comparison with sequences in the GenBank database.

Pulsed-Field Gel Electrophoresis (PFGE)

PFGE typing of 40*Shigella* isolates was performed in accordance with the standardized PulseNet method.[26] Agarose-embedded DNA was digested by the XbaI restriction enzyme (Takara) for 4 h at 37°C, followed by gel electrophoresis (Bio-Rad). The gel was stained for 30 min and then transferred for developmental exposure. PFGE restriction spectrums were analyzed using the BioNumerics software (version 7.6, Applied Maths). The isolates were considered to have originated from the same clone at similarity 85%.[27]

Statistics Analysis

Descriptive statistics were used to calculate detection rates and resistance rates for *S. flexneri* isolates and related variables. Rates are presented as counts and percentages. Differences in resistance rates among serotypes were assessed using Fisher's exact test due to small sample sizes. All statistical analyses were performed using SPSS version 20 (IBM Corp., Armonk, NY, USA). A P value < 0.05 was considered statistically significant.

Results

Serotype Distribution and Temporal Trends of Shigella flexneri Isolates

In this study, we examined 40*Shigella flexneri* isolates collected from nine administrative districts in Wuhan, China, between 2011 and 2022. These isolates were classified into six distinct serotypes. The highest detection rate was in the Jianghan District, accounting for 30% (12/40), followed by Dongxihu District at 22.5% (9/40), and Qiaokou District at 17.5% (7/40). In Qiaokou District, the predominant serotype is 1b (71.4%, 5/7), while in Jianghan District, the serotype X is the most prevalent (50.0%, 6/12). In Dongxihu District, the detection rates of various serotypes are approximately equal (Figure 1A). Overall, serotype 2a was the most frequently identified, with 12 isolates detected, accounting for 30% of the total. Serotype X followed with 8 isolates, representing 20%. Serotype 2b was found in 7 isolates, making up 17.5%, while serotype 1b was identified in 6 isolates, comprising 15%. Additionally, serotype 4c was present in 4 isolates, corresponding to 10%, and serotype 4a was the least common, with 3 isolates detected, accounting for 7.5%. These findings are detailed in Figure 1. It is worth noting that the detection of various serotypes has also changed over the years. From 2011 to 2013, the 2a serotype was most prevalent detected (2/5, 2/3, 6/6, respectively), followed by a gradual increase in the frequency of serotype X. In the past five years, the proportion of serotypes 1b, 4c, and others has steadily risen (Figure 1B).

Antimicrobial Resistance Patterns and Serotype Distributions

Antibiotic susceptibility testing revealed high resistance rates among the *S. flexneri* isolates to several commonly used antibiotics (Table 1). Ampicillin and tetracycline both showed resistance rates of 75.0%, trimethoprim-sulfamethoxazole 55.0%, and ceftriaxone 35.0%. Notably, resistance to ciprofloxacin and levofloxacin was mainly observed in serotypes 2a and 4a, while piperacillin and third- and fourth-generation cephalosporin resistance was primarily found in serotypes 4c and x. In contrast, serotypes 1b and 2b exhibited markedly lower resistance rates. Overall Fisher's exact tests confirmed significant differences in resistance rates among serotypes for multiple antibiotics, including piperacillin, ceftriaxone, cefepime, minocycline, ciprofloxacin, and tobramycin ($P < 0.05$, Table 1). In total, 67.5% (27/40) of isolates were

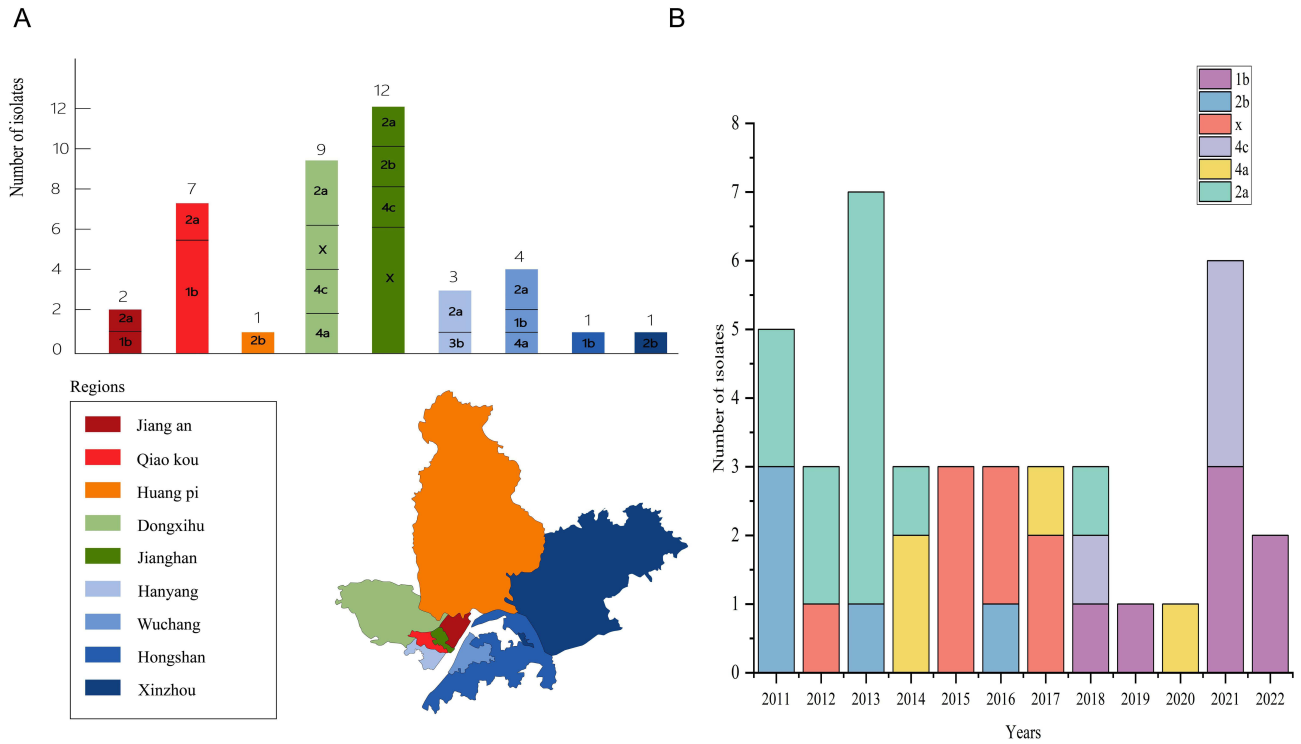

Figure 1 (**A**) Distribution of *Shigella flexneri* serotypes among isolates from different administrative districts in Wuhan (2011–2022). (**B**) Temporal trends of different serotypes of isolated *Shigella flexneri* strains.

