Vital Surveillances

Molecular Epidemiology of Type F *Clostridium perfringens* Among Diarrheal Patients and Virulence-Resistance Dynamics — 11 Provinces, China, 2024

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ABSTRACT

Introduction: Type F *Clostridium perfringens* (*C. perfringens*) represents a significant pathogen in human gastrointestinal diseases, primarily through its *cpe* gene encoding *C. perfringens* enterotoxin (CPE). This investigation examined the prevalence, antimicrobial resistance patterns, and genetic characteristics of Type F *C. perfringens* within the Chinese population.

Methods: The study analyzed 2,068 stool samples collected from 11 provincial hospitals in 2024. Antimicrobial susceptibility testing was conducted following Clinical & Laboratory Standards Institute (CLSI) guidelines, while whole-genome sequencing provided detailed genetic profiles. Evolutionary relationships and clonal transmission patterns were investigated through phylogenetic and genetic environment analyses.

Results: The prevalence of Type F *C. perfringens* was 2.38%, with isolates predominantly identified in human clinical samples and higher detection rates in gastroenterology departments. Notably, 47.1% of isolates demonstrated high resistance to metronidazole, while all exhibited intermediate resistance to erythromycin. Phylogenetic analysis revealed high similarity among isolates from patients within the same province (single-nucleotide polymorphism (SNPs)<100), and genetic environment analysis indicated potential horizontal gene transfer between animal and human strains.

Conclusions: This investigation predominantly identified Type F *C. perfringens* in human clinical cases, with sporadic detection in pets and food products. These findings highlight the emergence of Type F *C. perfringens* outbreaks among diarrheal patients, emphasizing the necessity for targeted interventions as virulence factors increase.

Clostridium perfringens (*C. perfringens*) is ubiquitously distributed across diverse environments, including soil, water, and animal gastrointestinal tracts (*1*). Based on the differential production of four major extracellular toxins (α, β, ε, and ι), toxin-producing strains are classified into five distinct toxinotypes (A through E) (*2*). Among these, Type F *C. perfringens* is particularly significant due to its *cpe* gene, which encodes enterotoxin CPE and is associated with non-foodborne gastrointestinal diseases (*3–4*). Type F *C. perfringens* has been implicated in large-scale diarrheal outbreaks, with strains harboring both *plc* and *cpe* genes identified in cases such as those reported in Beijing (*5*). Global epidemiological data indicate that Type F *C. perfringens* accounts for a substantial proportion of foodborne disease outbreaks in both developed and developing nations (*4*). In the United States alone, Type F food poisoning affects approximately 1 million individuals annually, resulting in economic losses exceeding $310 million (*6*). These infections can prove fatal even in otherwise healthy individuals (*7*).

The extensive deployment of antimicrobial agents has escalated antibiotic resistance among *C. perfringens* strains. Resistance mechanisms include β-lactamase production, multidrug efflux pumps, and plasmid-mediated gene transfer (*8*). Agricultural isolates demonstrate high resistance to multiple antibiotics, particularly tetracyclines and fluoroquinolones (*9*). In China, 13.8% of *C. perfringens* isolates exhibit resistance to six antibiotics, with 54.4% harboring multiple resistance genes (*10*). Similar multidrug resistance patterns have been documented globally, significantly impacting both animal and human health (*11–12*).

The investigation of Type F *C. perfringens* is therefore crucial, particularly in the context of diarrheal illness. Beyond its role in widespread foodborne outbreaks, this strain's capacity to cause severe gastrointestinal disorders represents a significant public health concern. This study aims to elucidate the molecular epidemiology and pathogenic mechanisms

of Type F *C. perfringens* in patients across 11 provincial-level administrative divisions (PLADs) in China, employing bioinformatics analysis to characterize resistance and virulence genes. This comprehensive approach is essential for addressing the challenges posed by *C. perfringens* and protecting both animal and human health.

