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CASE REPORT

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Prevalence and multilocus sequence typing of *Clostridium perfringens* isolated from retail chicken products and diseased chickens in Tai'an region, China

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Abstract

Background: *Clostridium perfringens* is an important zoonotic microorganism, which can cause diseases in animals and humans under suitable conditions. Contamination of *C. perfringens* in chicken products has been reported worldwide, but the genetic diversity and relationship of isolates were seldom analyzed.

Objectives: The current study was undertaken to investigate the prevalence of *C. perfringens* from retail chicken products and sick chickens with suspected necrotic enteritis (NE) in Tai'an area, China.

Methods: In total, 295 samples were collected from Tai'an large poultry retail market and veterinary hospital in 2018, then the isolates were tested for toxin genes, drug resistance and multilocus sequence typing (MLST).

Results: Overall, 138 (46.78%) samples were determined to be positive for *C. perfringens*, and 99.37% of the isolates were identified as *C. perfringens* type A, with the remaining isolates being type F; 18.99% of the isolates were positive for *cpb2* gene. Antimicrobial susceptibility testing revealed that 52.27% of the isolates from poultry retail market and diseased chickens showed multiple antibiotic resistance. MLST results showed that 50 analyzed isolates can be divided into 39 sequences types (STs), clustered in three clonal complexes (CCs) and 23 singletons. Although most of the isolates belong to type A, considerable genetic diversity can be observed, with the Simpson's diversity index up to 0.9181. MLST results and phylogenetic analysis showed that a portion of the isolates from humans and chickens were assigned to the same clusters in the phylogenetic tree or found to be in the same CCs, indicating the chicken isolates and the human isolates are related in certain stratification.

Conclusions: This study showed that the contamination rate of *C. perfringens* in the local retail chicken products was relatively high. Most of the isolates exhibit broad-spectrum antimicrobial resistance. The high antibiotic resistance of *C. perfringens* isolates and the relationship between isolates from human and chicken indicated potential public health risks.

KEYWORDS
antimicrobial resistance, chicken necrotizing enteritis, chicken products, *Clostridium perfringens*, multilocus sequence typing

1 | INTRODUCTION

Clostridium perfringens is a common pathogen in humans and animals, which can cause human food poisoning and necrotic enteritis (NE) infected chickens (Hibberd et al., 2011). According to the main toxins produced by *C. perfringens* (α-, β-, ε-and ι-toxins), it was divided into five types (type A to type E) (Aschfalk & Müller, 2002). In 2018, a new toxin typing scheme was proposed. In addition to the above five toxinotypes, type F consists of isolates that produce α-toxin and *C. perfringens* enterotoxin (*cpe*), and type G is defined as isolates that produce α-toxin and *netB* toxin (Rood et al., 2018).

In poultry, *C. perfringens* constitutes a human health hazard through the food chain and is one of the most frequently isolated bacterial pathogens from chicken meat, constituting up to 70%–98% of cases (Hamza et al., 2017). Moreover, *C. perfringens* is the major etiological agent of NE, a disease in poultry which is estimated to cost the poultry industry between 2 and 6 billion USD worldwide annually (Mwangi et al., 2019). To eliminate safety hazards at the source, more and more countries banned antibiotics as feed additives (Mwangi et al., 2019; J. Wang et al., 2019; Y. Wang et al., 2020).With the reduction and prohibition of antibiotics in the feed, the incidence of NE increased in recent years (Lacey et al., 2016; Mwangi et al., 2019; Rajput et al., 2020).

In China, there are few reports on the prevalence and characteristics of *C. perfringens* in retail chicken products and chickens with NE. According to the latest report, type A was the predominant genotype of *C. perfringens* from broiler chickens and retail chicken meat samples in central China (Zhang et al., 2018). However, the genetic diversity and relationship of *C. perfringens* isolates were not analyzed. Multilocus sequence typing (MLST) is a high-resolution genotyping approach with the advantages of data preservation and comparison between laboratories. It had been successfully used to conduct epidemiological studies on *C. perfringens* (Jost et al., 2006).