Table 1 Rates of Resistance to Common Antibiotics by Different Serologic Shigella Spp

Antibiotics	Total (n,%) N=40	F2a N=12	F4a N=3	F1b N=8	F2b N=5	F4c N=4	Fx N=8	*P value*
Ampicillin	30(75.0)	11(91.7)	3(100)	2(25.0)	2(40.0)	4(100.0)	8(100.0)	0.471
Piperacillin	11(27.5)	0(0)	0(0)	0(0)	0(0)	3(75.0)	8(100.0)	**<0.05**
Ceftazidime	1(2.5)	0(0)	0(0)	0(0)	0(0)	0(0)	1(12.5)	0.425
Ceftriaxone	14(35.0)	2(16.7)	1(33.3)	0(0)	0(0)	3(75.0)	8(100.0)	**<0.05**
Cefepime	6(15.0)	0(0)	0(0)	0(0)	0(0)	3(75.0)	4(50.0)	**<0.05**
Tetracycline	30(75.0)	11(91.7)	3(100.0)	2(25.0)	2(40.0)	4(100.0)	8(100.0)	0.471
Minocycline	4(10.0)	0(0)	0(0)	0(0)	0(0)	0(0)	4(50.0)	**<0.05**
Ciprofloxacin	12(30.0)	9(75.0)	3(100.0)	0(0)	0(0)	0(0)	0(0)	**<0.05**
Levofloxacin	10(25.0)	8(66.7)	2(66.7)	0(0)	0(0)	0(0)	0(0)	0.104
Trimethoprim-sulfamethoxazole	22(55.0)	9(75.0)	0(0)	2(25.0)	0(0)	4(100.0)	7(87.5)	0.348
Gentamicin	9(22.5)	4(33.0)	0(0)	0(0)	0(0)	3(75.0)	2(25.0)	0.456
Tobramycin	5(12.5)	0(0)	0(0)	0(0)	0(0)	3(75.0)	2(25.0)	**<0.05**
MDR	27(67.5)	10(83.3)	3(100.0)	0(0)	0(0)	4(100.0)	8(100.0)	0.512

Notes: *P* values were calculated using Fisher's exact test to assess overall differences among serotypes. N indicates the total number of isolates per serotype. Bold values indicate statistical significance (P < 0.05).

multidrug-resistant (MDR). Serotypes 4a, 4c, and x each displayed a 100% MDR rate, higher than that of serotype 2a (83.3%) and substantially higher than serotype 1b (25%), with serotype 2b showing no MDR isolates. Additionally, we explored the association between resistance and the geographical and temporal distribution of *Shigella* serotypes in Wuhan. The results indicate that resistance patterns largely reflect serotype distribution across districts and over time (Figure 1A and B, Table 1). Together, these findings highlight that the local serotype landscape strongly influences *S. flexneri* resistance profiles, emphasizing the need for targeted surveillance and empiric therapy guidance.

Molecular Mechanisms of Antimicrobial Resistance

In order to explore the potential molecular mechanisms of antimicrobial resistance phenotypes based on antibiotic resistance genes (ARGs), we used PCR to detect common resistance genes in these bacterial strains. Among the 14 cephalosporin-resistant isolates, all were positive for the β-lactamase resistance gene *blaCTX-M* (Figure 2). Among the ampicillin-resistant isolates, 50% (15/30) tested positive for the *blaOXA* gene, and 40% (12/30) tested positive for the *blaTEM* genes. In the genes encoding trimethoprim resistance, *dfrA1* was detected in 90.9% (20/22) of isolates, while *dfrA14* was found in only 9.1% (2/22). For sulfonamide resistance genes, *sul1* was present in 18.2% (4/22) of isolates, and *sul2* was detected in 90.9% (20/22). Among the 30 tetracycline-resistant isolates, 90.0% (27/30) carried the *tetB* gene, while 20% (6/30) had the *tetA* gene. Among the 9 isolates resistant to aminoglycosides, only 1 isolate tested positive for the *aadA1* gene. For plasmid-mediated quinolone resistance, the *qnrS* gene was identified in one isolate (8.3%). Regarding quinolone resistance-determining regions, mutations in the *gyrA* and *par*C were found in all 12 quinolone-resistant isolates (Figure 2). Specifically, in the *gyrA*, four isolates exhibited the co-mutations *A221V, H211Y, D87N*, and *S83L*, while a few isolates also showed the mutations *D87G, I222N*, and *A221E*. In the *parC*, five isolates had the co-mutations *D197A* and *S80I*, and one isolate had the mutation *G35A*. Additionally, dual mutations in the *parE, S458A* and *G408*D, were found in two isolates, no mutation was seen in the *gyrB* (Table 2). In summary, the genetic analysis of antimicrobial resistance in *S. flexneri* isolates from Wuhan revealed a diverse range of resistance genes, with notable prevalence of β-lactamase, trimethoprim, and tetracycline resistance genes. Specific mutations in quinolone resistance-determining regions were also identified, underscoring the complexity of resistance mechanisms in these isolates.

Genetic Diversity and Resistance Profiles: Insights from PFGE Analysis

In order to further assess the homology and genetic relatedness among these *S. flexneri* isolates, PFGE was conducted. It revealed that these 40 *S. flexneri* isolates exhibited 36 distinct PFGE patterns (Figure 3 and Supplementary Figure S1), categorizing them into 8 different groups (P1-P8). This suggests a significant genetic diversity among the *S. flexneri*

