European Journal of Biology and Medical Science Research
Vol.5, No.6, pp.19-27, October 2017
Published by European Centre for Research Training and Development UK (www.eajournals.org)

BACTERIALOGICAL AND PARASITOLOGICAL ASSESSMENT OF FRESH MEAT MARKETED IN OWERRI, IMO STATE, NIGERIA

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ABSTRACT: *The bacteriological and parasitological assessment of some fresh meat marketed in Owerri, Nigeria was carried out using standard bacteriological and parasitological methods. The meat samples used for the study were flesh, towel, intestine and liver from goat, pork and chicken. There was no fungal and Salmonella - Shigella count in all the samples. The presence of Staphylococcus count was obtained in fresh goat meat and the viable bacterial counts ranged from 5.0× 10^5cfi/ml to 8.0× 10^6 cfu/ml while the total coliform counts ranged from 1.0× 10^5(cfu/ml to 4.0×10^6cfu/ml. The bacterial isolates obtained were: Staphylococcus aereus, Micrococcus species, Salmonella species, Shigella species, Corynebacterium species, Pseudomonas aureginosa, Bacillus species and Escherichia coli with Micrococcus species showing the highest occurrence. Among the parasites identified, Taenia spp had the highest occurrence. There is need for proper hygienic practices to be observed by the butchers in addition to beefing up the activities of Consumers Protection Council(CPC) to ensure the safety of meat available for public consumption.*

KEYWORDS: Fresh meat, Oocysts, Bacteria, Assessment, Parasites, Owerri

INTRODUCTION

Meat is an excellent source of high quality protein, fat, carbohydrate, vitamins and minerals and is delicious, palatable and easily digestible food item. This entire nutritional requirement can be met easily and efficiently if reasonable amount of meat is included in the diet. Meat is widely consumed by majority of people and it is one of the most perishable foods as its chemical composition is ideal for the growth of a wide range of spoilage and pathogenic bacteria. Contaminated raw meat is one of the main sources of food-borne illnesses (Bhandre *et al*, 2007). According to Clarence *et al* (2009), food-borne diseases result from ingestion of bacteria, toxins and/or cells produced by microorganisms present in foods. The intensity of signs and symptoms may vary with the amount of contaminated food ingested and the susceptibility of the individual to the toxin. Food security is therefore a complex issue, where animal proteins such as meat are generally regarded as high risk commodity in respect of pathogen contents, natural toxins and other possible contaminants and adulterants (Yousuf *et al*, 2008). Food-borne pathogens are the leading cause of illness and death in the developing countries such as Nigeria, causing billions of dollars in medical care and social costs (Fratamico *et al*, 2005). Public concern has risen due to numerous food scandals such as those surrounding bovine spongiform encephalopathy and foot and mouth disease epidemics (Hassan *et al*, 2010) and food-borne diseases have, therefore remained a substantial burden (Tauxe, 2002).

Print ISSN: ISSN 2053-406X, Online ISSN: ISSN 2053-4078

Pathogenic bacteria and other organisms are known to be most important food safety hazards associated with fermented meat products (Zakkpaa *et a*l, 2010). Food borne infections and illnesses are a major international health problem with consequent economic reduction (Okonko *et al*, 2008). It is the major cause of illness and death worldwide, and recognizing this, the World Health Organization (WHO) developed its Global Strategy for food Safety (Adak *et al*, 2009). In the developing world, food-borne infection leads to the death of many children and the resulting diarrheal disease can have long – term effects on children´s growth as well as their physical and cognitive development (Okonko *et al*, 2008). In the industrialized world, food borne infections cause considerable illness, heavily affecting health care systems (Adak *et al*, 2009).

The widespread distribution of meat therefore, makes the consequences of contamination with food poisoning microorganisms and disease causing parasites more serious. The state of health of animals prior to slaughtering and the prevailing circumstances in the slaughter houses can contribute to the quality of meat from such animals. In Nigeria particularly Owerri in Imo State, slaughtering of animals usually takes places under very unhygienic conditions. This coupled with the high ambient temperature, high humidity, shortage of portable water and poor handling practices, predisposes meat to microbial contamination and rapid deterioration. This work is therefore carried out to assess the prevalence of bacteria and parasites in fresh meat marketed in Owerri, Imo State, Nigeria.