In order to further understand the epidemic situation of *C. perfringens* from chickens in China, this study was undertaken to investigate the prevalence, toxinotype distribution, antibiotic resistance, genetic diversity of *C. perfringens* isolated from retail chicken products and suspected NE infected chickens in Tai'an region. It can not only provide data for public food security assessment, but also provide epidemiological reference for animal and human disease associated with this microorganism.

2 | MATERIALS AND METHODS

2.1 | Sample collection

In total, 225 samples of chicken products, intestinal contents and environment were collected from four major stores (the distance between each store varies from 10 to 50 m) in a large retail market of Tai'an, China, from April to December 2018. There are many stores in this market, with diverse products, wide sources and poor sanitary conditions. The intestines and chicken products (livers, hearts, gizzard, split meat, etc.) were rapidly transferred into sterile sampling bags after purchase, and the carcass and environmental samples were wiped with cotton balls containing 2 ml sterile PBS. Each sample of carcass was swiped on areas (at least 100 cm^2) including the inner surface and the outer surface and immediately transferred into the sterile sample bags after the wipe was completed. Fresh faeces samples ($n = 7$) from healthy shopkeepers and their employees were also collected. In addition, 63 samples were collected from diseased chickens with diarrhoea, intestinal contents samples and liver samples were collected aseptically and quickly placed in a centrifuge tube, marked and refrigerated. Samples were transported to the laboratory within 1 h in a freezer box. The number of samples collected is shown in Table 1.

2.2 | Isolation and identification of *C. perfringens*

The chopped 3 g chicken products, carcass and environmental samples in PBS (0.5–1 ml) were placed in 7 ml FTG (fluid thioglycollate medium) broth and incubated in anaerobic conditions (90% N_2, 10% CO_2) for 8 h at 42°C with shaking at 180 rpm. *C. perfringens* was identified by colony morphology, cell morphology and haemolytic characteristics (gram-positive bacterium under a microscope, black colonies on TSC (tryptose-sulfite-cycloserine) agar (Liu et al., 2019). At least one

TABLE 1 Positive rate of *Clostridium perfringens* from different samples

Sampling location	Source	Number of samples	Number of positives	Positive rate (%)
Retail market	Chicken liver	41	24	58.54
	Chicken heart	19	13	68.42
	Gizzard	20	11	55.00
	Split meat	33	19	57.58
	Carcass	30	11	36.67
	Intestinal tract	66	30	45.45
	Environment	16	7	43.75
	Healthy person	7	7	100.00
Veterinary hospital	Diseased chicken	63	16	25.40
Total	_	295	138	46.78

and at most four (*C. perfringens*) colonies from each positive sample were identified in this study.

2.3 | Antimicrobial susceptibility test

The Kirby-Bauer disk diffusion method was applied for the antimicrobial susceptibility test (Xing et al., 2015) in accordance with the guidelines of Clinical and Laboratory Standards Institute (CLSI). Antibiotics were selected based on their use in poultry and human health. These antibiotics include ampicillin (10 μg), penicillin (1 UI), ciprofloxacin (5 μg), tetracycline (30 μg), erythromycin (15 μg), gentamicin (10 μg), sulfamethoxazole (300 μg), bacitracin (10 μg) and lincomycin (20 μg). *C. perfringens* reference strain ATCC13124 was used as a quality control strain for the antimicrobial susceptibility test. Antimicrobial susceptibility tests were carried out on 50 isolates of the retail market, 20 isolates of diseased chickens and 18 isolates of humans. The inhibition zone was measured according to CLSI standards for antimicrobial susceptibility, standards that CLSI does not list refer to instructions from manufacturers (Hangzhou Microbial Reagent Co. Ltd).

2.4 | DNA preparation

Genomic DNA was obtained from *C. perfringens* isolates grown in FTG using the one-step bacterial genome extraction kit (Beijing Norbelai Biotechnology Company). DNA was dissolved in 50 μl double distilled water and stored at −20°C.

