



MOLECULAR INVESTIGATION OF *CLOSTRIDIUM SEPTICUM* IN THE GASTROINTESTINAL TRACTS OF CATTLE IN SOUTHWEST NIGERIA

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ABSTRACT

The study was conducted to determine the prevalence of *Clostridial species* and toxigenic *Clostridium septicum* in beef cattle present in abattoirs of southwestern Nigeria. Faecal samples were collected from 108 cattle in three abattoirs from Lagos and Oyo states, Nigeria. Isolation and tentative identification of anaerobic bacteria was performed according to standardized procedure, and served as inclusion criteria for further analysis. DNA was extracted from selected samples and polymerase chain reaction (PCR) performed using *Clostridia* 16SrRNA, triosephosphate isomerase (*tpi*), and *Clostridium septicum* alpha toxin (*csa*) primers. The PCR products were purified with *exo sap* and subjected to Sanger sequencing. A total of 64 samples were selected for analysis based on the isolation of obligately anaerobic Gram positive, catalase and oxidase negative, motile bacteria. A total of 21 (32.8%) *Clostridia species* were identified by PCR amplification of the 16SrRNA and *tpi* gene regions, and 19 toxigenic *C. septicum* identified by *csa* amplification. Uncultured *Clostridia*, and *Clostridium tetani* were identified by sequencing and a phylogenetic tree constructed with the nucleotide sequences. The presence of *C. septicum* and in the largest abattoir in West Africa poses a significant health risk to the inhabitants of the state.

Keywords: Beef, Cattle, Clostridiales, *Clostridium septicum*, Nigeria.

INTRODUCTION

The Agricultural sector, the engine of growth of the Nigerian economy, became relegated post-independence in favour of the petroleum sector upon discovery of commercial quantities of oil (Abubakar and Ibrahim, 2019). In recent times, the agricultural sector has begun regaining its former glory, contributing more to the nation's economy, 25.58% of gross domestic product (GDP) in 2022, than oil which contributed 5.67% to the GDP in 2022, and employing almost two-third of the total working population as at 2024 (National Bureau of Statistics, 2023; Central Bank of Nigeria, 2024). Cattle is the single most important livestock species in Nigeria and the biggest source of meat and milk, making cattle farming highly profitable, and a major aspect of the agricultural sector in terms of food security and contribution to the economy (Rekwot *et al.*, 2021).

The predominant method of cattle rearing in Nigeria is the nomadic approach where cattle are allowed to roam freely in search of pasture and water, often involving movement of cattle from northern Nigeria states such as Taraba, Adamawa, Jigawa, and Yobe where they are raised, to southern Nigeria where they enter the food chain, exposing the cattle to different environmental conditions and a host of potential pathogens (Ajayi *et al.*, 2023). *Clostridium septicum* is the highly pathogenic aetiology of myonecrosis, braxy, malignant edema (gas gangrene) and clostridial dermatitis in humans and animals, and is introduced into cattle through wounds caused by accident, castration, docking, insanitary vaccination, and parturition (Khiav and Paradise, 2020; Stämpfli and Oliver-Espinosa, 2021). Cattle could be at higher risk of *C. septicum* colonization and infection in nomadic farming due to increased likelihood of accidental injuries during movement, and poor hygiene of their forage environment. Data on the impact of clostridial infections in cattle in Nigeria is however limited, especially limited due to the practice of slaughtering sickly cattle for sale to prevent economic losses. Emergency slaughter of cattle with acute injury or disease is permitted in countries like the United Kingdom, Ireland, British



Columbia, and Canada, with strict guidelines ensuring that animals with diseases that make them unfit for human consumption do not enter the food chain after slaughter (McDermott *et al.*, 2022). In Nigeria, while there are regulations prohibiting slaughter of sick cattle for commercial purposes, cattle herders flout these laws and smuggle meat from their sick animals into the food chain often resulting in outbreak of diseases (Oyewole, 2013; Obi, 2019; Uti, 2023).

