PREVALENCE OF LISTERIA SPECIE IN LOCALLY PROCESSED MILK IN NASARAWA STATE, NIGERIA

BY

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DECLARATION

I hereby declare that the work in this thesis "PREVALENCE OF LISTERIA SPECIE IN LOCALLY PROCESSED MILK IN NASARAWA STATE, NIGERIA" was carried by me in the in the Department of Community Medicine, Faculty of Medicine under the supervision of Dr Mohammed Bello of the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria, Nigeria. The information derived from the literatures has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma in any educational institution or university.

Abdullahi Muhammad Musa _____ _____

Signature Date

CERTFICATION

This dissertation titled: "PREVALENCE OF LISTERIA SPECIES IN LOCALLY PROCESSED MILK IN NASARAWA STATE, NIGERIA" by Abdullahi Muhammad MUSA meet the regulations governing the award of the Degree of Masters of Public Health (Veterinary Epidemiology) of ABU Zaria, and is approved for its contribution to knowledge and literacy presentation.

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DEDICATION

I dedicate this work to Almighty God for protecting and guiding me all my life. I also dedicate this work in memory of my late friends and grandparents Bajjabo Ango (Abdullahi) and Hauwa Akajo (Dado).

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SUMMARY

Listeriosis is an emerging zoonoses requiring continuous surveillance in order to prevent outbreaks in humans. The disease can develop after consumption of a wide range of foods such as meat products, dairy products especially in ripened cheeses, delicatessen products, fish and seafood products and vegetables. It is a rare disease with high hospitalization rates for those who become ill and high mortality rate.

Consumption of locally processed milk is becoming more popular among low and medium income earners in Nasarawa state and checking for possible disease agents that could be ingested in locally processed milk is essential for prevention. This research estimates the risks of listeriosis associated with consumption of milk products in Nasarawa State and establishes science-based intervention strategies aimed at reducing these risks. The study determines association between socio-demographic characteristics of milk vendors and the occurrence of *Listeria species* in locally processed milk in Nasarawa State, Nigeria.

Four hundred and twenty (420) milk samples were collected from vendors after administering questionnaire on milk processing practices.

Milk samples were processed in the laboratory by culture and isolation, biochemical characterisation, agglutination and Microbact test. Antibiotic sensitivity was also conducted on samples that tested positive for listeria.

The socio-demographic characteristics of respondents showed that majoritywere within the age group 20-24 years, they were mostly females (92.4%), traders (53.1%), Muslims (99.5%); had no formal education (67.4%). Furthermore, majority of them earned between N6,000-N18,000 monthly (52.1%). Out of 420 samples collected and tested, 10.2% (43 samples) were positive for *listeria* through culture and isolation. *Listeria* species found in this

study are 100% susceptible to enrofloxacin and doxycycline; 77.8% to kanamycin but resistant to Penicillin (93.7%) and Amoxicillin (80%) and Sulphamethazole/trimethoprim (60%). Borrowing/sharing of milk processing equipment and refrigeration/addition of ice to processed milk are factors significantly found to be associated with presence of *listeria* in milk sample (with odd ratio of 3.07 and 5.54 respectively). Conversely, training on food safety and supervision during milk processing were found to be significantly protective from *listeria* in milk sample (with odd ratio of 0.14 and 0.18 respectively).

Processors of locally processed milk should be encouraged to undergo food safety training, observe strict supervision during processing of milk and be discouraged from the practice of adding ice to milk and borrowing or sharing of equipment.

CHAPTER ONE

INTRODUCTION

1.1. Background

Listeriosis caused by *Listeria* specie is an emerging zoonotic disease requiring continuous surveillance in order to prevent outbreaks in humans. The invasive form of listeriosis is observed primarily in high-risk population groups such as the elderly, individuals with lowered immunity, pregnant women and newborns. Ready-to-eat foods are the most important source of both sporadic cases and outbreaks of listeriosis in humans.[1] The disease can develop after consumption of a wide range of foods such as meat products [2], dairy products especially in ripened cheeses,[3] delicatessen products[4] fish and seafood products[5] and vegetables.[6] Animals are also susceptible to listeriosis but can carry *Listeria monocytogenes* asymptomatically.

First confirmed human case of listeriosis was reported in Nigeria in 1982,[7] food borne listeriosis has not been much documented. The organism causes infection (listeriosis) in man and in different animal species such as cattle, sheep and goats.[8] People at highest risk included the elderly, the fetuses of pregnant women, and the immunosuppressed. It is unique among foodborne pathogens since its incubation time is about seven days. Human infection is therefore more devastating in immunosuppressed individuals, pregnant women, infants, geriatrics, HIV-AIDS patients etc.[9] *Listeria monocytogenes* has been reported to be pathogenic for humans and animals [10;11]. It has recently been implicated in several food-borne epidemic listeriosis.[12] *Listeria monocytogenes* principally causes meningitides, encephalitis, abortion; in adult animals, septicaemia; in neonates,[13,14] ophthalmitis which is associated with bacterial contamination of the cornea from the feed source. *Listeria monocytogenes* has the potential to be present in all raw foods. Cooked foods can also be

contaminated, usually as the result of post-process contamination. The pathogen has been isolated from a very wide range of processed foods including pâtés, milk, soft cheeses, ice cream, ready-to-eat cooked and fermented meats, smoked and lightly processed fish products and other seafood. *Listeria monocytogenes* is usually found only in low numbers (less than 10/g) in foods. However, products such as pâtés and soft cheese have occasionally been found to contain levels of 10,000/g or more.

The contamination of foods could be due to many factors. One of the possible factors is via cross-contamination after the foods were cooked; although sometimes, the possibility is that the cooking process is not sufficient to inactivate these tough bacteria.

Milk is processed locally by fermenting milk product and acidification of milk, which results in changes in the physical and chemical properties of the milk (now yoghurt).[15] Recently, "yoghurt markers" have been urged to produce yoghurt under controlled conditions[16]. Several microorganisms have been incriminated to be present in raw milk and milk products (cheese and yoghurt) that are of public health importance. They are capable of causing food borne illnesses after ingestion of contaminated milk of these products, these organisms range from viruses, rickettsia, bacteria, protozoan and parasitic organisms to their toxins.[17] *Listeria monocytogenes*, *Escherichia coli* O157:H7 and *Salmonella* spp. are among the most dangerous food borne bacterial pathogens in terms of human health and disease[18]. *E. coli* and *L. monocytogenes* are important indicators of contamination of milk and milk products.

1.2. Problem Statement

Foods can become contaminated with *Listeria monocytogenes* along the continuum from farm to fork, in the produce rowing environment, during processing, or during handling and preparation in retail establishments and consumers' kitchens.[19] Listeriosis is a rare disease with high hospitalization rates for those who become ill and high mortality rate, causing

about 43 percent of the food poisoning deaths. *Listeria monocytogenes* is a leading cause of death attributed to food borne bacterial pathogens. It grows at refrigeration temperature (of 0-4^{o}C), which is of great concern especially with the emergence of the new generation of minimally processed foods relying only on refrigeration and intrinsic parameters as proliferation of undesirable microorganisms.[20]

This organism has been associated with a number of serious foodborne outbreaks and recalls of fresh produce [21; 22].In Nigeria, *Listeria* has been isolated in many ready to eat foods like raw milk, cheese, raw meat, roasted fish and vegetables. Good hygienic practices in milk processing to control food-borne diseases, including *Listeria* is not being strictly observed thus exposing milk consumers to listeriosis.[23]

This research will be relevant for estimating the risks of listeriosis associated with consumption of milk products in Nasarawa State and for establishing science-based intervention strategies aimed at reducing these risks, especially for pregnant women, children and immunocompromised individuals.

1.3. Justificationor Rational for the study

Consumption of locally processed milk is becoming more popular among low and medium income earners in Nasarawa state and checking for possible disease agents that could be ingested in locally processed milk is good so that people become aware of health risk involved.

Listeria has been found to be a contaminant of most ready-to-eat-food (RTE) - like locally processed milk and other RTEs in Nigeria. There has been documented evidence of this in some states in Nigeria including Plateau, Benue and Sokoto among others. In Nassarawa, the prevalence of *Listeria* in meat has been documented but supportive evidence of the presence of listeria in milk remains limited. World Health Organization (WHO) published in the

Lancet Infectious Diseases that an urgent effort is needed to fill in information on *Listeria* infections in developing countries, as countries accounting for 48 percent of the world's population do not report Listeria illnesses: while most *Listeria* cases are reported in high-income countries, while cases are much more likely to go unreported in developing countries

1.5. Research Questions

1. What is the prevalence and characteristics of *Listeria* in locally processed milk samples in Nasarawa State?
2. Which characteristics and practices of local milk sellers are risk factors for the presence of *Listeria* in locally processed milk in Nasarawa State?

1.6. Objectives:

1.6.1. General objectives:

To determine association between socio-demographic characteristics of milk vendors and the occurrence of *Listeria species*in locally processed milk in Nasarawa State, Nigeria.