2.5 | Toxin gene screening

Toxin genes including *plc*, *cpb*, *etx* and *iap* were detected by using a previously published multiplex polymerase chain reaction (PCR) assay (Yoo et al., 1997) for each isolate, and isolates were also assayed for the presence of *cpb2*, *cpe*, *tpeL* and *netB* genes (Wen et al., 2019). *C. perfringens* reference strains, including *C. perfringens* type A, National Collection of Type Culture (NCTC) 528 (*cpa*); type C, NCTC3180 (*cpb*), NCTC 4989 (*cpb*, *cpb2*); type D, NCTC 8346 (*etx*) and type E, NCTC8084 (*iap*, *cpe*) were used as positive controls of toxin typing.

2.6 | Sequencing of housekeeping genes

The primers of eight housekeeping genes *ddla* (D-Ala-D-Ala ligase), *dut* (dUTP nucleotidohydrolase), *glpk* (glycerol kinase), *gmk* (deoxyguanylate kinase), *plc* (phospholipase C (alpha toxin)), *sod* (superoxide dismutase), *recA* (DNA repair) and *tpiA* (tiosephosphate isomerase) were synthesized by using MLST scheme (Jost et al., 2006). In this study, 39 isolates of *C. perfringens* were selected for MLST analysis, of which 28 isolates from chicken products and environmental samples at different stores of the poultry market, six isolates from suspected NE infected chickens and five isolates from faeces of healthy people in the poultry market were selected. PCR assays were performed in final volumes of 25 μl, containing 10× Buffer (Mgcl2) 2.5 μl, dNTP 2 μl, each primer (10 mmol/L) 0.5 μl, Taq enzyme 0.5 μl, DNA template 2 μl and the remainder was supplemented with double distilled water. Reactions were performed with initial denaturation at 95°C for 5 min, followed by 35 circular arguments at 95 °C for 15 s, 50 °C or 55 °C for 15 s, 72 °C for 40 s, and a final extension at 72 °C for 10 min. Agarose gel electrophoresis [1% agarose, Tris-borate-EDTA (TBE)] was performed to confirm the presence and correct size of the PCR amplicon. Then, the PCR products were submitted to the sequencing company (Beijing Ruiboxing Technology Company) for sample purification, automated nucleotide sequencing in both directions.

2.7 | MLST and phylogenetic analysis

Genetic relationship of 50 strains was analyzed using MLST. These isolates were selected based on their origin (different stores and hosts) and antimicrobial resistance profiles. The nucleotide sequences of 11 reported strains of *C. perfringens* from chicken were downloaded from the National Center for Biotechnology Information (NCBI) database for analysis and comparison (Hibberd et al., 2011). Eight housekeeping genes successfully sequenced by bidirectional sequencing were assembled by DNASTAR software package (http://www.dnastar.com), and ambiguities were resolved during assembling. After which all examined genes were aligned and trimmed to an equal length by BioEdit software (http://bioedit.software.informer.com) according to reference sequence of each allele. After assembling, data of all examined genes (FASTA files) were imported into Bionumerics software (Bionumerics, version 7.6 (3); Applied Maths, Inc.) to create an allele database. After importing, sequences of all 50 isolates were first compared by alleles to obtain the allele numbers and then by alleles and profiles to obtain the sequence types (STs) using Bionumerics. CCs were defined as groups of independent isolates that shared identical alleles at six or more of the eight loci in this study, and each CC was arbitrarily assigned a number. Both STs and CCs were considered to be *C. perfringens* MLST subtypes (Hibberd et al., 2011).

The phylogenetic relationship of all examined strains and the allelic differences among different STs were identified and a minimum spanning tree was drawn by Bionumerics using MST method, sources of strains were indicated by different colours in the minimum spanning tree (Xiu et al., 2020). To better examine ST relatedness at the sequence-level revolution, the optimum inferred phylogenetic tree was generated by the neighbour-joining (NJ) and maximum composite likelihood (MCL) methods based on eight housekeeping genes which concatenated together to form the sequence of 2449 bp, then the concatenated sequence was imported into MEGA7.0 to estimate evolutionary distances, and the topology was validated by bootstrapping (1500 replicates) (Hibberd et al., 2011). To display antibiotic resistance profiles of examined isolates, each evolutionary cluster was attached to the corresponding resistance profile (heat map), which was constructed by an online software (https://evolgenius.info/evolview-v2/).

