Accepted Manuscript

Genetic variation among Clostridium perfringens isolated from food and faecal specimens in Lagos

Emelda E. Chukwu, Francisca O. Nwaokorie, Akitoye O. Coker, Mario J. Avila-Campos, Folasade T. Ogunsola

PII: S0882-4010(17)30365-0
DOI: 10.1016/j.micpath.2017.08.031
Reference: YMPAT 2421

To appear in: Microbial Pathogenesis

Received Date: 3 April 2017
Revised Date: 30 July 2017
Accepted Date: 30 August 2017

Please cite this article as: Chukwu EE, Nwaokorie FO, Coker AO, Avila-Campos MJ, Ogunsola FT, Genetic variation among Clostridium perfringens isolated from food and faecal specimens in Lagos, Microbial Pathogenesis (2017), doi: 10.1016/j.micpath.2017.08.031.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.
RESEARCH ARTICLE

Genetic variation among *Clostridium perfringens* isolated from Food and Faecal specimens in Lagos

Emelda E. Chukwu, 1* Francisca O. Nwaokorie, 2 Akitoye O. Coker, 3 Mario J. Avila-Campos 4 and Folasade T. Ogunsola 1

Corresponding author
Emelda Ebere Chukwu
Department of Medical Microbiology and Parasitology
College of Medicine, University of Lagos. Nigeria
Tel: 2348037444754
Email: emeldachukwu123@gmail.com

ABSTRACT

**Background:** *Clostridium perfringens* is an anaerobic Gram-positive bacterium which is commonly present in the gastrointestinal tract of man and animals and causes enteritic diseases in animals and food poisoning in humans. Previous studies have looked at the epidemiological relationship between *C. perfringens* isolates from outbreak source. In this study, the genetic diversity of *C. perfringens* strains from non-outbreak food and faecal specimens was investigated for epidemiological purposes.

**Methods:** We analyzed thirty-eight (38) *Clostridium perfringens* strains isolated from food and faecal specimens in Lagos State. Bacterial identification was done using colonial morphology, Gram stain reaction, conventional biochemical tests and PCR. Genetic analysis was performed using arbitrary primed polymerase chain reaction (AP-PCR) technique with oligonucleotide primer of random sequences (OPA-3) to determine the genetic diversity of *C.*
perfringens. The distance between the different bands produced were analyzed using numerical taxonomy and multivariate system software (NTSYS).

**Results:** Seventeen (44.7%) C. perfringens strains showed at least one polymorphic DNA patterns when genotyped. However, this method identified polymorphisms among the C. perfringens species from which four genetic groups (1, 2, 3 and 4) were established.

**Conclusions:** Our findings suggest that there may be faecal contamination of food products and similar clones of Clostridium perfringens may be incriminated.

**Keywords:** Clostridium perfringens; Food; Faeces; Genetic variation; Food poisoning; AP-PCR;

**INTRODUCTION**

Clostridium species are ubiquitous in nature found in soil, decaying vegetation, marine sediment, and the intestinal tract of humans. Clostridium perfringens, the most frequent clinical isolate of Clostridium, is classified into five types, A through E, according to four major lethal toxin produced (alpha, beta, epsilon, iota). They are responsible for a variety of infections including gas gangrene [1], skin and soft tissue infection [2], liver abscess [3], bacteremia and septic shock [1,4,5]. It is also an important pathogen of human gastrointestinal (GI) tract diseases such as food poisoning, antibiotic-associated, and sporadic diarrhoeas as well as nosocomial diarrhoeal disease outbreaks [6,7].

Toxinogenic C. perfringens has been isolated in foods, human faeces, and the environment [8-12]. High numbers may be recovered from food samples during an outbreak but the numbers present in non outbreak related food samples are usually few [11]. The strains involved sometimes vary from one region to another and this is put into consideration when
managing related cases especially under epidemic and emergency conditions. Understanding the diversity of toxigenic strains in food products and faecal specimen will lead to a greater appreciation of the epidemiology and pathogenesis of *C. perfringens* which may help in developing effective intervention methods for preventing transmission and outbreaks. Genotyping allocates distinctive labels to bacterial isolates and facilitate identification of transmission routes and sources [13]. Common typing methods that have been used to type *C. perfringens* include Multilocus sequence typing (MLST) [14], Multiple-locus variable-number tandem repeat analysis (MLVA) [15], Pulsed-field gel electrophoresis (PFGE) [16,17], Random amplified polymorphic DNA (RAPD) typing [18,19], Amplified Fragment Length Polymorphism [20] and Toxinotyping [12].

