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Analysis of Microbial Contamination of Slaughterhouses in Owo, Ondo State, Nigeria

EGBEBI, Adeola O[1]., OYAMA, Marshanty O[2]., ADEDEJI, Ebenezer[3]

Department of Food Microbiology[1] adeola@yahoo.com +234-806-7343-382

Department of Medical Microbiology[2]graciousdel@gmail.com +234-806-3242-615

Department of Biochemistry[3] lincsbog@gmail.com +234-7039-406-293

Afe Babalola University Ado-Ekiti, Ekiti State Nigeria

ABSTRACT

Twenty samples of meat from Owo, Ondo State, South-West Nigeria, were used for this study. The microbiological quality of the meat was assessed and the total viable count of bacteria was deduced as ranging between 2.9×10^3 cfu/g to 5.0×10^3 cfu/g and 2.5×10^3 cfu/g to 5.4×10^3 cfu/g on nutrient agar and chocolate agar respectively. Six genera were isolated, characterized and identified; namely, *Staphylococcus, Micrococcus, Proteus, Bacillus, Escherichia coli and Streptococcus.* The most prevalent microorganism observed was *Staphylococcus.* Microbial contamination of meat results in spoilage and in some cases pathogenicity. Due to poor hygiene of the meat handlers, contamination often occurs at slaughterhouses. The result revealed that the hygiene conditions of meat from the slaughterhouses used in this study were below acceptable standards for human consumption.

Keywords: Meat contamination, Slaughterhouses, Microbial analysis, Isolates.

INTRODUCTION

Meat is a widely consumed food item globally (Lawrie and Ledward, 2006). The consumption of meat dates back to prehistoric times (Wildman and Medeiros, 2000), when animals were hunted for meat and hide (Wildman and Medeiros, 2000). Civilization has however led to the domestication of animals which has allowed production of meat on an industrial scale. Meat mostly refers to skeletal muscles and associated fat, lungs, livers and a variety of other internal organs (Hammer, 2007). The flesh and tissues of aquatic animals are also considered as meat (Lawrie and Ledward, 2006). Meat is mainly composed of proteins, fats and water. It contains approximately 75 percent water, 19 percent protein, 2.5 percent intramuscular fat, 1.2 percent carbohydrates and 2.3 percent other soluble non-protein substances such as minerals, vitamins and cholesterol (Lawrie and Ledward, 2006). Although the nutritional composition varies between animal species and breed, meat is generally rich in proteins which contain all the essential amino acids (Schurgers and Vermeer, 2000).

The constituents of meat that makes it nutritious also causes its susceptibility to microbial infection (Kołożyn-Krajewska and Dolatowski, 2012). Meat contains amounts of carbohydrate which the bacteria can utilize for energy (Thomas, 2012). In addition, meat contains abundant amounts of proteins which putrefactive bacteria can metabolize to promote growth (Thomas, 2012). Furthermore, meat has a high water content that is an essential factor for bacterial growth, and its pH of 5.7 is within the acceptable range of most bacteria (Thomas, 2012). Microbial contamination of meat leads to spoilage resulting in economic losses (Komba *et al*., 2012). Typically, meat of healthy animals is sterile, however, contamination may occur during the various stages of slaughter, preparation and transportation (Ercolin *et al*., 2006). A variety of microbes can contaminate meat, although different species may become dominant depending on factors that include pH, oxygen, water availability and storage temperatures (Ercolin *et al*.,

2006; Weigand *et al*., 2007). Aside from spoilage, infection of meat can be pathogenic to the consumer. The level of pathogenicity differs according to the causative microorganism. The slaughterhouse, also known as an abattoir, is the site where the meat is prepared, cut into smaller pieces and then transported to retailers. The average Nigerian slaughterhouse may contain a variety of microbes partly due to illiteracy and lack of hygiene protocols. Therefore, microbial analysis is essential to identify and study the level of contamination by microbes in Nigerian slaughterhouses. This study aims to isolate, characterize and identify the microbial contaminants in meat from five separate slaughterhouses in Owo, Ondo state, Nigeria.

MATERIALS AND METHODS

Meat Sample Collection and Preparation

20g of raw meat samples (beef) were collected from each of the 5 slaughter houses. The samples were obtained in the early hours of the morning and within 8 hours post-slaughter in order to minimize the level of microbial contamination due to environmental temperatures. 10g of collected meat samples were weighed and transferred to sterile flasks containing 90ml of distilled water. Samples were homogenized using pestle and mortar under aseptic conditions.

Microbiological Analysis

Serial dilution of the meat sample was done by sterilizing six test tubes which were labeled 10^{-1} to 10^{-6} and kept in a test tube rack; 9ml of distilled water was then measured into the six test tubes. 1ml of diluted meat sample was introduced into the first test tube labelled 10^{-1} and mixed thoroughly, 1ml was taken from the test tube and transferred to the second test tube labelled 10^{-2}. This is continued until the 10^{-6} dilution is obtained. The 10^{-4}, 10^{-5} and 10^{-6} diluted meat samples were inoculated on nutrient agar (NA) and chocolate agar and incubated at 37^{o} C for 18-24 hours.