This study aimed to investigate the prevalence of *C. septicum* in cattle at abattoirs in Southwest Nigeria as nomadic rearing exposes cattle to increased risk of infection, and sickly cattle are often slaughtered swiftly and sold to avert economic loss.

MATERIALS AND METHODS

Sample Collection

Three abattoirs in South-West Nigeria were included in this study viz, the Lagos State Abattoir and Lairage Complex, Oko Oba, Agege, Lagos state (LSAC), Lion Unisco Abattoir, Ilaje, Bariga, Lagos state (LUAI) and Odo Eran Cattle Market and Abattoir, Sango Ota, Ogun state (OCMA), as they are major sources of meat in the region. Faecal samples were collected into sterile universal bottles from 108 cattle. Close-ended questionnaires were administered to their handlers to determine feeding, vaccination, and hygiene practices. The samples were analysed within 3 hours of collection or stored in a refrigerator at 4°C and analysed within 24 hours of collection.

Anaerobe Isolation

Samples were shocked in alcohol by mixing with equal volume of 95% ethanol then cultured on Anaerobic Agar (Brewer) (Biomark laboratories) supplemented with 10% defibrinated sheep blood. Cultures were incubated in an anaerobic jar with anaeroGEN bag (OXOID, UK) under an anaerobic atmosphere at 37°C for 48 hours, using resazurin strip as anaerobic indicator. Colonies with zones of haemolysis were subcultured on fastidious anaerobe agar (FAA) (LabM, Bury, Lancashire, England) supplemented with 10% defibrinated sheep blood in an anaerobic jar with anaeroGEN bag (OXOID, UK) and an anaerobic indicator under an anaerobic atmosphere at 37°C for 48 hours. Distinct colonies were stored in 50% glycerol Brain Heart Infusion broth at -20°C.

Identification and Confirmation

Isolates were subjected to Gram staining, as well as catalase, oxidase and motility tests. DNA was extracted from faecal samples from which Gram positive, catalase and oxidase negative, motile organisms were isolated, and assayed for the presence of Clostridial bacteria and *C. septicum* using PCR. Primers used include F- GAGAGTTTGATCCTGGCTCAG and R- GTGGACTACCAGGTATCTAATCC for 16SrRNA, F- GCWGGWAAYTGGAARATGMAYAA and R- TTWCCWGTWCCDATWGCCADAT for triosephosphate isomerase housekeeping gene (*tpi*), and F- TAGGATTTGGATGGTGC GGTTGGAA and R- TGCACGATACCCACTTGCATAAGG for *Clostridium septicum* alpha toxin (*csa*) as described by Dhalluin *et al.*, 2003 and Neumann *et al.*, 2010. DNA was extracted using Zymo Research Genomic DNATM – Solid tissue MiniPrep kit (Catalog No. D4068 & D4069), and PCR with Solis Biodyne 5x HOT FIREPol Blend Master mix. Amplicons were separated by electrophoresis on a 1.5% (w/v) agarose gel with 100bp DNA ladder as DNA molecular weight standard. After electrophoresis, DNA bands were visualized by ethidium bromide staining. Bio-Rad ChemiDoc™ MP Imaging System, UK) was used to capture the image using Gene Snap (version 6.00.22) software. 16SrRNA PCR products were purified with Exo sap and sent to Epoch Life Science (USA) for Sanger sequencing.

RESULTS AND DISCUSSION

Herders' Behaviour

All herders interviewed practiced open grazing, both while rearing in northern Nigeria and in the abattoirs in southern Nigeria, but used trailers in moving their cattle to the south. They also reported vaccination of all their cattle. The hygiene practices of the herders however, particularly in LSAC and OCMA, were not near sanitary as they cleaned the lairages an average of once a week and gather the faecal matter and other wastes for sale as manure.

