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# Multilocus sequence typing analyses of *Clostridium perfringens* type A strains harboring *tpeL* and *netB* genes

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ABSTRACT

*Clostridium perfringens* is an anaerobic bacterium ubiquitous in various environments, especially in soil and the gastrointestinal tract of healthy humans and animals. In this study, multilocus sequence typing protocol was used to investigate genotypic relationships among 40 *C. perfringens* strains isolated from humans and broiler chicken with necrotic enteritis [NE]. The results indicated a few clonal populations, mainly observed in human strains, with 32.5% of all strains associated with one of three clonal complexes and 30 sequences types. The CC-1 cluster showed an interesting and unexpected result because it contained seven strains [six from animals and one of human origin]. Detection assays for toxin genes *tpeL* and *netB* were also performed. The *netB* gene was only observed in 7.5% of the strains from healthy human. The toxin gene *tpeL* was detected in 22.5% of the *C. perfringens* strains isolated from three individuals and in six broilers with NE. Our study describes the role of some *C. perfringens* strains of human origin acting as reservoirs of virulence genes and sources of infection. In addition, the strains of human and animal origin were found to be genetically distinct but phylogenetically close, and the human strains showed more diversity than the animal strains.



## 1. Introduction

*Clostridium perfringens* is an anaerobic, endospore-forming, gram-positive rod ubiquitous in various environments, especially in soils and the gastrointestinal tract of healthy humans and animals [1]. This species produces at least 17 different toxin and according to the production of major toxins alpha, beta, epsilon and iota it has been classified into five toxinotypes (A, B, C, D and E) [2]. *C. perfringens* type A causes gangrene and food poisoning in humans, diarrhea in foals and pigs, and necrotic enteritis (NE) in chickens [3,4].

The α-toxin produced by *C. perfringens* type A is phospholipase C which hydrolyzes phospholipids causing the release of inflammatory mediators and acute cell death [5]. Strains of *C. perfringens* produce low, intermediate or high levels of α-toxin which depends on the growth conditions, such as the medium type and pH [6].

Large clostridial cytotoxins produced by *Clostridium* spp. are important virulence factors in pathogenesis of myonecrosis and intestinal diseases. They include *Clostridium difficile* toxins A (TcdA) and B (TcdB), *C. sordellii* lethal toxin (TcsL) and hemorrhagic toxin (TcsH), *C. novyi* alpha toxin (TcnA), and more recently discovered *C. perfringens* large cytotoxin (TpeL) [7,8].

The TpeL toxin was initially detected in culture supernatants of *C. perfringens* type C isolated from swine. It is lethal to mice and cytotoxic to Vero cells, resulting in enlarge, rounded cells forming aggregates, which eventually detach from the plate [8,9]. This toxin has been detected in *C. perfringens* Type A isolated from avian hosts; and it may potentiate or contribute to the pathogenesis of NE [2].

The necrotic enteritis toxin B like (NetB) of *C. perfringens* type A was first discovered in a chicken with NE from Australia [10]. These toxins form pores, which disrupt the phospholipid membrane bilayer of both human and animal cells, causing ion influx that may lead to osmotic cell lyses [11,12]. The TpeL and NetB toxins have not yet been reported in humans.

Certain molecular typing techniques are used to verify bacterial diversity of *C. perfringens*. The methods include pulsed-field gel electrophoresis [13], multilocus variable number of tandem repeats analysis [14], and multilocus sequence typing (MLST) [15]. Such phylogenetic studies have elucidated distinct lineages of

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*C. perfringens* strains associated with specific diseases [16,17].

MLST is an excellent method for bacterial characterization based on sequencing of several housekeeping genes [18]. Genes are amplified by polymerase chain reaction [PCR] and sequenced, and the differences in DNA sequences define the allelic profile for each isolate, known as the sequence type (ST). Genetic events such as point mutations or recombinations result in different alleles and subsequently, in different allele combinations [19]. Most bacterial species have sufficient variation within housekeeping genes to provide many alleles per locus, allowing multiple STs to be identified using a limited number of loci [20]. This global approach would be of great value for the study of the diseases-causing *C. perfringens*.

In this study, the phylogenetic relationships and niche partitioning were verified in a diverse collection of *C. perfringens* isolated from humans and broiler chickens using the MLST protocol described by Jost et al. [15].