Identification of bacteria was carried out using standard microbiological/biochemical methods. Tests including Gram staining, oxidase, citrate, indole, motility, carbohydrate fermentation tests (glucose, sucrose, and lactose), catalase, methyl red and Voges Proskauer test were conducted according to Cheesbrough, 2009.

Nutrient agar and chocolate agar plates were streaked using the 10^{-4} serial dilution. Samples A-E were obtained from five separate slaughter houses.

RESULTS

Table 1. Total viable count (cfu/g) and morphology of the isolates

Samples	NA count (10^{-4})	CA count (10^{-4})	Colour	Shape	Edge
A	3.2×10^3	4.2×10^3	Milk/Yellow	Circular	Irregular
B	2.9×10^3	2.5×10^3	Milk	Circular	Irregular
C	5.0×10^3	5.4×10^3	Milk	Circular	Irregular
D	4.4×10^3	3.2×10^3	Milk	Circular	Irregular
E	4.8×10^3	4.3×10^3	Milk	Circular	Irregular

Table 2. Biochemical identification of isolates

Isolates	Gram Stain	Gram Shape	Motility Test	Catalase Test	Citrate Test	Indole Test	Oxidase Test	Methyl Red	Voges Proskauer Test	Glucose Test	Lactose Test	Sucrose Test	Probable Microorganisms
S1	+ve	Cocci	NM	+ve	-ve	-ve	-ve	+ve	+ve	A	A	A	*Staphylococcus*
S2	+ve	Cocci	NM	+ve	-ve	-ve	+ve	-ve	-ve	-ve	A	-ve	*Micrococcus*
S3	+ve	Cocci	NM	-ve	+ve	+ve	-ve	+ve	+ve	A	A	A	*Streptococcus*
S4	+ve	Cocci	NM	-ve	+ve	+ve	-ve	+ve	+ve	A	A	A	*Streptococcus*
S5	+ve	Cocci	NM	+ve	-ve	-ve	-ve	+ve	+ve	A	A	A	*Staphylococcus*
S6	+ve	Rod	M	+ve	+ve	-ve	+ve	-ve	+ve	A	A	A	*Bacillus*
S7	-ve	Cocci	M	+ve	-ve	-ve	-ve	+ve	-ve	AG	-ve	AG	*Proteus*
S8	+ve	Cocci	NM	+ve	-ve	-ve	-ve	+ve	+ve	A	A	A	*Staphylococcus*
S9	+ve	Cocci	NM	+ve	-ve	-ve	+ve	-ve	-ve	-ve	A	-ve	*Micrococcus*
S10	+ve	Cocci	NM	+ve	-ve	-ve	-ve	+ve	+ve	A	A	A	*Staphylococcus*
S11	-ve	Cocci	M	+ve	-ve	-ve	-ve	+ve	-ve	AG	-ve	AG	*Proteus*
S12	+ve	Cocci	NM	+ve	-ve	-ve	+ve	-ve	-ve	-ve	A	-ve	*Micrococcus*
S13	+ve	Cocci	NM	+ve	-ve	-ve	-ve	+ve	+ve	A	A	A	*Staphylococcus*
S14	-ve	Cocci	M	+ve	-ve	-ve	-ve	+ve	-ve	AG	-ve	AG	*Proteus*
S15	+ve	Cocci	NM	+ve	-ve	-ve	-ve	+ve	+ve	A	A	A	*Staphylococcus*
S16	-ve	Rod	M	+ve	-ve	+ve	-ve	+ve	-ve	AG	AG	-ve	*Escherichia coli*
S17	+ve	Cocci	NM	+ve	-ve	-ve	-ve	+ve	+ve	A	A	A	*Staphylococcus*
S18	+ve	Cocci	NM	-ve	+ve	+ve	-ve	+ve	+ve	A	A	A	*Streptococcus*
S19	+ve	Cocci	NM	+ve	-ve	-ve	+ve	-ve	-ve	-ve	A	-ve	*Micrococcus*
S20	+ve	Cocci	M	+ve	-ve	-ve	-ve	+ve	-ve	AG	-ve	AG	*Proteus*
S21	+ve	Rod	M	+ve	-ve	+ve	-ve	+ve	-ve	AG	AG	-ve	*Escherichia coli*
S22	+ve	Cocci	NM	+ve	-ve	-ve	-ve	+ve	+ve	A	A	A	*Staphylococcus*
S23	+ve	Cocci	NM	-ve	+ve	+ve	-ve	+ve	+ve	A	A	A	*Streptococcus*
S24	+ve	Cocci	M	+ve	-ve	-ve	-ve	+ve	-ve	AG	-ve	AG	*Proteus*
S25	+ve	Cocci	NM	+ve	+ve	-ve	-ve	+ve	+ve	A	A	A	*Staphylococcus*

KEY: A= Acid Production; AG= Acid and Gas Production; -ve= Negative; +ve= Positive; M= Motile; NM= Non-Motile