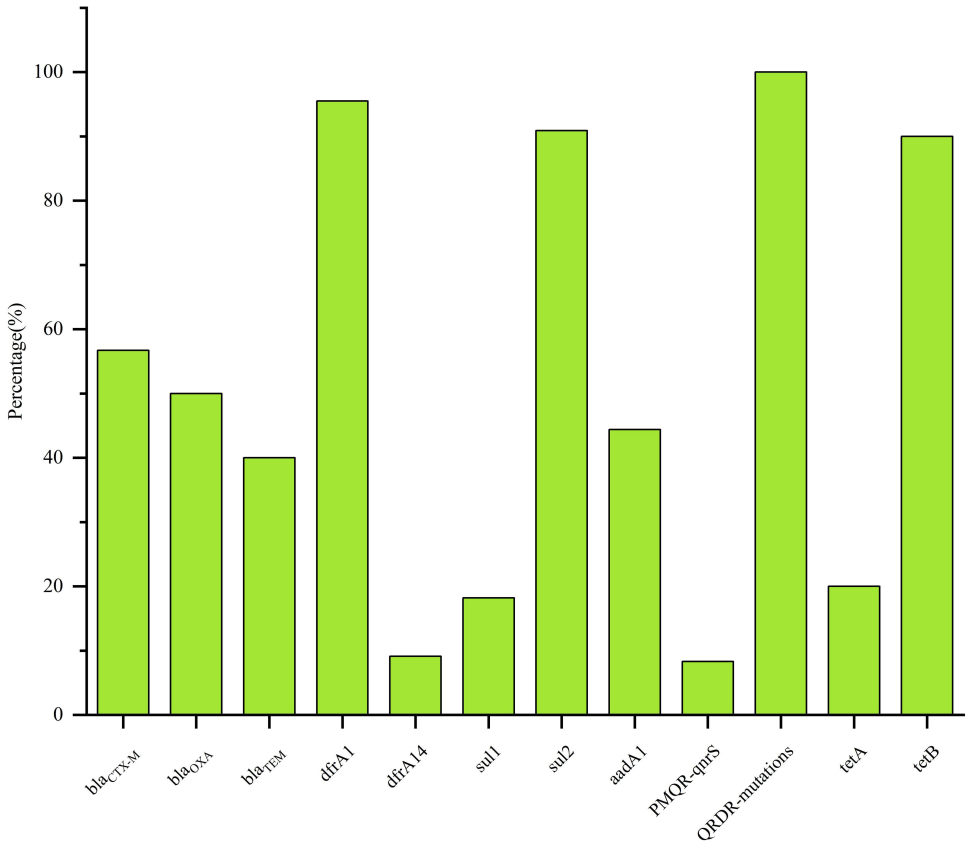

Figure 2 Genetic mechanisms of antibiotic resistance in *Shigella flexneri* isolates.

isolates from different regions and years in Wuhan. Notably, groups P1 and P4 were the predominant PFGE types of *S. flexneri* in Wuhan. Additionally, in group P1, the predominant serotypes were 2a; in group P3, they were 2b; in group P4, they were x and 4c; and in group P5, all isolates were 1b serotypes (Figure 3).

Among the strains isolated from Jianghan District, P1 and P4 each accounted for 50% (6/12). In Qiaokou District, P5 was the predominant type (57.1%, 4/7), whereas in Dongxihu District, the proportions of P4 and P1 were approximately equal (55.6% and 44.4%, respectively, Figure 4). Our results also revealed that within three PFGE clone groups, some patterns exhibited 100%

Table 2 Characteristics of Shigella Isolates Analyzed in This Study (n=40)

Isolate ID	Organism (Serotypes)	Resistant Pattern	MDR	Acquired Resistance Genes	Mutation in QRDR			
					gyrA	*gyrB*	*parC*	*parE*
WH301	*S. flexneri* 2a	AMP-CRO-TET	NO	*blaCTX-M, tetB*	–	–	–	–
WH323	*S. flexneri* 2a	AMP-CRO-TET-CIP-LVX	YES	*blaCTX-M, tetB*	*H21IY,D87N, S83L*	–	*S80I*	–
WH266	*S. flexneri* 2a	AMP-SXT-TET	YES	*tetB,dfrA1,sul2*	–	–	–	–
WH267	*S. flexneri* 2a	AMP-SXT-TET-GEN	YES	*blaTEM,tetB,dfrA1, sul1,sul2,aadA1*	–	–	–	–
WH302	*S. flexneri* 2a	AMP-TET-CIP-LVX	YES	*blaTEM*	*A22IV,H21IY, D87N,S83L*	–	*D197A, S80I*	–
WH348	*S. flexneri* 2a	AMP-SXT-TET-CIP-LVX	YES	*tetB,dfrA1,sul2*	*A22IV,H21IY, D87N,S83L*	–	*D197A, S80I*	–

(*Continued*)

Table 2 (Continued).

Isolate ID	Organism (Serotypes)	Resistant Pattern	MDR	Acquired Resistance Genes	Mutation in QRDR			
					gyrA	*gyrB*	*parC*	*parE*
WH357	*S. flexneri* 2a	AMP-SXT-TET-CIP-LVX	YES	*tetB,dfrA1,sul2*	*A221V,H211Y, D87N,S83L*	–	*S80I*	–
WH360	*S. flexneri* 2a	AMP-SXT-TET-GEN-CIP	YES	*blaTEM,tetB,dfrA1, sul1,aadA1*	*A221V,H211Y, D87N,S83L*	–	*D197A, S80I*	–
WH361	*S. flexneri* 2a	AMP-SXT-TET-GEN-CIP-LVX	YES	*blaTEM,tetB,dfrA1, sul1,aadA1*	*I222N,A221E, H211Y,D87N, S83L*	–	*S80I*	–
WH371	*S. flexneri* 2a	AMP-SXT-TET-CIP-LVX	YES	*tetB,dfrA1,sul2*	*A221E,H211Y, D87N,S83L*	–	*S80I*	–
WH481	*S. flexneri* 2a	AMP-SXT-TET-GEN-CIP-LVX	YES	*blaTEM,dfrA1,sul2, aadA1,qnrS*	*H211Y,S83L*	–	–	–
WH322	*S. flexneri* 2a	SXT-CIP-LVX	NO	*sul1*	*D87N,S83L*	–	*S80I, G35A*	*S458A, G408D*
WH392	*S. flexneri* 4a	AMP-TET-CIP	YES	*tetB*	*H211Y,D87N, S83L*	–	*S80I*	–
WH393	*S. flexneri* 4a	AMP-TET-CIP-LVX	YES	*blaOXA,tetB*	*A221E,H211Y, D87G,S83L*	–	*D197A, S80I*	–
WH512	*S. flexneri* 4a	AMP-CRO-TET-CIP-LVX	YES	*blaCTX-M, blaTEM, tetA,sul2*	*A221E,D87N, S83L*	–	*D197A, S80I*	*S458A, G408D*
WH458	*S. flexneri* 1b	AMP-SXT-TET	YES	*blaCTX-M, blaOXA, tetB,dfrA14,sul2*	–	–	–	–
WH487	*S. flexneri* 1b	AMP-SXT-TET	YES	*blaTEM,tetB,dfrA1, dfrA14,sul2*	–	–	–	–
WH262	*S. flexneri* 2b	AMP-TET	NO	*blaOXA,tetB*	–	–	–	–
WH263	*S. flexneri* 2b	AMP-TET	NO	*blaOXA,tetB*	–	–	–	–
WH479	*S. flexneri* 4c	AMP-PIP-CRO-FEP-SXT-TET-TOB-GEN	YES	*blaCTX-M, blaTEM, blaOXA,tetA,tetB, dfrA1,sul2*	–	–	–	–
WH538	*S. flexneri* 4c	AMP-SXT-TET	YES	*blaCTX-M, blaOXA, tetB,dfrA1,sul2*	–	–	–	–
WH539	*S. flexneri* 4c	AMP-PIP-CRO-FEP-SXT-TET-TOB-GEN	YES	*blaCTX-M, blaTEM, blaOXA,tetA,tetB, dfrA1,sul2*	–	–	–	–
WH544	*S. flexneri* 4c	AMP-PIP-CRO-FEP-SXT-TET-TOB-GEN	YES	*blaCTX-M, blaTEM, blaOXA,tetA,tetB, dfrA1,sul2*	–	–	–	–
WH287	*S. flexneri* x	AMP-PIP-CAZ-CRO-FEP-TET	YES	*blaCTX-M,tetB*	–	–	–	–
WH409	*S. flexneri* x	AMP-PIP-CRO-SXT-TET-MNO	YES	*blaCTX-M, blaOXA, tetB,dfrA1,sul2*	–	–	–	–
WH413	*S. flexneri* x	AMP-PIP-CRO-SXT-TET-MNO	YES	*blaCTX-M, blaOXA, tetB,dfrA1,sul2*	–	–	–	–
WH415	*S. flexneri* x	AMP-PIP-CRO-SXT-TET-MNO	YES	*blaCTX-M, blaOXA, tetB,dfrA1,sul2*	–	–	–	–
WH430	*S. flexneri* x	AMP-PIP-CRO-SXT-TET-MNO	YES	*blaCTX-M, blaOXA, tetB,dfrA1,sul2*	–	–	–	–
WH443	*S. flexneri* x	AMP-PIP-CRO-FEP-SXT-TET	YES	*blaCTX-M, blaOXA, tetB,dfrA1,sul2*	–	–	–	–