In Nigeria, food poisoning is not often investigated because many patients tend not to go to hospital. We therefore assessed the occurrence and diversity of pathogenic *C. perfringens* from food and faecal specimens of patients with suspected food poisoning for epidemiological purposes.

**MATERIALS AND METHODS**

**Study design and Sample collection**

Four hundred and twenty (420) samples comprising 220 food products and 200 faecal specimens were randomly selected from food commodities and faecal specimens of patients with suspected food poisoning indicated by diarrhoea. The study sites include six local government areas (Epe, Shomolu, Mushin, Ikeja, Surulere and Lagos Island) randomly selected, two from each of the three senatorial districts in Lagos state (Lagos east, Lagos west and Lagos central). Faecal specimens were collected from the General hospitals located in the
selected local governments while food products were collected from different markets and
grocery stores located in the six local governments. All samples were collected into a pre-
reduced transport medium (Thioglycollate broth) or sterile plastic bag (for food products) and
transported to the laboratory for processing within 2 hours of collection.

**Bacterial strains**

Bacterial colonies showing a double zone of beta-haemolysis on blood agar (Fastidious
Anaerobic agar- Lab M), black colonies on Tryptose Sulphite Cycloserine agar-TSC and/or
*Clostridium perfringens* agar-CPA (Oxoid, Basingstoke- UK), were Gram stained and
identified using conventional biochemical tests. The isolates were further confirmed to be *C.
perfringens* by the presence of alpha toxin using PCR as described in our previous study [21].

**Toxin typing of *C. perfringens* using multiplex PCR assay**

Bacterial DNA was extracted using GeneJET Genomic DNA Purification kit (Thermo
Scientific #K0722 Lot 00232256). The presence of genes encoding the toxins α, β, ε, ι and
enterotoxin were detected by a multiplex PCR assay as described in our previous study [21].
Alpha-toxin positive *C. perfringens* ATCC 13124 was used as control.

**Antimicrobial Susceptibility Testing**

The antimicrobial susceptibility of thirty-eight *C. perfringens* isolates to 5 commonly used
antibiotics; Amoxicillin, Clindamycin, Erythromycin, Tetracycline (Luper Ind Farm Ltd, Sao
Paulo, SP-Brazil) and Metronidazole (Aventis Farm Ltd, Sao Paulo, SP-Brazil) was
determined by agar dilution method as recommended by Clinical Laboratory Standard
Institute [22]. The antibiotics were reconstituted according to the manufacturer’s instructions and serial two-fold dilutions (ranging from 0.125-64 µg/ml) were prepared on the day of the test and added to Wilkins-Chalgren agar supplemented with hemin (5 µg/ml), vitamin K (1 µg/ml). A final inoculum concentration of $1.5 \times 10^5$ cfu/spot was delivered using a Steers replicator (Cefar Ltd, Sao Paulo, Brazil). Control plates without drugs were inoculated before and after each drug containing plates. All the plates were incubated in anaerobic atmosphere (90 % N$_2$, 10 % CO$_2$) at 37 $^\circ$C for 48 hours. A Reference strain *C. perfringens* ATCC 13124 was included in each batch as control.

**Determination of genetic diversity of *C. perfringens***

Genetic analysis was performed using arbitrary primed polymerase chain reaction (AP-PCR) technique with a 10-base oligonucleotide of random sequences (OPA-3: AGTCAGCCAC) as described by Llanco *et al.* [19]. Amplifications were carried out in final volumes of 25 µL in a thermocycler programmed to 1 cycle of 94$^\circ$C for 5 minutes, followed by 35 cycles of 94$^\circ$C for 1 minutes, 42$^\circ$C for 2 minutes, 72$^\circ$C for 2 minutes, and a final extension cycle of 72$^\circ$C for 10 minutes. A negative control without a DNA template was included in each AP-PCR run. Amplified products (20 µL) were separated using 1% agarose gel electrophoresis in TAE buffer (40 mM Tris-acetic, 2 mM EDTA [pH 8.3]) at 70V for 2.5 hours. Gels were stained with 0.5 µg/ml of ethidium bromide for 45 minutes and washed with water for 20 minutes. Stained gels were examined under ultra-violet (UV) trans-illuminator and photographed using a digital Kodak Science 120 system. A DNA ladder digest of 1kb plus and 100 bp (Invitrogen, SP-Brazil) was used as a molecular weight marker.
AP-PCR data collection and Statistical analysis