METHODS

Sample Collection

From January 2 to May 28, 2024, we conducted a cross-sectional study to determine *C. perfringens* prevalence among inpatients at 11 provincial hospitals across China. The study included hospitals in Shandong (*n*=230), Guangxi (*n*=100), Henan (*n*=196), Gansu (*n*=190), Shaanxi (*n*=243), Fujian (*n*=238), Hunan (*n*=104), Guangdong (*n*=177), Jilin (*n*=200), Jiangxi (*n*=300), and Zhejiang (*n*=350) PLADs. Participating departments included Gastroenterology and Neurology. A total of 2,068 fecal or rectal swab samples were collected using ESwab^{TM} collection kits (Copan, Brescia, Italy). For *C. perfringens* isolation, we processed either a small fecal sample or 0.2 mL of transport medium with 50% ethanol, followed by centrifugation and plating on TSC agar for anaerobic incubation. Suspected colonies underwent further purification on blood agar and definitive identification using MALDI-TOF MS (Bruker Daltonik GmbH, Bremen, Germany).

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing of *C. perfringens* isolates was performed using the Etest method following Clinical & Laboratory Standards Institute (CLSI) guidelines (M100-S29:2019). Nine antimicrobial agents were evaluated: metronidazole, penicillin, amoxicillin, tetracycline, ciprofloxacin, cefoxitin, linezolid, clindamycin, and erythromycin. For erythromycin and ciprofloxacin testing, we applied breakpoints equivalent to clindamycin and fluoroquinolones, respectively, due to the absence of specific CLSI guidelines for *C. perfringens*. *C. perfringens* ATCC 13124TM served as the quality control strain.

Whole-Genome Sequencing (WGS) and Analysis

Genomic DNA extraction was performed using PureLink Genomic DNA Mini Kit (Invitrogen, Carlsbad, CA, USA). DNA libraries were indexed using TruSeq DNA PCR-free Sample Preparation Kit (Illumina, Inc., San Diego, CA) and sequenced on the Illumina HiSeq X Ten System, generating 300-bp paired-end reads with minimum 150-fold coverage per isolate. Raw reads underwent trimming and assembly using SPAdes v3.11.1, followed by targeted analysis of AMR and virulence genes using ABRicate against relevant databases, employing thresholds of >90% identity and >75% coverage.

Phylogenetic Analysis

Type F *C. perfringens* isolates were retrieved from the National Center for Biotechnology Information (NCBI) database on August 27, 2024, using specific search criteria: "Toxin_genotypes: *cpe* & *plc*" and "species_taxid:1502." 91 Type F *C. perfringens* isolates, with their sources, countries of origin, and accession numbers are documented in Table 1 (*13*). Single-nucleotide polymorphisms (SNPs) were identified through sequence alignment using Snippy v4.6.0 (https://github.com/tseemann/snippy) (*14*), which generated a core genome alignment profile. Pairwise SNP distances were calculated using Snp-dists v0.6. We constructed a phylogenetic tree based on core-genome SNPs using Parsnp within the Harvest suite, with midpoint rooting and visualization enhanced through iTOL v6.25 (*15*).

Statistical Analysis

Clinical data were extracted from the hospital information system. We employed the Wilcoxon test to analyze differences in antimicrobial resistance and virulence genes, while Pearson chi-square and Fisher's exact tests were used to evaluate statistical significance (P<0.05) in gene frequencies and resistance phenotypes.

Data Availability

All supporting data for this study are included in this article and its Supplementary Information. The genome assemblies of *C. perfringens* have been deposited in NCBI under BioProject accession number PRJNA1154412. Additional data are available from the corresponding authors upon reasonable request.

RESULT

Epidemiological Information for Type F *C. perfringens* Isolates from China

Among 2,068 non-duplicated stool specimens

TABLE 1. Updated summary of the pathogenicity mechanisms of the currently identified/ characterized *Clostridium perfringens* toxins.