## 2. Materials and methods

### 2.1. Strains origins

Forty intestinal strains of *C. perfringens* isolated from children and broilers were used. Twenty strains were obtained from 20 healthy asymptomatic children (without diarrhea, from 3 to 11 years old) living in Sao Paulo city, Brazil. The other 20 strains of *C. perfringens* were obtained from eight broilers with NE, which belonged to eight farms in five different Brazilian states (2 in Ceara - CE, Parana - PR, 1 in Rio Grande do Sul - RS, 1 in Santa Catarina - SC, and 1 in Sao Paulo – SP). Clinical signs observed in sick chickens represented marked depression, decreased appetite, ruffled feathers, enteritis, and diarrhea. Briefly, approximately 2 cm of intestine showing severe injuries were transferred to broth meat (Difco Laboratories, USA) and incubated at 37 °C for 48 h under anaerobic conditions (90% $N_2$, 10% $CO_2$). Aliquots (0.1 mL) were streaked onto trypticase soy agar (Difco Laboratories, USA) enriched with 5% defibrinated horse blood agar. Bacterial identification was performed by colonial and cell morphology, biochemical tests, and other characteristics (short gram-positive bacilli, dual haemolysis, and gelatinase and lectinase producing).

### 2.2. DNA preparation

Genomic DNA was obtained from *C. perfringens* strains grown in brain heart infusion broth using the QIAmp DNA Mini kit (Qiagen Inc., Valencia, CA, USA). DNA was eluted with 50 μL of ultrapure water and stored at −30 °C until use.

### 2.3. Toxinotyping

All *C. perfringens* strains were toxinotyped for the presence of the *plc, cpb, etx, iap, cpb2, cpe, tpeL,* and *netB* toxin genes using PCR assays [10,21]. Two reference strains *C. perfringens* ATCC 13124 and strain 13 harboring genes for toxin types, were included as positive controls in the toxinotyping scheme.

### 2.4. Sequencing of housekeeping genes

The presence of eight housekeeping genes (*ddlA, dut, glpK, gmK, plc, recA, sod,* and *tpi*) was determined by amplification of gene segments in accordance with Jost et al. [15]. PCR assays were performed in final volumes of 25 μL, containing: 1 X PCR buffer, 50 mM $MgCl_2$, 0.2 mM dNTP mix, 0.4 mM each primer, 0.5 U of high fidelity platinum *Taq* polymerase [Invitrogen, Sao Paulo, SP, Brazil] and 1 ng of DNA. Reactions were performed with initial denaturation at 94 °C for 2 min, followed by 35 cycles at 94 °C for 60 s, 50 °C for 60 s, and 72 °C for 60 s, and a final extension at 72 °C for 5 min. PCR products were analyzed by 1% agarose gel electrophoresis to confirm the presence and correct size of the PCR amplicon. Then, the PCR products were purified (Illustra GFX, GE Healthcare Life Sciences, Sao Paulo, SP, Brazil) and sequenced in both directions.

### 2.5. Multilocus sequence typing and phylogenetic analysis

Bidirectional sequencing reads for segments of each of the eight housekeeping genes were aligned and trimmed to a uniform length (BioNumerics, version 7.0; Applied Maths, Inc., Austin, TX, USA). Ambiguities were resolved, when appropriate, using the CAP3 sequence assembly program (http://pbil.univ-lyon1.fr/cap3.php). Nucleotide sequences of *C. perfringens* ATCC 13124 (accession number NC_008261.1) and strain 13 (accession number NC_003366.1), were obtained from the NCBI repository and included in subsequent analyses for comparison. After imported, aligned, and trimmed, sequences from all 40 isolates were first compared by allele and then by allelic profile using the BioNumerics, at this point, the data were used to assign each isolate to an ST. Besides, the START2 software package (available at http://pubmlst.org/software/analysis/start2/) was used to assemble and analyze concatenated sequence information for each ST [22]. Based on a representative of each ST, the Maynard-Smith index of association (*IA*) was calculated to evaluate the recombination, and the ratio of synonymous to non-synonymous mutations (*dN*/*dS*) was computed by the Nei-Gojobori method as a measure of selection [23]. The eBURST tool for MLST analysis [available at http://eburst.mlst.net/default.asp] was used to identify clonal complexes (CCs) [defined by seven or more identical loci], singleton STs, and possible ancestral genotypes [24]. Both STs and CCs were considered to be *C. perfringens* MLST subtypes. Concatenated sequence data for a representative of each distinct ST were imported into the MEGA 6.0 software package [http://www.megasoftware.net/] in order to examine the strain and ST relatedness at the sequence-level resolution. After complete deletion of alignment gaps, a total of 2449 positions were used in each concatenated sequence as a data set for phylogeny calculations. An evolutionary phylogeny was constructed in MEGA 6 using the neighbor-joining method and maximum composite likelihood (MCL) to estimate evolutionary distances, and the topology was validated by bootstrapping (1500 replicates) [25–27]. To establish evolutionary relevance, *C. perfringens* strain 13 (ST-32) was used as the tree root. The optimum tree generated was condensed where the bootstrap support for the clustering of taxa was <50% of the replicates [28]. Additionally, *C. perfringens* sequences from broilers and turkeys previously analyzed by Chalmers et al. [20] (ST-1, ST-10, and ST-18) and Hibberd et al. [17] (ST-2, ST-8, ST-14, and ST-31) were also included.