TABLE 2 Multilocus sequence typing (MLST) typing results of *Clostridium Perfringens* isolates from different sources

Source	Host (n)†	STs (n)†	CCs (n)†
Booth A	Chicken products (7)	ST4 (2), ST20 (1), ST21 (1), ST22 (1), ST23 (1), ST24 (1)	CC2 (3)
Booth B	Chicken products (3)	ST29 (1), ST30 (1), ST31 (1)	CC3 (1)
	Environment (3)	ST32 (1), ST33 (1), ST34 (1)	
Booth C	Chicken products (9)	ST4 (5), ST35 (1), ST36 (1), ST37 (1), ST38 (1)	CC1 (1), CC2 (5)
	Environment (1)	ST39 (1)	
Booth D	Chicken products (5)	ST3 (1), ST25 (1), ST26 (1), ST27 (1), ST28 (1)	CC1 (1), CC3 (1)
Veterinary hospital	Diseased chicken (6)	ST2 (4), ST3 (1), ST5 (1)	CC1 (1), CC2 (5)
Retail market	Human (5)	ST15 (1), ST16 (1), ST17 (1), ST18 (1), ST19 (1)	CC1 (1), CC2 (1)

Abbreviations: CCs, clonal complexes; STs, sequence types.
†The number of strains contained.

TABLE 3 Prevalence (%) of antibiotic resistance in 88 strains of *Clostridium perfringens* isolated from retail market and veterinary hospital in Tai'an

	Number (%) of antibiotic resistance isolates			
Antibiotics	Diseased chicken	Retail market	Humans	Total
Bacitracin	18/20 (90.00)	35/50 (70.00)	18/18 (100.00)	65/88 (73.86)
Sulfamethoxazole	11/20 (55.00)	32/50 (64.00)	5/18 (27.78)	48/88 (54.55)
Lincomycin	12/20 (60.00)	31/50 (62.00)	17/18 (97.40)	60/88 (68.18)
Tetracycline	4/20 (20.00)	13/50 (26.00)	5/18 (27.78)	22/88 (25.00)
Erythromycin	12/20 (60.00)	27/50 (54.00)	16/18 (88.89)	55/88 (62.50)
Ciprofloxacin	0/20 (0.00)	4/50 (8.00)	0/18 (0.00)	3/88 (3.41)
Ampicillin	0/20 (0.00)	0/50 (0.00)	0/18 (0.00)	0/88 (0.00)
Multidrug-resistant rate	12/20 (60.00)	25/50 (50.00)	9/18(50.00)	46/88 (52.27)

2.8 | Data analysis

Sequence analysis, STs and CCs determinations were performed using self-built database for ST designation. Simpson's index of diversity was used to determine the discriminating power of MLST in typing isolates of *C. perfringens* according to the following formula described by Hunter and Gaston (1988).

3 | RESULTS

3.1 | Occurrence of *C. perfringens*

One hundred thirty-eight (46.78%) samples were determined to be positive for *C. perfringens*, and a total of 115 isolates of *C. perfringens* were isolated from 225 samples collected from the retail market, with a positive rate of 51%. Eighteen isolates were isolated from seven samples of human faeces with a positive rate of 100%. A total of 25 isolates were isolated from 63 samples of diseased chickens, with a positive rate of 25%. The positive rates of various samples can be seen in Table 1.

3.2 | Toxin gene screening

The *cpe* gene (type F) was found in one (5.56%, $n = 18$) human isolate, and 99.37% (157/158) of isolates were identified as *C. perfringens* type A, which means that *cpb*, *etx*, *iap* and *netB* genes were not found in any isolates. The *cpb2* gene was found in 30 (18.99%, $n = 158$) isolates in which the *cpb2* gene positive rates of retail chicken isolates, sick chicken isolates and human isolates were 12.17% (14/115), 40% (10/25) and 33.33% (6/18), respectively. And the *tpeL* gene was not found in any isolates.

3.3 | Antibiotic resistance profiles

Antimicrobial susceptibility testing showed that broad antibiotic resistance was observed in strains isolated from retail market and veterinary hospital. The resistance rate of the isolates to each antibiotic was shown in Table 2. Strains which are resistant to three or more classes of antibiotics are defined as multidrug resistant. The proportion of multidrug resistant isolates was 52.27% (46/88) in which the multiple antibiotic resistant rates of diseased chicken, retail chicken products and human isolates were 60%, 50% and 50% (Table 3), respectively.