	Toxins	Gene	Toxin name	Alternative name	Mechanism of pathogenicity
1	Typing toxins	*plc/cpa*	Phospholipase	α-toxin	Disruption of cell membrane
2		*cpb*	β-toxin	–	Pore-formation
3		*etx*	ε-toxin	–	Pore-formation
4		*iap*	ι-toxin component Ia	–	Cytoskeleton disruption
5		*ibp*	ι-toxin component Ib	–	Cytoskeleton disruption
6		*cpe*	Enterotoxin (CPE)	–	Pore-formation and tight-junction disintegration
7	Non-typing toxins	*netB*	NetB	–	Pore-formation
8		*cpb2*	β2 toxin	–	Pore-formation
9		*lam*	λ-toxin	–	Potent protease
10		*pfo/pfoA*	Perfringolysin O	θ-toxin	Pore-formation
11		*cpd*	δ-toxin	–	Pore-formation
12		*ccp*	Clostripain	–	Digestion of collagen
13		*colA*	Microbial collagenase	κ-toxin	Digestion of collagen
14		*nanI*	Sialidase	–	Mucolysis
15		*nanJ*	Exo-α-sialidase	–	Mucolysis
16		*nanH*	Neuraminidase	–	Mucolysis
17		*nagH*	Hyaluronidase	μ-toxin	Digestion of connective tissue
18		*tpeL*	Glucosylating toxin	–	Induction of apoptosis
19		*becA*	Binary Enterotoxin Component A	–	Pore-formation
20		*becB*	Binary Enterotoxin Component B	–	Pore-formation
21		*netE*	NetE	–	Pore-formation
22		*netF*	NetF	–	Pore-formation
23		*netG*	NetG	–	Pore-formation

Note: "–" means no alternative toxins.

collected from patients across 11 provincial tertiary hospitals in 2024, 17 Type F *C. perfringens* isolates were identified, yielding a prevalence rate of 2.38% [95% (confidence interval) *CI*: 1.95%, 2.91%]. These isolates were distributed across 6 PLADs, with isolation rates varying from 0.9% in Shandong to 2.0% in Henan, Jilin, and Guangxi PLADs.

The demographic distribution of Type F *C. perfringens* cases closely mirrored the overall study population, with cases showing a mean age of 54.00±24.00 years and a gender distribution of 52.9% female versus 47.1% male (compared to the overall study population: age 37.51±11.98 years, 45.48% female versus 54.52% male). Notably, 64.7% of Type F *C. perfringens* isolates were recovered from patients presenting with diarrhea in gastroenterology departments.

Antimicrobial Susceptibility Profiles

Antimicrobial susceptibility testing revealed that most Type F *C. perfringens* isolates demonstrated susceptibility to linezolid, cefoxitin, and amoxicillin. However, 47.1% of isolates exhibited high resistance (>32 μg/mL) to metronidazole, and all strains showed intermediate resistance to erythromycin (Figure 1 and Supplementary Table S1, available at https://weekly.chinacdc.cn/).

Genomic Characteristics of 17 Type F *C. perfringens* Isolates

Phylogenetic analysis (Figure 2) revealed distinct clonal clusters of Type F *C. perfringens* isolates, each characterized by specific virulence and resistance determinants. Whole-genome sequencing identified

