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Incidence of Clostridium perfringens in Retail Meat Samples from Ikorodu, Lagos, Nigeria

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ABSTRACT
Meat is a widely consumed source of protein that is typically cooked or processed to enhance shelf life and ensure safety. However, contamination with *Clostridium perfringens* is a major cause of foodborne illness due to the ingestion of large numbers of viable vegetative cells following meat consumption. This study investigated the incidence of *Clostridium perfringens* in retail meat samples from selected markets from Ikorodu, Lagos, Nigeria. A total of 75 samples, comprising 30 beef and 45 chevon, were purchased. Detection and isolation of *C. perfringens* were carried out on reinforced clostridial agar supplemented with blood under anaerobic conditions after initial pre-enrichment, and the identification was based on morphological and biochemical characteristics using standard microbiological procedures. The overall incidence of *C. perfringens* in the meat samples was 29.3% (22/75), with an occurrence rate of 31.1% (14/45) in chevon and 26.7% (8/30) in beef. No statistically significant difference (χ^2 = 0.17, p = 0.68) in occurrence rates between the meat types. These findings highlight the presence of *C. perfringens* in retail meat sold in markets from Ikorodu and underscore the need for stringent hygiene practices across all stages of meat handling, from slaughter to sale, to reduce the risk of *C. perfringens* foodborne illness.

Keywords:
Meat,
Clostridium perfringens,
Occurrence,
Foodborne illness

INTRODUCTION

Clostridium perfringens is a Gram-positive, rod-shaped, anaerobic, spore-forming bacterium that inhabits the gastrointestinal tract of humans and animals, as well as different environments including soil, water, and sewage (El Bayomi *et al*., 2020; Grenda *et al*., 2023). It is a major pathogen owing to its ability to produce different toxins. *C. perfringens* is known to produce four (4) major toxins viz., alpha (α, cpa), beta (β, cpb), epsilon (ε, etx) and iota (ι, iap) toxins. Beyond these, more than 15 other minor toxins such as enterotoxin (CPE), perfringolysin O (PFO), necrotic enteritis B-like toxin (NetB), beta-2 toxin (CPB2), and others are known to be produced by this bacterium (Hailegebreal, 2017). Based on the ability to produce a combination of the major toxins, *C. perfringens* has been classified into seven toxinotypes: A, B C, D, E, F and G (Grenda *et al*., 2023; Rood *et al*., 2018).

Clostridium perfringens is involved in different intestinal and systemic diseases in humans and animals. The notable among them are gas gangrene or myonecrosis, enterotoxemia, human food poisoning, non-food-related diarrhoea, and necrotic enteritis, among others (Camargo *et al*., 2024; Kiu & Hall, 2018).

C. perfringens foodborne diseases may occur due to consumption of food contaminated with about 10^6 CFU/g of the pathogen and improper cooking and storage that allow the spores to survive, germinate, and produce vegetative cells and toxins (Issimov *et al*., 2022; Sabry *et al*., 2016). A wide variety of food items especially meat and meat products have been implicated in *Clostridium perfringens* foodborne diseases (El Bayomi *et al*., 2020; Hailegebreal, 2017; Issimov *et al*., 2022).

Among Nigerians, meat constitutes a major source of dietary proteins, and due to its richness in protein, vitamins, and minerals, it is an excellent source of nutrients and an environment for microbial growth (Issimov *et al*., 2022; Khalifa *et al*., 2020). Although other food products such as spices, raw vegetables, dairy products, seafoods, and fish have been implicated in *C. perfringens* foodborne disease, poultry meat, chicken, beef, pork, gravies, and other meat products are the main reservoirs and are commonly implicated in food poisoning outbreaks worldwide (Cortés-Sánchez, 2018; Grass *et al*., 2013; Hailegebreal, 2017). Spores of some *C. perfringens* are highly resistant near boiling temperatures (Xiao *et al*., 2025), and they some can survive at 71°C for 2 hours (El Bayomi *et al*., 2020).

