OCCURRENCE AND ANTIBIOGRAM OF *SALMONELLA* SPECIES IN RAW AND LOCALLY FERMENTED MILK (*NONO*) IN ZARIA AND ENVIRONS, NIGERIA

BY

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BY

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DEPARTMENT OF VETERINARY PUBLIC HEALTH AND PREVENTIVE MEDICINE,
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OCTOBER, 2015

DECLARATION

I declare that the work in this thesis entitled **"Occurrence and Antibiogram of *Salmonella* Species in Raw Milk and Locally Fermented Milk (*Nono*) in Zaria and Environs, Nigeria''**has beencarried out by me in the Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, under the supervision of Dr. M. Bello and Prof. M. A. Raji. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diplomaat this or any other institution.

ZainabTAMBA	.....	.....
Name of student	Signature	Date

CERTIFICATION

This dissertationentitled "OCCURRENCE AND ANTIBIOGRAM OF *SALMONELLA* SPECIES IN RAW MILK AND LOCALLY FERMENTED MILK (*NONO*) IN ZARIA AND ENVIRONS, NIGERIA" byZainabTamba meets the regulations governing the award of the degree of Master of Science (M.Sc) in Veterinary Public Health and Preventive Medicine of Ahmadu Bello University, Zaria and is approved for its contribution to scientific knowledge and literary presentation.

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Prof. K. Bala Dean, School of Postgraduate Studies, A.B.U, Zaria	..... Signature	..... Date

DEDICATION

My profound gratitude goes to Almighty God for making this work possible. I am dedicating this work in memory of my Late motherHajiyaAsama'uTamba, who inspired me through my academic pursuit and also, my fatherCol. S.M. Tamba (Rtd) who also continued to encourage and support me through this program. I remain eternally grateful for the huge gift from these my parents.

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ABSTRACT

Salmonellosis is an important food-borne disease affecting both humans and animals. This study determined the presence and antibiogramof *Salmonella* species from raw and fermented milk. A total of 350 samples comprising 174 raw milk and 176 fermented milk samples were collected, using convenience sampling from different points/markets and nomadic (Fulani) herds in Zaria and environs. The Samples were examined for total aerobic count plate count (TAPC) while conventional biochemical method, Microbact 12E kit system and *Salmonella* polyvalent antisera were used to characterize and confirm *Salmonella* species. In addition, antibiotics susceptibility test was carried out on the isolates and minimum inhibitory concentration evaluation (MICE) was also conducted on the resistant isolates. The raw milk samples had TAPC with values that ranged between 0-9.5 $\log_{10}$CFU/ml, with a mean value of 7.42±0.14 $\log_{10}$ CFU/ml while fermented milk samples had TAPC values ranging between 0-7.9 $\log_{10}$CFU/ml, with a mean value of 7.22±0.19 $\log_{10}$ CFU/ml. A total of 14 (4%) *Salmonella* species were confirmed to be *Salmonellaarizonae* using the *Salmonella* polyvalent antisera from the total samples collected. Of these, 8 (4.6%) and 6 (3.4%) *Salmonella* species were isolated from raw and fermented milk samples respectively. Antibiotic susceptibility testing indicated that all the 14 isolates showed resistance to one or more antibiotic(s). Ten of the fourteen isolates were randomly selected and MICE test using strip containing amoxicillin and erythromycin antibiotics was carried out on 10 of the 14 *Salmonella* isolates. Only 1(10%) isolate

was sensitive to amoxicillin strip with a value of 0.12µg/ml whereas the remaining 9 (90%) isolates showed resistance to the antibiotic, with the highest value being 256µg/ml. In conclusion, the high bacterial load beyond the permissible level in the milk samples, the presence of *Salmonella* species in the milk and also multidrug resistant strains of *Salmonella* species are of serious public health significance. Therefore, there is need for the nomads to take corrective measures through public health enlightenment so as avoid milk contamination by *Salmonella* species.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Food safety and quality is a global topic of public concern. *Salmonella* is a Gram negative bacteria, rod shaped, aerobic or facultative anaerobic, belonging to the Family *Enterobacteriaceae* and it is a major pathogenic bacterium,'inhabiting the intestinal tract of humans and animals (Holt *et al.*,1994). *Salmonella* spp are important causes of bacterial contamination of the environment and the food chain (Ponce *et al*., 2008), and are the leading causes of acute gastroenteritis in several countries.In developing countries, contaminated vegetables, water and human-to-human transmission are believed to contribute to comparatively larger proportion of human cases than those in industrialized countries (Acha and Szyfres, 2003; Soltan *et al*., 2009). Salmonellosis due to *Salmonella* spp is the most common food-borne disease in both developing and developed countries, although incidence rates vary according to human and animals (Gerosa and Skoet, 2013).