Sample Selection

Based on the isolation of obligately anaerobic Gram-positive catalase and oxidase negative bacteria from the samples, a total of 64 (59.3%) samples qualified for further analysis, with the majority of the samples from female cattle (Table 1). The highest prevalence (60.7%) of suspected clostridial bacteria was observed in samples from OCMA. The higher prevalence of bacteria in OCMA than in the Lagos state abattoirs could be indicative of improved management in the Lagos state abattoirs as the Lagos state government commissioned the Lion Unisco Abattoir in 2021 to curb illegal and unhygienic meat butchering, and shut down the LSAC in June 2025 due to unsanitary practices (Omoike, 2021; Akoni, 2025).

Table 1: Samples Selected for Analysis based on Isolation of Anaerobic Gram-positive Catalase and Oxidase Negative Motile Bacteria

Sampling Area	Cattle	Samples Collected	Samples Selected (%)
Lagos State Abattoir Complex, Oko Oba, Lagos	Calves 0-8 months	10	2 (20)
	Males 9-24 months	20	15 (75)
	Females 9-24 months	20	12 (60)
	All Cattle	50	29 (58)
Odo Eran Cattle Market and Abattoir, Ota, Ogun	Calves 0-8 months	6	2 (33)
	Males 9-24 months	13	5 (38.5)
	Females 9-24 months	13	13 (100)
	All Cattle	32	20 (62.5)
Lion Unisco Abattoir, Ilaje, Bariga, Lagos	Calves 0-8 months	6	1 (16.7)
	Males 9-24 months	10	9 (90)
	Females 9-24 months	10	5 (50)
	All Cattle	26	15 (57.7)
Total	Calves 0-8 months	22	5 (22.7)
	Males 9-24 months	43	29 (67.4)
	Females 9-24 months	43	30 (69.8)
	All Cattle	108	64 (59.3)

Clostridia Identification

A total of 21 Clostridia were identified by PCR amplification, with 10 (16.7%) and 11 (18.3%) identified by amplification of the 16SrRNA and tpi gene regions respectively (Figure 1). Female cattle had a higher prevalence of clostridial bacteria than male cattle, with 12 (40%) Clostridia identified from their samples (Table 2). The highest prevalence (60%) of Clostridial bacteria was however observed in calves. Calves younger than 6 months old have been reported to have higher prevalence of Clostridial colonization which reduces as they grow older due to weakened immune system at their young age (Bandelj *et al.*, 2016). The higher prevalence of Clostridial bacteria in female cattle than their male counterparts corroborate reports that gender also plays a role in the microbial composition of cattle gastrointestinal tract. Studies have shown increased microbial diversity in older cattle, and different microbial flora structure between same age male and female cattle fed the same diet (Li *et al.*, 2019; Pan *et al.*, 2024). Among the different abattoirs, LUAI had the least prevalence of *Clostridial species* with only 3 (20%) identified by tpi amplification and none with 16SrRNA amplification. This is indicative of the higher hygiene standards maintained by LUAI in comparison with other abattoirs sampled.

