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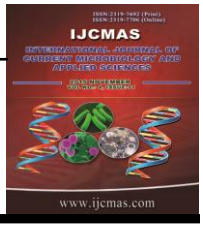

Original Research Article

Isolation and Biochemical Identification of *Clostridium perfringens* from Raw Beef Sold in Retail Outlets in Zaria Metropolis, Nigeria

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A B S T R A C T

Clostridium perfringens is a leading cause of bacterial food-borne illness in countries where consumption of meat and poultry is high. The bacteria being saprozoonotic causes food poisoning and wound infections in humans and enterotoxaemia in animals. The aim of this study was to determine the occurrence of *C. perfringens* in raw beef. A total of 400 raw beef samples were collected from seven different retail markets and zango abattoir in Zaria, and were screened for the presence of *C. perfringens* using fluid thioglycolate broth for enrichment and reinforced clostridial agar to which egg yolk tellurite was added for selective isolation. Nitrate reductive test, Sugar fermentation test and egg yolk emulsion for lecithinase test was used for biochemical identification of the organism. A total of 14 (3.5%) beef samples were tested positive for the presence of *C. perfringens*. The result revealed that beef tested positive for *C. perfringens* had potential to cause food poisoning in the populace. Even those beef samples tested negative could be contaminated from the environment due to poor handling and processing. In conclusion, this study has demonstrated the occurrence of *C. perfringens* in raw beef in Zaria, Nigeria. Therefore, hygiene should be maintained at all levels of meat preparation in order to prevent food-borne illness caused by *C. perfringens*.

Introduction

Members of the genus *Clostridium* are anaerobic, spore forming, rod-shaped gram-positive bacteria. They exist as free-living bacteria as well as pathogens that infect both humans and animals. Most pathogenic *Clostridium* species are known to produce toxins, which are responsible for a wide range of diseases (Hathaway, 1990).

Clostridium perfringens can grow between the temperature of 15^0C and 50^0C with an optimum of 45^0C for many strains. The

generation time (Gt) for most strains at temperatures between 33^0C and 49^0C is below 20 minutes, while Gt of 8 minutes has been reported (Labbe, 2000). The organism can produce over 13 different toxins, although each bacterium only produces a subset of these toxins (Petit, 1999).

Clostridium perfringens is more widely spread than other pathogenic bacteria, its principal habitat is soil, water and the intestinal contents of man and animals (Hayes, 1992). Anaerobic bacteria constitute an important group of microorganisms which are responsible for many public health hazards as well as spoilage due to lack of oxygen. *Clostridia* are the most anaerobic organisms which contaminate food due to production of their resistant spores (Barnes, 1985). It has great effect on the human health causing food poisoning and a number of human diseases ranging from necrotic enteritis to wound infection and life threatening gas gangrene.

Food outbreaks caused by *C. perfringens* are usually those presenting with high counts in the meat or meat products due to high contamination. *Clostridium perfringens* may be from different sources; mainly internally from animal after slaughtering as post-mortem invasion or externally from contaminated hands, skin of animals, soil, water and processing equipment (Satio, 1990).

Clostridium perfringens produces enterotoxin during sporulation of vegetative cells in the host intestine and formation of the spore coat layers, has a unique four step membrane action that binds to receptors on intestinal epithelial cells and exerts its intestinal action resulting in the characteristics diarrhoea and abdominal cramping symptoms associated with *C. perfringens* food poisoning (McClane, 1996).

Despite several works in the area regarding food-borne pathogens, there were no previous works in the study area which showed the importance of *Clostridium perfringens* as a food borne risk from raw beef. The organism being ubiquitous has the potential to contaminate meat even after processing. Hence the study was significant as it could give background information for subsequent studies in the area.

So, the aim of the present study was to determine the presence of *C. perfringens* in retail raw beef in Zaria Metropolis, Nigeria.

Materials and Methods

Study area

The study area is Zaria metropolis in Kaduna State, Nigeria. It lies between Latitude 11^0 7′12″N and Longitutide 7^0 41′ 50″ E. It shares border with Giwa Local Government to the west, Kudan Local Government area to the North and Soba Local Government area to the South. It occupies an area of 300 square kilometers. The climate of Zaria has a mean annual rainfall of 1092.8mm and 28^0C respectively. Zaria metropolis has a population of 408,198 (NPC, 2006). The vegetation is characterised by Guinea Savannah.

Sampling and sample collection

A total of 400 raw beef samples were collected from seven different markets; Samaru, Palladan, Kwangilla, Sabon gari, Tudun wada Zaria city Danmagaji and Zango abattoir in Zaria metropolis immediately after slaughter using convenience sampling technique (Thrusfield, 1997). The samples were placed in sterile polyethene bag and were labelled appropriately based on sampling locations and then transported on ice in a Coleman

box to the Bacterial Zoonoses Laboratory of the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University Zaria, until required.