1.6.2. Specific objectives:

1. To determine the proportion of locally processed milk samples testing positive for *Listeria* specie in Nasarawa State, Nigeria.
2. To identify different species of *Listeria* found in locally processed milk in Nasarawa State, Nigeria.
3. To determine the socio-demographic characteristics of locally processed milk vendors
4. To identify the practices and characteristics that could be risk factors of *listeria* contamination of locally processed milk in Nasarawa State.
5. To determine the susceptibility of listeria found in milk in Nasarawa state to commonly-used antibiotics.

CHAPTER TWO

LITERATURE REVIEW

2.1. History of *Listeria*Species

Listeriosis also called tiger river disease, silage sickness, leukocytosis, cheese sickness. Interestingly, Gustav Hülphers first discovered the bacteria and named it *Bacillus hepatltis*. However, he did not preserve his bacterial strains, and the bacterium was later recognized as *Listeria monocytogenes*[24] named after Joseph Lister. Later, it was identified as bacteria that cause of monocytosis in rabbit and guinea pig.[25] *Listeria monocytogenes*, the causative agent of listeriosis, was discovered and named by E.G.D. Murray and J. Pirie but named after Joseph Lister.[26]

It is a Gram positive, non-spore forming, aerobic to facultative anaerobic psychrotrophic bacteria with low C and G content[27] and high mortality rate.[28] It has the ability to cause severe diseases in humans and animals; and is ubiquitous, found in foods, water, soil, vegetables as well as animals and humans.[29,30]

In the early 1980's listeriosis was classified under anthropozoonoses, which was changed to amphixenoses in the late 1990s. The genus *Listeria* belongs to the Clostridium sub-branch, together with Staphylococcus, streptococcus, Lactobacillusand Bronchothrix. This phylogenetic position of Listeria is consistent with its low G+C DNA content (36-42%). As Gram-positive, non- spore forming, catalase-positive rods, the genus *Listeria* was classified in the family Corynebacteriaceae through the seventh edition of Bergey's Manual. 16S rRNA cataloging studies demonstrated that *Listeria monocytogenes* was a distinct taxon within the ***Lactobacillus-Bacillus*** branch of the bacterial phylogeny. In 2001, the Family **Listeriaceae** was created within the expanding Order **Bacillales**, which also includes Staphylococcaceae,

Bacillaceae and others. Within this phylogeny there are six species of *Listeria*. The only other genus in the family is *Brochothrix*.[31]

It lacks its true definition of Zoonotic disease because of involvement of an inanimate reservoir (food and Environment). Up to 1961, *Listeria monocytogenes* was regarded as the one and only species of the genus *Listeria* but later other species have been identified. Listeriosis is of great public health concern because of its high mortality (20 to 30%) and its common source epidemic potential. Animals may be diseased or asymptomatic carriers of *Listeria monocytogenes* shedding the organism in their faeces. Thus, earlier it was believed that *Listeria monocytogenes* was causing disease by direct transmission from animals to humans. Today it is generally considered that ingestion is the main mode of infection with food and water being the main vehicles of infection. *Listeria monocytogenes* causes one of the most severe forms of foodborne infection even though it is a relatively rare disease. The overall mortality rate associated with the disease is 30 %, but it can be as high as 40% in vulnerable individuals; hospitalisation is more likely than for other common foodborne infections. Those most at risk of acquiring the disease are pregnant women (20 times greater risk than healthy individuals), the elderly and the immuno-compromised, although healthy individuals can develop listeriosis, particularly if the food is heavily contaminated.[27,31]

In 2010, Listeria monocytogenes was estimated to infect 23,150 people worldwide. It killed 5,463 of them, or 23.6 percent, according to a new study by World Health Organization and a new study World Health Organization (WHO) published in The Lancet Infectious Diseases reported that*Listeria* infections in developing countries, as countries accounting for 48 percent of the world's population do not report Listeria illnesses.Listeria caused the highest burden on quality of life in Latin American regions. The least affected region was Eastern Europe, stretching from Poland to Turkey. Other highly affected areas included Southeast Asia, Africa, Polynesia and India.

Listeria contamination has been reported in many states of Nigeria

2.2. Causative Agent

Listeria monocytogenes is a Gram-positive, facultative anaerobic, non-spore-forming, rod-shaped bacterium that belongs to the genus *Listeria* and family *Listeriaceae*[32] which uses flagella for movement. It is catalase-positive and oxidase-negative, and expresses a beta hemolysin, which causes destruction of red blood cells. Other members of the genus include *Listeria ivanovii*, *Listeria innocua*, *Listeria welshimeri*, *Listeria seeligeri* and *Listeria grayi*. Although the first four of these have all been implicated in human infection nearly all cases of *Listeria* infection are caused by *Listeria monocytogenes*. At least 13 different serotypes of *Listeria monocytogenes* are known. All can cause the disease listeriosis in humans, but most cases are caused by serotypes 1/2a, 1/2b and 4b. The majority of reported foodborne outbreaks have been caused by serotype 4b.[32,33]

Listeriamonocytogenes is the causative agent of listeriosis and is transmitted to susceptible individuals via consumption of contaminated foods.[34] The major population group at risk for invasive listeriosis are the immune-compromised such as pregnant women, new born babies, elderly people and AIDS patients.[35]

Although *Listeria monocytogenes* is actively motile by means of peritrichous flagella at room temperature (20−25°C), the organism does not synthesize flagella at body temperatures (37°C)[36,37] (Todar, 2008). It tolerates environmental stress.

Listeria monocytogenes is quite hardy and resists deleterious effects of freezing, drying and heat remarkably well for a bacterium that does not form spore.[37] The bacteria organism is ubiquitous in nature - widely present in plant, soil, sediments and surface water samples, sewage, human and animal faeces,[38] animal products such as raw milk and raw meat[39] due

to unsanitary practices during milking and slaughtering,[40] roasted fish,[41] raw and decaying vegetables,[31] plants, feeds and silage, as well as from the environment in food industry plants and from foods.[21,42] *Listeria monocytogenes*infection is widespread in domestic animals in Nigeria It can be present in raw milk and foods made from raw milk. It can also live in food processing plants and contaminate a variety of processed meats. In Nigeria, listeria has been reported in milk and milk products, ice cream, fermented and local butter.[43]

2.3. Listeriosis Outbreaks

The first outbreak of *Listeria monocytogenes* that could be definitely linked to food was caused by commercially prepared coleslaw in Canada in 1981 (at least 41 cases with 7 deaths). Manure from *Listeria*-infected sheep had been used as a fertilizer when growing the cabbages used to prepare the salad; but reported cases *Listeria* infections increased dramatically during the 1980s, During the first decade of the 21st Century there were a number of large *Listeria* outbreaks caused by ready-to-eat (deli) poultry products in North America.[44] Contamination of raw and pasteurised milk by *Listeria* increases possibility of human listeriosis.[45]

Listeria monocytogenes, an intracellular bacterium has become an emerging food borne pathogen that causes listeriosis; a zoonotic disease, posing serious risks to public health; in livestock industries, pregnant mothers, neonates and in immunocompromised individuals.[40] *Listeria monocytogenes* is the most pathogenic (able to cause illness) member of the *Listeria* genus. Other members of the genus collectively referred to as *Listeria* species (spp.) are unlikely to cause illness but can often be found growing under the same conditions. Therefore finding any type of *Listeria* species e.g. *Listeriainnocua* can be a warning that the pathogenic bacteria could also be present.*Listeria monocytogenes* causes the illness, listeriosis. About

90% of cases of listeriosis are associated with the consumption of food contaminated with *Listeria monocytogenes*.[32,33]

2.4. Epidemiology of Listeriosis

Listeria is unlike many other germs because it can grow even in the cold temperature of the refrigerator. *Listeria* can be denatured by cooking and pasteurization.

It was not until the outbreak in California from January to August 1985 that food was recognized as an important vehicle of *Listeria* transmission.[11]

Ready-to-eat foods have been implicated in several outbreaks of listeriosis.[46] The largest outbreaks of human listeriosis have involved dairy products.[47]

The public health significance of detecting pathogens in the samples of pasteurised milk and other milk products studied cannot be ignored. This is because it has been estimated that of diseases due to milk and milk products among food-borne diseases recorded in France and in other countries since 1980, a total of 17,405 cases of disease due to *Listeria monocytogenes*, *Salmonella* spp., *E. coli* or *S. aureus* were linked to pasteurised milk with 22 deaths.[48] Outbreaks of listeriosis linked to pasteurised milk have been reported in Massachusetts.[10,49]

Isolation of *Listeria monocytogenes*, *Salmonella* spp in RTE foods could posea health risk to consumers.[50]

Listeria can grow at low temperatures. So while refrigeration will slow down or prevent the growth of most food-poisoning bacteria, the *Listeria* bacteria may continue to grow, albeit more slowly at lower temperatures. Very little if any growth will occur if foods are dry (water activity under 0.90) or are acidified. *Listeria* bacteria may survive in frozen food, but they will not grow. However once the food is thawed, growth may occur if the conditions are right. *Listeria* is also able to continue to survive and grow in most types of packaging where

the normal atmosphere has been changed or removed.[32,33] There is a large range of RTE foods that may be associated with foodborne listeriosis and it is hard to provide a simple list of higher-risk foods. Nevertheless it is evident that these RTE foods tend to have some common features, such as that they support the growth of *Listeria* or there is the potential for contamination following a listericidal step and before final packaging.[33]