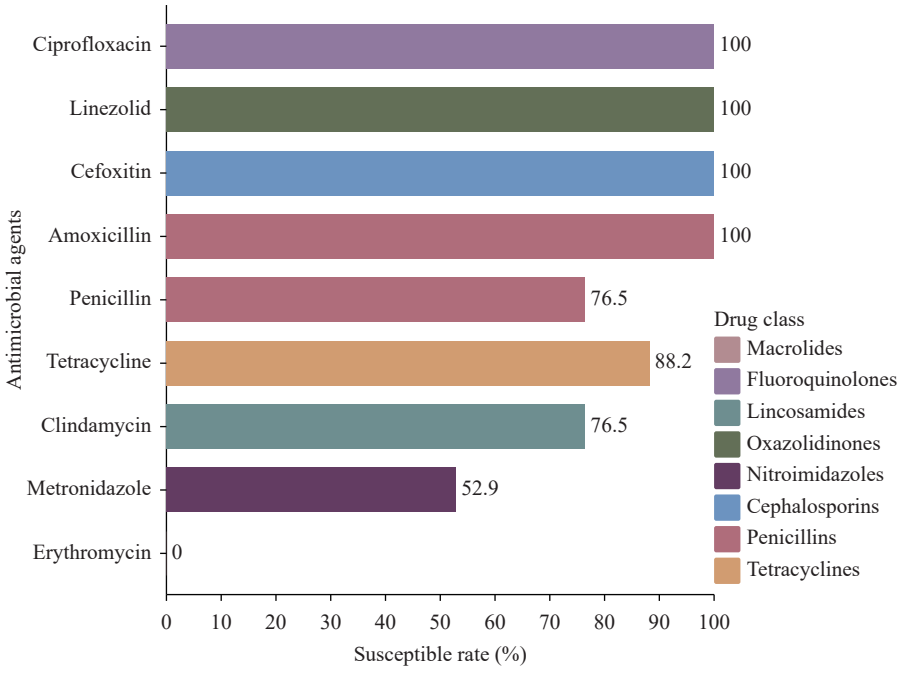

FIGURE 1. Distribution of antimicrobial resistance patterns among 17 F toxinotype *C. perfringens* isolates against 9 antimicrobial agents across 8 distinct categories.