Laboratory procedures

Isolation and identification of *Clostridium perfringens*

Ten grams each of the beef samples were aseptically cut with sterile scissors and forceps into small pieces. Ninety milli litres of 0.1% peptone water autoclaved at 121^0C for 15 min was added to each weighed samples and homogenized using laboratory stomacher 400 (Seward, UK) for 1-2 minutes at 10,000g. The homogenates of each sample were placed in a water bath for 10-15 minutes at 80^0C (heat shocking) to kill non-spore forming aerobic bacteria and 1ml was inoculated deep into 9ml of fluid thioglycolate medium (Merck KGaA, Germany) and incubated at 37^0C for 24 hours for enrichment. A loopful of thioglycolate inoculum was streaked unto reinforced clostridial agar to which egg yolk tellurite was added (Oxoid Ltd, England) in a sterilized petridish. The inoculated agar plates were placed in anaerobic jar containing gas generating kits (AnaeroGen™ Oxoid Ltd, England) and incubated at 37^0C for 48hours. The plates were observed for growth of black colonies with characteristics typical for *C. perfringens*. The suspected colonies on the agar plates were subjected to biochemical tests.

Biochemical test

Egg yolk emulsion for lecithinase test

Egg yolk emulsion (2ml) was mixed with nutrient broth and 1% Sodium chloride added for clearance of the media. The inoculating loop was thrusted with the sample into the egg yolk media and was incubated anaerobically for 24 hours at 37^0C (Jeffrey and Stanley, 2001).

Nitrate reductive test

The test organism was inoculated into nitrate broth and incubated at 37^0C for 24 hours. Five drops each of BactiDrop™ Nitrate A and B (REF.R21536 Remel, UK) was added. The broth was observed for development of red colour. When there was no development of red colour, a pinch of zinc powder was added to confirm for the presence of *C. perfringens* in the inoculated nitrate broth.

Sugar fermentation test

Sugar such as mannitol, sucrose, maltose and lactose were prepared at 1% concentration. Five (5) milli litres was dispensed into test tubes and autoclaved. They were inoculated with the test organism and incubated aerobically at 37^0C for 48 hours, followed by observation for change in colour (Eyre, 2009).

Statistical analysis

Data obtained in the study were subjected to Graph pad prism 5.03. Descriptive statistics was used to express the data as percentages. Chi-square was used to test for association between the presence of the organism and the markets sampled for raw beef ($P<0.05$).

Results and Discussion

In the present study, isolation of *Clostridium perfringens* was attempted from raw beef samples. The isolates were identified as *C. perfringens* on the basis of their cultural, morphological and biochemical characteristics. A total of 400 raw beef

samples were screened for isolation of *C. perfringens.* Bacteriological examination revealed the presence of the organism in 14 (3.5%) out of 400 raw beef samples as depicted in table 1. The highest prevalence of *C. perfringens* was found in Samaru Market 7 (14%), followed by Zango abattoir 4 (8%) and Zaria City Market 3 (6%). Beef samples from other sources of sampling failed to reveal the presence of the suspected organism. Significant association ($p<0.05$) was observed between the presence of the organism in beef and the source of samples as presented on table 2.

Table.1 Isolation rate of *Clostridium perfringens* from beef in different markets in Zaria metropolis

Markets	**No. of samples Screened**	**No. of Samples Positive for *C. perfringens* (%)**
Samaru	50	7 (14.00)
Zango abattoir	50	4 (8.00)
Palladan	50	0 (0.00)
Kwangilla	50	0 (0.00)
Sabon gari	50	0 (0.00)
Tudun Wada	50	0 (0.00)
Zaria City	50	3 (6.00)
Dan Magaji	50	0 (0.00)
Total	400	14 (3.50)

Table.2 Frequency distribution of raw beef contamination with *C. perfringens* based on markets' samples

Markets	**No. of samples Screened**	**No. of Samples Positive for *C. perfringens* (%)**
Samaru	50	7 (14.00)
Zango abattoir	50	4 (8.00)
Palladan	50	0 (0.00)
Kwangilla	50	0 (0.00)
Sabon gari	50	0 (0.00)
Tudun Wada	50	0 (0.00)
Zaria City	50	3 (6.00)
Danmagaji	50	0 (0.00)
Total	400	14 (3.50)

$X^2 = 26.09$, df = 7, P- value = 0.0005

Table.3 Lecithinase, Nitrate reductive and sugar fermentation tests by *Clostridium perfringens* isolates from raw beef sampled from some markets in Zaria

		Number positive for tests		
Markets	**No. of samples screened**	**Lecithinase**	**Nitrate reduction**	**Sugar fermentation**
Samaru	50	7	7	7
Zango abattoir	50	4	4	4
Zaria City	50	3	3	3
Total	150	14	14	14

Plate.I Carbohydrate fermentation test with *clostridium perfringens* produces acid in mannitol, sucrose lactose and maltose.