According to the Centers for Disease Control and Prevention (CDC, 2011); the genus *Salmonella* contains two species, *Salmonella enterica* and *Salmonella bongori. S. enterica* consists of six subspecies (Popoff and Le Minor, 2001) namely; *S. enterica* subsp *enterica*, *S. enterica* subsp *salamae*, *S. enterica* subsp *arizonae*, *S. enterica* subsp *diarizonae*, *S. enterica* subsp *houtenae*, *S. enterica* subsp *indica.* A relatively small number of these serotypes have been documented to be host specific, characteristically producing systemic disease in a restricted number of animal species. These serotypes include: *S.* Gallinarium *and S.* Pullorumin chickens; *S.* Dublinin cattle; *S.* Cholera-suisprimarily in pigs (Zhang-Barber *et al*., 1999).

Milk is often described as fresh, clean and normal mammary secretion obtained by milking of one or more dairy animals that are properly fed and kept or also defined as the fluid secreted by mammals for the nourishment of their young (Rani and Mahesh, 2012). In dairy animals, cow milk is the most consumed milk world-wide and in 2011, Food and Agricultural Organisation (FAO) estimated 85% of all milk world-wide was produced from cows (Gerosa and Skoet, 2013). The cow milk is a good source of animal proteins, fats, vitamins and minerals to the human body (Rani and Mahesh, 2012). Nutritionally less useful substances like enzymes are also present in normal cow milk, and some of these enzymes are used as indices in screening or quality control tests for fresh milk (Anonymous,2012). The principal components of milk are water, fats, proteins and lactose. High water activity, moderate pH (6.4-6.6) and ample nutrients make milk an excellent culture medium for microbial growth at suitable temperature (Adams and Moss, 2008).

Pathogenic bacteria in raw milk have been a major public health concern. The main sources of contamination are the dairy cattle; food handlers and dairy equipment (Muehlhoff *etal*., 2013). Consumption of raw milk is considered to be the main cause of several outbreaks of*Salmonella* spp.,*Listeria monocytogenes* and *Escherichia coli* O157:H7 (Grant *et al*., 1995). Raw milk products have posed the greatest human health problems in various countries (Lejeune andRajala-Schultz, 2009). However, raw cow milk forms the basis for most commonly sold local milk products in Nigeria which include locally fermented skimmed milk known as “Nono”, locally fermented full creamed milk “Kindirmo”, local milk butter “main Shanu”or ‘‘ghee’’, and cheese “Wara”(Waters-Bayer, 1994).

Nono is the Fulani word for fermented cow’s milk sold predominantly by Fulani women. It is mostly available in the northern part of Nigeria (Yahuza, 2001). Nono is rich in protein,

notably essential amino acids; phosphorous and vitamins (Nebedun and Obiakor, 2007). Lactic acid bacteria are mostly associated with the production of fermented milk products and play a key role in producing nice flavour, aroma and good physical appearance in fermented milk products (Widyastuti *et al*., 2014). Although, lactic acid is the principal product of the fermentation lesser amount of flavouring substance diacetyl is also produced (Reyee *et al*., 2003; Tannock, 2004).

1.2 Statement of Research Problem

Salmonellae are zoonotic enterobacteria that are responsible for outbreaks of both human and animal diseases called salmonellosis and have important health significance worldwide, with several transmission routes of which majority of human infections are being derived from the consumption of contaminated foods (Yuk and Schneider, 2006) such as insufficiently cooked meat or improperly pasteurized milk and milk products (Jackson *et al.,* 2007). Salmonellosis is of significant public health concern, because humans and animals can become infected from consumption of food/feed and drinking water contaminated with *Salmonella* spp. from faeces of infected animals and raw milkproducts which have not been adequately pasteurized (Jackson *et al*., 2007).