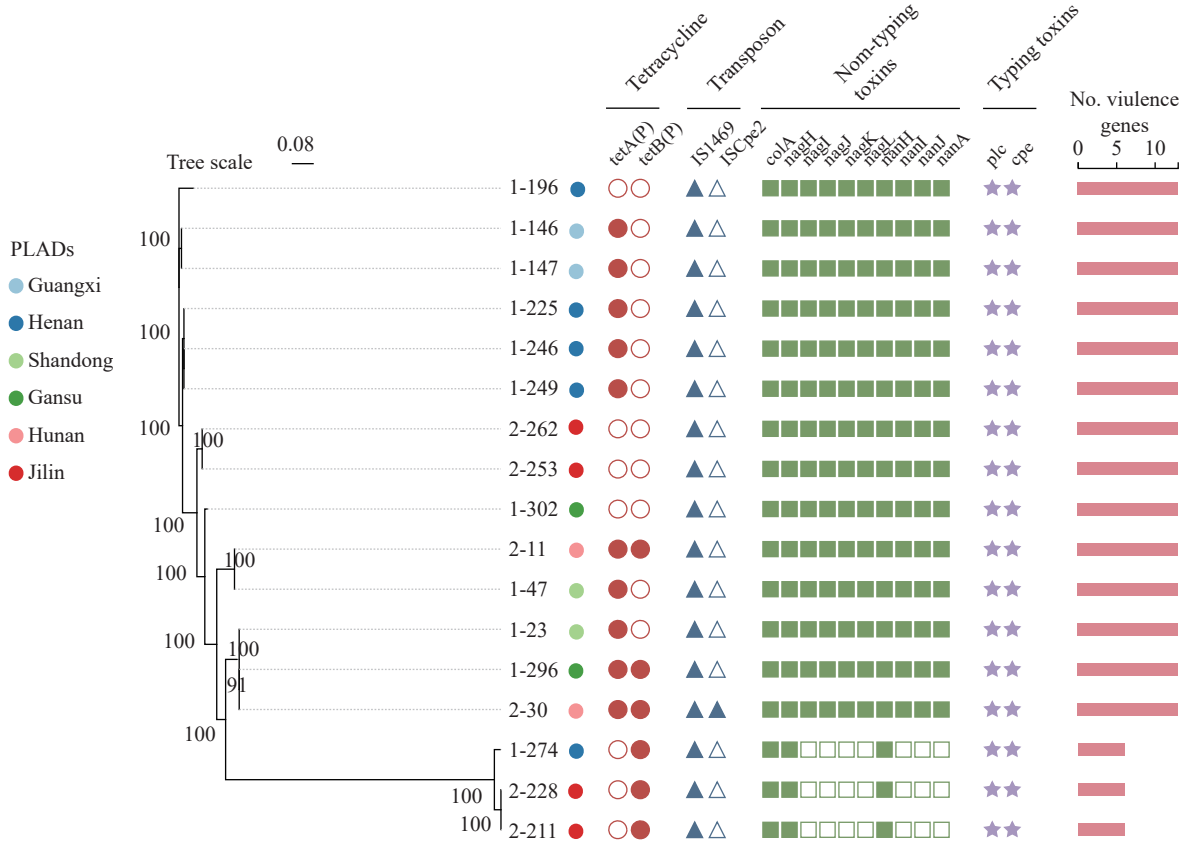

FIGURE 2. Phylogenetic relationships and corresponding antimicrobial resistance phenotypes, virulence characteristics, and genotypic profiles of 17 *C. perfringens* isolates from China.
Abbreviation: PLADs=provincial level administrative divisions.

tetracycline resistance genes [*tet*(A) and *tet*(B)], which corresponded with observed phenotypic resistance patterns. Among the analyzed isolates, we identified 12 distinct virulence factors: *colA, nagH, nagI, nagJ, nagK, nagL, nanH, nanI, nanJ, pfoA, plc*, and *cpe*. All isolates harbored the essential virulence determinants *cpe* (encoding enterotoxin CPE) and *plc* (encoding α-toxin). Notably, 18.0% (3/17) of isolates lacked *nagI, nagJ, nagK, nagL, nanI, nanJ*, and *pfoA* genes. The clustering patterns suggested the emergence of distinct clonal lineages, with an apparent inverse relationship between resistance gene carriage and virulence factor repertoire, indicating a potential fitness trade-off between resistance and virulence mechanisms.

Genetic Environment of the Type F *C. perfringens*

Analysis of the genetic environment (Figure 3) revealed high sequence homology between the IS*1469*-*cpe*-*hp*-IS*1151* gene cluster in our isolates (82.4%, 14/17) and sequences from a diarrheal canine isolate (strain D13122 plasmid pD13122_cpe, accession No. MG456815.1). This homology suggests potential horizontal gene transfer events between animal and human strains. The *cpe* gene, flanked by mobile genetic elements including transposons and insertion sequences (IS*1469*, IS*1151*), was found integrated into the chromosomal DNA of multiple isolates, indicating a mechanism for stable inheritance of virulence factors.

Phylogenetic Analysis of Type F *C. perfringens* Isolates in Global

Our 17 Type F *C. perfringens* isolates were analyzed in comparison with 91 Type F *C. perfringens* strains from the NCBI database, representing 13 countries and diverse sources (Figure 4). Comparative genomic analysis revealed that none of the NCBI database strains exhibited SNP distances less than 100 from our study isolates, suggesting distinct evolutionary trajectories. Notably, isolates 1-296 from Henan Province showed close genetic relatedness (SNPs<100) to isolates 2-30 and 1-23 from Hunan and Shandong, respectively. Within Henan Province, isolates 1-246, 1-225, and 1-249 demonstrated remarkable genetic similarity with less than 5 SNPs difference. Similarly, in Jilin Province, isolates 2-253 and 2-211 exhibited 100% sequence identity with isolates 2-262 and 2-228, respectively. Analysis of antimicrobial resistance genes revealed a relatively low prevalence of resistance determinants, with *tet(A)* and *tet(B)* being the most common at 46.7% and 15.2%, respectively.

CONCLUSION

This study characterized the epidemiological landscape of Type F *C. perfringens* across PLADs, revealing a 2.38% isolation rate. Although this prevalence appears relatively low, the exclusive detection of Type F *C. perfringens* in human samples, particularly from gastroenterology departments, emphasizes its clinical significance in human gastrointestinal health. The predominant isolation from gastroenterology departments aligns with established associations between Type F *C. perfringens* and diarrheal diseases, corroborating previous research linking these strains to gastrointestinal pathology through the *cpe* gene (*16*).