MATERIALS AND METHODS

Study Area

This study was conducted in Owerri, Imo State, South – eastern Nigeria. Owerri lies between latitudes 5°29E and longitude 7°2E of the equator. It has an altitude of 152M and a population of about 400,000 people comprising of both civil servants and business men. Although with such high population of humans, there is no functional pipe borne water in the city, few bore holes can be seen in some places. Also there is a slaughter house located in Egbu in Owerri and another in a neighboring community, Mbaise where meat sold to the populace is obtained in addition to cold storage rooms for preservation of chicken parts, fish, amongst others.

Sterilization of materials

All the glass wares used for the experiment were sterilized in the laboratory autoclave at temperature of 121°C for 15 minutes at 15psi. The wire loop was sterilized over the burning flame till it was red hot, while glass spreader was sterilized by dipping into 70% ethanol and passing over Bunsen flame. The media used in this study: Nutrient agar; MacConkey agar, Simmon citrate agar, Triple sugar ion agar, etc were prepared according to manufacturers´ instructions and sterilized using the autoclave at a temperature of 121°C at 15psi for 15 minutes and were allowed to cool at 45°C and about 20 milliliters of this was poured into sterile Petri – dishes. The plates were allowed to cool and set for inoculation.

Fresh meat sample collection

Samples of raw meat were collected from different butchers´ open shops in Owerri markets, Imo State. The raw meat samples collected were: pork, beef, chicken and goat meat. Different parts of the above named samples collected were the intestines, the liver, the towel and the

Print ISSN: ISSN 2053-406X, Online ISSN: ISSN 2053-4078

muscles. The collected samples were immediately transported in insulated ice containers to the Microbiology laboratory of Federal University of Technology, Owerri for analysis.

Fresh meat sample Preparation

The method described by Dutta *et al* (2012) was adopted in the preparation of the meat samples. Ten grams of each of the solid samples was weighed and aseptically taken into a steeled jar containing 90ml sterile normal saline. It was homogenized with sterile blender at 3000rpm for 10minutes. 1ml aliquot of homogenized was transferred to a test tube containing 9ml of sterile water to make a ten – fold serial dilution and shaken vigorously. Sterile dilutions up to 10^{-5} were prepared for the microbiological analysis.

Microbiological Analysis

The microbiological quality and safety of the meat were assessed on the basis of: Total Viable Bacterial Count (TVBC); Total Coliform Count (TCC), Total Salmonella and Shigella Count (TSSC), Total Fungal Count(TFC) and Total Staphylococcus Count(TSC) using Nutrient agar, MacConkey agar, Mannitol Salt agar, Salmonella – Shigella agar and Salbouraud dextrose agar respectively. Diluted meat samples in normal saline were spread onto these agar plates and incubated at 37°C for 24 hours except for detection of fungi, which were incubated at 25°C for 5 days.

Microbial Plate Count

After incubation of the plates, the different colonies formed on the media were counted using the digital colony count. The total population of the colonies was expressed as colony forming unit per gram(CFU/g).

Purification and Preservation of isolates

After the various colony counts, bacterial isolates were picked with a wire loop based on their morphological appearances and were sub – cultured onto freshly prepared nutrient agar plates to obtain pure cultures. They were further incubated for 24 hours at 37°C after which pure cultures were stored in McConkey bottle in a refrigerator at 4°C. Fungal isolates were sub – cultured onto freshly prepared Sabouraud Dextrose medium.

Lactophenol Cotton Blue Staining Technique

The fungal isolates were identified by morphological characteristics on Sabouraud Dextrose agar(SDA) and microscopic examination using lactophenol cotton blue staining technique. Each of the fungal isolates were separately collected with a sterile wooden stick and teased out on a drop of lactophenol cotton blue stain on a clean glass slide. The wet mount preparations were then viewed under the microscope for branched and unbranched hyphae (Fawole and Oso, 2004).

Parasitological Analysis

Ten gram of the meat samples, that is pork (muscle, live, and intestine); cow (muscle, liver, intestine and towel), chicken and goat (liver, muscle, intestine and towel) respectively was weighed and aseptically taken into a sterile jar. It was homogenized with sterile blender at 300rpm for 10 minutes. 1ml each of the homogenate was smeared on different portions of the slide (I.e. two smears on each slide).One of the smears was stained with Lugol´s iodine while

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the other was unstained. The slide was viewed under the microscope for parasitic cysts, oocysts, eggs and larvae using ×10 and × 40 objective lens.