(*Continued*)

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Table 2 (Continued).

Isolate ID	Organism (Serotypes)	Resistant Pattern	MDR	Acquired Resistance Genes	Mutation in QRDR			
					gyrA	*gyrB*	*parC*	*parE*
WH456	*S. flexneri* x	AMP-PIP-CRO-FEP-SXT-TET-TOB-GEN	YES	*blaCTX-M, blaTEM, blaOXA,tetA,tetB, dfrA1,sul2*	–	–	–	–
WH465	*S. flexneri* x	AMP-PIP-CRO-FEP-SXT-TET-TOB-GEN	YES	*blaCTX-M, blaTEM, blaOXA,tetA,tetB, dfrA1,sul2*	–	–	–	–
WH504	*S. flexneri* 1b	S	–	–	–	–	–	–
WH534	*S. flexneri* 1b	S	–	–	–	–	–	–
WH540	*S. flexneri* 1b	S	–	–	–	–	–	–
WH565	*S. flexneri* 1b	S	–	–	–	–	–	–
WH570	*S. flexneri* 1b	S	–	–	–	–	–	–
WH543	*S. flexneri* 1b	S	–	–	–	–	–	–
WH269	*S. flexneri* 2b	S	–	–	–	–	–	–
WH359	*S. flexneri* 2b	S	–	–	–	–	–	–
WH431	*S. flexneri* 2b	S	–	–	–	–	–	–

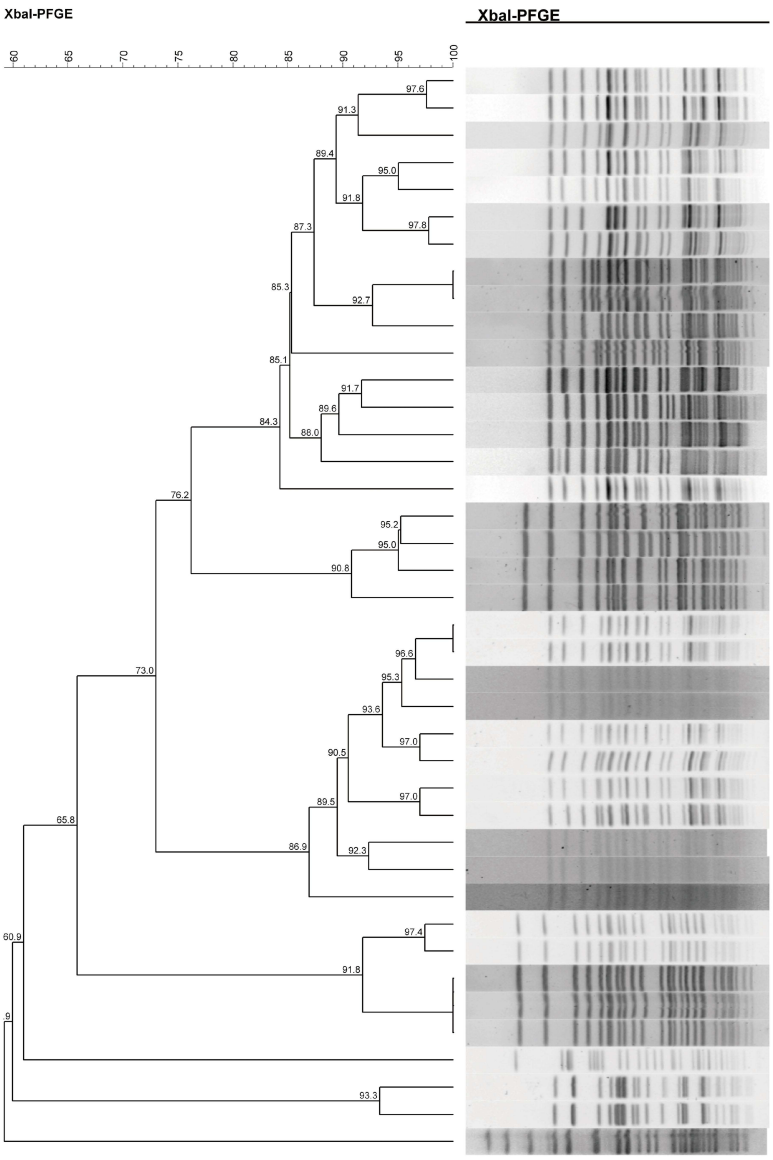
Notes: —, not applicable or not detected.
Abbreviations: MDR, multidrug resistance; S, susceptible; QRDR, quinolone resistance-determining region.

similarity, specifically between P1-8 and P1-9, P4-1 and P4-2, and P5-3, P5-4, P5-5 (Figure 3). P1-8 and P1-9 were both identified in Jianghan District in 2011, whereas P5-3, P5-4, and P5-5 were found in Wuchang and Qiaokou districts during 2021–2022. P4-1 and P4-2 were detected in Jianghan District from 2016 to 2017. Interestingly, while the first two groups did not show any change in antibiotic resistance during the outbreak, the latter group acquired resistance to aminoglycoside antibiotics during its spread.