The enterotoxin CPE is a crucial virulence determinant in Type F strains (*17*), with historical studies reporting CPE detection rates of 40%–70% in gastroenteritis outbreaks (*18*). While research in Japan demonstrated a predominance of plasmid-mediated CPE with downstream IS*1151* sequences in food poisoning outbreaks (*19*), our analysis revealed a different pattern. The majority of our CPE-positive isolates (82.0%, 14/17) carried chromosomally-encoded CPE associated with IS*1469*, though some isolates harbored plasmid-borne *cpe*-IS*1151* loci, suggesting potential involvement in extraintestinal *C. perfringens* infections.

Antimicrobial susceptibility profiles revealed a concerning trend: while most Type F *C. perfringens* isolates maintained susceptibility to common antibiotics, 47.1% exhibited high resistance to metronidazole, a critical first-line treatment for anaerobic infections (*20*). The universal intermediate resistance to erythromycin among isolates suggests that antibiotic selective pressure in clinical settings may be driving the emergence of resistant strains, potentially compromising future therapeutic options.

Phylogenetic analysis revealed highly virulent strains (harboring both *cpe* and *plc* genes) with relatively few resistance elements, particularly tetracycline-associated transposons (IS*1469*, IS*cp2*). Core SNP analysis demonstrated evidence of clonal transmission within specific geographic regions, particularly among isolates from Henan, Hunan, Shandong, and Jilin provinces, suggesting localized spread patterns among diarrheal patients.

The identification of genetic similarities between human clinical isolates and those from a diarrheal dog