### 2.6. Nucleotide sequence accession numbers

The locus nucleotide sequences obtained in this study were submitted to GenBank under accession numbers KT020415 to KT020454 (*ddlA*), KT020455 to KT020494 (*dut*), KT020495 to KT020534 (*glpK*), KT020535 to KT020574 (*gmK*), KT020575 to KT020614 (*plc*), KT020615 to KT020654 (*recA*), KT020655 to KT020694 (*sod*), and KT020695 to KT020734 (*tpi*).

## 3. Results

### 3.1. Toxinotyping

All 40 strains were toxinotyped as type A (Table 1). The *tpeL* toxin gene was detected in three (15%) and six (30%) *C. perfringens* strains from children and broilers, respectively. The *netB* gene was

identified in three (15%) strains isolated from children. The presence of both *tpeL* and *netB* toxin genes was observed in one human strain (7H). The sequences from all strains showed 99% similarity to those of the *tpeL* gene (AB262081.1) and *netB* gene (JQ655731.1) of *C. perfringens*.

### 3.2. Analysis of alleles

The average number of alleles for all loci analyzed was 10.2. The *glpK* gene with 15 alleles was the most polymorphic, and the *gmk* gene with four alleles was the least polymorphic (Table 2). The polymorphism index was determined by the percentage of polymorphic sites per allele. The maximum percentage of polymorphism was found for the *plc* gene (7.3% of sites), and the minimum percentage of polymorphism was found for the *gmk* gene (1.9% of sites) (Table 2). All allelic sequences examined were coding sequences; thus, the ratio of nonsynonymous to synonymous mutations was used as a measure of selective pressure on each allele. Based on this analysis, all genes possessed the *dN*/*dS* ratio of 1, indicating purifying selection (Table 2). The *sod* and *tpi* genes presented *dN*/*dS* equal to 0; besides, all mutations observed in these genes were identified as synonymous. A significant linkage disequilibrium between the genes examined was demonstrated by a Maynard-Smith $I_A$ = value of 1.3424 ($P = 0.000$).

### 3.3. Sequence types and eBURST analysis

Among the 40 isolates typed by MLST, 30 unique STs were identified (Fig. 1). *C. perfringens* ATCC 13124 and strain 13 segregated into individual STs containing a single isolate each, thus bringing the total number of STs to 32. In the human strains, multiples STs were identified, resulting in 20 STs containing one strain each. No ST contained strains of both human and animal origin. Among the animal isolates, 2/10 STs (20%) contained strains from different farms. Thus, ST-21 contained a strain from farm 1 (PR) and another one from farm 4 (CE); ST-23 contained strains from farms 1 and 2 (PR), as well as from farm 8 (SC). Among the STs containing the *tpeL* and/or *netB* genes, 2/30 (6.6%) contained only the *netB* gene (human strains), 6/30 (20%) harbored only the *tpeL* gene (two human and six animal isolates), and 1/30 (3.33%)