Based on the above findings, we further explored the correlation between PFGE spectrums and antimicrobial resistance patterns. The results indicated that the P1 type predominantly exhibited resistance to ciprofloxacin and levofloxacin, while the P4 type was resistant to piperacillin and higher-generation cephalosporins (third and fourth generations). Additionally, the P4 and P1 types demonstrated high rates of MDR, whereas the P5 and P3 types generally exhibited lower resistance rates, reflecting a more susceptible antimicrobial profile (Figure 5). Overall, the PFGE analysis revealed significant genetic diversity among *S. flexneri* isolates in Wuhan, with distinct PFGE groups displaying unique antimicrobial resistance patterns and varying multidrug resistance rates.

Discussion

Shigella, a leading cause of dysentery, presents significant public health challenges in developing regions. Alarmingly, there is an increasing trend in resistance to third-generation cephalosporins, which are commonly used to treat infections caused by *Shigella*. The regional specificity of *Shigella* outbreaks in Wuhan highlights key epidemiological patterns that warrant further investigation. The concentration of cases within the Dongxihu, Jianghan, and Qiaokou districts, which are geographically contiguous, suggests that physical proximity may play a significant role in the transmission dynamics of *Shigella*. These areas might share common socio-economic factors or public health challenges that facilitate the spread of infectious diseases. It is plausible that factors such as population density, sanitation practices, and local healthcare infrastructure contribute significantly to the observed patterns. For instance, higher population density can enhance the transmission of pathogens through closer human contact. Additionally, variations in water quality and waste management practices across different districts could influence the prevalence of *Shigella*. The distinction in serotype prevalence, with P4 predominantly in Jianghan and Dongxihu and P5 exclusively in Qiaokou, may indicate serotype-specific niches or differing susceptibility to environmental conditions or interventions. Understanding these dynamics is crucial for tailoring public health interventions aimed at controlling *Shigella* outbreaks in the region. The spatial and temporal



Strain NO.	Origin	Serotypes	Year	PFGE Type
WH360	dong xi hu	2a	2013	P1-1
WH361	dong xi hu	2a	2013	P1-2
WH323	wu chang	2a	2013	P1-3
WH357	han yang	2a	2013	P1-4
WH301	jiang han	2a	2012	P1-5
WH392	dong xi hu	4a	2014	P1-6
WH348	jiang an	2a	2013	P1-7
WH262	jiang han	2b	2011	P1-8
WH263	jiang han	2b	2011	P1-9
WH430	jiang han	x	2016	P1-10
WH544	jiang han	4c	2021	P1-11
WH481	wu chang	2a	2018	P1-12
WH266	qiao kou	2a	2011	P1-13
WH267	jiang han	2a	2011	P1-14
WH302	dong xi hu	2a	2012	P1-15
WH371	han yang	2a	2014	P2-1
WH269	huang pi	2b	2011	P3-1
WH359	xin zhou	2b	2013	P3-2
WH543	hong shan	1b	2021	P3-3
WH431	han yang	2b	2016	P3-4
WH443	jiang han	x	2016	P4-1
WH456	jiang han	x	2017	P4-2
WH415	jiang han	x	2015	P4-3
WH413	jiang han	x	2015	P4-4
WH465	dong xi hu	x	2017	P4-5
WH479	jiang han	4c	2018	P4-6
WH538	dong xi hu	4c	2021	P4-7
WH539	dong xi hu	4c	2021	P4-8
WH287	dong xi hu	x	2012	P4-9
WH409	jiang han	x	2015	P4-10
WH393	dong xi hu	4a	2014	P4-11
WH504	qiao kou	1b	2019	P5-1
WH534	qiao kou	1b	2021	P5-2
WH540	wu chang	1b	2021	P5-3
WH565	qiao kou	1b	2022	P5-4
WH570	qiao kou	1b	2022	P5-5
WH512	wu chang	4a	2020	P6-1
WH458	jiang an	1b	2017	P7-1
WH487	qiao kou	1b	2018	P7-2
WH322	qiao kou	2a	2013	P8-1

Figure 3 PFGE pattern analysis of 40*Shigella flexneri* isolates from Wuhan.

distribution of *Shigella* serotypes and resistance patterns in Wuhan highlights the critical need for robust surveillance systems to monitor emerging trends and guide public health interventions. Targeted strategies, such as region-specific sanitation improvements, vaccination programs, and tailored antibiotic stewardship, could significantly enhance outbreak prevention and control. These findings provide a foundation for optimizing treatment protocols and strengthening *Shigella* management in Wuhan.

The antibiotic resistance profiles of *S. flexneri* isolates underscore the critical challenge of antimicrobial resistance in managing shigellosis, Over the decades, the principal antibiotics used to treat *Shigella* infections have included tetracycline, chloramphenicol, ampicillin, trimethoprim-sulfamethoxazole, and nalidixic acid.[28] This study reveals high resistance rates to ampicillin (75.0%), tetracycline (75.0%), and Trimethoprim-sulfamethoxazole (TMP–SMX) (55.0%) among *Shigella* isolates, suggesting that these antibiotics may no longer be effective for empirical therapy in regions with similar resistance patterns. Although the resistance rate for ceftriaxone is relatively high, the resistance rate for ceftazidime remains low at only 2.5%. Although both ceftriaxone and ceftazidime are third-generation cephalosporins, our data show a striking difference in their resistance rates among *Shigella* isolates. This can be explained by several factors. First, most *Shigella* strains in China harbor *CTX-M* type extended-spectrum β-lactamases (ESBLs), particularly