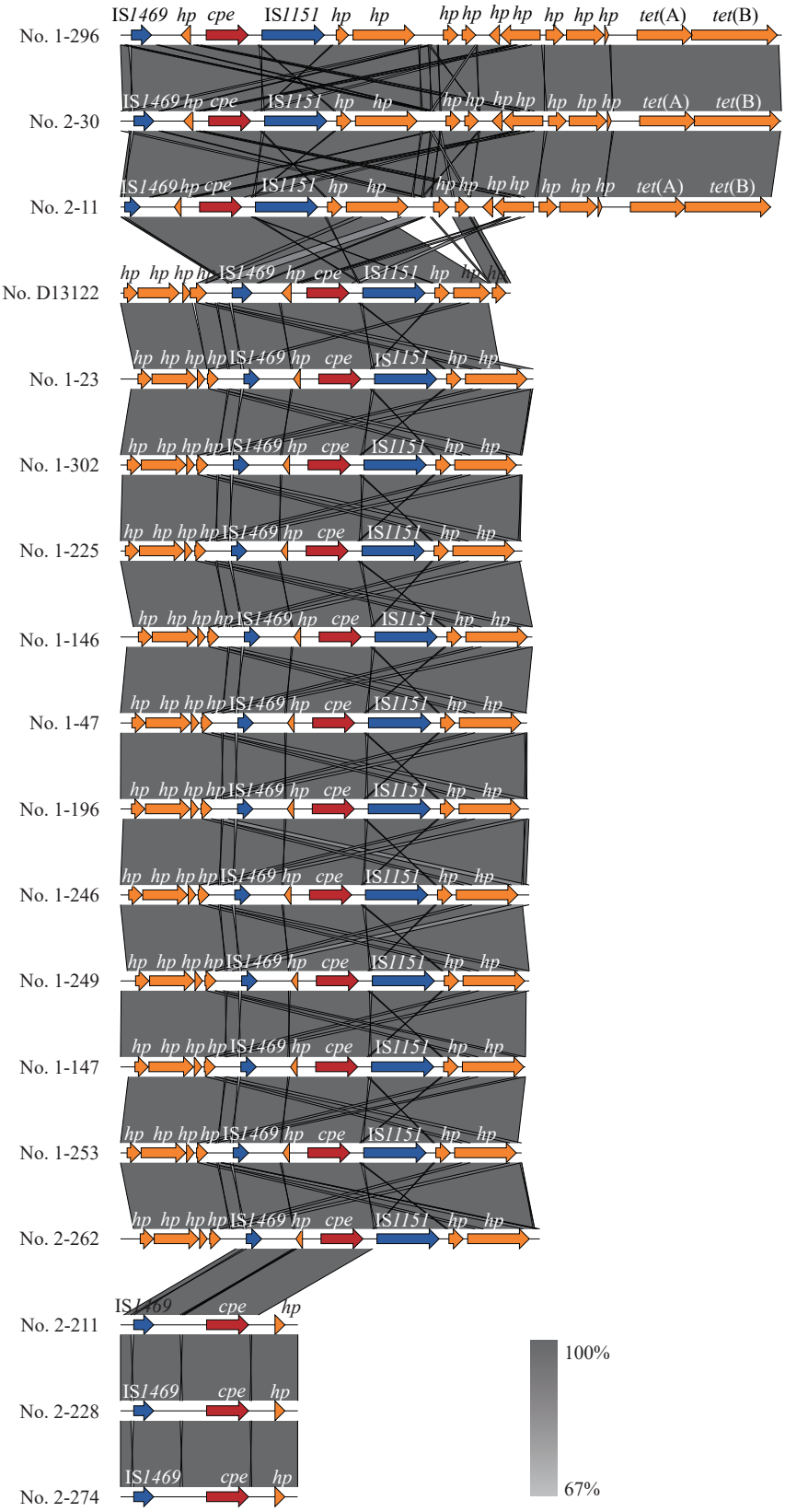

FIGURE 3. Genetic organization of the *cpe* locus in *Clostridium perfringens*.
Note: Arrows indicate gene orientation and function: red (toxin gene *cpe*), blue (mobile genetic elements), and orange (other protein-encoding genes).