**Table 1**
Bacterial origin, disease association, sequence type and toxin genes.

| Strains | Brazilian States | Origin | Disease association | Sequence types | Toxin genes | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | *plc* | *cpb* | *etx* | *iap* | *cpb2* | *cpe* | *tpeL* | *netB* |
| 1H | SP | Human | Healthy | 1 | + | − | − | − | − | − | − | − |
| 2H | SP | Human | Healthy | 2 | + | − | − | − | − | − | − | − |
| 3H | SP | Human | Healthy | 3 | + | − | − | − | − | − | − | − |
| 4H | SP | Human | Healthy | 4 | + | − | − | − | − | − | − | − |
| 5H | SP | Human | Healthy | 5 | + | − | − | − | − | − | + | − |
| 6H | SP | Human | Healthy | 6 | + | − | − | − | − | − | − | + |
| 7H | SP | Human | Healthy | 7 | + | − | − | − | − | − | + | + |
| 8H | SP | Human | Healthy | 8 | + | − | − | − | − | − | − | − |
| 9H | SP | Human | Healthy | 9 | + | − | − | − | − | − | − | − |
| 10H | SP | Human | Healthy | 10 | + | − | − | − | − | − | − | − |
| 11H | SP | Human | Healthy | 11 | + | − | − | − | − | − | − | + |
| 12H | SP | Human | Healthy | 12 | + | − | − | − | − | − | − | − |
| 13H | SP | Human | Healthy | 13 | + | − | − | − | − | − | − | − |
| 14H | SP | Human | Healthy | 14 | + | − | − | − | − | − | − | − |
| 15H | SP | Human | Healthy | 15 | + | − | − | − | − | − | − | − |
| 16H | SP | Human | Healthy | 16 | + | − | − | − | − | − | − | − |
| 17H | SP | Human | Healthy | 17 | + | − | − | − | − | − | − | − |
| 18H | SP | Human | Healthy | 18 | + | − | − | − | − | − | − | − |
| 19H | SP | Human | Healthy | 19 | + | − | − | − | − | − | − | − |
| 20H | SP | Human | Healthy | 20 | + | − | − | − | − | − | + | − |
| 1A | PR | Broiler [farm 1] | NE | 21 | + | − | − | − | − | − | − | − |
| 2A | PR | Broiler [farm 1] | NE | 21 | + | − | − | − | − | − | − | − |
| 3A | PR | Broiler [farm 1] | NE | 22 | + | − | − | − | − | − | − | − |
| 4A | PR | Broiler [farm 1] | NE | 23 | + | − | − | − | − | − | − | − |
| 5A | PR | Broiler [farm 2] | NE | 23 | + | − | − | − | − | − | − | − |
| 6A | PR | Broiler [farm 3] | NE | 24 | + | − | − | − | − | − | − | − |
| 7A | PR | Broiler [farm 3] | NE | 25 | + | − | − | − | − | − | − | − |
| 8A | PR | Broiler [farm 3] | NE | 24 | + | − | − | − | − | − | + | − |
| 9A | PR | Broiler [farm 3] | NE | 24 | + | − | − | − | − | − | + | − |
| 10A | CE | Broiler [farm 4] | NE | 21 | + | − | − | − | − | − | + | − |
| 11A | SP | Broiler [farm 5] | NE | 26 | + | − | − | − | − | − | − | − |
| 12A | SP | Broiler [farm 6] | NE | 27 | + | − | − | − | − | − | − | − |
| 13A | SP | Broiler [farm 6] | NE | 28 | + | − | − | − | − | − | + | − |
| 14A | SP | Broiler [farm 6] | NE | 27 | + | − | − | − | − | − | + | − |
| 15A | SP | Broiler [farm 6] | NE | 27 | + | − | − | − | − | − | + | − |
| 16A | RS | Broiler [farm 7] | NE | 29 | + | − | − | − | − | − | − | − |
| 17A | SC | Broiler [farm 8] | NE | 23 | + | − | − | − | − | − | − | − |
| 18A | SC | Broiler [farm 8] | NE | 23 | + | − | − | − | − | − | − | − |
| 19A | SC | Broiler [farm 8] | NE | 30 | + | − | − | − | − | − | − | − |
| 20A | SC | Broiler [farm 8] | NE | 23 | + | − | − | − | − | − | − | − |
| ATCC 13124[a] | − | Human | G | 31 | + | − | − | − | − | − | − | − |
| Strain 13[a] | − | Soil | − | 32 | + | − | − | − | + | − | − | − |

H: human; A: animal; NE: necrotic enteritis; G: gas gangrene.
+: Presence of the gene.
-: Absence of the gene.
[a] *Clostridium perfringens* reference strains.