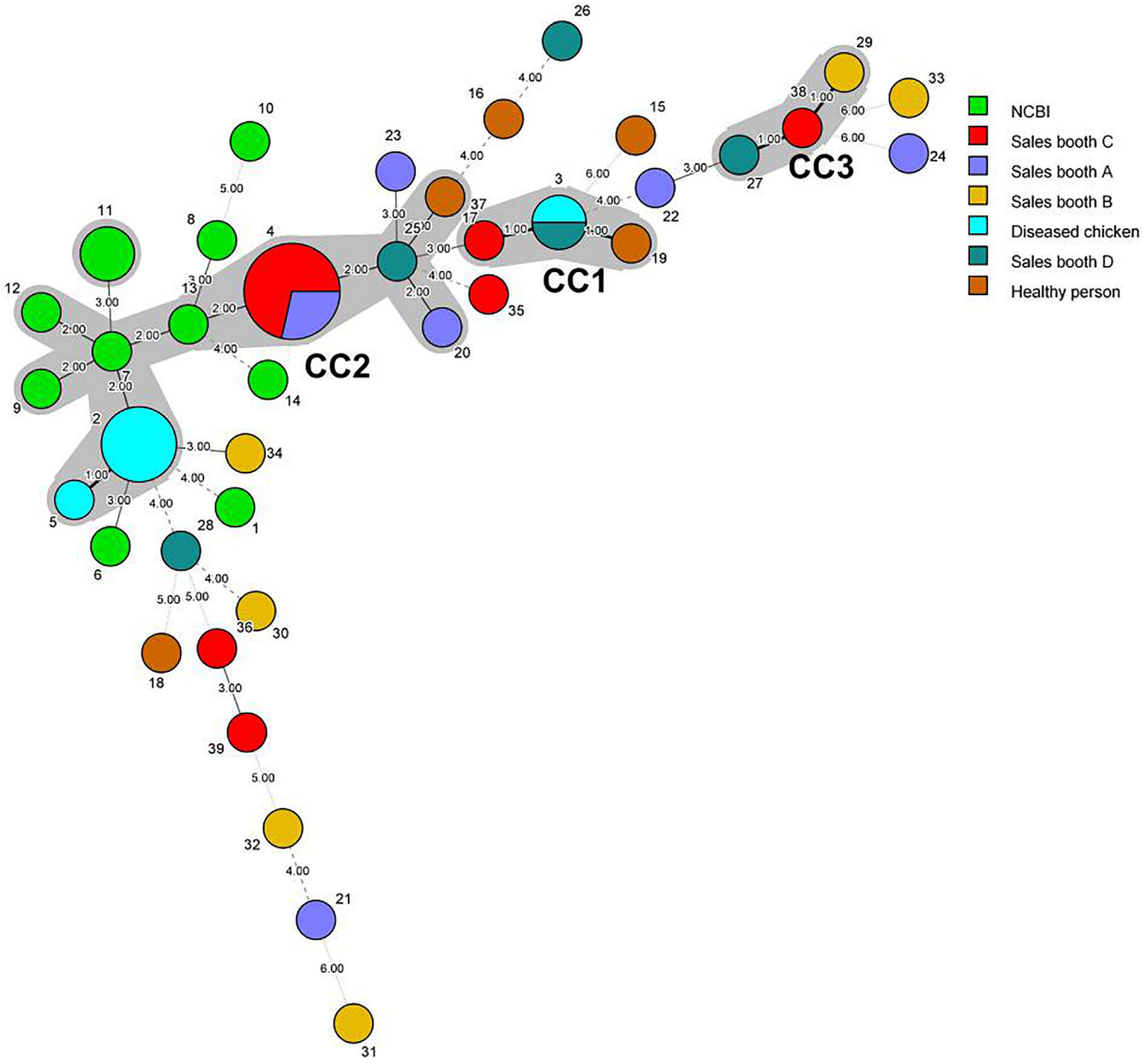

FIGURE 1 The minimum spanning tree constructed by Bionumerics software (Bionumerics, version 7.6 (3)). Notes: The shaded section represents three clonal complexes (CCs). The area of the circle represents the number of strains, different colours represent different sources, and the number on the branch represented the difference of alleles. Fifty strains of *Clostridium perfringens* from different sources were analyzed by MLST -minimal spanning tree