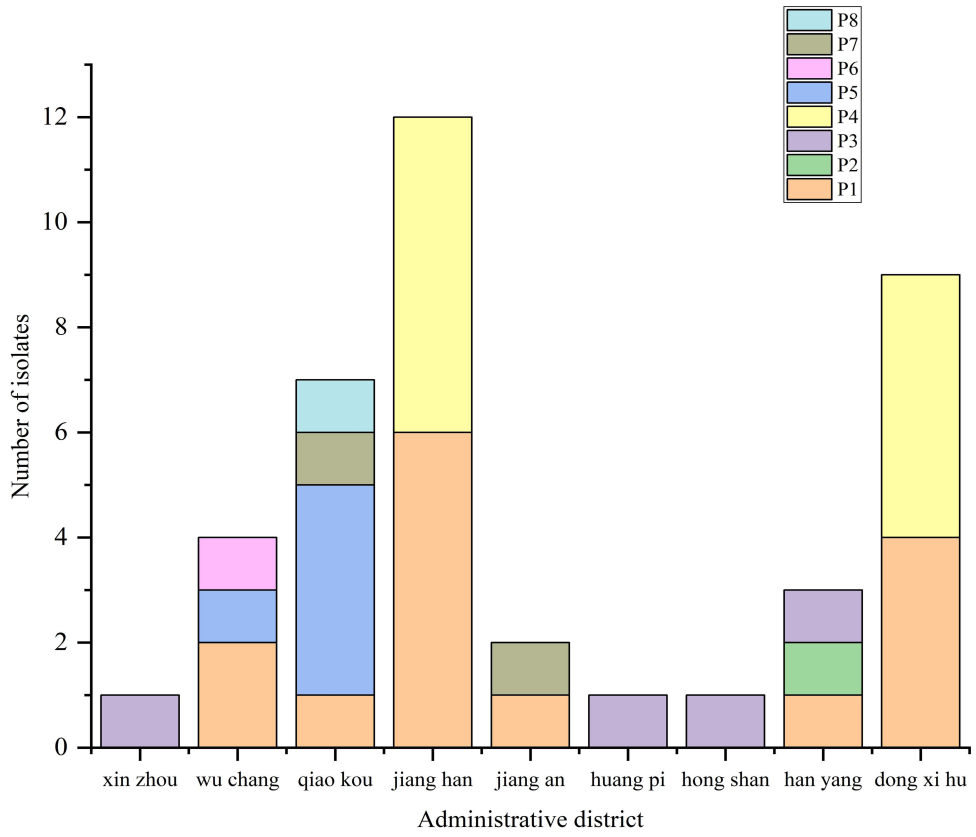


Figure 4 The distribution of PFGE patterns of *Shigella flexneri* across different administrative districts in Wuhan.

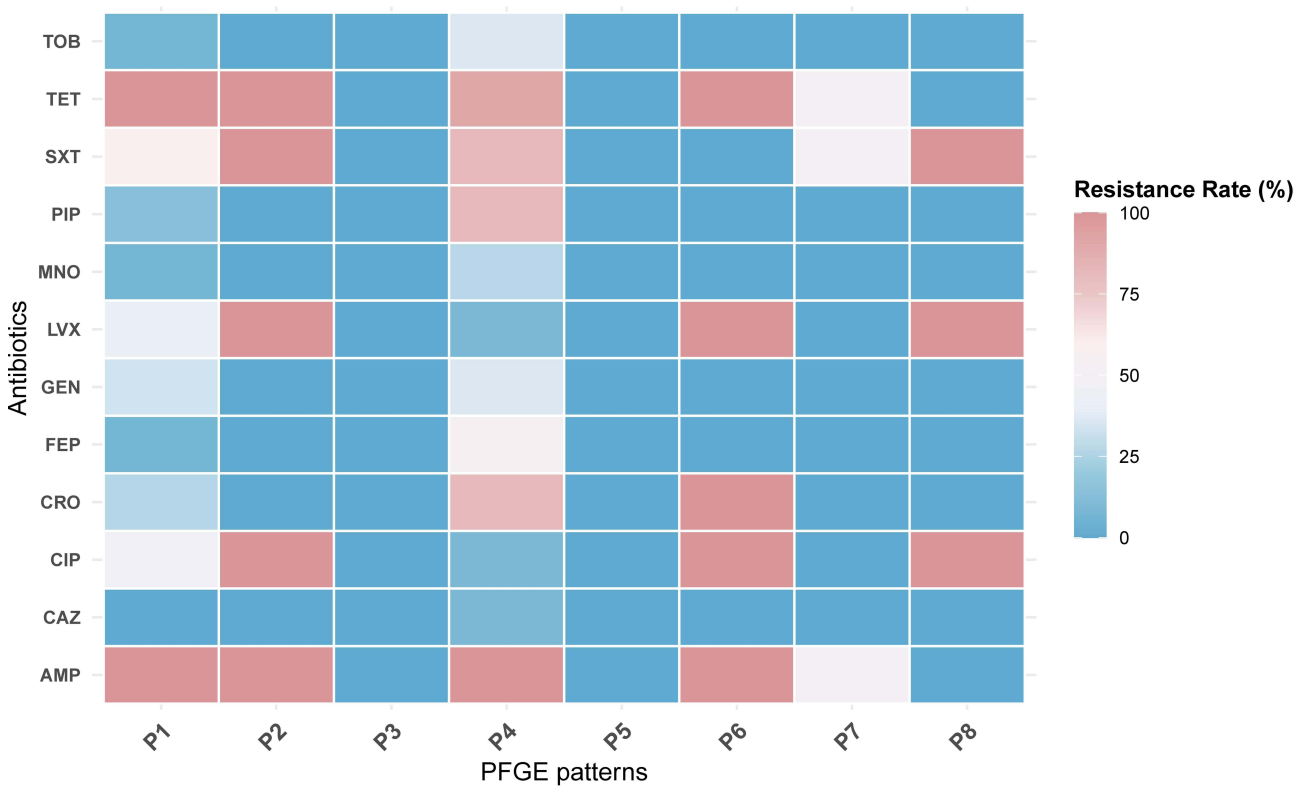


Figure 5 A heatmap displaying the correlation between PFGE typing and antibiotic resistance rates of these *Shigella flexneri* isolates.

the *blaCTX-M-15* variant, which preferentially hydrolyzes cefotaxime and ceftriaxone, but exhibits limited activity against ceftazidime due to its bulkier side chain.[29,30] Second, ceftriaxone is widely used in pediatric and empirical therapy for shigellosis, especially as resistance to fluoroquinolones rises,[31] thereby creating strong selective pressure for resistance. In contrast, ceftazidime is rarely used for treating enteric infections such as shigellosis and is often reserved for nosocomial pathogens like *Pseudomonas aeruginosa*, resulting in lower exposure and thus lower resistance rates.[32] These biochemical and clinical factors together explain why ceftriaxone resistance is frequently higher than that of ceftazidime in *Shigella*, despite both being in the same antibiotic class. Therefore, as other studies have reported,[33] third-generation cephalosporins are considered the drugs of choice for treating *Shigella* infections.