Table 3. Frequency of occurrence of microbes

Organisms	Frequency of Occurrence	Percentage of Occurrence (%)
Staphylococcus	9	36
Streptococcus	4	16
Micrococcus	4	16
Proteus	5	20
Bacillus	1	4
Escherichia coli	2	8
Total	25	

DISCUSSION

Bacteria that commonly infect meat include *Salmonella spp.*, *Shigella spp.*, *Escherichia coli*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Clostridium perfringens*, *Bacillus spp* and S*treptococci spp* (Lawrie and Ledward, 2006; Pennacchia *et al*., 2011). Certain microorganisms like *Staphylococcus, Escherichia, Streptococcus, Micrococcus*, and *Bacillus* are pathogens, while others like *Pseudomonas spp.*, *Enterobacteriaceae*, and *Brochothrix thermosphacta* cause spoilage and therefore reduction in the shelf-life of the meat (Pennacchia *et al*., 2011). Spoilage can be halted by lowering the temperature of the meat (< 5-7.2 C) (Samelis and Blackburn, 2006; Frazier and Westhoff, 2008). The reduced temperature causes reduction in enzymatic activity and consequently inhibition of the growth of microorganisms (Frazier and Westhoff, 2008). Infection by pathogenic strains, however, is not affected by freezing (Doyle, 2002). It has been reported that bacteria are prevalent on almost all types of environment (Biers *et al*., 2009) which includes the environment of slaughterhouses. Each of the microorganism isolated can cause urinary tract infection. This could mean that unwashed hands after unsanitary practices may be a source of contamination. *Micrococcus* and *Bacillus* are part of the normal flora of the soil (Gill *et al*., 2000). *Bacillus* produces toxins that cause food poisoning (Gill *et al*., 2000) whereas *Micrococcus* is an opportunistic pathogen (Nuňez, 2014). The *Staphylococcus* contamination may be due to handlers touching the meat without gloves or as a result of aerosols from sneezing, talking or coughing. *Staphylococcus* is part of the skin and nose flora and it is also found in soil and air (Gilbert and Harrison, 2001). The most prevalent among the isolates is *Staphylococcus* which is known to be a Gram-positive and catalase positive cocci (Becker *et al*., 2004). *Staphylococcus* is an opportunistic pathogen that could cause life-threatening diseases. Most *Staphylococcus* strains also secrete enterotoxins, superantigen toxic shock syndrome toxins, and exfoliative toxins which are associated with pathogenicity (Becker *et al*., 2004).

The source of the *Micrococcu*s contamination may be the skin of the dead animal or the handlers because skins of warm-blooded animals are reservoirs for *Micrococcus* (Kocur *et al*., 2006). Meat is prone to contamination by *Escherichia coli* (Fraizer and Westhoff, 2008). Pathogenicity of *Escherichia coli* depends on its class (Pandey, 2014). Enteroinvasive, enterohemorrhagic, enterotoxigenic, enteroaggregative, and enteropathogenic *E. coli* have been described and are reportedly prevalent in developing countries due to large population and poor hygiene (Trabulsi *et al*., 2002; Nataro *et al*., 2007; Todar, 2008). A large amount of *E. coli* is required for pathogenicity, for example 10^6 microorganisms of enteropathogenic *E. coli* are required to cause illness (Todar, 2008). In certain developed countries, the recommended medium for qualitative analysis of food contaminated by coliforms such as *E. coli* is lauryl sulfate tryptose (LST) broth (Downes and Ito, 2001). The sodium lauryl sulfate content of LST is a selective inhibitory agent which ensures that only coliforms would grow on the medium (De Boer, 2014). Another advantage of LST broth is that an indole test can be conducted directly on the medium. Generally, presence of *Escherichia* is regarded as an indicator of the unsanitary quality of food (Pandey,

2014). Different strains of *Streptococcus* cause serious diseases that are communicable (Brock, 2000). Air-borne contamination of the meat by *Streptococcus* may occur because the bacteria can be transmitted by respiratory droplets (Bessen, 2009). Meat handlers with strep throat may be another source of contamination. *Streptococcus* can also be transmitted by contact (Bessen, 2009) which can be prevented by the wearing of gloves. Handlers with illnesses should be excluded from work until recuperation. *Proteus spp*. is present in the flora of the gastrointestinal tract (GIT) of animals, soil and polluted water. Outside the GIT, *Proteus spp*. cause infectious diseases that occur worldwide (Abbott, 2007).

The meat used in this study have significant amounts of bacteria ($>10^5$ cfu) such that consumption is not advisable. The high amount of bacteria suggests that contamination of the meat may have occurred outside the slaughterhouse.

CONCLUSION

Meat constitutes a great source of protein which is essential for body building and repair of worn-out tissues in humans hence, the sterility of meat is very important. Care and adequate precautions must be taken to prevent contamination and spoilage by microorganisms. The microbes isolated demonstrates that international standards of hygiene are not practiced in most Nigerian slaughterhouses. Aseptic techniques should be properly employed in slaughterhouses especially in the process of handling meat so that microbial load of meat can be reduced for safe consumption. In addition, consumers should ensure that purchased meat is properly cooked so that contaminants can be removed therefore preventing food-borne infections.

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