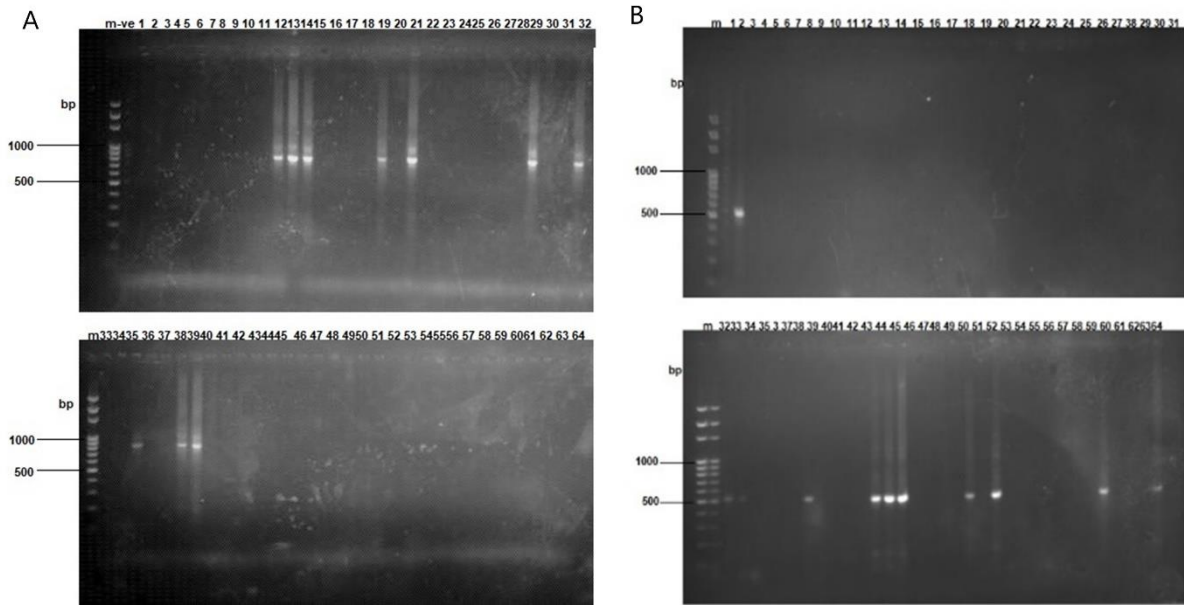


Figure 1: Identification of *Clostridium* species. A – PCR amplification of 16SrRNA gene region; B – PCR amplification of tpi housekeeping gene region. Lanes 1-29 are from the Lagos State Abattoir Complex, Agege, 30-49 from Odo Eran Cattle Market and Abattoir, Ota, and 50-64 from Lion Unisco Abattoir, Ilaje, Bariga.

Table 2: *Clostridial* species Detected in the Different Samples Selected

Sampling Area	LSAC	OCMA	LUAI	Total (%)
Calves 0-8 months	1	2	0	3 (60)
Males 9-24 months	3	2	1	6 (20.7)
Females 9-24 months	3	7	2	12 (40)
Total (%)	7 (24.1)	11 (55)	3 (20)	21 (32.8)

LSAC – Lagos State Abattoir Complex, Oko Oba, Agege, Lagos state, Nigeria. OCMA – Odo Eran Cattle Market and Abattoir, Sango Ota, Ogun state, Nigeria. LUAI – Lion Unisco Abattoir, Ilaje, Bariga, Lagos state, Nigeria.

***Clostridium septicum* Identification**

Amplification of the *csa* gene region revealed the presence of 19 (29.7%) *C. septicum* (Figure 2). All were from samples obtained at LSAC, with 2 (10.5%) from calves, 11 (57.9%) from male cattle, and 6 (31.6%) from female cattle. This finding is highly concerning as the Lagos State Abattoir and Lairage Complex Oko Oba, Agege, Lagos is the biggest abattoir in West Africa, with a capacity of 3000 cattle slaughter per day, accounting for over 30% of the meat processed daily for consumption in Lagos, the second largest city in Africa with over 21 million inhabitants (Ajayi, 2024). Meat for human consumption can get contaminated with the cattle’s gastrointestinal microflora during slaughter and evisceration, posing a significant food safety concern which is exacerbated by low hygiene levels in the abattoirs. *C. septicum* contamination of meat is a potential cause of disease outbreak as the bacteria is sporulating and can survive traditional cooking. An outbreak of foodborne disease from contaminated meat in the LSAC would be particularly devastating due to the dense population and high meat consumption of Lagos state. Although the LSAC has undergone several government interventions over the years to improve its hygienic practices, illegal slaughtering and other unhygienic practices are still rampant in the abattoir (Ajayi, 2024; Akoni, 2025).