**Table 2**
Characteristics of the housekeeping genes evaluated and the analyzed alleles.

| Genes (Function) | Sequence (bp)[a] | N° of alleles | (%) of alleles[b] | Polymorphic sites[c] N° (%) | *dN/dS*[d] |
|---|---|---|---|---|---|
| *ddlA* (D-Alanine-D-Alanine ligase) | 265 | 13 | 32.5 | 18 (6.8) | 0.0807 |
| *dut* (deoxyuridine triphosphatase) | 259 | 9 | 22.5 | 10 (3.9) | 0.0811 |
| *glpK* (glycerol kinase) | 446 | 15 | 37.5 | 17 (3.8) | 0.0741 |
| *gmK* (deoxyguanylate kinase) | 321 | 4 | 10 | 6 (1.9) | 0.0502 |
| *plc* (phospholipase C – alpha toxin) | 327 | 14 | 35 | 24 (7.3) | 0.1149 |
| *recA* (recombinase) | 298 | 8 | 20 | 14 (4.7) | 0.0180 |
| *sod* (superoxide dismutase) | 265 | 13 | 32.5 | 16 (6.0) | 0.0000 |
| *tpi* (triose phosphate isomeras) | 268 | 6 | 15 | 7 (2.61) | 0.0000 |

[a] Length of aligned sequences.
[b] Percentage: n° of alleles/strains analyzed (n = 40).
[c] Percentage: n° of polymorphic sites/size of alleles (bp).
[d] Calculated in the START2 by method of Nei-Gojobori.

contained both *tpeL* and *netB* genes (human strain). The BURST (eBURST implementation) analysis defined a CC as comprising isolates for which seven of eight alleles were identical. Overall, three CC subtypes containing 13/40 total strains, were identified; the largest (CC-1) comprised a total of seven strains. Twenty-four STs were identified as singletons, with no CC associations. CC-1 grouped human and animal strains (ST-4, ST-23, and ST-30); CC-2 contained only animal strains (ST-24 and ST-25); and CC-3 contained two single strains (ST-9 and ST-17) of human origin. All other STs were clustered singly by the eBURST analysis. Based on the ratios of single- and multiple-locus variants, eBURST analysis is able to identify probable founding or ancestral genotypes for CCs with more than two members. eBURST identified ST-23 as the probable ancestral genotype for CC-1, but no ancestral genotypes were predicted for CC-2 and CC-3.

### 3.4. Phylogenetic analysis

A phylogenetic tree was generated by the neighbor-joining and MCL methods. The CCs were generally, but not exclusively, identified by ST profile-based eBURST analysis, clustered together in the tree (Fig. 2) and were often interspersed with singleton STs. Generally, STs containing only one strain were scattered throughout the phylogenetic tree, moreover two closely related clusters containing STs from human and animal strains were observed. The dendrogram was found to be dominated by four large clusters, which contained CC-1, CC-2, and CC-3, as well as a substantial number of closely related STs. ST-1 was more distant from the other STs, forming a unique clade. *C. perfringens* ATCC 13124 (ST-31) had an ST most closely related to ST-2 [human strain] while *C. perfringens* strain 13 (ST-32) was used as the root of the tree. Furthermore, STs kindly donated by Hibberd et al. [17] and Chalmers et al. [20] did not cluster with the animal or human strains used in this study or between them.

## 4. Discussion

In this study an epidemiologic analysis *C. perfringens* was performed using the MLST scheme previously published by Jost et al. [15]. Based on the allelic polymorphism of the housekeeping genes examined here, considerable genetic diversity exists in our collection of *Clostridium perfringens* strains. The MLST scheme that formed the methodological basis for this work enabled highly specific comparisons of *C. perfringens* populations isolated from humans and animals.

The literature data varies in terms of the average numbers of alleles, STs, CCs, and isolates evaluated. Jost et al. [15] analyzed 132 *C. perfringens* strains of human and animal origin and found an average of 24.4 alleles, 80 STs and three CCs. Chalmers et al. [20] and Hibberd et al. [17] evaluated 61 and 139 isolates, respectively, and found average of 5.9 and 12.2 alleles, 22 and 41 STs, and both six CCs each. In our study, which used 40 strains isolated from children and chickens, we found an average of 10.25 alleles, 30 STs, and three CCs; whereas ST-21, ST-23, ST-24, and ST-27 were more frequents.