A rectangular binary data matrix was obtained by scoring each DNA fragment as a discrete variable and statistical analysis was performed using the Numerical Taxonomy and Multivariate Analysis System NTSYS-pc statistical package [23]. Pair wise similarities were computed by the NTSYS program (Applied Biostatistic, inc. version 2.21) using UN1 coefficient of similarity and unweighted pair group method with arithmetic averages (UPGMA) clustering. A dendogram was developed and a batch mode of NYSYS-pc was used to show the inter-relationship and genetic similarity matrix among the strains. Cross contamination was assessed based on the similarities of the fingerprints of *C. perfringens* isolated from the different sources. Data obtained was used to assess the health risk of these contaminated food products.

Discriminating index

Simpson’s index of diversity was used to determine the discriminating power of AP-PCR in typing local isolates of *C. perfringens* according to the following formula described by Hunter and Gaston [24].

\[
D = 1 - \frac{S}{N(N-1)} \sum_{j=1}^{S} n_j (n_j -1)
\]

Where N is the total number of strains in the sample population for which data were obtained. S is the total number of types/ subtypes described. And n is the number of strains on the jth type.
RESULTS

Thirty-eight *C. perfringens* strains harbouring alpha toxin were obtained and included in this study, 29 (76.3%) from food and 9 (23.7%) from faecal specimens. Thirty-six (94.8%) of these were typed as *C. perfringens* toxin type A, one (2.6%) *C. perfringens* type C and one (2.6%) *C. perfringens* type D. One (2.8%) of the 36 strains of *C. perfringens* identified as Type A, harboured the enterotoxin gene. All *C. perfringens* strains were susceptible to Metronidazole but showed varying degrees of resistance to Amoxicillin (3.3%), Tetracycline (16.7%), Erythromycin (33.3%) and Clindamycin (36.6%). Table 1

AP-PCR typing of *C. perfringens* using the primer OPA 3 was able to type 17/38 (44.7%) strains. The typeable strains were differentiated into four distinct groups (1, 2, 3 and 4) showing genetic differences that exists among the different strains of *C. perfringens* (Fig.1). The discriminating capability of this method was high (0.95) as calculated using the Simpson’s index of diversity but the degree of amplification by OPA-3 was low (44.7%). S1

Fig. shows fingerprints of *Clostridium perfringens* obtained using primer OPA-3. In general, all amplified and typeable isolates had approximately 20% similarity coefficient. The genetic relatedness of the strains ranged from 30 to 100% indicating a wide genetic variation. In this study, any band differences between two strains were considered sufficient to distinguish between two AP-PCR types and sub-types. Based on the food source, presence of toxin genes and common antimicrobial resistance; the typeable strains of *C. perfringens* were categorized into groups and sub-groups (Table 2).

Group 1 comprises two subgroups A and B. Subgroup A (1A) showed two strains from food and stool with 60% similarities while subgroup B (1B) consists of two strains also from stool and food with about 65% similarity. Group 2 was divided into two sub-groups 2A and 2B. Sub-group 2A harboured four strains which were further sub-divided into two; 2Ai consisting...
of two strains with 75% similarity and 2Aii with two strains from food belonging to the same
clone (100% similarities). Sub-group 2B contains only one strain. Group 3 harboured two
strains from yoghurt and stool with 30% similarity (Table 2). Group 4 consist of six strains
which were divided into two sub-groups 4A and 4B. Group 4A harboured four strains with
about 90% similarity. Three of the strain which were obtained from food and stool had AP-
PCR profiles with indistinguishable pattern (100% similarity), while the fourth strain (4Aii)
obtained from raw meat (identified as C. perfringens type D) was multi-resistant and
belonged to a separate clone. Sub-group 4B contained two similar strains possessing exactly
the same genetic makeup and are epidemiologically related. Interestingly, both strains were
obtained from stool samples of two male diarrhoea patients from two different local
government areas (Epe and Ikeja respectively).