Statistical Analysis

Statistical analyses were carried out using GraphPad Prism 5.0 using one way analysis of variance (ANOVA) $P<0.05$. Data were expressed as Mean ± SEM.

Identification of bacterial isolates

The bacterial isolates from the plates were identified by gram staining and other biochemical tests such as catalase, oxidase, indole, sugar fermenting tests, according to Cheesebrough(2003).

RESULT

A total of 12 meat samples were collected from different meat vendors at Ekeonuwa market in Owerri, Imo State, Nigeria and analyzed bacteriologically and parasitologically. Table 1 shows the microbial load of meat based on total viable count, total coliform count, total Salmonella – Shigella count, total *Staphylococcus aureus* count and total fungal count.

Table 1: The variation of microbial loads of fresh meat samples.

Meat samples	TVBC(cfu/ml)	TCC (cfu/ml)	TSS(cfu/m)	TSAC (c/ml)	TFC (cfu/ml)
FGT	1.1×10^6	1.0×10^5	ND	2.0×10^6	ND
FGI	8.0×10^5	1.0×10^5	ND	ND	ND
FGL	6.0×10^5	1.0×10^5	ND	ND	ND
FGF	8.0×10^6	2.8×10^6	ND	ND	ND
FCI	1.4×10^6	9.0×10^5	4.0×10^5	ND	ND
FCL	2.3×10^6	1.0×10^5	ND	ND	ND
FCF	5.3×10^6	2.1×10^6	1.1×10^6	ND	ND
FCT	3.0×10^6	4.0×10^6	ND	ND	ND
FCK	1.4×10^6	ND	ND	ND	ND
FPI	9.0×10^5	2.2×10^6	ND	ND	ND
FPF	1.5×10^6	1.2×10^6	ND	ND	ND
FPL	5.0×10^5	1.0×10^6	ND	ND	ND

KEY: TVBC: Total viable bacteria count, TCC: Total coliform count, TSSC: Total Salmonella – Shigella count, Total *Staphylococcus aureus* count, TFC: Total fungal count, ND: Not detected, cfu/ml: Colony forming unit per milliliter, FGT: Fresh goat towel, FGI: Fresh goat intestine, FGL: Fresh goat liver, FGF: Fresh goat flesh, FCI: Fresh cow intestine, FCL: Fresh cow liver, FCF: Fresh cow flesh, FCT: Fresh cow towel, FCK: Fresh chicken , FPI: Fresh pork intestine, FPF: Fresh pork flesh, FPL: Fresh pork Liver.

Eight genera of bacteria were isolated from the fresh meat samples and they were identified as *Escherichia coli*, *Corynebacteria species*, *Staphylococcus species*, *Micrococcus species*,

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Shigella species, *Salmonella species*, *Pseudomonas species* and *Bacillus species* by comparing their morphological and biochemical characteristics with standard reference organisms.

The frequency and percentage incidence of the bacterial isolates from fresh meat marketed in Owerri showed that *Micrococcus spp* 11(44%) is the most predominant bacteria pathogen. This was followed by *Corynebacterium spp* 4(16%), *Escherichia coli* 3(12%) and finally *Salmonella spp*, *Staphyllococcus aureus* and *Pseudomonas auriginosa* had the same frequencies and percentage of I(4%)(Table 2).

Table 2: Prevalence of the Bacterial isolates from fresh meat samples.

Pathogens	Frequency	Percentage
Escherichia coli	3	12
Corynebacterium spp	4	16
Staphylococcus aureus	1	4
Micrococcus spp	11	44
Shigella spp	2	8
Salmonella spp	1	4
Pseudomonas auriginosa	1	4
Bacillus spp	2	8
Total	25	100

The result of the variation of microbial loads for chicken, goat, pork, cow meat samples showed that samples cultured between 24 hours in MacConkey agar plate had no significant effect on TCC when compared with the TVBC samples. The Salmonella – Shigella agar plate for chicken sample had no significant effect on the TSSC when compared with TVBC sample. The cow sample significantly (P<0.05) decreased in TSSC when compared with TVBC while that of goat and pork samples significantly (P<0.01) decreased in TSSC when compared with the TVBC samples. The Mannitol salt agar plate for chicken samples had no significant effect on TSAC samples when compared with TVBC samples, while that of goat, cow and pork samples significantly (P<0.001) decreased on TSAC when compared with the TVBC samples. The Sabouraud dextrose agar plate for chicken sample had no significant effect on TFC samples when compared with TVBC, while goat, cow and pork samples significantly (P< 0.001) decreased in TFC compared with the TVBC sample.