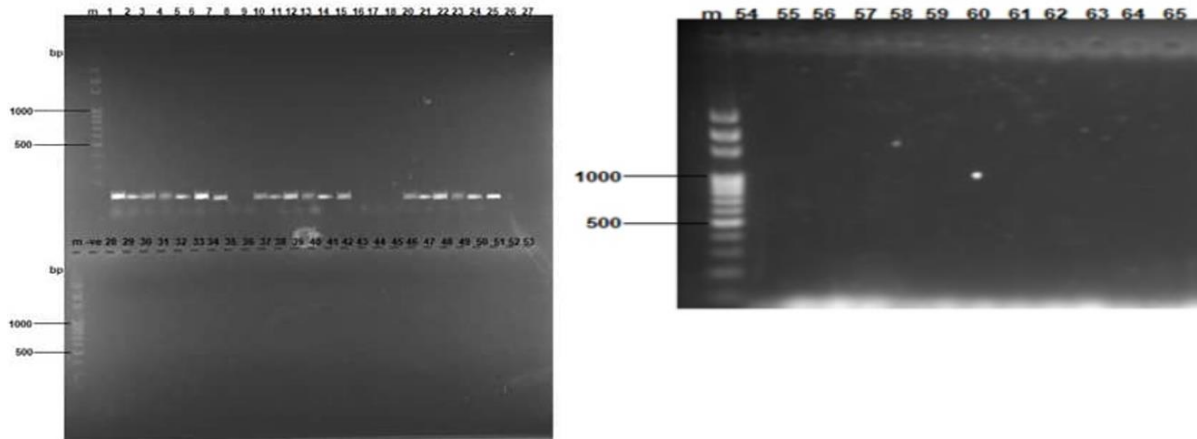


Figure 2: Agarose gel showing PCR detection of the *Clostridium septicum* alpha toxin gene. Lanes 1-29 are from the Lagos State Abattoir Complex, Agege, 30-49 from Odo Eran Cattle Market and Abattoir, Ota, and 50-64 from Lion Unisco Abattoir, Ilaje, Bariga.

Phylogeny Analysis

Several uncultured clostridia and *Clostridium tetani* were identified by Sanger sequencing of the PCR products (Table 3), and are shown in a phylogenetic tree constructed with their nucleotide sequences (Figure 3). Studies have shown that uncultured Clostridiales are significant occupants of cattle rumen (Li *et al.*, 2019; Pan *et al.*, 2024). While they do not appear to pose a threat to the wellbeing of the cattle, their impact on human health and the environment is largely unknown as means to culture and study these clostridial bacteria is presently unavailable. The presence of *C. tetani* however poses a major concern as it is a pathogen of both humans and animals. Other than contamination of meat, *Clostridium tetani* can contaminate the environment due to practices in the LSAC abattoir. Open grazing in which cattle roam the state depositing faeces wherever they go, and the sale of cattle waste as manure for growing agricultural produce puts the environment at risk of contamination. Furthermore, liquid waste from the abattoir is washed into the public draining system, including open roadside drains, thereby releasing these sporulating pathogens into the environment, and placing the humans in the abattoir and its environs in higher risk of clostridial infection.

Table 3. Bacterial Species Identified from Sequencing of PCR Products

Identity Similarity	Identified organism	Sequenced ID
82%	Uncultured bacterium clone T2WK15B77	HQ716700
85%	Uncultured <i>Clostridium</i> sp. Clone GKJWQY101AAXLR	JQ997850
83%	Uncultured <i>Clostridiales</i> bacterium clone BW	JN540211.1
86%	Uncultured <i>clostridia</i> bacterium clone OTU4622	KP840259
96%	<i>Clostridium tetani</i> E88 plasmid pE88	AF528097