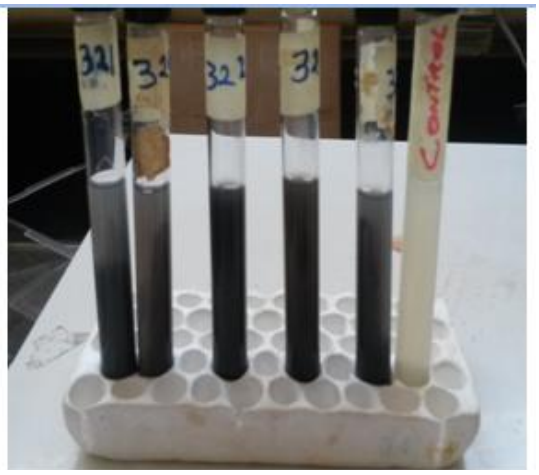

Plate.II Shows a typical blackening of egg yolk media with opaque white zone surrounding the colony as a result of lecithinase activity.

Plate.III Nitrate reduction test showing development of red colour after addition of BactiDrop Nitrate A and B to Nitrate broth

Plate.IV Opaque opalescent colour shown after addition of zinc powder to Nitrate broth showing positive for *C. perfringens*

The overall prevalence of *C. Perfringens*, 3.5% is low when compared to similar works done in other places (Agarwal *et al.,* 2000; Bachhil and Jaiswal, 1989; Singh *et al.,* 2005; Elham and Nahla, 2011). The low prevalence in the present study could be attributed to the heat shock in the medium during isolation in order to kill non-spore forming aerobic bacteria. This is in agreement with the work by Qiyi and McClane (2004) who reported *C. perfringens* at a rate of 2% and 29% in heat shocked and non-heat shocked samples. *Clostridium perfringens* isolates were detected in approximately 1.4% of 900 surveyed non-outbreak American retail Endale food (Qiyi and McClane, 2004). It was suggested that vegetative cells were killed by heat shocking. This is contrary to the findings of Endale *et al.* (2013), who isolated *C. perfringens* from another other than beef samples.

Some enterotoxigenic *C. perfringens* strains were estimated to represent less than 5% of the global *C. perfringens* isolates (Smedley and McClane, 2004), accounting for their rare isolation and detection in meat. Strains of *C. perfringens* that causes food poisoning occurs

It has been firmly established that an enterotoxin produced in the intestine following sporulation of ingested vegetative cells is responsible for the illness (Duncan, 1973). In recent decades, many surveys have been conducted on the prevalence of *C. Perfringens* in raw and processed meat and poultry. These reports indicate widespread occurrence of the organism in meat and poultry (Labbe and Doyle, 1989; Labbe *et al.,* 2000).

In the present investigation, specific and standard biochemical test were used for identification of *C. perfringens.* All the 14 isolates of *C. perfringens* obtained were subjected to lecithinase, nitrate reduction and sugar fermentation tests (Table 3). Acid was produced by change in colour from red to pink (Plate I). In egg yolk media, *C. perfringens* colonies were black with 2-4 mm opaque white zone surrounding the colonies in broth culture due to lecithinase activity (Plate II). Nitrate reduction test reveals the presence of *C. perfringens* in samples inoculated into nitrate broth shown by the organisms' ability to reduce nitrate to nitrite. Addition of BactiDrop Nitrate A and B to nitrate broth, resulted in the instant development of red colour (Plate III). In samples unable to show development of red colour, zinc powder was subsequently added. This reacts with potassium nitrate (KNO_3) in the broth, reduce nitrate to free nitrogen and ammonia to form an opaque opalescent colour indicating the presence of *C. perfringens* (Plate IV).

Meat items irrespective of species may be contaminated with spores of *Clostridia* during slaughtering process and subsequent handling. Since *C. Perfringens* is a normal microflora of the intestinal tract of animals, contamination of the carcass from the intestinal contents as well as soil, dust or from workers is virtually unavoidable as supported by McClane (2004).

The present study has demonstrated the presence of *C. perfringens* from raw beef samples sold in seven different markets and Zango abattoir in Zaria metropolis Kaduna State. It is therefore recommended that the sanitary measures should be adequately observed to prevent subsequent contamination of raw beef sold in retail markets and Zango abattoir in Zaria Metropolis, Nigeria.

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