Table 3: Variation of Microbial loads of fresh meat samples

Samples	TVBC	TCC	TSSC	TSAC	TFC
GOAT	6.153± 0.2552	5.360± 0.3600^{ns}	0.00± 0.00$^{* **}$	1.325±1.325$^{** *}$	0.00± 0.00$^{* **}$
COW	6.423±0.1206	5.968±0.349^{ns}	2.910±1.682*	0.00± 0.00$^{* **}$	0.00± 0.00$^{* **}$
CHICKEN	0.00±0.00	0.00±0.00^{ns}	0.00±O.OO^{ns}	0.00±0.00^{ns}	0.00± 0.00 ^{ns}
PORK	5.937± 0.1387	6.150±0.0953^{ns}	0.00±0.00$^{* **}$	0.00± 0.00$^{* **}$	0.00± 0.00$^{* **}$

Values are expressed in Mean ± SEM, n = 4, *(P<0.05) vs TVBC.

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Parasite eggs, cysts, oocysts and larvae were also isplated from the fresh meat samples. 6 genera of parasites were isolated and were identified as follows: *Eschericia spp, Taenia spp, Entamoeba hystolytica, Cryptosporidium spp, Trichinella spiralis, Toxoplasama gondii* by comparing their morphological characteristics by the standard reference organisms Table 3.

The frequency and percentage incidence of the parasites eggs, cysts, oocysts, and larvae isolated from meat samples marketed in Owerri showed that *Taenia spp* 3(3.33%) was the most predominant parasite. This was followed by *Echinococcus spp* 2(2.22%), *Cryptosporidium spp, Trichinella gondi* and *Entameba histolytica* showed the same frequencies and percentage incidence of 1(11.1%)

Table 4: Frequency and Percentage Incidences of eggs, cysts, oocysts and larvae of parasites in fresh meat samples.

Parasitic pathogens	Frequency	Percentage(%)
Taenia spp	3	33.3
Entamoeba *hystolytica*	1	11.1
Cryptosporidium spp	1	11.1
Trichnella spiralis	1	11.1
Toxoxoplasls gondi	1	11.1
Echinococcus spp	2	22.2
Total	9	100

DISCUSSION

Microbial population that comes in contact with meat during slaughtering, dressing and processing presents a challenging problem to the meat industry. The presence of bacteria and parasites in meat has been widely reported from different parts of the world. However, pathogenic and non – pathogenic bacteria have their standard load specifications and non-pathogenic microorganism when in amounts above the recommended standards may pose some health hazards (Nanachi *et al*, 2014).

The presence of these organisms on meat parts could be attributed to the fact that meat contains an abundant nutrients required for the growth of bacteria in adequate quantity. The high total viable count recorded in this study showed the diversity (differences in form or species) in fresh meat marketed in Owerri, condition of the market and hygienic practice employed by meat sellers and butchers. The higher microbial load reflects the unhygienic and improper handling of animals during slaughter, dressing and evisceration. The usual practice of washing the meat with the same water in which intestine or offal has been washed was considered as one of the possible reasons for increased microbial counts of the meat samples. Also, some of the butchers might not be enlightened and therefore could lack proper personal hygiene, which might make it difficult to handle samples in a hygienic manner in the course of slaughtering and retailing (Nnachi *et al*, 2014).

On assessing the bacterial contamination, the result obtained is on the high side. This is an indication of recontamination in food handling and hygiene techniques (Clarence *et al*, 2009). Similar values were reported by Yousuf *et al* (2008). A total of 25 bacterial isolates comprising of 8 genera were detected including *Eschericia coli, Corynebacterium spp, Staphylococcus aureus, Micrococcus spp, Shigella spp, Salmonella spp, Pseudomonas auroginosa* and *Bacillus*

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spp by comparing their morphological and biochemical characteristics with standard reference organism. The parasites found on the fresh meat samples are identified as *Echinococcus spp*, *Taenia spp*, *Entamoeba hystolytica*, *Cryptosporidium spp*, *Trichinella spiralis* and *Toxoplasma gondii*. The parasites were found as cysts, oocysts, eggs and morphology was compared to standard reference organisms.