Although considerable polymorphism existed in the loci analyzed, the characteristics of the sequence data measured in this study also suggested the existence of a few clonal populations, mainly observed in the human strains. A significant linkage disequilibrium was observed between all eight loci ($IA = 1.3424$), indicating, in accordance with Smith et al. [29], low recombination rates in the genomes of the *C. perfringens* isolates examined. This hypothesis is substantiated by the observation that 13 of the 40 isolates [32.5%] partitioned into three clonal complexes, stringently defined as groups of isolates sharing seven of the eight loci examined, which was supported by the eBURST analysis; moreover, two abundant sequence types, ST-24 and ST-23, contained three and five isolates from animals, respectively.

CC-1 showed an interesting and unexpected result because this cluster harbored seven strains (six from animals and one of human origin). Besides, one clone (ST-23) belonged to farms from two different Brazilian states (two farms in PR and another in SC), whereas two strains grouped with different clusters, but one of them was isolated from a healthy child living in SP. Although, our results show that these strains are genotypically different and belong to different intestinal ecosystems (healthy humans and chickens with NE), these strains shared seven out of the eight evaluated alleles. This fact indicates that ST-23 is a strong candidate for a possible common ancestor of these strains.

Toxinotyping of *C. perfringens*, including evaluation of the *tpeL* and *netB* genes, is an important complement to the phylogenetic characterization in the population evaluated. All 40 *C. perfringens* strains evaluated here and isolated from humans and animals belonged to type A.