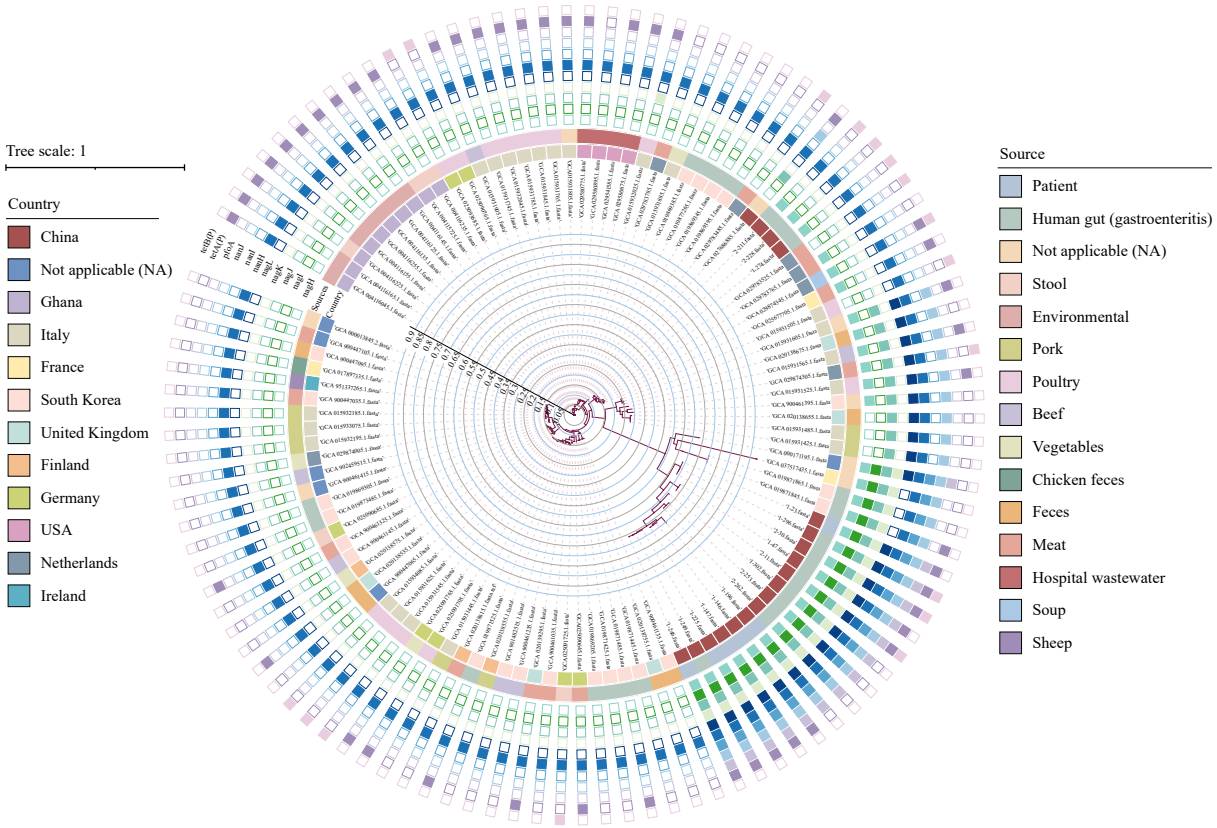

FIGURE 4. Global phylogenetic analysis of 108 F type *Clostridium perfringens* isolates based on core genome SNPs.

in an antibiotic-prevalent setting underscores the significance of horizontal gene transfer in virulence factor dissemination across species. Furthermore, the exclusive detection of Type F *C. perfringens* in human cases within our study suggests potential human-specific adaptation or a preferential ecological niche for human colonization. This host specificity, combined with the pathogen's virulence capabilities, emphasizes the importance of investigating its transmission dynamics, particularly regarding its persistence in human populations despite relatively low isolation rates.

This study's limitations include a relatively modest sample size that may not fully represent the diversity of China's population, potentially affecting the generalizability of the findings to different regions and demographic groups. Additionally, the potential selection bias introduced by hospital participation could skew the results, as the hospitals involved might differ from others in terms of patient characteristics, treatment protocols, and care quality.

In conclusion, our findings highlight the significant public health implications of virulence and antibiotic resistance patterns in Type F *C. perfringens*. The predominant detection of this pathogen in human cases emphasizes its clinical relevance and raises important questions about its transmission mechanisms and host adaptation. These observations underscore the necessity for targeted surveillance and preventive strategies to mitigate potential risks in both clinical and community settings.

Conflicts of interest: No conflicts of interest.

Ethics approval and consent to participate: Ethical approval was given by the Zhejiang University ethics committee (number 2024–0994). Informed patient consent was waived as samples were taken under a hospital surveillance framework for routine sampling. The research conformed to the principles of the Helsinki Declaration.

Funding: Supported by the National Key Research and Development Program of China (No. 2022YFD1800400) and the National Natural Science Foundation of China (No. 22193064).

doi: 10.46234/ccdcw2025.013

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Submitted: September 04, 2024
Accepted: November 13, 2024
Issued: January 17, 2025

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SUPPLEMENTARY MATERIAL

SUPPLEMENTARY TABLE S1. Antimicrobial susceptibility profiles of 17 F toxinotyping *C. perfringens* strains

Drug class	Antimicrobial agents	MIC50 μg/mL	MIC90 μg/mL	Range μg/mL	R%	I%	S%
Macrolides	Erythromycin	2	2	1 to >2	0.0	100.0	0.0
Fluoroquinolones	Ciprofloxacin	0.25	0.5	0.064 to 1	0.0	0.0	100.0
Lincosamides	Clindamycin	0.5	2	≤0.064 to 2	0.0	23.5	76.5
Oxazolidinones	Linezolid	1	1	0.5 to 1	0.0	0.0	100.0
Nitroimidazoles	Metronidazole	1	>32	0.5 to >32	47.1	0.0	52.9
Cephalosporins	Cefoxitin	0.5	4	0.064 to 1	0.0	0.0	100.0
Penicillins	Penicillin	≤0.064	1	≤0.064 to 1	0.0	23.5	76.5
	Amoxicillin	≤0.064	0.064	≤0.064	0.0	0.0	100.0
Tetracyclines	Tetracycline	1	4	≤0.064 to 16	5.8	5.9	88.2

Note: MIC90 and MIC50 values were defined as the lowest concentration of the antibiotic at which 90% and 50% of the isolates were inhibited, respectively.
Abbreviation: S=susceptible; I=intermediate resistant; R=resistant.