Our study revealed a high MDR rate of 67.5% among *S. flexneri* isolates in Wuhan, which is consistent with the regional Asian average (~68.7%),[34] but notably exceeds the national Chinese average (41.6%).[9] Certain provinces such as Xinjiang (90.4%) and Shanxi (91.1%) report even higher MDR rates.[9,35] Globally, recent XDR *Shigella sonnei* outbreaks in Europe and the US reflect the growing international relevance of this issue.[36] The high MDR rates in Wuhan are likely driven by local antibiotic prescribing practices. In Hubei Province, where Wuhan is located, 44.28% of primary care prescriptions include antibiotics, with 9.28% involving multiple antibiotics.[37] External factors, such as patient pressure and time constraints on prescribers, significantly influence overprescription, outweighing intrinsic factors like physician knowledge.[37] This overuse creates selective pressure, fostering the emergence and spread of resistant *Shigella* strains. While specific antibiotic prescription data for shigellosis in Wuhan are unavailable, regional patterns suggest that empirical use of ampicillin and cephalosporins contributes to resistance.[38] Public reporting interventions have shown promise in reducing unnecessary antibiotic prescriptions in Hubei (where Wuhan is located),[39] suggesting that integrating stricter hospital guidelines and public education into local practices could enhance public health relevance and curb AMR. Although clinical outcomes were not assessed in our study, the high MDR burden raises concerns about potential treatment failure and adverse outcomes, especially in pediatric populations. Infections caused by MDR *Shigella* strains have been linked to prolonged hospitalization, delayed recovery, and increased healthcare costs. For instance, during an MDR *S. flexneri* outbreak in New Mexico, approximately 70% of affected patients required hospitalization with a median stay of 4 days.[40] Similarly, in Vancouver, the hospitalization rate among affected populations rose from 14% to 61% during MDR shigellosis outbreaks.[41] These clinical burdens underscore the urgency of strengthening local surveillance and optimizing empirical treatment strategies. Together, these findings emphasize the pressing need to integrate local resistance trends into stewardship frameworks.

Notably, our study also identified unexpectedly low resistance rates in serotypes 1b and 2b, with no MDR strains detected. This contrasts sharply with previous reports, which documented complete resistance to tetracycline in serotype 1b, alongside high resistance rates to ampicillin (94%) and trimethoprim-sulfamethoxazole (72%),[18] Additionally, another study reported that 85% of serotype 1b strains were MDR.[42] In contrast, serotype 2b has been found to exhibit substantial resistance to quinolones (74.6%) and trimethoprim-sulfamethoxazole (54.6%), with MDR prevalence rates for serotypes 2b and 1b at 37.68% and 19.5%, respectively.[43] The distinct resistance patterns observed, such as serotype 2a showing resistance to ciprofloxacin and levofloxacin, and serotypes 4c and X exhibiting resistance to piperacillin and higher-generation cephalosporins, suggest the need for tailored antibiotic stewardship programs in Wuhan. These findings highlight not only the evolving serotype-specific resistance landscape but also the potential for regional variation in strain evolution and antibiotic exposure.[42] Taken together, Wuhan's antimicrobial resistance profile shares key features with national trends—such as widespread detection of *blaCTX-M* and quinolone resistance mutations—but also presents distinct serotype-specific patterns that underscore the need for localized therapeutic strategies. This dual pattern positions Wuhan as a representative yet uniquely challenging area within China's broader *Shigella* resistance landscape.

This study revealed a complex array of resistance mechanisms within bacterial pathogens, characterized by widespread resistance genes and specific mutations. All 14 cephalosporin-resistant isolates carried the *blaCTX-M* gene, mirroring findings from Iran where Bialvaei et al reported a 66.7% prevalence of *blaCTX-M* among extended-spectrum β-lactamase-producing *Shigella* isolates.[44] This high prevalence underscores the global challenge posed by β-lactamase-mediated resistance, which is further highlighted by the substantial occurrence of *blaOXA* and *blaTEM* genes in our ampicillin-resistant isolates. Contrastingly, our results identified *dfrA1* and *sul2* as the primary resistance genes for trimethoprim-sulfamethoxazole in our region, differing from Phiri et al's findings, which did not detect *dfrA1* and

highlighted *sul1, sul2*, and *sul3* as key resistance genes.[45] This discrepancy suggests regional variations in resistance gene distribution and highlights the importance of localized antimicrobial resistance surveillance. Furthermore, our research confirmed that the *tetB* gene is the predominant tetracycline-resistance determinant among *Shigella* isolates, corroborating with high resistance levels previously reported in Brazil and Chile.[46] Although 30% of our isolates were resistant to quinolones, only 8.3% harbored the *qnrS* PMQR gene, indicative of the limited role of plasmid-mediated mechanisms compared to chromosomal mutations in quinolone resistance. We identified well-known QRDR mutations, such as *gyrA (S83L, D87N, D87G), parC(S80I)*, and *parE(458A)*,[47–49] and discovered novel mutations in *gyrA (H221Y, A221V, A221E, I222N), parC(D197A)*, and *parE(G408D)*. These findings enhance our understanding of the evolving landscape of quinolone resistance, emphasizing the urgent need for continuous surveillance and targeted therapeutic strategies to manage the dynamic nature of *Shigella* antibiotic resistance effectively. However, functional validation such as MIC correlation studies, site-directed mutagenesis, or plasmid-based transfer assays was not performed in this study. Future investigations are warranted to determine the phenotypic impact and resistance-contributing role of these novel QRDR mutations. Importantly, resistance to ceftazidime was rare (2.5%), suggesting its viability for empirical treatment. However, given the potential for resistance evolution under selective pressure, dynamic monitoring of minimum inhibitory concentrations (MICs) is recommended when ceftazidime is used in pediatric severe cases. The co-detection of *blaCTX-M, blaTEM*, and QRDR mutations aligns with national patterns,[9,35] but the unique serotype-specific resistance profiles observed in Wuhan warrant localized surveillance and therapeutic planning. In summary, the identification of novel QRDR mutations, region-specific resistance genes (eg, *dfrA1, tetB*), and low ceftazidime resistance highlight the need for regionally informed antibiotic stewardship and empirically optimized therapy. Wuhan's resistance profile, while reflecting broader national trends, shows distinct genetic and serotype-linked features, emphasizing the value of targeted interventions. Future longitudinal surveillance and integration with clinical outcome data are essential to improve AMR mitigation strategies.