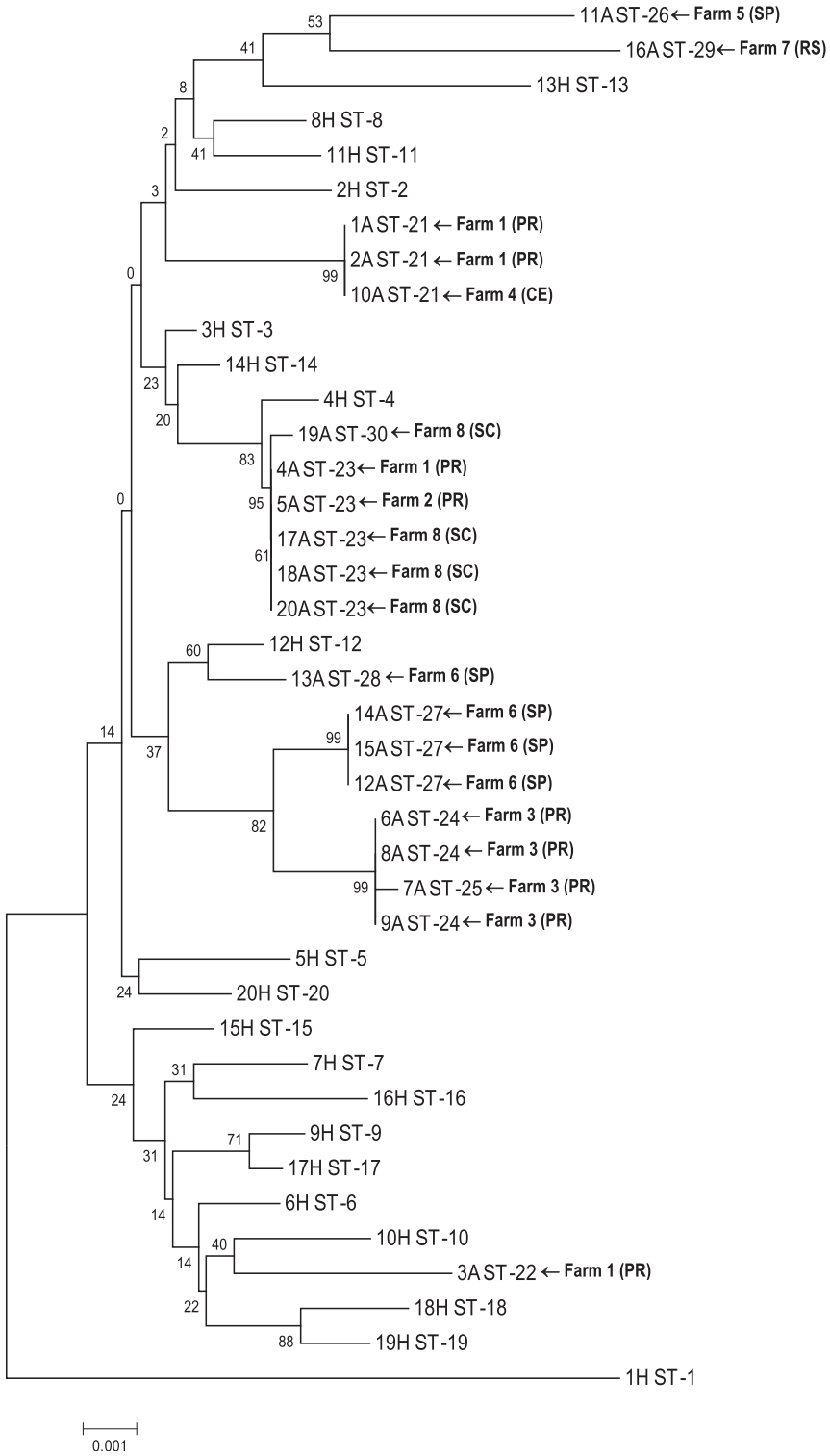


**Fig. 1.** Dendrogram of sequence type from 40 *C. perfringens* from human (H) and animal (A). The dendrogram was generated using the Neighbor-Joining and maximum composite likelihood (MCL) method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1500 replicates).

Detection of virulence genes in the genome has an epidemiological relevance since these genes are largely conserved and stably inherited. The virulence factors produced by *C. perfringens* and associated with a specific disease can be either lost or acquired, and this is an environment dependent process and association with other bacteria [17,30,31].

The *tpeL* gene was detected in 22.5% of the *C. perfringens* isolated from three humans and six broilers with NE. Since *tpeL* was detected in animal strains, it is reasonable to speculate that its presence contributes to the pathogenesis of NE in chickens [2]. However, the presence of this gene in human strains can play a role in the intestinal microbiota of healthy individuals, and thus further