Salmonella have been associated with documented food-borne illness episodes in the past 20 to 30 years and their numbers appear to be increasing (Fratamico *et al.,* 2005). Generally, the morbidity and mortality of attendant cases of food borne diseases are on the increase (Mead *et al.,* 1999).

Salmonellosis is a bacterial disease with a rising prevalence in the cattle industry. It is most common in dairy calves one to ten weeks of age, but can also be seen in adult dairy cows and beef cattle (Randall, 2001).

Salmonellosis has a serious economic impact on the cattle industry causing livestock mortality, abortions, reduced milk production, and reduced consumer confidence (Jackson *etal*., 2007). These result in extra labour and increased veterinary expenses (Santos *et al*., 2003; Nielsen *et al.*, 2004). Determination of food-borne disease estimates require continued and improved active surveillance (Mead *et al*., 1999), because despite many advances in food technology, it is still difficult to ensure food safety from stable to table (Duffy and Schaffner, 2002) with animals serving as reservoirs of many food borne bacterial pathogens (Cooke, 1990).

Acquired antibiotic resistance is a growing worldwide problem due to abusive use of antibiotics in humans, animals and agriculture.

Food contamination with antibiotic resistant bacteria can be a major threat to public health, as the antibiotic resistant determinant can be transferred to other bacteria of human significance. This is in view of the fact that the prevalence of antibiotic resistance among food borne pathogens has increased during past decades (Holt *et al*., 1994; Van *et al.*, 2007).

In Nigeria, studies have been reported from Plateau State on raw milk and locally-fermented milk with a prevalence of 0.8% and 6.4% of *Salmonella*spp respectively (Karshima *et al*. 2013) and Olatunji *et al*. (2012), also reported a prevalence of 17.06 % *Salmonella*spp in raw milk sold in Gwagwalada, F.C.T., Abuja.

The global problem of antimicrobial resistance is particularly pressing in developing countries with reservoirs of resistance present in healthy human and animal populations and also the increasing resistance to flouroquinolones by*Salmonella* spp as reported in Lagos, (Akinyemi *et al*. 2007), which is of public health concern.

1.3 Justification for the Study

Salmonella species are excreted through animal's faeces into the environment and can contaminate milk during milking. Also, the hands of the milkers who are suffering from salmonellosis could also be a source of contamination to the milk due to unhygienic practices. Therefore, milk and milk products derived from animals can be important vehicles of human salmonellosis (Wong *et al*., 2009; D'Aoust, 1998). Studies in Plateau State on contamination of raw milk and locally-fermented milk have reported prevalence of 0.8% and 6.4% of *Salmonella* spp respectively (Karshima *et al*. 2013) with Olatunji *et al*. (2012), also reporting a prevalence of 17.06 % *Salmonella* spp in raw milk in Gwagwalada, the Federal Capital Territory-Abuja. However, there is paucity of information on *Salmonella* spp. and their antibiogram associatedwith milk sold in Zaria.

1.4 Aim

The aim of this study was to isolate and identify *Salmonella* species from raw milk and locally- fermented milk "Nono" in Zaria, Nigeria.

1.5 Objectives

The specific objectives of the study are to:

1. determine the total aerobic plate count in raw and fermented milk samples from Zaria
2. isolate and characterize *Salmonella* spp from raw milk and locally-fermented milk "Nono" in Zaria, Nigeria.
3. determine the occurrence of *Salmonella* sppin raw milk and locally-fermented milk "Nono" in Zaria, Nigeria.

4. determinethe antimicrobial resistance patterns of *Salmonella* isolates from raw milk and nono in Zaria, Nigeria to commonly used antibiotics.
5. Evaluate the Minimum Inhibitory Concentrations (MIC) of selected antibiotics on resistant *Salmonella* isolates from raw milk and Nono in Zaria, Nigeria

1.6 Research Questions

i. What is the estimated bacterial load of raw and fermented milk samples in Zaria?
ii. Is *Salmonella* spppresent in raw milk and locally fermented milk "Nono" in Zaria?
iii. Are *Salmonella*isolates resistant to commonly used antibiotics?
iv. What are the antimicrobial resistance patterns of *Salmonella* isolates from raw milk and nono in Zaria, Nigeria?
v. What is the Minimum Inhibitory Concentrations (MIC) of selected antibiotics on resistant *Salmonella* isolates from raw milk and Nono in Zaria, Nigeria?