Discussion

The high prevalence of C. perfringens type A recorded in this study is consistent with
previous reports [25-27]. Approximately 2.6% of the investigated C. perfringens strains
possessed the gene encoding the enterotoxin and therefore potentially pathogenic. Studies
have shown that the occurrence of cpe in non-outbreak strains is generally low compared
with out-break strains of C. perfringens [10,28,29]. In an Iranian study, none of the C.
perfringens type A isolated from non-outbreak food and human sources harbored the
enterotoxin gene [30]. In the present study, the estimated risk for perfringens food poisoning is less than estimated on the basis of the prevalence of the microorganism judging by the low prevalence (2.6%) of cpe positive C. perfringens recorded. An encouraging susceptibility pattern was observed for Metronidazole (100%) and Amoxicillin (96.7%) which are recommended as the drugs of choice for treating Clostridial infections (Table 1). These results are in keeping with the findings of Kouassi and colleagues [31] who reported Metronidazole and Penicillin G as the most potent agents against Clostridium perfringens and Clostridium difficile isolated from cooked beef sold in Cote d'Ivoire. Nonetheless, there is need for periodic antimicrobial susceptibility testing and surveillance to detect geographic or temporal changes in the resistance trends.

Several molecular methods have been used for typing C. perfringens in attempts to differentiate pathogenic strains from commensals and as an epidemiology tool to identify clusters or strains that are associated with disease outbreak [15,17,19,20]. Leflon-Guibout et al., [18] assessed the discriminatory capability of AP-PCR for typing C. perfringens strains and observed a discriminatory index of 0.97. The authors suggested that AP-PCR can be used as an epidemiological tool for C. perfringens. The aim of the present study was to demonstrate the general genetic diversity of C. perfringens isolated from food and faecal specimens in Lagos state, Nigeria. In this study, 44.7% of the strains were typeable with OPA-3 with high (0.954) numerical index of discrimination. Contrary to our findings, Llanco and workers [19] recorded 100% typeability for Clostridium perfringens Type A using OPA-3. They recommended OPA 3 primer for Arbitrary primed PCR typing of Clostridium perfringens. The low typeable recorded in this study highlights the need for the development of primers locally using our local strains.
Cluster analysis based on the genetic distance identified and established four distinct groups (1, 2, 3 and 4). The wide genetic diversity recorded among *C. perfringens* isolates in this study was not unexpected considering the wide variety of samples analyzed and the fact that the samples were non-outbreak random samples. However, a number of strains showed high genetic similarity and clonal relationship. Comparison of the AP-PCR pattern of food and faecal isolates revealed clonal relationship among some isolates (group 1A&B, 4Ai) which cluster together in the dendogram (Fig. 1) suggesting the possibility of faecal contamination of food. The result obtained could not establish any definite relationship between the source of isolates, the origin (local government areas) and the positions in the dendogram.

Of particular concern is strain (4Aii) obtained from raw meat (*C. perfringens* type D) which was multi-resistant (Amoxicillin, Clindamycin, Erythromycin, Tetracycline) and belonged to a separate clone from the group. *Clostridium perfringens* type D has been reported to cause severe, often fatal enterotoxaemia mainly in sheep, goat and cattle [32]. Its presence in raw meat may be suggestive of contamination of the meat with animal litter. Unfortunately, there is paucity of literature on the role of *C. perfringens* type D in human infections. Based on the results from this study, some of the isolates from different sources may have originated from a single clone and transmitted through faecal contamination of food.

Previous studies have defined different clonal types among *C. perfringens* species [19,33]. Afshari et al [30] used Randomly Amplified Polymorphic DNA (RAPD) technique to investigate the relatedness among 49 *C. perfringens* type A isolates from food and human source. They were able to divide the strains into 5 major groups based on genomic diversity. Different commercial OPA primers have been used to study bacterial cells but not every arbitrary primer produced useful banding pattern following amplification [34]. We decided to use OPA-03 based on high discriminating capacity and typeability observed in a previous study [19]. Based on the Simpson’s index of diversity [24] this primer was quite rapid and
discriminatory but showed low level of typeability because visible bands were observed in only 44.7% (17 / 38) of isolates studied. Further studies can be aimed at evaluating other typing techniques for their suitability for typing our local strains of *C. perfringens*.

**Conclusion**

Although high level of clonal differences was observed among species, some of the isolates were genotypically identical and belonged to the same clone. Our findings suggest that there may be faecal contamination of food products and these similar clones may be associated with *Clostridium perfringens* food intoxication. However, there is a need for further verification.

**Ethics approval and consent to participate**

Ethical approval for this study was obtained from ethical Research Grants and Experimentation Ethics Committee of College of Medicine, University of Lagos (CM/COM/08/VOLXXV, 2014) All participants signed an informed consent form.

**Consent for publication:** Not Applicable

**Availability of data and material**

All data generated or analysed during this study are included in this published article [and its supplementary information files].
Competing interests

The Authors declare that no competing interest exists.