The most important control measure for a food operator is to prevent *Listeria* from entering the production and manufacturing premises. Where prevention is not possible, then minimising the impact of those that are present is essential.[33]

Listeria monocytogenes causes one of the most severe forms of foodborne infection but is a relatively rare disease. The overall mortality rate associated with the disease is 30 %, but it can be as high as 40% in vulnerable individuals and hospitalisation is more likely than for other common foodborne infections. Those most at risk of acquiring the disease are pregnant women (20 times greater risk than healthy individuals), the elderly and the immuno-compromised, although healthy individuals can develop listeriosis, particularly if the food is heavily contaminated. This group who is at risk from the severe form of listeriosis are typically referred to as 'vulnerable consumers' or YOPIs (the young, old, pregnant and immuno-compromised).[33] People now live longer through better social and working conditions and medical advances in the treatment of chronic diseases prolonging life expectancy; this means that over time there will be more vulnerable consumers. The trend for more cases of severe listeriosis in the older population is already being seen in some parts of the world.[33] There is a large range of RTE foods that may be associated with foodborne listeriosis and it is hard to provide a simple list of higher-risk foods. Nevertheless it is evident that these RTE foods tend to have some common features, such as that they support the growth of *Listeria* or there is the potential for contamination following a listericidal step and before final packaging.[33]

Listeria monocytogenes has the potential to be present in all raw foods. Biofilm is a factor that enhances the resistance of *Listeria monocytogenes* to cleaning and sanitation, thereby promoting its transmission by food. A biofilm is a population of microbial cells growing on a surface and enclosed in an amorphous extracellular matrix of primarily polysaccharide materials and cannot be removed by gentle rinsing.[51] Numerous studies have shown that *Listeria monocytogenes* is capable of adhering and forming biofilm on metal, glass, rubber and plastic surfaces.

Cooked foods can also be contaminated, usually as the result of post-process contamination. The pathogen has been isolated from a very wide range of processed foods including pâtés, milk, soft cheeses, ice cream, ready-to-eat cooked and fermented meats, smoked and lightly processed fish products and other seafood. *Listeria monocytogenes* is usually found only in low numbers (less than 10/g) in foods. At least 13 different serotypes of *Listeria monocytogenes* are known. All can cause the disease listeriosis in humans, but most cases are caused by serotypes 1/2a, 1/2b and 4b. The majority of reported foodborne outbreaks have been caused by serotype 4b.[33]

Studies has shown that *listeria* survives in milk products for several weeks like yoghurt, buttermilk and cheese;[52] and also survive manufacture and storage is able to survive the manufacture and storage conditions of several cheeses [53; 54]; thus confirming the potential health risks associated with post processing contamination of even low levels of *Listeria monocytogenes* in several dairy products.[55]

Some lactating ruminants may also have clinical mastitis associated with listeriosis, only a few cases of listeria mastitis have been reported in the literature.[56,57] These observations indicate that either the organism does not readily invade the udder or cases of listerial mastitis

are not being detected in routine laboratory examination of mastitic milk samples. However, when it occurs, the animal may have prolonged shedding of the bacteria in the milk.[56,57]

There is a large range of RTE foods like milk that may be associated with foodborne listeriosis and it is hard to provide a simple list of higher-risk foods. Nevertheless it is evident that these RTE foods tend to have some common features, such as that they support the growth of *Listeria* or there is the potential for contamination following a listericidal step and before final packaging.[33] They are prepared at very dirty surroundings with waste water and garbage disposed nearby, providing nutrient and breeding ground for rodents and vermin. In most cases running water is not available at milk vending sites, washing of hands and crockery are done in bowls or buckets and sometimes without soap.[58] Furthermore the conditions under which street food like locally processed milk is prepared and vended are worsened by weak implementation of relevant environmental and public health regulations.[58,59] Poorly regulated RTE operations raise serious questions about food safety and hygiene standard, as well as monitoring by relevant authority.[58,59]

Numerous studies conducted concerning various aspects of food hygiene over the past decade, have revealed poor food hygiene knowledge and attitudes of street food vendors, with personal hygiene least observed by the least educated.[59,60,61] There is strong statistical evidence that 70% of all bacterial food poisoning is caused by caterers. This is greater than occurrences reported from any other food sector. Most of these food poison outbreaks are due to the inadequate time and temperature control of food.[58,59]

The infective dose is uncertain, although it is generally considered to be high for healthy individuals, with food contamination rates of more than 1,000 cells/g being required. Due to the length of the incubation period, it can be difficult to determine the numbers of bacteria in foods at the time of consumption[33].

While small numbers of *Listeria monocytogenes* (less than 100cfu /g) in food are unlikely to cause illness, as the numbers of bacteria increase, consumers will be at risk of becoming ill. If foods are contaminated with very large numbers (over 100,000cfu/g) of *Listeria monocytogenes* consumers may become ill with listeriosis. For most people the illness is likely to be mild, often people will experience flu-like symptoms or an illness similar to other types of 'tummy bugs'. However for some people, even a lower number of bacteria (around 1,000) will result in a severe illness and may even lead to death.

Some lactating ruminants may also have clinical mastitis associated with listeriosis, only a few cases of listeria mastitis have been reported in the literature.[56,57] These observations indicate that either the organism does not readily invade the udder or cases of listerial mastitis are not being detected in routine laboratory examination of mastitic milk samples. However, when it occurs, the animal may have prolonged shedding of the bacteria in the milk.[56,57]

Deliberate or accidental contamination of food during large production might endanger the health of consumers, and have very expensive repercussions on a country, as such outbreaks feature prominently in national statistics [62] which could have been avoided by safe food handling practices.[63]

2.4.1. Incubation period of listeriosis

This varies from 1 to 90 days (mean 30 days). The two main clinical manifestations of listeriosis are sepsis and meningitis which is often complicated by encephalitis. The onset of illness is typically marked by flu-like symptoms (fever and headache), and sometimes by nausea, stiff neck, confusion, weakness, vomiting sometimes preceded diarrhoea. In some cases these symptoms develop into meningitis and septicaemia. Infection in pregnant women can lead to infection of the foetus, which can result in miscarriage, stillbirth, or the birth of an

infected infant, although the mother usually survives. Duration of illness varies from days to weeks.[33]

Kitchen and food processing environments, particularly those that are cold and wet, can be reservoirs for *Listeria.* The organism can be particularly persistent and difficult to control because of its resistance to unfavourable environmental conditions and ability to grow at low temperature. Animals such as sheep, goats and cattle are recognized carriers of *Listeria*, often acquired from the consumption of contaminated (usually poor quality) silage. Healthy humans can also be carriers.[33]

2.4.2. Source of infection

Almost all cases of listeriosis is as a result of consumption of *Listeria monocytogenes* in any of the followings:

1. Ready-to-eat deli meats and hot dogs
2. Refrigerated pâtés or meat spreads
3. Unpasteurized (raw) milk and dairy products
4. Soft cheese made with unpasteurized milk, such as queso fresco, Feta, Brie, Camembert
5. Refrigerated smoked seafood
6. Raw sprouts[19]

2.4.3. Primary routes of transmission

The primary route of transmission is through the ingestion of contaminated food. The International Life Sciences Institute in 2005 described high-risk foods for causing listeriosis as those with the following properties:

a. have the potential for contamination with *Listeria monocytogenes*;
b. support the growth of *Listeria monocytogenes* to high numbers;
c. are ready-to-eat;

d. require refrigeration and

e. are stored for an extended period of time.[11]

2.5. The Increasing Problem of *Listeria*

Human listeriosis is a relatively recent disease. *Listeria monocytogenes* came to international prominence as a foodborne pathogen in 1985 following a large outbreak in the USA. The bacteria have always been present but food production has changed and with the greater availability and range of chilled, long shelf-life RTE foods the risk of illness from *Listeria monocytogenes* is increasing.

In addition, people are living longer through better social and working conditions and medical advances in the treatment of chronic diseases prolonging life expectancy; this means that over time there will be more vulnerable consumers. The trend for more cases of severe listeriosis in the older population is already being seen in some parts of the world.

The contamination of ready–to-eat-foods (RTE) could be due to many factors. One of the possible factors is via cross-contamination after the foods were cooked. It cannot be ruled out the possibility that the cooking process is not sufficient to inactive these tough bacteria. Recent outbreaks of food-borne listeriosis in USA and other countries and the high prevalence of *L. monocytogenes* in local foods have drawn the attention of local authorities on the possible widespread of *L. monocytogenes* in the country[33].

In developing countries, a wide variety of local foods sold by street hawkers is a major source of RTE foods for the locals. However, the hygiene status of street hawkers is questionable. Previous studies conducted many developing countries found high a prevalence of foodborne pathogens in street foods.[64,65,66,67] Therefore, the occurrence of this pathogen in various types of RTE foods, sold by hawkers could pose a significant public health risk to the consumers.