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 History of *Salmonella*

Salmonella was named after Daniel Elmer Salmon an American veterinary pathologist, who together with Theobald Smith first discovered the *Salmonella* bacterium in 1885 in swine suffering from hog cholera (Zee, 1994). The generic name *Salmonella* was given to this bacterium in 1900 by Lignieresi in honour of Dr. Daniel Elmer Salmon, who at that time was chief of Bureau of Animal Industry of the United States Department of Agriculture. The name has been universally used in 1933 (Edwards and Ewing, 1986).

Salmonella has been the subject of many studies throughout the world since 1885, when Salmon and Smith, first isolated the organism from swine in association with ‘’swine flu’’. William Bud who during his research work on origin of infectious diseases stated that typhoid fever was not spread by stench but was an alimentary disease in which the infective material was found in faeces, contaminated water, milk and the hands of those who attended to the sick (Ewing, 1972; Burrows, 1973).

In 1888, when 58 people became ill in Frankenhausen, Germany the outbreak of gastroenteritis was associated with consumption of beef and bacterium Enteritidis was isolated from the outbreak (Jordan, 1917).

2.2 Microbiology of *Salmonella*

Salmonella are facultative aerobic, gram negative rod-shaped bacteria belonging to the family enterobacteriaceae. Most of the members of this genus are motile by peritrichous flagella except *Salmonellaenterica* serovar Pullorum and *Salmonella entrica* serovar

Gallinarum, and non-motile strains resulting from dysfunctional flagella (D'Aoust, 2001). *Salmonella* are straight, non-spore forming rod measuring 0.7-1.5x2.0-5.0um, and are non-capsulated(Craig and James, 2006).

Typical *Salmonella* colonies on agar media are about 2-4mm in diameter with round smooth edges, slightly raised and glistening. They are aerobic to facultative anaerobes. The optimum temperature for growth is 37^{o}C but some can grow at a temperature range of 8^{o}C-45^{o}C(Craig and James, 2006) and the pH for growth is 4 to 8. The visibility of *Salmonella* can be maintained for years in simple media such as peptone or nutrient agar (Gast and Beard, 1990). They usually produce acid and gas from dextrose and carbohydrates such as lactose, sucrose or salicin.

However, *Salmonella* Typhi never produces gas, a point of some relevance to the blowing of cans (Christie, 1974). In a clinical laboratory, they are usually isolated on MAC Conkey, agar, Xylose Lactose Dextrose (XLD) agar or Desoxycholate (DCA) agar (Zee, 1994). There is no reason to doubt that members of *Salmonella* group are primarily intestinal parasites though they may also be isolated from the blood and internal organs of animals. They are frequently found in sewage, rivers, sea-waters and certain foods. They may survive for varying periods of time but it is doubtful whether they can exist indefinitely in any environment outside the animal's body (Cornell University,1997).

The chief reservoirs of infection in Europe and North America are probably fowls and pigs, but in tropical and sub-tropical countries the organisms are widespread in cold-blooded animals (Pui *et al*., 2011). A few species such as the typhoid bacillus in man and

*S.*Gallinarium in fowls are restricted to one or a few closely related host species. The majority, however, have a wide range of possible hosts (Wilson and Miles, 1966).

Salmonella organisms cause intestinal infections and are greatly outnumbered by the bacteria normally found in the healthy bowels. Primary isolation requires the use of a selective medium, thusthe use of a relatively non-selective medium such as Cystine Lactose Electrolyte Deficient (CLED) agar is not often practiced. Numbers of *Salmonella* may be so low in clinical samples that stools are routinely also subjected to "enrichment culture" where a small volume of stool is incubated in a selective broth and selective medium, such as Rapapport vassiliadis broth andSoya peptone broth. These media are inhibitory to the growth of the microbes normally found in the healthy human bowel, while allowing Salmonellae to become enriched in numbers. Salmonellae may then be recovered by inoculating the enrichment broth in one or more selective media. On blood agar, they do not ferment lactose (Zee, 1994).