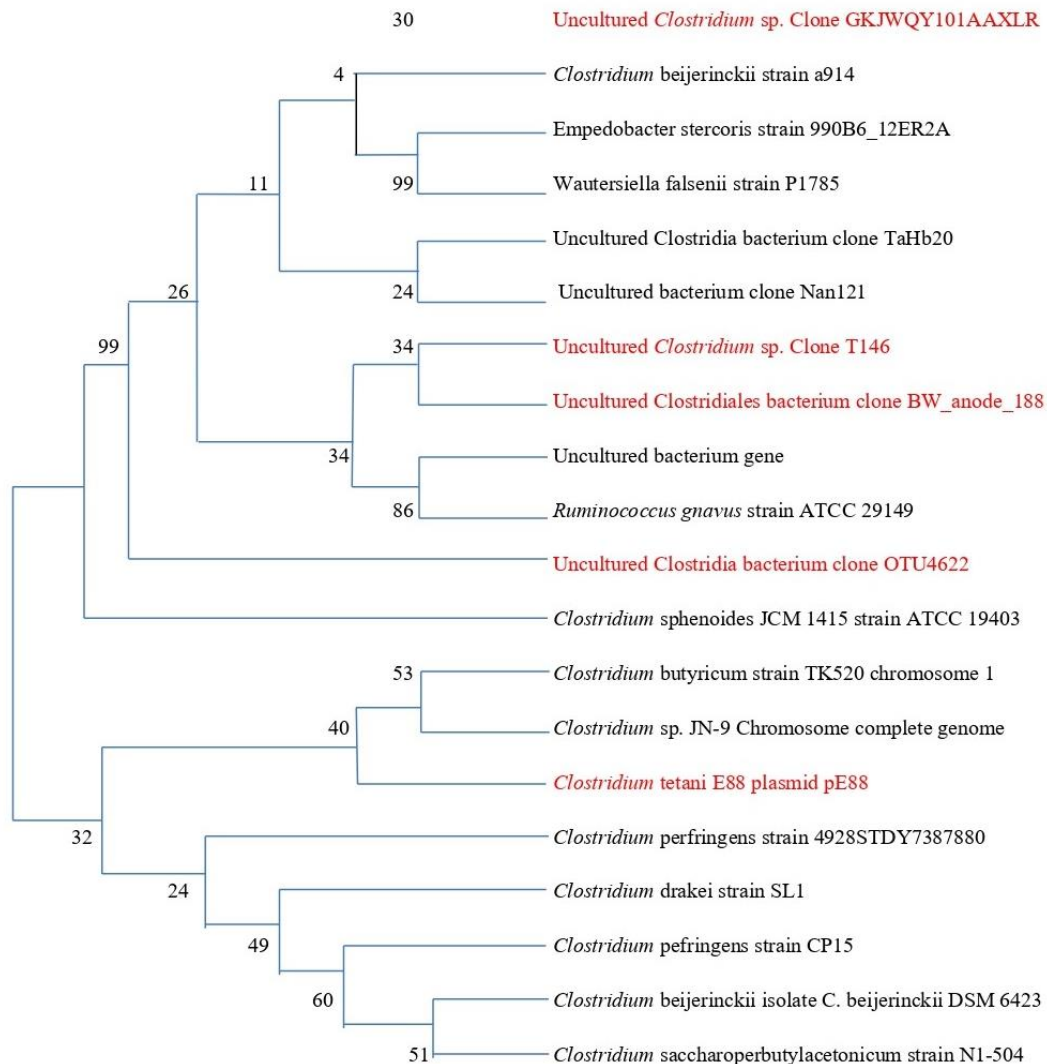


Figure 3. Phylogenetic tree built from the alignment of sequences corresponding to *Clostridium* spp.

CONCLUSION AND RECOMMENDATION

The gastrointestinal tract of cattle of different ages and gender in the Lagos state abattoir complex, Oko Oba, Agege, Lagos serves a reservoir for *Clostridium septicum* and other species of clostridial bacteria. The impact of the uncultured *Clostridium* spp. on human and animal health is unknown due to their uncultured status, while *Clostridium septicum* and *C. tetani* pose significant risk to human and animal health. This is especially concerning as animal and waste handling practices in this abattoir, the largest in West Africa, ensure the release of these pathogens into the environment. Stricter hygiene enforcement is necessary, particularly in the Lagos State abattoir and lairage complex, Oko Oba, Lagos to prevent disease outbreak in this metropolis with over 21 million inhabitants. Community outreach programmes educating cattle herders, slaughterhouse staff, and other meat handlers on the dangers of improper hygiene to the people themselves, the cattle, meat consumers, and other people in the environment.

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