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Research Paper

MICROBIAL EVALUATION OF *CLOSTRIDIUM BOTULINIUM* IN DIFFERENT MEAT TYPES SOLD IN AMAI-OGUME MARKET

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An investigation was carried out on the detection and enumeration of *Clostridium botulinum* from two different meat samples from the rayal market in Ekpomam Edo State using Nutrient agar and MacConkey agar for presumptive test detection and fermentative tests for confirmation. It was found that samples contained *Clostridium botutimum,* the count ranged between 10^5 and 10^8 cells per gram of meat sample. However, this work was carried out to reveal the presence of *Clostridium botutimum* in meat eaten and hence can be used as a parameter to ascertain the suitability of fresh meat for consumption.

Keywords: Microbial Evaluation, Clostridium botulinium, Presumptive test, Fermentative test

INTRODUCTION

Clostridium botulinium is widely distributed in soils shores and bottom deposits of lakes and streams as well as ocean. It can also be found in fruits and vegetables as these items are often in contact with soil. It also colonizes the gastrointestinal tract of fishes, birds and mammals and have been incriminated in meat products (Cherington, 1998), *Clostridium botulinium* produces a potent neurotoxins (Brown, 2000). The spores are heat resistant and can survive in food that correctly or minimally processed. Food borne botulism is a severe type of food poisoning caused by the infection of foods containing the potent neurotoxin formed during the growth of the organism. The toxin is heat labile and can be destroyed if heated at 120 ^oC for 30 min or longer.

The incidence of the disease is low, but the disease is of considerable concern because of its high mortality rate if not immediately and properly treated. The infective dose is very small (a few nano grams). The onset of symptoms occurs 18 to 36 h after ingestion. *Clostridium botulinum* toxins causes paralysis of blocking motor nerve terminals at the myonemal function. The resulting asphyxia cause death (Lior *et al.,* 2003). The severe nature of diseases caused by this microbe makes it study in meat and meat product imperative considering the meat handling nature and processing methods obtainable in our

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environment. Throughout the world, and particularly in Africa, there is a need for modernization of the livestock processing industry. Hygienic human slaughter houses are an essential requirement of the meat industry sub-sector in all countries and the quality and availability of these facilities in developing countries vary significantly. In Nigeria, few Abattoirs have been constructed to reasonably good standard, but have frequently bee left without adequate funds to maintain the essential services and standards of hygiene (Adeyemo *et al*., 2009). In "clean and dirty" operations are kept this means that separate rooms are required for the reception of animals, slaughter, washing, chilling, cutting and packing freezing and storage, dispatch and by product processing.

In design an Abattoir, the plan should show a smooth flow of products along the processing line, with minimum distances between all operations including those which require other material to be used in the process. There should also be minimum interference between other operation and cross flow operation should be kept to the absolute minimum. Equipment to be used must conform to the local standard of construction and safety it must also be designed to be cleaned properly after use. Wash hand basin should be made in stainless steel, sterilizer for knives and hand tool equipment should used. *Clostridium botulinium* is a gram positive obligate, anaero-bicspore forming slightly covered rod-shaped bacterium. It is motile with peritrichous flagella.

MATERIALS AND METHODS

Collection of Samples

Two samples were collected from Ekpoma market. The samples collected was fresh beef and chicken. The purpose for this was to determine the bacteria load of *Clostridium botulinium* and to identify the organism associated with the samples collected from the market. The samples were transported to the laboratory for analysis.

Sterilization of Materials Used

The glass-ware used were conical flask, measuring cylinder, test-tubes. These were sterilized in hot air oven at 160 ^{o}C for 1 h other material include: wire gauge, Bunsen-burner, test tube racks, slide, Petri dishes. The inoculating loops and scalpel blades were sterilized by flaming until red hot.

Media Used

The media used include

1. Mannitol salt agar
2. Nutrient agar
3. MacConkey agar
4. Nutrient broth
5. Peptone water sugar

The constituents and method of preparation of all the media and reagents mentioned in this work was described at the appendix. 1 g of each meat sample was macerated with sterile blade and was suspended in a test tube containing 9 mL of nutrient broth. The meat suspension was shaken vigorously and allowed to stand for 20-30 min. This gave a meat suspension of 1:10 (w/v) dilution from which serial dilution was carried out in test tubes (arranged in rack).