2.5.1. How *Listeria* is introduced into production facility and food

Listeria monocytogenes is widespread in the environment. Small numbers of the bacteria can often be found on unprocessed foods, and in and around a processing facility. It can be introduced into a processing operation on people's shoes, clothing and body, in dust, on equipment such as tools and vehicles and on ingredients and packaging. Once in the processing plant, the bacteria will find suitable niches, in particular damp spots in which to reside and multiply, including hidden equipment surfaces. If the cleaning and sanitation procedures are not thorough the bacteria may then form a biofilm and become difficult to remove. These niches can be a major source of contamination during food processing.

If this contamination occurs after a processing step designed to eliminate pathogens e.g. pasteurisation or cooking, the bacteria may grow particularly rapidly as other competing bacteria will have been significantly reduced by the process.[32,33]

2.6. Listeria Management Programme (LMP)

A *Listeria* management programme is intended to identify the control measures that a food operation has in place to prevent *Listeria* contamination. The documented plan should be a living document subject to constant review and responsive to events that could alter the control requirements. It will be unique to each food operation. In general LMPs are likely to cover procedures that specifically target the control of *Listeria* through the application of good operating practice (GOP) which enables the production of safe and suitable food. GOP is a system made up of a collection of procedures that set out how you operate your business to produce safe and suitable food. GOP is fundamental to a risk-based programme, such as a food safety programme (FSP) or a risk management programme

2.6.1. Developing LMP for food operation

Food operators should begin by reviewing their operation in terms of the potential for the RTE foods produced and/or packaged to be contaminated with *Listeria monocytogenes*.

Listeria control measures will include any activities relating to:

1. The introduction of *Listeria* into the processing environment e.g. in ingredients or on packaging or by people or equipment and by air and water
2. Reducing the opportunity for *Listeria* to become established in the processing environment e.g. by using good process design, appropriate building materials
3. Ensuring effective cleaning and sanitising of all processing and environmental surfaces to eliminate any build up of *Listeria.*
4. Ensuring product formulations and processing steps e.g. pasteurisation achieve the expected effects in killing or preventing the growth of *Listeria*.

2.6.1.1. Tools that can be used to achieve these controls could include:

I. Restricting the movement of people, equipment, air, water and materials and product within the processing environment
II. Educating staff on what could happen if contaminated food is produced and how this can be prevented
III. Cleaning and sanitation programmes and monitoring
IV. Environmental and product monitoring programmes
V. Building and equipment design and workflow
VI. Clothing /footwear /personal hygiene policies and protocols
VII. Microbiological limits or customer specifications for *Listeria monocytogenes* in ingredients and products.

The key risk factors for *listeria* in foods are:

1. The characteristics of the ready-to-eat-food (RTE) food, i.e. whether or not *Listeria monocytogenes* is able to grow.
2. The potential for *Listeria* to be present in the ingredients.
3. The type of processing (e.g. is there a listericidal step).

4. The likelihood of contamination (exposed to the environment or not) before final packaging.
5. Who is intended to eat the food, i.e. the general population or a specific sub-set, e.g. infants.[19]

2.8. Prevention of Listeriosis

Strategies to reduce the incidence of *Listeria* infections were implemented in many countries during the 1990s resulting in a reduction in the incidence of the disease. However, outbreaks have continued to occur and the incidence in the last decade has again risen in some countries.

The following practices prevent cases of listeriosis:

1. Do not drink raw (unpasteurized) milk, and do not eat foods that have unpasteurized milk in them.
2. Wash hands, knives, countertops, and cutting boards after handling and preparing uncooked foods.
3. Rinse raw produce thoroughly under running tap water before eating.
4. Keep uncooked meats, poultry, and seafood separate from vegetables, fruits, cooked foods, and ready-to-eat foods.
5. Thoroughly cook raw food from animal sources, such as meat, poultry, or seafood to a safe internal temperature.
6. Wash hands, knives, countertops, and cutting boards after handling and preparing uncooked foods.
7. Consume perishable and ready-to-eat foods as soon as possible.
8. Persons in higher risk groups should heat hot dogs, cold cuts, and deli meats before eating them.

CHAPTER THREE

METHODOLOGY

3.1. Study Area

The study was conducted in Nasarawa state of Nigeria. Nasarawa State is located in the North Central geopolitical zone of Nigeria. It lies between latitude $08^{0}35^{'}$N and longitude $08^{0}36^{'}$E. It is bounded to the North-west by Federal Capital Territory (FCT), Abuja, and to the North-East by Plateau State, to the South-East by Taraba and to the North by Kaduna State, to the South by Benue State and to the South-West by Kogi State.

Nasarawa State has a land mass of 27, 117 square kilometres with a population of 2,100,000, making it the 10^{th} largest State in Nigeria with population density of 75 persons per kilometre.[68]

Nasarawa State is made up of 13 Local Government Areas; Awe, Akwanga, Doma, Karu, Keffi, Keana, Kokona, Lafia, Nasarawa, Nasarawa-Eggon, Obi, Toto and Wamba. Locally processed milk is consumed all over the State.[68]

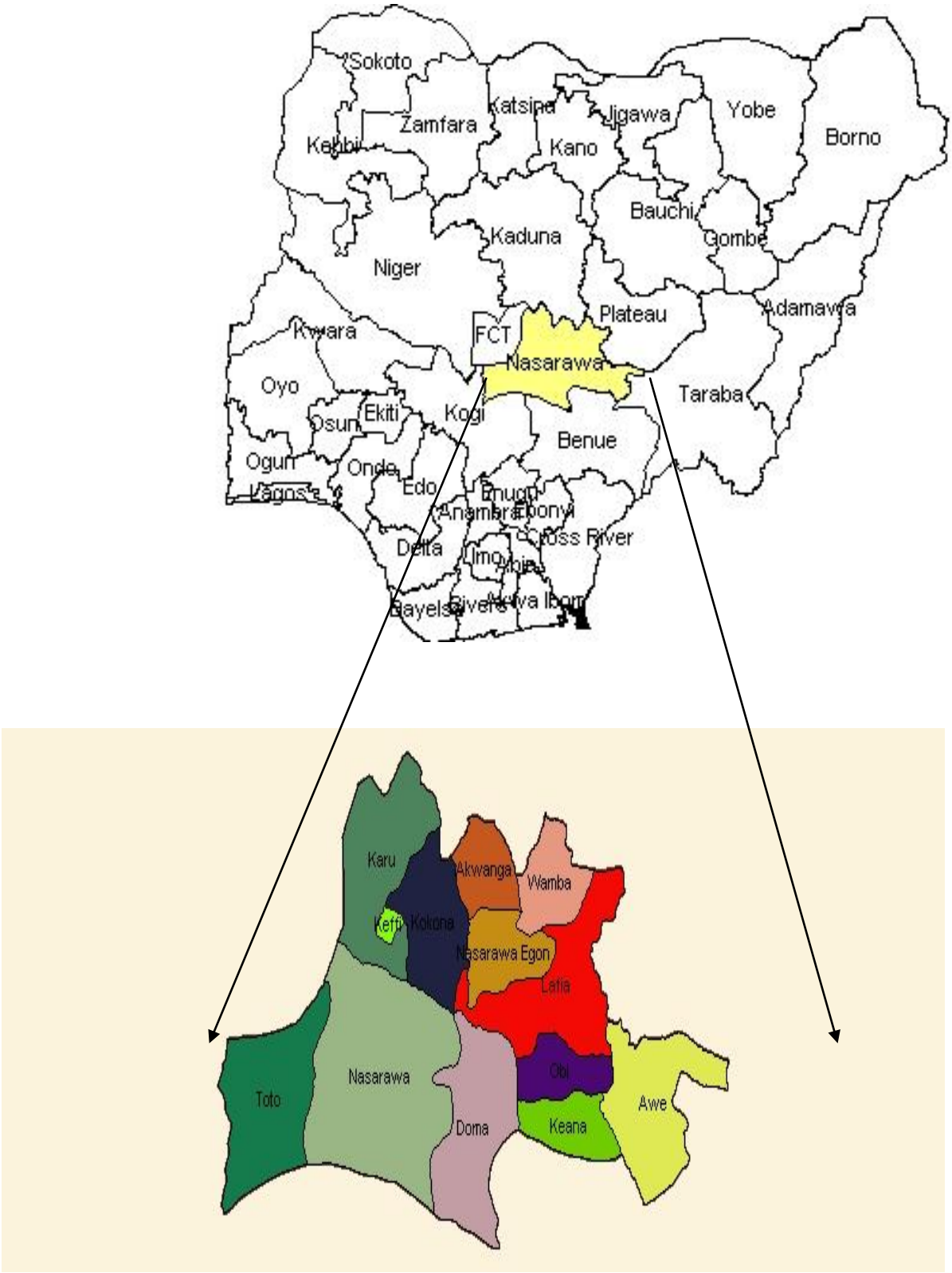


Figure 3.1: Map of Nigeria showing Nasarawa state[68]

3.2. Study Population:

The study population are vendors of locally processed milk in Nasarawa State.

3.3. Study Design:

The study is a cross sectional descriptive study.