Environmental sources of *Salmonella* include, water, soil, insects, kitchen surfaces, animal faeces raw meat, raw sea-foods to name only a few (FDA, 1998).

2.3 Classification of *Salmonella*

Scientific classification

Kingdom	:	Bacteria
Phylum	:	Proteobacteria
Class	:	Gamma Proteobacteria
Order	:	Enterobacteriales
Family	:	enterobacteriaceae

Genus : *Salmonella*

Species :***S. bongori* and *S. enterica***

The family entrobacteriaceae to which *Salmonella* belongs consists of five groups namely: Salmonellae, *Echerichiae*,Klebsiellae, Proteae, and Edwardsiellae (Tindall *et al*., 2005).

Taxonomy of *Salmonella* is complicated and classification of its species has been controversial for several years. However, the taxonomy of this genus is now established on a scientific basis and a corresponding nomenclature has been proposed by the World Health Organization (WHO) collaborating centre for references and research on *Salmonella* (Le Minor and Poppof,1987). The genus *Salmonella* is divided into 5 biochemically distinct subgenera:the various motile and non-host adapted serotypes of subgenus 1 are often refered to as paratyphoid salmonellae. Because the degree of genetic relatedness among the salmonellae is so great, researchers suggest that the genus actually consists of only a single species (Edwards and Ewing, 1986). However, the names of individual serotypes remain in common usage to facilitate diagnostic classification and epidemiological analysis.

As at 7th Dec., 2005, there were two species within the genus: *S. bongori* (previously subspecies V) and *S. entrica* (formerly called *S.* Cholerasuis) which is divided into six subspecies.

i. *enterica* - I
ii. *salamae* - II
iii. *arizonae* - IIIa
iv. *diarizonae*– IIIb
v. *houtenae* - IV

vi. obsolete (now designated *S. bongori*)- V

vii. *indica* - VI

The six subspecies of *Salmonella enterica* may be differentiated using DNA-DNA hybridization or biochemical properties. The first four subspecies have been considered as subgenera by Kauffman. Kauffman's subgenus III contains the *arizona* group but to avoid confusion, Kauffman's subgenus designation have been kept, but it is now clear that the taxon formally called Arizona or *Salmonella* subgenus III contains two clearly district subspecies called *S. arizonae* and *S. diarizonae* (*S. arizonae* includes the monophasic strains). Members of this subspecies have been isolated majorly from cold blooded animals (reptiles such as, snakes and lizards) have been reported to be major reservoirs of this subspecies. Although, isolation of *S.enterica* subspecies I from capture or freeliving reptiles is also common (Briones *et al*., 2004), it does not cause disease in them. *Salmonellaarizonae* and *Salmonelladiarizonae* are poorly maintained in warm blooded animals, but humans can be affected (Briones *et al*., 2004).

A correlation has been found between DNA-DNA hybridization, biochemical and serological characters for these two subspecies. Two new subspecies VI and VI have been characterized recently. Each subspecies is divided into serovars according to the O and H antigens specificities of the strain (Antigenic formulae of *Salmonellae*) (WHO, 2007).

There are numerous (totaling over 2,500) serovars within both species, which are found in a disparate variety of environments and which are associated with many different diseases. For the sake of simplicity, the Centers for Disease control and prevention(CDC, 2011) recommend that *Salmonella* species be referred to only by their genus and serovar e.g. *S.*

Typhi instead of the more correct designation,*Salmonellaenterica* subspecies *enterica* serovar Typhi. *Salmonella* isolates are most usually classified according to serology (Kauffman-White classification). The traditional Kauffman-White scheme for antigenic classification of *Salmonella* is based on both somatic and flagella antigens (Edwards and Ewing, 1986).

The main division is first by the somatic O antigen. The somatic antigens are determined by polysaccharides associated with the body of the cell and are identified by Arabic numerals. Then by flagella or H-antigens which are formed from structural proteins which make up the flagella that endows the organisms with motility. H antigens are further divided into phase 1 phase 2. The full description of a *Salmonella* isolate is given as (O antigens, VI: H antigen phase 1: H antigen phase). Note that, with the exception of typhoid and paratyphoid, *Salmonella* is not a common blood-related infection as is commonly believed (WHO, 2007).