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In the United State, *C. perfringens* is a leading foodborne pathogen and ranks second after *Salmonella* among bacterial causes (Grass *et al*., 2013; Grenda *et al*., 2023). However, in Nigeria and other developing countries, *C. perfringens*-associated foodborne diseases are rarely investigated, and it is probable that many outbreaks go unreported because the food implicated and patient faeces are not routinely tested for *C. perfringens* or its toxin. In 2016, a severe local food poisoning outbreak caused by *C. perfringens* resulted in 13 deaths and 10 survivors (Adebowale, 2016; AfricaNews, 2016; Premium Times, 2016). Similarly, an evidence-based study by Tizhe *et al*. (2015) demonstrated a 3.5% occurrence of *C. perfringens* in raw beef from retail markets and abattoir-linked sampling in Zaria, Nigeria. In another study on food commodities and faecal specimens, Chukwu *et al*. (2015) reported the isolation of *C. perfringens* with alpha (cpa) toxin gene from meat products in Lagos. These present the evidence of meat and meat product contaminations and the risk of food poisoning due to *C. perfringens*. Therefore, this study was aimed at determining the incidence of *C. perfringens* in retail meat samples from selected markets from Ikorodu, Lagos, Nigeria.

MATERIALS AND METHODS

Sample collection

A total of seventy-five (75) raw meat samples comprising of thirty (30) beef and fourty-five (45) chevons samples were purchased from different meat vendors in Ikorodu, Lagos, Nigeria. The samples were collected inside sterile zip-lock bags, labelled appropriately, and transported to the Microbiology Laboratory, Department of Biological Sciences and Biotechnology, Caleb University, Lagos, for analysis.

Sample enrichment and pre-treatment

The initial sample enrichment was carried out by inoculating the meat samples into sterile Brucella broth (Himedia, India). The samples were then pre-treated at 80^0C for 15 minutes in a water bath and then incubated anaerobically in anaerobic jar with a CO_2 generating kit (Anaerogen, Oxoid, UK) for 48 hours at 37^0C.

Isolation of *Clostridium perfringens*

The enriched, pre-treated meat samples were plated out on blood agar plated by streaking a loopful of the aliquots on Reinforced Clostridial agar supplemented with blood. The plates were then incubated anaerobically in an anaerobic jar with a CO_2 generating kit (Anaerogen, Oxoid, UK) at 37^0C for 48 hours. After incubation, the plates were examined for colonies with a double zone of haemolysis with transparent, flat, leaf-like morphology characteristics of *Clostridium perfringens*, and the colonies were sub-cultured until pure isolates were obtained.

Biochemical characterisation and identification of the isolates

The pure isolates were subjected to biochemical characterisation by carrying out Gram's staining, catalase, oxidase, endospore staining, growth under anaerobic condition only, lipase, lecithinase, motility, indole production, urease production, casein hydrolysis, starch hydrolysis, gelatin hydrolysis, and sugar fermentation (glucose, lactose, fructose, sucrose, and mannitol) tests as described by Jousimies-Somer *et al*. (2002) and Barrow and Feltham (2010). The true probable identity of the isolates was confirmed using the results obtained based on the classical morphological and biochemical characteristics of the isolates.

Statistical analysis

Statistical analysis was performed using Statistical Package for Social Science (SPSS) software version 21 (IBM Corp., USA). Prevalence (%) was calculated as the proportion of positive samples. A chi square (χ^2) test of independence was carried out to determine the association between the meat type and the occurrence of *C. perfringens*. Odd ratios with 95% confidence intervals were calculated to estimate the strength of the association, and a p-value of < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The occurrence of *C. perfringens* in beef and chevon samples is presented in Table 1. Out of the 75 meat samples analysed, 22 (29.3%; 95% CI: 19.0-39.6%) were positive for *C. perfringens*. Chevon samples showed a slightly higher occurrence rate (31.1%) compared to beef samples (26.7%). However, statistical analysis revealed there was no significant difference between the occurrence rate of *C. perfringens* in the two meat types ($\chi^2 = 0.17$, df = 1, $p = 0.68$). However, the odds of contamination were marginally higher in chevon than in beef samples (OR = 1.24; 95% CI: 0.46 - 3.34).