3.4. Sample Size Determination:

Sample size was determined using Fisher's formula

$$n = \frac{Z_{\alpha}^{2} pq}{d^{2}}$$ [69]

where

Z_{α} = the standard normal deviation at 95% confidence interval= 1.96

p = is the proportion of outcome = 21.5% (0.215) [41]

q = complementary probability of p= 78.5% (0.785)

d = the desired level of precision=0.05

Therefore, $n = 1.96^{2}$ x0.215x0.785/0.05x0.05

n = 3.84x0.17/0.0025 =0.65/0.0025=259

Add 10% (26) non-response rate gives a total of 285 respondents.

Therefore we collected 285 milk samples and administered 285 questionnaires- for every milk sample collected a questionnaire was administered to the milk seller. The sample size was increased from 285 to 420.This is because higher sample size increases the significance level of the findings, since the confidence of the result are likely to increase with a higher sample size. This is to be expected because larger the sample size, the more accurately it is expected to mirror the behavior of the whole group.

3.5. Sampling Technique

Multi-stage sampling technique was used.

Stage 1: One Local Government Area (LGA) was selected from each of the three senatorial districts in the State (West, North and South), by simple random sampling (SRS) using balloting. The following LGAs were thus selected: Keffi (West), Wamba (North) and Awe (South).

Stage 2: One ward was also selected, by SRS using balloting, from each of the earlier selected LGAs (in stage 1 above). The following wards were selected: Sabon Gari (Keffi), Farin Ruwa (Wamba) and Mahanga (Awe).

Stage 3: Markets where locally processed milk was sold were identified and milk samples collected and questionnaires administered systematically to every other milk seller (systematic random sampling). Selected markets were: Kara market (Sabon Gari- Keffi LGA), Farin Ruwa (Farin Ruwa market – Wamba LGA) and Mahanga market (Mahanga – Awe LGA).

3.7. Data Collection Method

3.7.1. Data collection

A semi-structured interviewer administered questionnaire was used to study practices of milk sellers during processing, storage and sales of milk.

3.7.2. Collection of samples:

3.7.2.1. *Laboratory*

A total of 420 milk samples were collected at random (from places where locally processed milk was sold in selected LGAs of Nasarawa State. Twenty milliliters (20ml) milk sample was collected in Universal Sample Bottles. The 20ml samples were collected using

appropriately labelled sterile universal bottles, and transported in a cold box with ice packs to the laboratory for bacteriological analyses in the Veterinary Public Health Laboratory of Ahmadu Bello University, Zaria.

3.7.2.2. *Field*

Relevant information (demography, milk preparation and handling practices) were obtained through face to face interview and use of questionnaire. Data were recorded on the questionnaire. Both of them were cross-checked for error and missing variable and corrected.

3.7.3. Laboratory procedures

3.7.3.1. Culture and isolation

Using culture and identification method as recommended by the International Organization for Standardization (ISO 11290-1, 1996), 1ml of milk sample was inoculated into 9mls of One Broth-Listeria (Oxoid, Hamphire United Kingdom) and incubated aerobically at 30^{o}C for 48 hours. The now broth-enriched sample was inoculated onto Oxoid *Listeria* Agar (selective differential media - (Oxoid, Hamphire United Kingdom). This was incubated aerobically at 37^{o}C for 24 – 48 hours.

Brown colonies on the media that expressed gram positive coccobacilli on gram staining were presumed to be *Listeria* species.

3.7.3.2. *Biochemicalcharacterisation*

Biochemical characterization as recommended in OIE 2008 terrestrial manual was conducted (Christie, Atkins, and Munch-Peterson test) in order to differentiate the various *Listeria* species.

The sugars (glucose, Rhamanose, Xylose and manitol) were prepared by suspending 1g of each of the sugars and 1.5g andrade peptone in 100m of distilled warm water to dissolve

completely. 3mls were dispensed into the bottles and autoclave at 115^{o}C for 15minutes. The representative colonies from the Oxford Listeria Agar were inoculated into the sugars and incubated at 37^{o}C for 24-48hours. The result was read and interpreted - showing red to pink colour indicating positive reaction while any other colour indicates negative reaction.

3.7.3.3. *Agglutinationreaction*

Oxoid Listeria Test Kit reagents were first left to reach room temperature prior to use.

Specimen: Selective and/or enrichment broths or solid media can be tested with the Oxoid Listeria Test Kit.

Controls: The following controls should be performed each time the kit was used.

a. Reagent controls

Add 1 drop of the Oxoid Listeria Latex Reagent to 1 drop of saline in the same circle on a reaction card, mix and observe for agglutination for 2minutes. No agglutination should occur. If this control shows agglutination, either the Latex Reagent or the saline probably contaminated and should be discarded.

b. Positive control

Place a drop of the positive control antigen suspension on a reaction card. Place 1 drop of Latex Reagent in the same circle next to the drop of positive control suspension. The dropper was not allowed to touch the positive control. Mix the Latex Reagent and the positive control with a clean mixing stick surface of the circle. Gently rock and examine for agglutination within a maximum of 2minutes. Easily discernable agglutination with the Latex Reagent indicates normal reagent function.

Assay Procedure

1. Method for identification from the selective solid media.

a. Place a reaction card on the work bench.
b. Add 1 drop of saline within one circle on the reaction card.
c. Using a mixing stick or inoculating loop, emulsify the suspect colony in the drop of saline to produce a heavy smooth suspension. Suspensions should be made from colonies with morphology resembling *Listeria* specie. Observe the suspension for auto-agglutination. If the suspension remains smooth, proceed to step 'e'.
d. Gently mix the Oxoid Listeria Latex Reagent by inverting and place a drop of the reagent in the same well adjacent to the suspension. The dropper was not allowed to touch the organism suspension. Mix the Latex Reagent and organism suspension with a clean mixing stick, spreading the mixture over the entire area of the well and gently rock the reaction card.
e. Examine for agglutination for a maximum of 2minutes.

2. Method for testing broths
    a. Using a suitable pipette, 1 drop of culture was removed.
    b. A single drop of the broth culture was place within one circle of the reaction card.
    c. Oxoid Listeria Latex Reagent was mixed gently by inverting and placed a drop of adjacent to the broth in the same well on the card without allowing the dropper to touch the drop of the broth culture. Mixing stick was used to mix thoroughly, spreading the suspension over the entire area of the well. Reaction card was gently rocked.
    d. Agglutination was observed within maximum of 2minutesafter reading,, the used slides were discarded into suitable disinfectant.

3.7.3.4. Microbact test

The isolates earlier identified through culture were subjected to the Microbact for further identification. The kit was used according to the manufacture's instruction (Oxoid Ltd). Briefly, four colonies were picked using a sterile wire loop and emulsified in3ml of listeria suspension media. 100microlitre of the bacteria suspension was added to all wells of each strip. The overlay of the mineral oil was added to well 7 then inoculated at 37^{o}C for 24hours.

Then drop of fast blue reagent was added the well number 12 and observed for colour change to all the wells. The result shown from the wells was read and recorded on to the Microbact report form provided by the manufacture in the kit. The code which was entered into the Microbact software to interpret the results and the organisms was named and percentage probability recorded.

3.7.3.5. Drug sensitivity test

The sensitivity of the isolates weretested using Kirby-Bauer disk diffusion method. The antimicrobial agents and concentration used were Sulphamethazole/trimethoprim, penicillin, amoxicillin, enrofloxacin, mipenem, erythromycin, Chloramphenicol, kanamycin, Gentamycin, doxycycline, ceftriazole and ofloxacin.

The colonies of each isolate to be tested were picked and suspended in 5ml normal saline and made up to a 0.5Mcfarland standard. The diluted bacteria inoculums is swabbed onto Mueller-Hinton Agar plate. Each antimicrobial disk was applied on the surface of the inoculated media using a disc dispenser (Oxoid) and incubated at 37^{o}C for 24hours. The zone of inhibition for each antibiotic was measured in millimeters.

The zonesof inhibition of growth of *Listeria*were interpreted using Clinical and Laboratory Standard Institute (CLSI) 2011. The isolate was classified as Susceptible, Intermediate or Resistant.

3.7.4. Field

Data obtained was analysed using Epi Info and excel. A p-value of 0.05 was accepted as statistically significant. Descriptive statistics, bivariate and multivariate analysis was done.

3.8. Quality Assurance:

Standard OperatingProcedures were developed and maintained throughout the study period to ensure uniformity of the outcome.

3.9. Ethical Consideration

The research was performed according to the guidelines for human experimentations in clinical research stated by Federal Ministry of Health Nigeria. Confidentiality was maintained. Informed consent was obtained verbally from each participant and they could withdraw at any point of the study if they so wish. The study was beneficent and non-maleficent and has no potential harm to participants.

Approval of the Scientific and Ethical committee of Nasarawa State Ministry of Health was obtained.