3.4 | MLST analysis

MLST results (Figure 1) showed that 50 analyzed isolates were successfully divided into 39 STs, and the Simpson's index was 0.9616 in which the 33 isolates of the retail market were divided into 27 STs with the Simpson's index of 0.9311. The six isolates of the NE-infected chicken were divided into three STs with the Simpson's index of 0.5000. The five human isolates were divided into five STs. The 11 isolates of chicken source in the NCBI database were downloaded to form 10 STs. Among them, ST-4 was the most common in the minimal spanning tree (Figure 1) and contained seven isolates, accounting for 14% (7/50) of the total analysis isolates, of which five isolates were from booth C in the retail market and two isolates were from liver and heart samples in booth A. ST-2 contains four isolates, accounting for 8% (4/50) of the total analyzed isolates, which were isolated from the liver, jejunum, cecum and ileum of the same diseased chicken. ST-3 contains two isolates from diseased chicken and retail chicken products.

In total, three clonal complexes subtypes (CC-1, CC-2 and CC-3) were identified, containing 52% (26/50) of the examined isolates from different sources. Twenty-three STs were identified as singletons with no observed CC associations. CC-2, the largest CC, contained diseased chickens isolates ($n = 5$), retail chicken products isolates ($n = 9$), human

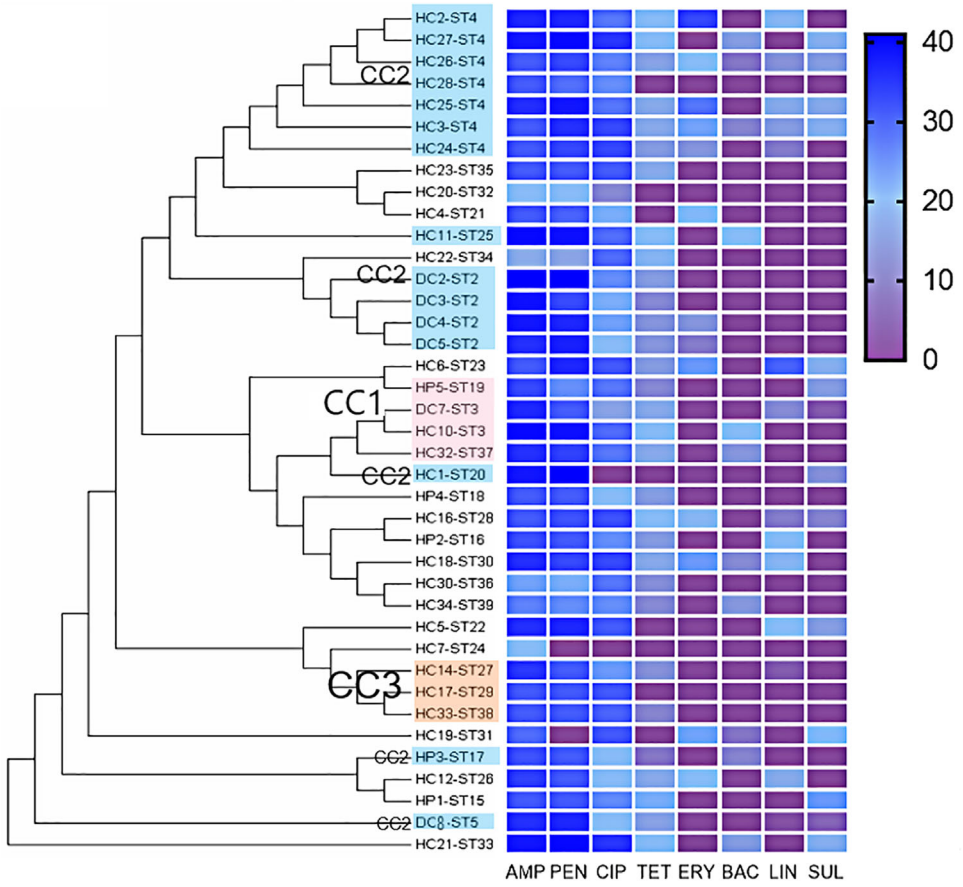

FIGURE 2 Phylogenetic trees of sequence types (STs) of 39 *C. perfringens* for MLST analysis and heat map (antibiotic resistance profiles). The heat map was constructed by an online software (https://www.graphpad.com/register/confirmation/); 0 to 40 stands for inhibition zone. AMP, Ampicillin; PEN, penicillin; CIP, ciprofloxacin; TET, tetracycline; ERY, erythromycin; SUL, sulfamethoxazole; BAC, bacitracin; LIN, lincomycin

isolate (n = 1) and chicken source isolates (n = 4) of American and 10 STs (ST-2, ST-4, ST-5, ST-7, ST-9, ST-12, ST-13, ST-17, ST-20, ST-25), with a total of 19 isolates which accounted for 38.00% (19/50) of all examined isolates. Four isolates of CC-1 (ST-3, ST-19, ST-37) were isolated from retail chicken products, humans and diseased chickens. Three isolates of CC-3 (ST-27, ST-29, ST-38) were isolated from chicken livers of booth A, gizzards of booth B and carcasses of booth C in retail market, respectively. The distribution information of STs and CCs is shown in Table 2.

3.5 | Phylogenetic analysis