Examples of *Salmonella* serovars are;

- *Salmonella* Enteritidis

  (The O antigens present are 1,9 and 12: the H antigens are g and m)

- *Salmonella* Typhi (9,12 Vid-)

  The Vi antigens is associated with the bacterial capsule, which acts as a virulence factor, hence its name).

In a clinical laboratory, only a small number of serovars are looked for (the remainder being rare or not clinically significant). The Health protection Agency recommends testing for the following antigens routinely: they are;

- O antigens 2,4,6,7,8,9 and 3,10
- Phase 1H antigens: 1,2,3,4,5,6,7
- Phase 2H antigens: a b c d E G i r

Isolates that cannot be identified using this panel are sent to the reference laboratory for identification (Tindall *et al.*, 2005).

2.4 Biochemical Properties of *Salmonella*

The common biochemical characters of salmonellae are stated below. Although most strains conform to this pattern, many of the characters are subject to variation; no organism should therefore be removed from the group on the result of a single test. The usual reactions include;

i. Fermentation of glucose, maltose, mannitol and sorbitol with the production of acid and gas.
ii. Absence of fermentation of lactose, sucrose, salicin and adonitol.
iii. Failure to produce indole, to hydrolyse urea, to deaminate phenyl alanine, to use malonate, or to grow in potassium cyanide medium.
iv. A positive methyl-red reaction and a negative Voges-Proskauer reaction (Hendriksen, 2003).
v. Most strains acidify dulcitol promptly but exceptions include *S.* Typhi and some of the d-tartarate-positive paratyphoid. Gas is not formed by *S.* Typhi, *S.* Gallinarum or by occasional stains of a number of other serotypes: maltose is not usually fermented by Pullorum strains and *S.* Typhi-suis does not attack mannitol. Occasional strains which ferment lactose, sucrose or salicin (e.g. *S. arizonae*,

Subgroup III and *S. houtenae*) or produce indole have been recorded. The urease, phenyl alanine, potassium cyanide, methyl-red and Voges-proskauer reactions appear, however to be constant (Hendriksen, 2003).

Most *Salmonella* grow with citrate as the sole carbon source, though important exceptions include the typhoid and paratyphoid A bacilli, of *S*. Gallinarum (Wilson and Miles, 1966).

Salmonella reduce trimethylamine oxide to trimethylamine and nitrate to nitrite. They are catalase positive, oxidase negative and normally do not liquefy gelatin, pectate and alginate. Inositol is utilized by numerous strains. Sodium malonate is not utilized and acid is produced in Jordan's tartarate medium. They form arginine dihydrolase, esculin is not hydrolysed, and growth occurs in sodium acetate medium (Buchanam and Gibbons, 1974; Al-hindawi and Rished, 1979).

The statement is often made that the production of H_2S is characteristic of the *Salmonella* group. Practically all enterobacteria form some H_2S under optimal conditions of growth. The ability to cause blackening of certain media in which heavy metallic salts are incorporated in an agar base is however, confined to the *Salmonella*, *Citrobacter* and *Proteus* groups(Thomas *et al*., 2011).

Thus, most salmonellae give a positive reaction for H_2S in Kligler's medium and in ferrous chloride gelatin, but exceptions include *S*. Paratyphi A, the diphasic variety of *S*. Choleraesuis, *S*. Typhi-sius, *S*. Sendai, *S*. Beria and some strains of the typhoid bacillus. Few of the common salmonellae liquefy gelatin, and only three (*S*. Abortus-bovis, *S*. Schleissheim and *S*. Texas) do so rapidly (Thomas *et al*., 2011). According to Lautrop (1956), "the gelatinase of the rapid liquefiers require the presence of calcium ions, has a low

temperature optimum, is inhibited by toluene, and is probably extracellular". That of the slow liquifiers is independent of calcium, is a little influenced by temperature, and accelerated by treatment of the cells with toluene (Thomas *et al*., 2011).