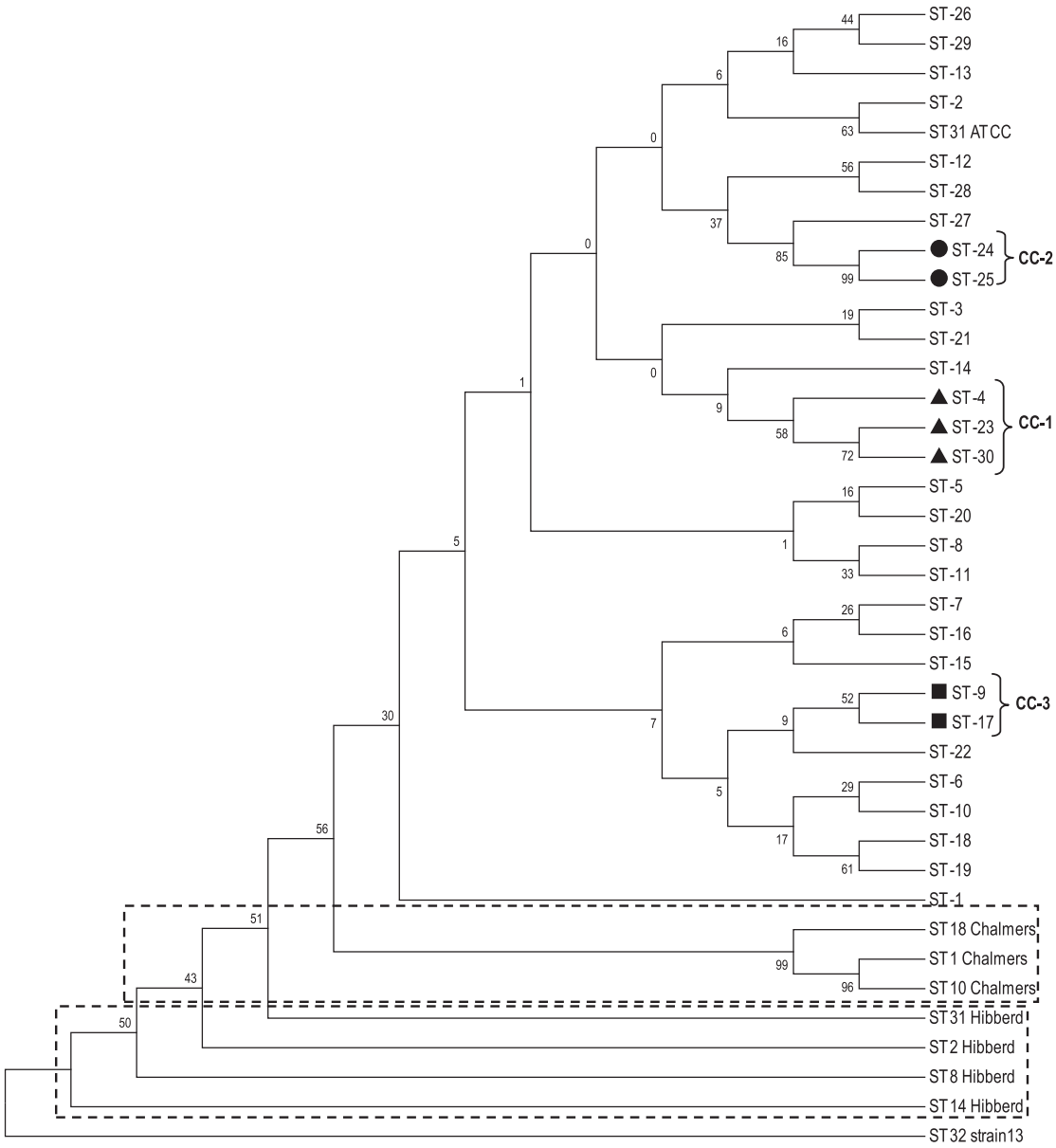


**Fig. 2.** Phylogenetic tree of the *C. perfringens* strains. The tree was generated by Neighbor-Joining and maximum composite likelihood method and was condensed where bootstrap support was <50% replicates. STs from the study of Chalmers et al. [20] and Hibberd et al. [17] are labeled. Clonal complexes: CC-1 (▲), CC-2 (●) and CC3 (■).

studies are required to assess its contribution to the virulence of *C. perfringens* strains.

Similarly, the *netB* gene was observed only in 7.5% of the strains from healthy human. Previous studies [20,32,33], have shown the presence of distinct clones within *C. perfringens* populations able to cause NE outbreaks, which are usually affected by one of these clones. Although *netB* is highly correlated with NE-associated isolates [12,34]; the absence of this gene in our NE-associated strains suggests that other virulence genes or microorganisms not detected here, can play an important role in NE appearance, which needs more studies.

Smyth [35] has concluded that *netB*-positive *C. perfringens* are necessary to produce NE, and *netB*-negative isolates should not be involved in NE cases, since that strains are found in coccidiosis infections produced by *Eimeria necatrix*, and it can induce to a misdiagnosis. On the other hand, studies have shown that *C. perfringens* isolated from healthy avian are not enough to induce the disease; and *netB*-negative strains isolated from diseased chickens can lost this plasmid gene during isolation and culturing, and it cause confusion to a suitable diagnosis [36,37].

Our study describes the role of some humans that act as reservoirs of virulence genes and possible sources of infection with *Clostridium perfringens.* The presence of *C. perfringens* harboring *tpeL* and *netB* genes in our healthy human subjects supports this suggestion. In several countries, the frequency of *tpeL* and *netB* in humans has not been reported. Even though Deguchi et al. [38] described an MLST scheme characterizing *C. perfringens* type A isolated from humans, animals, and food from different geographical regions (Japan, Europe, and USA) there has been no report about *tpeL* and *netB* strains.

In conclusion, the MLST protocol described by Jost et al. [15] was successfully used to subtype 40 human and animal *C. perfringens* strains using DNA sequencing and allelic profile data. The MLST and sequence-based phylogenetic inference identified a polymorphic,

predominantly clonal population [mainly in animal strains], with a phylogeny characterized by a dominant CC and ST, while the remaining strains existed as high-abundance STs.

This is the first report in Brazil showing an MLST scheme for *C. perfringens*. However, our strains [of human or animal origin] showed unique profiles because none of them clustered within the STs described by Chalmers et al. [20] or Hibberd et al. [17]. This demonstrates a highly diverse population of *C. perfringens* represented by our strains. Our results show that the strains of human and animal origin are genetically distinct but phylogenetically close. In addition, strains of human origin are more diverse than animal strains.

## Conflict of interest

The authors declare no conflict of interest.

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