3.10. Data Quality

Questionnaires were translated in hausa language (a language understood by all paticipants). Data were stored under lock and key and the electronic version was stored in password protected format.Data collectors were carefully selected and trained

Data were collected from the field were cross checked for accuracy and consistency and double entry of data was carried out to minimize errors

3.11. Data Management

Epi-Info version 3.5.4 was used to carry out analysisand p-value of 0.05 accepted as statistically significant. We calculated frequencies, proportions, bivariate and multivariate

3.12. Planned Use of Data

This study was carried out as there is currently no report on a comprehensive assessment of all the bacterial health risk posed to consumers of ready-to-eat (RTE) food products like locally processed milk sold in Nasarawa State.The studyis to be used to validate a treatment or process that aims to reduce or eliminate the presence of the pathogen

This study provides inference regarding the effectiveness of the strategies used to control *listeria* in milk because microbial load and the presence of the bacterial pathogens (including *listeria*) in foods are a good indication of the food quality and the potential health risk they pose to consumers.[70]

This research will be shared with food safety regulators and government inspection agencies like National Agency for Food and Drug Administration and Control (NAFDAC) in their evaluation of the design and interpretation of challenge studies involving *Listerias* in locally processed milk.

3.13. Limitation

Security challenges in Nasarawa State especially those involving farmers and pastoralists who make bulk of vendors of locally processed milk.

CHAPTER FOUR

RESULTS

The result of socio-demographic characteristics of respondents in this study, showed that there were more within the age group 20-24 years while only 9% were within15-19 years; there were mostly female (92.4%); mostly traders (53.1%); mostly Muslims (99.5%); more of them not educated (36.4%) while the least were those with tertiary education (1.9%); most of them earns between N6,000-N18,000 monthly (52.1%) and those that earn monthly income of below N6,00 were least (8.3%); most of them don't have additional occupation but sell milk only (53.6%).(Table 4.1).

Out of all the 420 samples collected and tested, 10.2% (43 samples) were positive for *listeria*through culture and isolation (Table 4.2).

Table 4. 1. Socio-demographic Characteristics vendors/processors of locally processed milk in Nasarawa state

Characteristics	Frequency (n=420)	Percent (%)
Age Group (Years)		
15-19	38	9.0
20-24	146	34.8
25-29	53	12.6
30-34	103	24.5
35+yrs	80	19
Sex		
Males	32	7.6
Females	388	92.4
Occupation		
Pastoralist	194	46.2
Trader	226	53.8
Additional Occupation		
Sells Milk Only	225	53.6
Have Other Occupations	195	46.4
Religious Belief		
Christian	2	0.5
Islam	418	99.5
Educational Level		
None	153	36.4
Qur'an	130	31.0
Primary	104	24.8
Secondary	25	6.0
Tertiary	8	1.9
Income		
<N6,000	35	8.3
N6,000-N18,000	219	52.1
>N18,000	166	39.5

Table 4.2. Milk samples positive to Listeria Species in milk using culture and isolation in Nasarawa state

Proportion	Frequency (n=420)	Percent (%)
Negative	377	89.8
Positive	43	10.2

Table 4.3. Distribution of Listeria species in milk (using culture and isolation) in Nasarawa State

Zone	Frequency (n=43)	Percent (%)
North	15	34.9
South	11	25.6
West	17	39.5

Table 4.4: Various species of Listeria found in locally processed milk samples in Nasarawa State through MICROBACTtest

Species	Frequency (n=18)	Percent (%)
Liseria welshimeri	3	16.7
Listeria grayi	3	16.7
Listeria ivanovii	11	61.1
Listeria monocytogenes	1	5.6

Table 4.5: Biochemical Characterisation of *listeria* species found in milk in Nasarawa state through biochemical test

Species	Frequency (n=20)	Percent (%)
Liseria welshimeri	4	20
Listeria grayi	4	20
Listeria ivanovii	11	55
Listeria monocytogenes	1	5

Table 4.6 showed varying susceptibility of *listeria* specie to different drugs. They were 100% susceptible to enrofloxacin and doxycycline; 77.8% to kanamycin but resistance to Penicillin is as high as 93.7%; to Amoxicillin and Sulphamethazole/trimethoprim 80% and 60% respectively.

From the Table 4. 7, it will be seen that that borrowing/sharing of milk processing equipment and refrigeration/addition of ice to processed milk are factors significantly found to be associated with presence of *listeria* in milk sample (with odd ratio of 3.07 and 5.54 respectively). Conversely, training on food safety and supervision during process were found to be significantly protective from *listeria* in milk sample (with odd ratio of 0.14 and 0.18 respectively).

Table 4.6: Drug resistance to *Listeria* specie found in milk samples in Nasarawa State

S/No	Drug	Susceptible %	Moderate %	Resistant %
1	Sulphamethazole/trimethoprim	13.3	20.0	60.0
2	Penicillin	6.7	-	93.7
3	Amoxicillin	20	-	80
4	Enrofloxacin	100	-	-
5	Imipenem	71.4	28.5	14.3
6	Erythromycin	46.7	6.7	26.7
7	Chloramphenicol	64.3	7.1	14.3
8	Kanamycin	77.8	11.1	11.1
9	Gentamycin	64.3	-	35.7
10	Doxycycline	100		
11	Ceftriazole	-	80	20
12	Ofloxacin	100	-	-

Table 4.7: Bivariate Analysis of risk factors of listeria among vendors/processors of locally processed milk in Nasarawa state

Risk Factors	O.R.	95% C.I.	p-value
Borrow/sharing of milk processing equipment	3.07	1.57-5.99	0.001
Conducting microbial testing before processing milk	0.41	0.09-1.76	0.22
Training of staff on food safety to staff processing milk	0.14	0.07-0.31	0.001
Supervision of staff during milk processing	0.18	0.03-0.43	0.001
Hand-washing practices by staff processing milk	0.98	0.51-1.86	0.94
Availability of toilet facility within processing area	0.53	0.12-2.28	0.38
Cleaning of milk processing equipment after use	3.63	0.68-19.30	0.11
Addition of ice to processed milk	5.54	2.13-14.39	0.001
Milk containers touching the ground	1.58	0.36-6.89	0.54
Having formal education among milk vendors	2.61	1.22-5.60	0.011
Milk vendors earning above N18,000 monthly	2.03	0.99-4.15	0.05

Table 4.8: Logistic regression of risk factors of listeria among vendors/processors of locally processed milk in Nasarawa state

Predictors	AOR	95% C.I.	p-value
Not supervising staff during milk processing	12.7	5.0 - 33.3	<0.01
Borrow/sharing of milk processing equipment	3.3	1.4 - 7.3	<0.01
Addition of ice to processed milk	6.6	2.3 - 18.8	<0.01
Not providing food safety training	10.8	5.0 - 20.0	<0.01

CHAPTER FIVE

DISCUSSION

Prevalence of *Listeria*by culture and isolation was 10.2% (43/420) but biochemical test with gave 4.76% (20/420) and both microbact and latex agglutination test gave 4.29% (18/420).

In all cases these figures (10.2%, 4.76% and 4.29% are lower than what was found in Plateau state which was 21.5%;[41] 33.8% in Port Harcourt;[71] 25% in Sokoto;[72] in EU it was 10.3%,[73] 17.9% in Malaysia.[74] The prevalence is higher when compared with what was found in Onitsha (0%) and China;[75,76] also higher than what was found in ready-to-eat foods in supermarkets in Trinidad (1.7%)[50] and 0.8%pre-processed cows' milk in Morocco.[77]

This prevalence in locally processed milk is similar to prevalence in locally processed milk in Fez region of Morocco which was 5.9%.[78]

In Nigeria, presence of *Listeria species* may have resulted from contamination from post processing and during storage [72] or poor sanitary condition and handling.[79]

Most of the respondents in this study had no formal education 67.4%; while those with tertiary education were only 1.9%; but a study in Benin Nigeria, showed respondents with tertiary education, 38.5% and primary education, 27.3%.[79]

In my study, most of vendors are female (92.4%) which is similar to what was found in Benin city with 90.2% of food vendors being females,[79] also Abeokuta;[80] Owerri;[81] the predominance of female food vendors is a means of complementing family income;[79] but in South Africa & Kenya there were more male vendors.[82,83]

Respondents in a similar study in Northwest Ethiopia, 78.9% were female and 21.10% were male. The mean age of the respondents was 23.53 years with standard deviation of 5.40 years (23.53 ± 5.40) and range 33 years (15 – 48 years). About 87.10 % of the respondents were

Orthodox Chrisians. 56.30% of the respondents were attending secondary education and 75.9% were not married. Similar study in Dakhar City, Bangladesh demonstrated that 25% street food vendors are illiterate and cannot write their names and have no formal education.

In an earlier study, protective factors observed were food safety training, knowledge food hygiene laws[80] while refrigeration was found to be predisposing factor.[84,76] This similar to this study where protective factor was food safety training and supervision during processing while risk factors were refrigeration of milk and sharing of equipment used for processing.

Milk products as a vehicle for the transmission of various diseases including *listeria*, especially in countries where hygienic standards are not strictly enforced has been well documented.[84] This agree with this study which revealed that unhygienic practices like sharing equipment like containers and leaving containers touch ground are significantly responsible for transmission of *listeria.*

Disinfection and cleaning of equipment was found to decrease the risk of contamination[85] which is a similar finding to this study.