Viewing the whole phylogenetic tree (Figure 2), the dendrogram was found to be dominated by different clusters, which contained all CCs, as well as a substantial number of closely related STs. The result of phylogenetic tree is basically consistent with the minimum spanning tree, but not completely. Isolates in the same CC were usually clustered together, isolates in CC-1 and CC-3 were clustered together, but there were exceptions; for example, 15 isolates in CC-2 (the blue part) were assigned to different branches of the tree, and STs of containing only one isolate scattered (Figure 2). Moreover, we also found that the isolates of the chicken and the human source were assigned to the same clusters in the phylogenetic tree (Figure 2), for example, ST-15 and ST-26, ST-16 and ST-28, ST-19 and ST-23.

The relationship between the corresponding resistance phenotype, STs and phylogenetic tree is shown in Figure 2. Antibiotic susceptibility testing showed that the antibiotic resistance of ST4-strains was not exactly the same (Figure 2), similar phenomena also exist in other STs or CCs, such as ST-2, CC-1 and CC-3.

4 | DISCUSSION

Since the 1940s, food poisoning caused by the contamination of *C. perfringens* has been reported (Yibar et al., 2018). The contamination rate of *C. perfringens* in retail meat products from different regions is different; for example, the contamination rate of *C. perfringens* in chicken products of this study is 51.11%, which is higher than the results of retail chicken meat samples in central China (15.1%) (Zhang et al., 2018), Korea (19.0%) (Jang et al., 2020) and the United States (30.00%) (Lin et al., 2003), and lower than the positive rate of Canadian retail

chickens (67%) (Nowell et al., 2010). Food poisoning caused by *C. perfringens* is often related to contaminated meat products that are subjected to inadequate temperature control during cooking, cooling and storage (Hu et al., 2018). Many meat-based food products were cooked to temperatures sufficient to inactivate vegetative cells of *C. perfringens*, but spores of this bacterium can survive, germinate, and grow in these products if sufficient time, temperature, and other variables exist (Taormina & Dorsa, 2004). Therefore, the contamination of *C. perfringens* in chicken products of the retail market may pose a threat to public health and should be cared.

Toxinotyping results showed that 99.37% of the isolates were identified as *C. perfringens* type A, only one isolate was of type F, and it is consistent with the results of other reports in China and other countries (Fan et al., 2016; Zhang et al., 2018;). It shows that the toxinotypes of isolates from chickens mostly are type A in Tai'an area. A recent study in France revealed that 84% of *C. perfringens* from diarrhoea patients were type A (Mahamat et al., 2019). *C. perfringens* isolates involved in many cases of food poisoning are type A. Compared with the *netB* gene, the *cpb2* toxin gene is common in isolates. In this study, the *cpb2* positive rates of isolates from different sources were different, among which the positive rates were 40% and 12.17% in the isolates of diseased chickens and retail chicken products, respectively.