Funding

This work was supported in part by the Sigma Delta Epsilon / Graduate Women in Science (SDE/GWIS) Nelly Mondy Fellowship award 2014 (www.gwis.org). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Authors' contributions

The main contributor (EEC) designed the study, collected samples, carried out the conventional culturing and molecular analysis, wrote the draft of the manuscript. The second author (FON) participated in molecular assays and supervised the work. The authors AOC, FTO and MJA supervised the work, reviewed the drafts and provided suggestions. All authors contributed to the literature searches and approved the final manuscript.

Acknowledgements

The Authors acknowledge Luis Llanco of the Anaerobe Laboratory, Department of Microbiology, Institute of Biomedical Sciences, University of São Paulo-USP, Brazil; for his technical assistance.

Authors' information

1Department of Medical Microbiology and Parasitology, College of Medicine, University of Lagos, Nigeria;

2Department of Medical Laboratory Sciences, College of Medicine, University of Lagos, Nigeria;
References


10. Carman, R. J., Sayeed, S., Li, J., Genheimer, C. W., Hiltonsmith, M. F., Wilkins, T.
    D., McClane, B. A. *Clostridium perfringens* toxin genotypes in the feces of healthy
    Prevalence and characterization of enterotoxin gene-carrying *Clostridium perfringens*
    *perfringens* and *Clostridium botulinum* from Food sold in Lagos, Nigeria. *Anaerobe*
13. van Belkum, A., Tassios, P. T., Dijkshoorn, L., Haeggman, S., Cookson, B., Fry, N.
    K., Fussing, V., Green, J., Feil, E., P. & other authors (2007). Guidelines for the
    validation and application of typing methods for use in bacterial epidemiology. *Clin
    Microbiol Infect Dis* 13 (Suppl. 3), 1–46.
    *Clostridium perfringens* of porcine origin as determined by multilocus sequence
    evolution and population structure, *Anaerobe* 12, 23–43.
    electrophoresis of the genetic diversity among *Clostridium perfringens* isolates from
    prevalence of netB in *Clostridium perfringens* isolated from a broiler flock affected by
    *Clostridium perfringens* strains by use of Random Amplified Polymorphic DNA (RAPD)
    Genetic Diversity in *Clostridium perfringens* Type A Isolated from Chicken with
20. McLauchlin, J., Salmon, J. E., Ahmed, S., Brazier, J. S., Brett, M. M., George, R. C.,
    & Hood, J. (2002). Amplified fragment length polymorphism (AFLP) analysis of


Table 1: Susceptibility pattern of *Clostridium perfringens* isolates to five commonly used antibiotics

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>(^a)CLSI (MIC (µg/ml))</th>
<th>Range (µg/ml)</th>
<th>(^c)MIC (_{50}) (µg/ml)</th>
<th>(^d)MIC (_{90}) (µg/ml)</th>
<th>Resistance (%)</th>
<th>Susceptible (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin</td>
<td>≥2</td>
<td>0.125 – 8</td>
<td>≤ 0.25</td>
<td>0.5</td>
<td>3.3</td>
<td>96.7</td>
</tr>
<tr>
<td>Metronidazole</td>
<td>≥32</td>
<td>0.125 – 8</td>
<td>1.0</td>
<td>4</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>≥8</td>
<td>0.25 – 64</td>
<td>1.0</td>
<td>≥64</td>
<td>36.6</td>
<td>63.4</td>
</tr>
<tr>
<td>Tetracyclin</td>
<td>≥16</td>
<td>0.125 – 32</td>
<td>2.0</td>
<td>8.0</td>
<td>16.7</td>
<td>83.3</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>≥8</td>
<td>4 – 64</td>
<td>2.0</td>
<td>64</td>
<td>33.3</td>
<td>66.7</td>
</tr>
</tbody>
</table>

Key:

\(^a\) Clinical Laboratory Standard Institute

\(^b\) Minimum Inhibitory Concentration

\(^c\) MIC at which growth of 50% of the isolates was inhibited

\(^d\) MIC at which growth of 90% of the isolates was inhibited

Fig. 1: Dendogram showing the genetic distance of *C. perfringens* obtained with the use of OPA-3. The tree was constructed using NTSYS statistical program (Applied Biostatistic, inc. version 2.21) to compute the pair wise similarities. The isolates were placed into groups 1, 2, 3, and 4
Table 2: AP-PCR types detected among *Clostridium perfringens* from food products and faecal specimens in Lagos State