The PFGE analysis conducted in our study reveals significant genetic diversity among *S. flexneri* isolates in Wuhan, categorizing them into eight distinct groups based on 36 unique PFGE patterns from 40 isolates. This diversity reflects the adaptive genetic variations among the *S. flexneri* populations, potentially driven by evolutionary pressures such as horizontal gene transfer and environmental challenges. The prevalence of specific PFGE groups, particularly P1 and P4, which predominantly consist of serotypes 2a, x, and 4c, suggests that these genetic lineages may have a selective advantage in the regional pathogenic landscape of Wuhan. This could be due to their enhanced ability to evade host immune mechanisms or their increased fitness in the local human population. The correlation between PFGE types and serotypes within these clusters underscores the complex interaction between genetic evolution and phenotypic expression in bacterial pathogens. Notably, some isolates with identical PFGE patterns exhibited distinct serotypes, raising the possibility of serotype switching. Serotype switching in *S. flexneri* has been previously attributed to horizontal gene transfer and O-antigen modification mediated by serotype-converting bacteriophages. These phages carry genes such as gtr and oac, which modify the O-antigen via glucosylation or acetylation, thereby altering the serotype and potentially enhancing immune evasion capacity.[50,51] For instance, Allison and Verma reported that phages SfV and SfX contribute to such conversions, while Wang et al demonstrated that sequential phage infections could create novel serotypes through chromosomal integration events.[50,51] Although our current study lacks whole-genome sequencing data, the observation of genetically identical yet phenotypically distinct strains supports this possibility. Future genomic investigations targeting serotype-converting loci are needed to confirm these findings. In parallel, historical surveillance data from China indicate a notable shift in the prevalent *S. flexneri* serotype from 1a to 2a over the period from 1972 to 2010.[52] A study of *S. flexneri* isolates in China from 2003 to 2013 revealed that serotype 2a remained predominant, while the serotype X variant emerged as a significant new serotype, reflecting shifts in serotype distribution.[53] Additionally, a novel serotype 4s strain, thought to have evolved from the serotype X variant, has been reported in China. This strain exhibits genomic changes that help it adapt to changing environmental conditions.[54] Serotype conversion in *S. flexneri*, driven by the addition of acetyl, glucosyl, or phosphatidylethanolamine groups to the O-antigen backbone and the horizontal transfer of these groups, plays a key role in the emergence of new serotypes and enables evasion of the host immune response.[55] These findings highlight the adaptive nature of serotype distribution, driven by environmental pressures and immune evasion mechanisms. Furthermore, the identification of identical PFGE patterns among isolates from different

years and regions suggests persistent clonal spread within specific districts of Wuhan. The unchanged resistance profiles in the P1 and P5 clone groups indicate stable antibiotic resistance traits over time within these clusters. However, the acquisition of aminoglycoside resistance by the P4 clone group between 2016 and 2017 is particularly noteworthy. This adaptation may reflect selective pressure from antibiotic use in the region, underlining the dynamic nature of bacterial resistance evolution. Such findings emphasize the importance of continuous surveillance and targeted antibiotic stewardship to preemptively manage emerging resistance trends in *Shigella* outbreaks.

Geographically, *S. flexneri* exhibited significant spatial clustering in Wuhan, with 70% of isolates detected in the central districts of Jianghan, Dongxihu, and Qiaokou. Jianghan District, the urban core, has a population density exceeding 25,000 residents/km² and contains major transportation hubs and densely populated residential neighborhoods.[56] Importantly, it is home to Tongji Hospital and Union Hospital—two of the largest tertiary care centers in Wuhan and primary referral sites for pediatric patients.[57] These institutions contribute to high outpatient volumes, especially among children, with antibiotic prescription rates often exceeding 40% in central clinics. This confluence of dense population, concentrated healthcare services, and frequent antibiotic use likely facilitates the selection and transmission of MDR strains.[58] Although formal spatial modeling was not conducted, our findings underscore the potential impact of population and environmental factors on *Shigella* epidemiology and resistance development. Future studies integrating geographic information systems, antibiotic utilization data, and district-level healthcare statistics are warranted to further delineate AMR spatial dynamics and support targeted stewardship strategies in central Wuhan.

In conclusion, our study provides a comprehensive analysis of *S. flexneri* across a broad spectrum of isolates from Wuhan, underscoring the significance of genetic diversity and adaptive resistance mechanisms. The observed regional specificity of serotype distribution and antibiotic resistance profiles reflects the influence of local environmental and sociodemographic factors on the epidemiology of *Shigella* infections. Notably, the high prevalence of multidrug resistance among these isolates, especially in certain serotypes, highlights the challenges of managing shigellosis in the face of escalating antibiotic resistance. Moreover, the PFGE analysis revealed significant genetic diversity among *S. flexneri* isolates in Wuhan, with distinct PFGE groups displaying unique antimicrobial resistance patterns. The diversity in PFGE patterns and serotype variability within groups indicates ongoing genetic evolution and potential serotype switching among *S. flexneri*, suggesting that these bacteria are continually adapting to overcome host immunity and antibiotic pressure. This adaptive capacity necessitates sustained surveillance and tailored public health strategies to mitigate the spread of these pathogens effectively. However, this study has limitations, including the focus on a single geographic region, which might limit the generalizability of the findings to other settings with different antimicrobial usage and public health practices. Additionally, the retrospective design restricts our ability to capture temporal changes in resistance patterns and the impact of interventions over time, and the relatively small sample size further limits the statistical power to perform more robust inferential analyses. Future research should aim to expand the geographic scope of surveillance and include prospective studies—potentially incorporating whole-genome sequencing (WGS)—to monitor the evolution of resistance, validate serotype-switching events, and identify mobile genetic elements. This approach would support more dynamic and targeted public health strategies, including antimicrobial stewardship and vaccine development.

Ethical Approval

We confirm that our study strictly adhered to all ethical standards set forth in the Declaration of Helsinki. The study protocol was approved by the Ethical Review Committee of Wuhan Children's Hospital. Informed consent was obtained from the participants before the start of the study and the study did not interfere with standard medical care or violate patients' rights, nor did it pose any additional risks to the participants. We ensure that patient identity is protected through coding and that medical records are properly maintained and accessible only to researchers. The results of the study will be published in an anonymous, summarized form Data.

Consent for Publication

All authors declared no conflict of interest existed in this work. All authors are aware of and agree to the content of the paper and their being listed as a co-author of the paper.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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