Nearly all salmonellae produce lysine, arginine and ornithine decarboxylase, but no glutamic acid decarboxylase (Moller, 1954). The only notable exceptions are the typhoid bacillus and some stains of *S.* Paratyphi B var odense which form no decarboxylase for ornithine(Acharya, 2015). Moller (1955) drew attention to the value of tests for the fermentation of organic acids.

Most salmonellae rapidly attack d-tartarate, citrate and mucate, though there are many exceptions. Several types, notably *S.* Paratyphi A, *S.* Sendai and the human type of *S.* Paratyphi B are d-tartarate negative, and numerous others attack this substance late and irregularly (Kemal, 2014).

Arizonae strains generally differ from salmonellae in that they attack lactose, have no action on dulcitol. Liquefy gelatin, and fail to ferment d-tartarate, citrate or mucate in 24 hours. With a few exceptions they are malonate positive (Lautrop, 1956). Many gelatin liquefying salmonellae of Africa origin are however, also able to use malonate and are slow or irregular in their attack on the organic acids (Wilson and Miles, 1966) indicating that these Arizona-like strains should form a separate subgenus within the genus *Salmonella*.

Some other biochemical tests are at times useful for the identification of individual serotypes, or for subdividing them further. They include the fermentation of rhamnose, xylose, arabinose, trehalose and inositol and most strains attack these sugars, and it is the occasional absence of fermentation which is of significance (Wilson and Miles, 1966).

The confirmation method of *Salmonella* detection is the serological method; Enzyme linked immunosorbent assays (ELISA) can be used to detect either the organism or a humoral immune response to the organism. Since culture may take 3-7 days to identify the organism, ELISA can detect the organism in a much shorter period of time, usually one day or less. The detection of antibodies to the O antigen of *Salmonella* has been used successfully in pigs (Neilsen *et.al.,* 1994). The antibody ELISA using mixed purified lipopolysaccharide (LPS) from both *S.* Choleraesius and*S.* Typhimurium has been used for routine screening in breeding, multiplying and slaughtering herds in Denmark since 1994 (Anon., 2010).

For screening purposes, serological testing provides an indication of exposure to *Salmonella* (Lo- Fo-Wong *et.al.,* 2003).

2.5 Salmonellosis as a zoonoses

Animals and humans discharge a large number of *Salmonella* organisms into the environment through faecal contamination of water and soil as part of the epidemiological cycle and can contaminate, for example, milk which then also becomes a source of food-borne human infections(Murray, 1991).The principal source of human food-borne *Salmonella* infections is through faecal/intestinal contamination of carcasses with the exception when *Salmonella* is directly transmitted into the food product, for example, into milk (Henzler and Opitz, 1992).Raw or processed milk is a well-known good medium that supports the growth of several microbes including *Salmonella* with resultant spoilage of the product or infections/intoxications in consumers (Murinda*et al*., 2004; Oliver *et al*., 2005). Salmonellosis caused by *Salmonella*organisms is the most common food-borne bacterial disease in the world, and a significant pathogen for food-producing animals and these animals are the primary source of salmonellosis(Forshell and Wierup, 2006).

2.6SalmonellaInfections

2.6.1 Human *Salmonella* infections

The incubationperiod ranges from five hours to seven days, butclinical signs usually begin 12 hours to 36 hours after ingestion ofa contaminated food. Clinical signsinclude diarrhoea, nausea, abdominal pain, mild fever andchills. Vomiting, prostration, anorexia, headacheand malaise may also occur (Anon., 1999). The syndrome usually lasts fortwo to seven days. Systemic infections sometimes occur,and usually involve the very young, the elderly or theimmuno-compromised. A fatal outcome is rare (Anon., 1999).This problem is particularly evident in developed countries like England and Wales, where the most frequently reported out-breaks were salmonellosis associated with the consumption of raw milk and products (De Buyser*et al*., 2001).

All Salmonellae are of public health concern having the ability to produce infection ranging from a mild self-limiting form of gastroenteritis to septicaemia and life threatening typhoid fever (Oliver *et al*., 2005).Lingathurai and Vellathurai, (2010) reportedthe incidence of *Salmonella*spp in local raw milk in Madurai, South India. They reported low isolation rate, as only 8 out of 60 milk samples were found positive for this organism. Thus, the occurrence of *Salmonella* in local milk was low; they still pose a health risk to consumers if milk is consumed without any heat treatment.