The use of antibiotic feed additives has led to the increase of antimicrobial resistance of some intestinal flora, and some zoonotic pathogens have developed multiresistance (Osman & Elhariri, 2013). A previous report showed that 30 tested strains of *C. perfringens* were completely resistant to five antibiotics and partially resistant to six antibiotics (Park et al., 2015). In this study, the antimicrobial susceptibility results showed that the isolates from chicken in Tai'an had broad spectrum drug resistance (Table 2). The multiresistance rate of the isolates in this study was 52.27%, and 46.59% of isolates were resistant to at least four classes of antibiotics, indicating spread of drug resistance and public health risks.

The literature data varies in terms of the average numbers of alleles, STs, CCs, and isolates evaluated. Jost et al. (2006) analyzed 132 *C. perfringens* strains of human and animal origin and found an average of 24.4 alleles, 80 STs and three CCs. Hibberd et al. (2011) evaluated 139 isolates and found average of 12.2 alleles, 41 STs and six CCs. In this study, 39 *C. perfringens* strains found an average of 12.13 alleles, 29 STs and three CCs (Figure 1). Among all STs, the most popular is ST-4, which contains 17.95% (7/39) of the analysis isolates [booth C ($n = 5$), booth A ($n = 2$)]. It contains isolates from two booths, which may also be due to cross-contamination between different booths. The most prolific CC (CC-2), accounting for 38% of all isolates, contained isolates mainly from diseased chicken, retail chicken products, human and diseased chickens from other countries. Figure 2 shows that the antibiotic resistance of ST4-strains was not exactly the same, indicating that the prevalence of ST-4 might not be directly related to the drug resistance of the strain, similar phenomena also exist in other STs or CCs, such as ST-2, CC-1, CC-2 and CC-3.

The human isolates are closely related to some isolates from chicken source in the CC-1 and CC-2, and the phylogenetic tree (Figure 2) also showed that some isolates of retail chicken products were closely related to human isolates (ST-15 and ST-26, ST-16 and ST-28 etc), indicating that some isolates of *C. perfringens* from chicken were related to the isolates from human in certain stratification and may be transmitted to humans. In the future, more methods can be used to further verify this conclusion.

In this study, the isolates from suspected chickens with NE were classified into three STs by MLST. The Simpson's index was 0.5000, while the isolates in the retail market were divided into 27 STs, and the Simpson's index was 0.9311. This further indicates that despite different genotyping methods, strains isolated from chickens infected with NE had a lower genetic diversity than those isolated from healthy chickens (Cakmak et al., 2006). The isolates from different parts of one NE infected chicken were of the same ST (ST-2) (Figure 1). It indicated that the isolates diffused in the suspected chickens with NE were the dominant strains, which were most likely the main pathogen of the disease. It is consistent with other countries research reports (Lacey et al., 2016; Nauerby et al., 2003) and can provide reference for prevention and control of *C. perfringens* related diseases.

5 | CONCLUSION

The result showed that the *C. perfringens* contamination rate of retail chicken products from Tai'an retail market was relatively high, and the isolates showed broad spectrum antibiotic resistance. Although 99.37% of isolates were type A, the Simpson's index of STs showed considerable genetic diversity. MLST results and phylogenetic analysis showed that a portion of the isolates from human and chicken were assigned to the same clusters in the phylogenetic tree or found to be in the same CCs, indicating some chicken isolates and the human isolates are related in certain stratification. The high resistance of *C. perfringens* and the relationship between strains from humans and chickens indicate potential public health risks.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICS STATEMENT

The authors confirm that the ethical policies of the journal, this study does not involve live animals.

AUTHOR CONTRIBUTIONS

Investigation, methodology and writing-original draft: Wenping Xu. *Data curation, formal analysis and software*: Huining Zhang. *Investigation*: Zixin Hu. *Methodology*: Zengmin Miao. *Resources*: Yuanrui Zhang. *Methodology, project administration, supervision and validation*: Hairong Wang.

DATA AVAILABILITY STATEMENT

All data used to support the findings of this study are available from the corresponding author upon request.

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