Table 1: Occurrence of *C. perfringens* in beef and chevon samples

Meat type	No examined	No Positive	No negative	Prevalence (%)	95% CI (%)	OR (95% CI)	χ^2	df	*p*-value
Beef	30	8	22	26.7	10.8 - 42.6	1.00 (Ref.)	-	-	-

Chevon	45	14	31	31.1	17.6 - 44.6	1.24 (0.46 - 3.34)	0.17	1	0.68
Total	**75**	**22**	**53**	**29.3**	**19.0 – 39.6**	-	-	-	-

CI - confidence interval; OR - odds ratio; df – degree of freedom; Ref. – reference category; χ^2 - Pearson's chi-square statistic.
A *p*-value < 0.05 was considered statistically significant.

C. perfringens is one of the most common foodborne pathogens that thrives in high-protein foods worldwide (Sabry *et al*., 2016). In this study, the occurrence of *Clostridium perfringens* indicates the likely contamination of both beef and chevon samples sold by retail meat vendors in Ikorodu, Lagos. The contamination of the meat samples is believed to have emanated from the carcasses during processing and handling processes such as dehiding/skinning, evisceration, equipment contact, storage, and distribution from abattoirs to the retailers (El Bayomi *et al*., 2020; Gutema *et al*., 2021; Khalifa *et al*., 2020).
Previous studies on the incidence of *C. perfringens* in meat and meat products support the findings in this study and suggest a moderate level of contamination that warrants attention from public health authorities. The observed overall occurrence rate of 29.3% in this study is similar to the 30% reported by Issimov *et al*. (2022) from a study in Kazakhstan. However, it is comparatively lower than 47.5% reported by Coşansu & Ersöz (2021) and higher than 13.53% reported by Hassani *et al*. (2022) from studies carried out in Turkey and Iran, respectively. This finding also suggests that retail meat samples may serve as potential sources and reservoirs of food poisoning illness. Although the mere presence of *C. perfringens* in food is insufficient to cause illness (Hailegebreal, 2017), the presence of the right strain and the right handling conditions can signal food poisoning risk. Not only that, but the dose also matters. According to Issimov *et al*. (2022), consumption of meat contaminated with *C. perfringens* at a level of 10^6 cfu/g may result in food poisoning. Therefore, the presence of this organism in meat poses potential health risks, particularly when the meat is inadequately cooked or improperly stored, as *C. perfringens* spores can survive cooking temperatures and germinate under favorable conditions, leading to toxin production and subsequent illness (Grenda *et al*., 2023).
Patel *et al*. (2022) and Birla *et al*. (2018) reported 16% (8/50) and 20% (10/50) incidence of *C. perfringens* in chevon samples from retail market shops in Gujarat and Jabalpur cities in India, respectively, which are lower compared to 31.1% (14/45) reported in this study. Similarly, Tizhe *et al*. (2015) and Jeong *et al*. (2017) reported 3.50% (14/400) and 4.88% (4/82) *C. perfringens* isolation rates in beef samples from markets in Zaria, Nigeria, and Seoul, Korea, respectively, both of which are lower than 26.7% (8/35) recorded in the current study. The slightly higher occurrence observed in chevon compared to beef may be attributed to differences in handling practices, slaughter conditions, or environmental exposure during processing. However, the lack of statistically significant difference between the meat types suggests that contamination may be influenced more by general hygiene practices rather than intrinsic differences between beef and chevon, underscoring the importance of hygienic control measures across the meat production and retail chain. The detection of *C. perfringens* in the meat samples in this study highlights the need for improved hygienic measures during slaughter, processing, and retailing. Similarly, adequate cooking and proper storage practices should be emphasised to minimize the risk of foodborne illness.

CONCLUSION

In conclusion, this study reveals the possible contamination and incidence of *C. perfringens* in retail meat samples. It is only a qualitative assessment; it neither quantifies the bacterium nor determines the toxigenicity of the isolated *C. perfringens*. However, the study presents the meat samples as a potential reservoir of *C. perfringens* food poisoning. It therefore emphasizes the need for improved hygiene measures during slaughter, processing, and retailing. Furthermore, adequate cooking and proper storage practices should be emphasized to minimize the risk of foodborne illness.

Conflict of interests

The authors declare that they have no actual, potential or perceived conflict of interests.

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