Serial Dilution Technique

Nine millileter of sterilized nutrient broth was pipette into three (3) different rest tubes arranged in a rack and labeled accordingly. 1 mL of each sample was drawn using sterile 1 mL syringe to

be transferred into the first test tube and was carried out for all samples.

Planting and Incubation Condition

Using a sterile wire loop, the second dilution was inoculated on MacConkey agar plate in order to identify the organism present (Table 1). The last dilution was inoculated into nutrient agar plate to determine the total coliform counts. The plate were then incubate at 37 °C or 24 h. The mannitol salt agar was also inoculated to isolate for the presence of *Clostridium botulinium*. Plates were labeled accordingly.

Identification of Isolates

The identification of isolates was based on the following:

Colonial Morphology

The colonial appearance of isolates at the end of incubation of MacConkey agar was noted. Those having pinkish colonies, those capable of fermenting lactose and those having golden-yellow colonies. The different colonies observed were picked and sub-cultured in MacConkey agar. Thus was incubated at 37 °C for 24 h. Isolates on mannitol salt agar plates gave dry golden- yellow colonies.

Gram Staining Reaction

A smear of the organism was made on clean grease free slides, allowed to air-dry and heat-fixed by quick passing it about two times over flames. This was allowed to cool and the slides were placed on a staining rack. The smear was flooded with crystal violet and allowed to act for 1 minute. Then rinsed with water. Iodine (1 or 2) drops was added to the slide for 30 s, Then rinsed with water. The slide was blotted dried and a drop of oil was added. Then the slide was examined under the microscope using ax100 oil immersion objective.

Biochemical and Other Test

Indole Test

Peptone water with the bacterial culture are inoculated and incubated at 37 °C for 24 h 0.5 mL of Kovac's reagent was added and shaken gently. After a minute it is examined for the formation of red color at the top of the peptone water culture. This is known as Kovac's Reagent inter-phase.

Motility

A drop of culture of the bacteria suspension in peptone water was placed on the centre of a clean dry cover slip. Using plasticine it was made on a clean dry grease free slide. The slide with the plasticine ring was gently place on the cover slip and carefully inverted so that the centre. It was then examined for motile bacteria actively using x 40 objective lens of a microscope.

Carbohydrate Fermentation Test

This bacteria isolate was inoculated into Andrades peptone water in which lactose, sucrose, glucose, mannitol and sorbitol have already been inoculated. Durham's tube was put iin an inverted position in the sterile bijoux bottle containing glucose. The bottles were incubated at 37 °C for 24 h and the test was observed for color change indicating acid production and formation on the Durham tube.

Hydrogen Sulphide Test

Inoculate into a tube containing Kligler iron agar using a sterile sterile straight wire. It was stabbed first and then streaked on the slop the tube was closed with loosed – fitting cap and incubated at 37 °C for 24 h. The bottle was observed for blackening—due to the production of hydrogen sulphite.

Oxidase Test

Filtered paper was moistened with a few drops of 1% tetramethyl-p-phenylene, diamine dehydro-

chloride with the spatular, growth from agar medium was smeared on the paper. A positive test was developed of a purple color with 10 s.

Culture Result

Following overnight inoculation, plates were examined and identified based on control appearance microscopy.

RESULTS AND DISCUSSION

Morphology

Vegetative Cells

Form and arrangement: straight or slightly curved rods.

Size: 2-10 m long

Gram stain: Positive

Flagella: Peritrichous

Chains: pairs, short chains

Color change due to production of reagent A and B develops a red to purple color. Counts of *Clostridium botulinum* from different fresh meat samples using nutrient agar medium (Table 3 shows the range of *Clostridium botulinum* counts obtained when nutrient agar was used for enumeration. All colonies isolated showed typical haemolysis depleting its pathogenicity.