Proportion of different species of *listeria* found in this study showed *Listeria welshimeri* is 16.7% (13/18); *Listeria gray*iis 16.7% (3/18) and *Listeria ivanovii* was 61.1% (11/18) while *Listeria monocytogenes* was only 5.6%(1/18) buta study in Sokoto [86] showed 1.3% were *Listeria innocua, 18.4% Listeria ivanovii, 22.4% Listeria monocytogenes, 5.3% Listeria welshimeri and 2.6% Listeria seeligeri.*

This study showed the sensitivity of *Listeria* to common antibiotic varies. Complete susceptibility was observed to doxycycline and enrofloxacin, 77.8% to kanamycin. An earlier study in Lagos, Nigeria showed complete susceptibility to Gentamycin. Here, Gentamycin susceptibility was lower (64.3%). In this study, resistance to Sulphamethazole/trimethoprim

and erythromycin was up to 60% and 46.7% respectively, but in the Lagos,[89] susceptibility to the same drug was as high as 71.4% and 92.9%.

When compared with a similar study in Sokoto, Chloramphenicol was highly susceptible, and resistance to Gentamycin was 20% in Sokoto[87] while it was 35.7% in this study.

Lagos study showed high susceptibility to commonly used antibiotics: Ciprofloxacin (100%), Pefloxacin (100%), Gentamycin (100%), Streptomycin (92.9%), Erythromycin (92.9%), Sulphamethoxazole (71.4%) and Amoxicillin (64.9%) but resistance to Ampiclox was encountered.[88]; but in Sokoto, it was revealed high resistance to Ampicillin (100%), and Streptomycin (80%), Ampiclox (70%), Tetracycline (30%), then Gentamycin (20%) while, there complete susceptibility to Ciprofloxacin and Chloranphenicol.[87]

Study in southwest Nigeria[89] show susceptible to Gentamycin, but resistant to Augmentin and Cloxacillin. The effectiveness was in this order; Gentamycin > Chloramphenicol > Erythromycin > Streptomycin > Tetracycline >Cotrimoxazole > Augmentin >Cloxacillin.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

Most of the vendors of locally processed milk had no formal education, females, Muslims, are of age group 20-24years and have monthly income of between N6,000 and N18,000. Out of the 420 milk sample collected, 10.2% were positive for listeria, with *Listeria ivanovii* being commonest while *Listeria monocytogenes* was least common. Enrofloxacin and erythromycin are shown in this study to be most effective against species of *Listeria* found, but *Listeria* species found were mostly resistant to penicillin.

Appropriate food safety training, supervision, good hygienic practices prevents *listeria* contamination in locally processed milk while addition of ice (to processed milk) and borrowing/sharing of equipment could pose a *Listeria* risk.

6.2. Recommendations

Processors of locally processed milk should be encouraged to undergo food safety training, observe strict supervision during processing of milk. Vendors of locally processed milk in Nasarawa state, being mostly nomads can be reached and organized through their cultural organization, *Miyetti Allah Cattle Breeders Association of Nigeria* (MACBAN) for licensing and training/public health enlightenment on how to reduce chance of occurrence of *listeria* specie in their milk.

Vendors and processors of locally processed milk should be discouraged from the practice of adding ice to milk and borrowing or sharing of equipment they use in processing milk.

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APPENDICES

Appendix 1: Biochemical Characterisation of *listeria* species found in milk in Nasarawa state through biochemical test

S/No	Sample No	Rhamanose	Manitol	Xylose	Glucose	*Listeria* specie
1	8	-	+	+	+	*L. ivanovii*
2	17	-	+	-	+	*L. ivanovii*
3	22	+/-	-	-	+	*L. ivanovii*
4	27	-	+	-	-	*L. ivanovii*
5	30	-	+	+	+	*L. ivanoviii*
6	32	+/-	-	+	+	*L. gravi*
7	42	-	+	-	+	*L. ivanovii*
8	87	+	+	+	+	*L. welshimeri*
9	88	-	-	+	+	*L. ivanovii*
10	129	-	-	+	+	*L. gravi*
11	132	-	-	+/-	+	*L. ivanovii*
12	205	-	-	+	+	*L. welshimeri*
13	226	-	+	+	+	*L. welshimerii*
14	227	+/-	-	+	+	*L. welshimeri*
15	267	+/-	-	+/-	+	*L. monocytogenes*
16	332	-	-	-	+	*L. ivanovii*
17	342	+	+	+	+	*L. gravi*
18	348	-	+	-	+	*L. ivanovii*
19	392	+	+	+	+	*L. welshimeri*
20	425	+	+	+	+	*L. welshimeri*

Appendix 2: Milk samples in Nasarawa State that tested positive through Listeria latex agglutination test (Oxoid product)

S/No	Sample No	Agglutination
1	8	+
2	17	+
3	22	+
4	27	+
5	30	+
6	32	+
7	42	+
8	87	+
9	88	+
10	104	+
11	129	+
12	132	+
13	205	++
14	226	+
15	267	+
16	326	-
17	332	+
18	342	-
19	348	+
20	425	++

Appendix 3: Milk samples in Nasarawa State that tested positive through MICROBACTtest

S/No	SAMPLE No	CODE	SPECIE	PERCENTAGE
1	8	6021	L. *ivanovii*	90.65
2	17	4001	L. *ivanovii*	96.33
3	22	6001	L. *ivanovii*	90.93
4	30	4001	L. *ivanovii*	96.33
5	32	6261	L. *grayi*	99.83
6	42	4001	L. *ivanovii*	96.33
7	87	7701	L. *ivanovii*	99.99
8	88	5201	L. *ivanovii*	99.84
9	129	1242	L. *grayi*	97.90
10	132	3314	L. *ivanovii*	99.99
11	205	7422	L. *welshimeri*	99.46
12	226	7060	L. *welshimeri*	99.28
13	227	7245	L. *ivanovii*	74.25
14	267	6447	L. *monocytogenes*	96.54
15	332	4001	L. *ivanovii*	96.33
16	342	6663	L. *grayi*	99.99
17	348	0001	L. *ivanovii*	96.33
18	425	7422	L. *welshimeri*	99.46

Appendix 4:Drug sensitivity Pattern in milk samples in Nasarawa State, Nigeria

S/No	SAMPLE No	SXT	P	AML	ENR	IPM	E	C	K	CN	DO	CRO	OFX
1	8	0	0	0			15	30	20	24			
2	17	0	0	0			0	12	14				
3	30	10	0	0	20	20	11		19	14	14	0	16
4	42	0	0	0			0	18	0	31			
5	87	30	10	29	28	29	10	24	20	22	30	12	27
6	88	0	0	0			0	0	0				
7	104	0	0	0		31	29	30	0				
8	132	0	0	0				0	0	0			
9	205	12	0	0		0	19	19	18				
10	226	11	0	0		11	26	15	16				
11	227	0	0	0	20	13	11	22	11	16	12	10	24
12	267	7	0	0			0	10	0	10			
13	342	30	8	23			29	20	30				
14	348	0	0	12	33	38	14	18	24	23	32	10	29
15	425	0	0	0	20	35	15	28	25	19	30	10	27

KEY

SXT = Sulphamethazole/trimethoprim; **P** = penicillin; **AML** = amoxicillin;

ENR= enrofloxacin; **IPM** = imipenem; **E** = erythromycin; **C** = Chloramphenicol; **K** = kanamycin; **CN** = Gentamycin;**DO** = doxycycline; **CRO** = ceftriazole; **OFX** = ofloxacin.