<table>
<thead>
<tr>
<th>Strain no</th>
<th>Source</th>
<th>Local government areas</th>
<th>Toxin genes</th>
<th>Resistance to antimicrobials</th>
<th>AP-PCR groups/subgroups</th>
</tr>
</thead>
<tbody>
<tr>
<td>117B</td>
<td>Stool</td>
<td>Surulere</td>
<td><em>cpa</em>+</td>
<td>Ery, Tet</td>
<td>1A</td>
</tr>
<tr>
<td>96</td>
<td>Sausage roll</td>
<td>Surulere</td>
<td><em>cpa</em>+</td>
<td>Tet</td>
<td>1A</td>
</tr>
<tr>
<td>107A</td>
<td>Stool</td>
<td>Surulere</td>
<td><em>cpa</em>+</td>
<td>-</td>
<td>1B</td>
</tr>
<tr>
<td>25B</td>
<td>Cabbage</td>
<td>Lagos Island</td>
<td><em>cpa</em>+</td>
<td>-</td>
<td>1B</td>
</tr>
<tr>
<td>89THI</td>
<td>Meat pie</td>
<td>Mushin</td>
<td><em>cpa</em>+</td>
<td>Clin, Ery</td>
<td>2Ai</td>
</tr>
<tr>
<td>95CPA</td>
<td>Sausage roll</td>
<td>Epe</td>
<td><em>cpa</em>+</td>
<td>Clin, Ery</td>
<td>2Ai</td>
</tr>
<tr>
<td>94CPA</td>
<td>Suya</td>
<td>Epe</td>
<td><em>cpa</em>+</td>
<td>-</td>
<td>2Aii</td>
</tr>
<tr>
<td>88CPA</td>
<td>Meat pie</td>
<td>Shomolu</td>
<td><em>cpa</em>+</td>
<td>-</td>
<td>2Aii</td>
</tr>
<tr>
<td>417</td>
<td>Stool</td>
<td>Surulere</td>
<td><em>cpa</em>+</td>
<td>Clin</td>
<td>2B</td>
</tr>
<tr>
<td>82CPA</td>
<td>Yoghurt</td>
<td>Ikeja</td>
<td><em>cpa</em>+</td>
<td>Tet</td>
<td>3</td>
</tr>
<tr>
<td>404THI</td>
<td>Stool</td>
<td>Mushin</td>
<td><em>cpa</em>+</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>63A</td>
<td>Sardine</td>
<td>Shomolu</td>
<td><em>cpa</em>+</td>
<td>Clin, Ery</td>
<td>4Ai</td>
</tr>
<tr>
<td>62B</td>
<td>Sardine</td>
<td>Mushin</td>
<td><em>cpa</em>+</td>
<td>Clin, Ery</td>
<td>4Ai</td>
</tr>
<tr>
<td>104B</td>
<td>Stool</td>
<td>Mushin</td>
<td><em>cpa</em>+</td>
<td>Clin, Ery</td>
<td>4Ai</td>
</tr>
<tr>
<td>52B</td>
<td>Raw meat</td>
<td>Mushin</td>
<td><em>cpa</em>+ <em>etx</em>+</td>
<td>Amx, Clin, Ery, Tet</td>
<td>4Aii</td>
</tr>
<tr>
<td>131A</td>
<td>Stool</td>
<td>Epe</td>
<td><em>cpa</em>+</td>
<td>Clin, Tet</td>
<td>4B</td>
</tr>
<tr>
<td>108</td>
<td>Stool</td>
<td>Ikeja</td>
<td><em>cpa</em>+</td>
<td>Clin, Tet</td>
<td>4B</td>
</tr>
</tbody>
</table>

Key:

- **Amx**: Amoxicillin
- **Ery**: Erythromycin
- **Tet**: Tetracycline
- **Clin**: Clindamycin
- **Cpa**: *Clostridium perfringens* alpha toxin
- **Etx**: *Clostridium perfringens* epsilon toxin
Supplementary material

**Figure S1:** AP-PCRfingerprints of *Clostridium perfringens* with OPA-3 primer. Lane M: Molecular weight marker (100 bp and 1 kb plus DNA ladder respectively). Lanes 1-8,10,11,18-22 show representative amplicons.
HIGHLIGHTS

- *Clostridium perfringens* type A prevalence of 94.8% was recorded
- Metronidazole and Amoxicillin showed encouraging susceptibility pattern 96.4% and 91.1% respectively
- Great polymorphism observed among the *C. perfringens* species
- Four genetic groups (1, 2, 3 and 4) and sub-groups were established
- Similar clones were found in food and faecal specimens confirming epidemiological relationship