Gram Staining Reaction

Clostridium botulinium are gram positive rods with oval sub-terminal spores that resemble a tennis racquet. Results in Table 4 shows that average count / g of meat revealed values of 7.9 x 10^5, 10.0 x 10^7 and 10 x 10^8 of *Clostridium botulinium* in fresh beef while values of 7.4 x 10^5 and 6.9 x 10^8 where observed in fresh chicken. It was also observed that values varied from sample to sample.

tables 1, 2 3 are not mentioned

Table 1: Cultural Characteristics of *Clostridium Botulinum* in Macconkey and Nutrient Agar

Features	MacConkey Agar	Nutrient Agar
Growth	Scanty	Copius
Forms	Small, pink	Moderately, large colonies
Surface	Smooth	Smooth
Edge	Entire	Irregular, entire
Elevation	Flat	Convex
Optical characteristics	Opaque	Opaque

Table 2: Characteristics of *Clostridium Botulinum* In Nutrient Broth

Features	Observation
Clouding	Present
Sediment	Present
Amount of sediment	Large (greenish)
Odor	Rancid or cheesy
Growth surface	Absent

Table 3: Fermentation of Sugar by *Clostridiuim botulinum*

Glucose	Maltose	Mannitol	Sucrose	Lactose
Gas	Gas	Mannital	Sucrose	Lactose
Acid	Acid	–	Acid	Acid

Table 4: *Clostridium botulinum* Count Using Nutrient Agar in Different Meat Samples

Sample Type	Sample No.	Count/Mg of Meat		Average Count/g of Meat
		1	2	
Fresh beef	1	1.9×10^8	11.6×10^6	10.1×10^8
	2	9.8×10^6	1.2×10^8	10.0×10^7
	3	2.5×10^4	7.6×10^5	7.9×10^5
Fresh chicken	1	7.0×10^8	1.3×10^9	6.9×10^8
	2	9.8×10^6	9.4×10^5	9.6×10^5
	3	8.1×10^4	1.4×10^6	7.4×10^5

DISCUSSION

The importance of detecting the presence of *Clostridium botulinium* even in the smallest amount lies in the fact that meat forms the basis of most food. *Clostridium botulinium* was detected in all the meat samples bought from open market. Taking into consideration the unhygienic environment in the meat samples the abattoirs, it is not surprising that meat sample should be contaminated. Contamination of meat according to Zlamalovia *et al*. (1980) occurs through the skin of the animal to the caresses at those sites at which slaughtering was carried out. Contamination also occurs in abattoirs due to inadequate facilities for slaughtering.

Sources of contamination also include the table on which the meat samples were displayed. The cracks on the tables serves as a suitable breeding place for the bacteria, the variation in average counts obtained range from 10^4-10^8 per g of fresh meat samples (Adams *et al.,* 1995) in their study concluded that 10^{-8} of *botulinium* toxin is for adult human. In view of the above statement, the range counts form 10^4 to 10^8 obtained in this work are capable of causing food poisoning. *Clostridium botulinium* was easily identified at least presumptively due to their colonial morphology on nutrient agar. They are β-haemolytic, however, the degree of pathogencity was not tested in any animal. From the literature cited, it could be seen that not much work has been done on *Clostridium botulinium* in this country, microbial examination of foods and meat in particular, may assist in the assessment of hygienic standards in our markets and give an insight on how best it can be improved.

CONCLUSION

On the basis of the data obtained from this investigation it is evident that the incidence of *Clostridium botulinium* in different fresh meat samples is high. The average count obtained ranged from 6.9×10^8 to 10.1×10^5 in fresh beef.

RECOMMENDATION

1. Most abattoirs make use of municipal sewerage systems. Where these facilities are not available, alternative arrangements must be made in consultation with officers of the department of water affairs contamination of natural streams and water sources should be avoided
2. There are basically two types of disposal system. They are the combined and separate

disposal system. The separate system is used for the ablution facilities utilizing a septic tank and a separate or common soil percolation system the combined disposal system for usually for Abattoir effluents. It incorporates the necessary solid/fat traps and sedimentation tank to remove solid. Effluent from this system can be discharged in a separate or the same common soil percolation system.

3. Septic maintenance tanks requires effective maintenance, when scum and sludge get discharged into the percolation trenches, the septic tank should be emptied and the silt and foam should be removed, if this is not due, the seepage system can be damaged perman-ently.

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