The total viable bacterial count showed the presence of viable bacteria on each sample and the total coliform count indicated that most of the organisms isolated are coliforms. From the result, the flesh and the intestine of different samples showed the highest microbial loads. The statistical analysis of the result showed that goat and pork samples for TCC had no significant effect while the TSSC, TSAC, and TFC significantly ($P< 0.001$) decreased when compared to TVBC. Cow samples for TCC had no significant effect, TSS significantly ($P < 0.05$) decreased and TSAC and TFC significantly ($P<0.001$). After 48 hours incubation of the chicken samples there was no significance on TCC, TSSC, TSAC and TFC when compared to the TVBC. The high coliform count suggests possible animal contamination (Raji, 2006). Moreover, the faecal coliforms as *Eschericia coli* are generally considered as indicators of faecal contamination from warm blooded animals (Yousuf et al, 2008). *E. coli* which is normal flora of the human and animal intestine has been identified as a leading cause of food borne illness all over the world. Gram negative bacteria such as *E. coli* are known to cause urinary tract infection and diarrhea in young children (Pelczar *et al*, 1999). *E. coli* and *E. coli* 0157: H7 strain has previously been isolated from meat samples (Hussein, 2007). Micrococcus was the most predominant bacteria species in this study. It is the normal floral of humans but can become an opportunistic pathogen. The species *Micrococcus liteus* has been reported to cause recurrent bacteriaemia, septic shock, intracranial abscess, pneumonia, septic arthritis, endocarditis and meningitis.

Salmonella species such as *Salmonella typhi* is a bacterium that causes typhoid fever (enteric fever), an acute, life threatening febrile illness (CDC, 2013). The disease is a cause of concern and a major public health problem in developing countries (Asia and Africa); especially in Nigeria due to poor sanitary conditions and lack of or inadequate portable water (Ibekwe *et al*, 2008). It is mainly transmitted through food or drink or water contaminated with urine or faeces of infected people or a chronic carrier (CDC, 2012. Ibekwe *et al*, 2008). Since 1987, *Salmonella enteritis* has been one of most frequently isolated Salmonellae associated with foodborne disease outbreaks, which have been linked to consumption of chicken, eggs and food that contain this organism and it presents an interesting challenge from an epidemiological perspective (Zheng *et al*, 2007). Infection with non typhoid Salmonella have increased during the last 3-4 decades, and although a decrease has been reported over the last decade, Salmonella infections continue to be a major public health concern in many countries. These zoonotic organism and the infections are generally foodborne (Helms *et al*, 2005). The reservoir of zoonotic Salmonella is food animals, and the main sources of infections in industrialized countries are animal - derived products, notably fresh meat products and eggs in different sectors of food animal production(swine, broiler chickens and particularly layer hens) has been suggested as the most important cause of this increase (Helms *et al*, 2005).

Corynebacterium diphtheriae is the pathogen causing diphtheria and it may be transmitted through food. This pathogen causes severe inflammation of the throat and other portions of the upper respiratory tract (Brock and Madigan, 1991).

The presence of parasitic eggs, cysts, oocysts and larvae in the fresh meat samples can be attributed to poor handling practice during slaughter and washing of animal carcass. Meat

Print ISSN: ISSN 2053-406X, Online ISSN: ISSN 2053-4078

producing animals may pick up parasitic cysts in the course of grazing and some of the animals serve as intermediate host to these parasites. In this study, *Taenia spp* showed the highest frequency. This could be as a result of faecal materials the animals and the meat come in contact with and it calls for immediate attention of public health agencies in order to reduce the risk of tape worm infestation. From the result obtained from this study, consumption of fresh meat marketed in Owerri could result to some foodborne diseases such as salmonellosis, toxoplasmosis, trichinellosis, taeniasis. Therefore, there is need for further research study that will aim at surveying the parasitic load among the butchers because they might be main source of distribution and not necessarily as a result their unhygienic practices

Acknowledgement

We wish to express our immense gratitude to entire technologists in Microbiology Laboratory of Federal University of Technology, Owerri especially Deaconess Mrs Ngozi Eze, Sister Chinwe for assistance in the analysis of the sample and identification of the organisms.

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