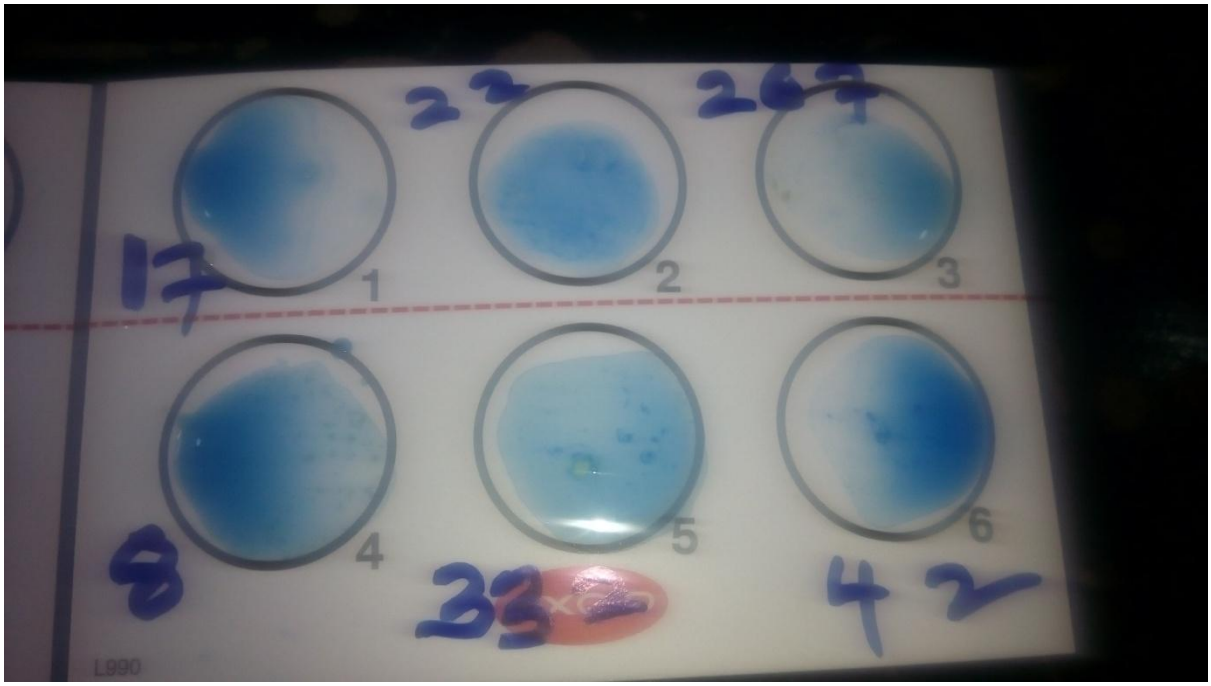




Plate 1: Agglutination Reaction

Questionnaire administered during the research

QUESTIONNAIRE ON RISK FACTORS AND PRCTICES OF *LISTERIA* IN SELLERS OF LOCALLY PROCESSED IN NASARAWA STATE

Good morning/Good day. I am Dr Abdullahi M. Musa, a student pursuing Masters in Public Health (MPH) in Ahmadu Bello University, Zaria in conjunction with the Nigeria Field Epidemiology and Laboratory program (NFELTP). I am carrying out a thesis to conduct a study to identify factors associated with listeriosis and assess practices related to listeriosis.

The aim of our study is to provide basic information/data that could be useful in controlling and preventing listeriosis and safeguarding health of consumers of locally processed milk as well as the general public.

You are not compelled to participate, however we would be grateful for your participation. The interview will last about 15-20 minutes. All information supplied will be treated as strictly confidential.

Questionnaire number _____

Interviewer code _____

Interview date (date/month/year) _____

SECTION A: Demographics

Sex: Male ☐ Female ☐

4. **Age** in years _____

5. senatorial zone of Nasarawa State_____

6. **Principal occupation of respondent** Farmer ☐ : Pastoralis☐

Trader/ Businessman ☐ Civil servant ☐

Others (specify)_____

7. Approximate income per month?

<N6,000 ☐ N6,000 – 18,000 ☐ >N18,000 ☐

8. Do you have any other occupation apart from selling milk ☐ **Yes** ☐ **No** ☐

9. **Level of education of Respondent**: None ☐ Primary ☐ Secondary Tertiary ☐

Qur'anic ☐ Others (specify) _____

10. **Religion**: Christianity Islam African tradition

Others(specify)_____ ☐ ☐ ☐

SECTION B: Practices and Risk Factors Associated with *Listeria monocytogenes* Contamination

11. Number of people/staff working in processing of milk:
1-4
5-9
10-20
20-30
>30

12. Do you own all of its own equipment or do you borrow some?
Yes
No

13. Do you perform any microbial testing?
Yes
No

14. What is tested?
Raw milk
Processed Milkt
Equipment
Other (list)

15. What organisms are tested for?
E. coli generic
E. coli O157
Listeria monocytogenes
Salmonella
Don't know
Other (list)

16. Who requires that you perform microbial testing?
No one (we see need to do it)
Govt Agency/ NAFDAC
Milk sellers
Retail buyers
Don't know
Other (list)

17. Is food safety training provided to workers?
Yes
No

18. Are workers supervised in milk processing?
Yes
No

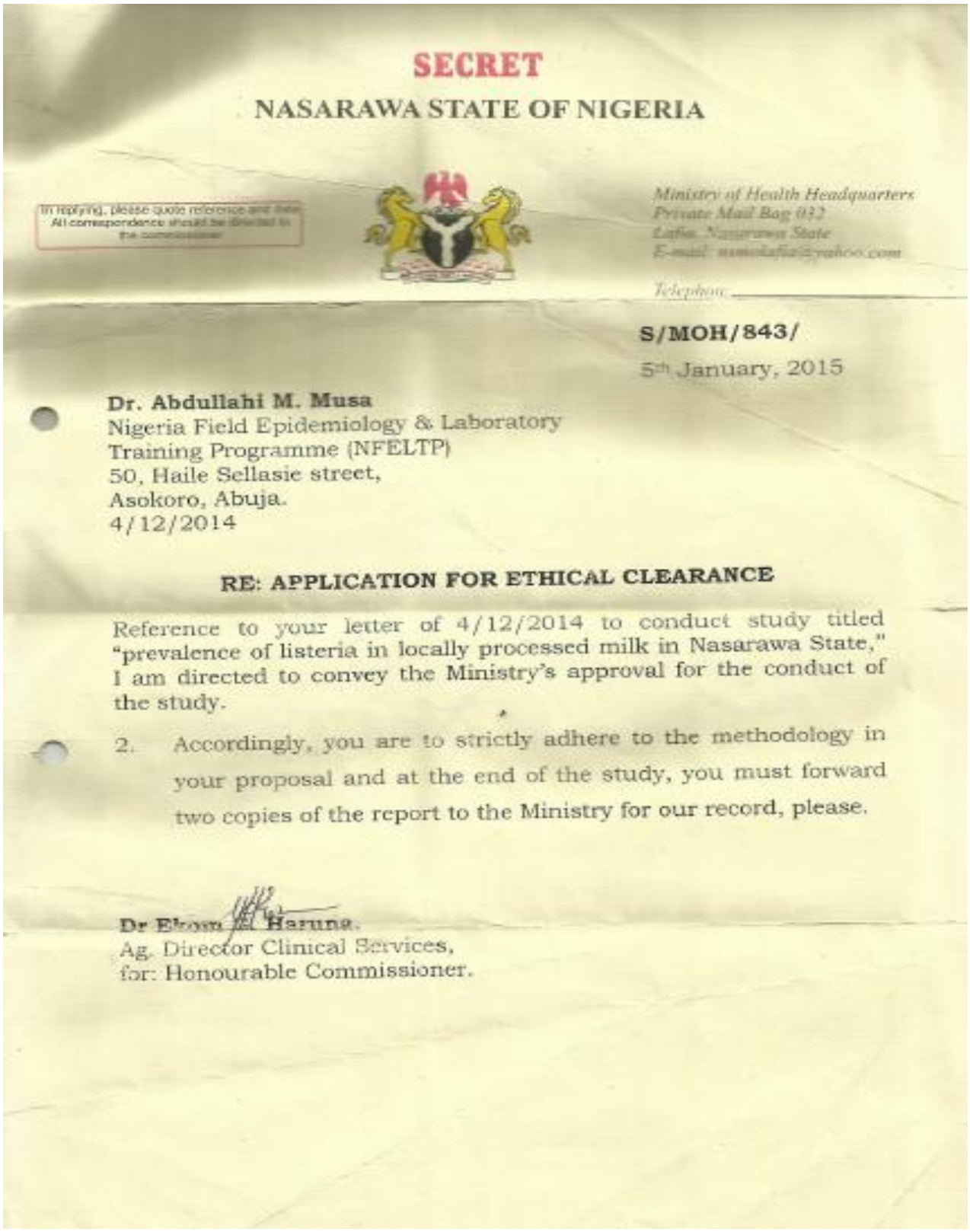
19. Is food safety training provided to workers in their native language?
Yes
No

20. How often do you clean milk processing equipment?
Every time - after use
Daily
2 times per week
Weekly
Other (list)

21. Are hand washing facilities available within milk processing areas?
Yes
No
22. What is the cleaning schedule for hand washing?
Every time (before and after milk processing)
Daily
2 times per week
Weekly
Other (list)
23. Are toilets facilities available within milk processing areas?
Yes
No
25. Is there any way of controlling insects like flies from contaminating the processed milk
Yes
No
26. After milk is processed, do containers of processed milk touch ground?
Yes
No
27. Do you add ice blocks to the processed milk?
Yes
No
Don’t know
9. How is milk stored after processing and during sales?
Refrigerated
Left at room temperature well covered
Left open at room temperature

30. How is processed milk dispensed during sales in the market?
Scooped from single container to all customers
Dispensed from individual sealed containers/satchets

ETHICAL CLEARANCE



SECRET

NASARAWA STATE OF NIGERIA

In replying, please quote reference and date. All correspondence should be directed to the commissioner

Ministry of Health Headquarters
Private Mail Bag 032
Lafia, Nasarawa State
E-mail: nsmoh...@yahoo.com

Telephone

S/MOH/843/

5th January, 2015

Dr. Abdullahi M. Musa
Nigeria Field Epidemiology & Laboratory
Training Programme (NFELTP)
50, Haile Sellasie street,
Asokoro, Abuja.
4/12/2014

RE: APPLICATION FOR ETHICAL CLEARANCE

Reference to your letter of 4/12/2014 to conduct study titled "prevalence of listeria in locally processed milk in Nasarawa State," I am directed to convey the Ministry's approval for the conduct of the study.

2. Accordingly, you are to strictly adhere to the methodology in your proposal and at the end of the study, you must forward two copies of the report to the Ministry for our record, please.

Dr Ekom [illegible] Haruna.
Ag. Director Clinical Services,
for: Honourable Commissioner.