2.6.2 *Salmonella* infections in cattle

Salmonella species are well documented to cause diseases in livestock industries, especially in dairy cattle (Sanchez *et al*., 2002; Guerin *et al*., 2005). Clinical signs are commonly seen when cattle are immuno-suppressed. The earliest signs include fever and diarrhoea (Gordon, 2008). Bacteremia or endotoxemia can occur leading to abortion in pregnant cows, (Lauretti and McKay, 2008). The risk period of salmonellosis in cattle varies, depending on the

serotype it could range from one week to several months of age (Murphy *et al*., 2008). Transmission and the spread of the disease are through the faecal-oral route and it is maintained within cattle populations by carrier and infected animals, contaminated environment and feeds (Mohler *et al*., 2009).

Salmonella has been seen as a food-borne pathogen for well over 10 decades (Newell *et al*., 2010). Its zoonotic potential is undebatable, human infections with *Salmonella* are quite common with more than 10 cases per 100,000 people (Mangen *et al*., 2010). Outcomes from the vast majority of people infected with *Salmonella* include mild to moderate, self-limiting gastroenteritis.

2.7 Serotyping

Salmonella species are classified into serovars (serotypes) based on the lipopolysaccharide (O), flagella protein (H), and sometimes the capsular (Vi) antigens. Within a serovar, there may be strains that differ in virulence. Based on the similarities in content of one or more O antigens, members of *Salmonella* are placed in groups designated A,B,C and so on. This, *S.* Hirschfeldii, *S.* Choleraesuis, *S.* Oranienberg and *S.* Montevideo are placed in group C, because they all have O antigens 6 and 7 in common. *S.* Newport is placed in group C2 due to its possession of O antigensand 8. For further classification, the flagella or H antigens are employed. These antigens are divided into 2 groups: Specific phase or phase 1 and group phase or phase 2. Phase 1 antigens are shared with only a few other species or varieties of *Salmonella*. Phase 2 may be more widely distributed among several serotypes. Any given culture may consist of organisms in only one phase or of organisms in both flagella phases. The H antigens of phase 1 are named with small letters, and those of phase 2 are designated

by Arabic numerals. Thus, the complete antigens analysis of *S.* Choleraesuis is as follows: 6, 7, c,1,5, where 6 and 7, refer to O antigens c to phase 1flagella antigens and 1 and 5 to phase 2 flagella antigens (Anon., 2010).

2.7.1 Serotypes of *Salmonella* species occurring in Nigeria.

As from the 1956-1960, 1005 strains of *Salmonella* species were identified and from time to time, these have been reported at the National *Salmonella* Centre for Nigeria (Collard and Sen, 1962).Kwaga *et al.* (1984) reported on the prevalence of *Salmonella* serotypes namely; *S.* Rough, *S.* Chester,*S. S*outhbank, *S.* Dublin, *S.* Bergeriand *S.* Langfordfrom cattle in some parts of Kaduna state.

A new *Salmonella* serotype *S. Zaria* was isolated from the mesenteric lymphnode of cattle slaughtered at Zaria (Kwaga *et.al.,* 1985). Twenty *Salmonella* serotypes were isolated from the lymph nodes of slaughtered cattle and market beef in Zaria namely *S.* Living stone, *S.* Saint Paul,

S. Schwarzengrund, *S.* Ealing, *S.* Ouakam, *S.* Abaetetuba, *S.* Waycross, *S.* Eppendorf, *S.* Montevideo, *S.* Hull, *S.* Tilene, *S.* Give, *S.* Stanleyville, *S.* Chester, *S.* Infantis, S. group E1 *S.* Garba, *S.* Widemarsh, *S.* Ikaji and *S.* Yaba (Kwaga *et al*., 1985).

In another study, three *Salmonella* serotypes namely *S.*Typhimurium, *S.* Eenteritidis and *S.* Derby were isolated from the environment of Jos municipal abattoir by Osiyemi and Agbonalahor, (1981). In Jos, *S.* Galinarium, *S.* Pullorum, *S.* Reading, *S.* Virchow, *S.* Infants and *S.* Ouakam, were isolated from indigenous fowls (Osiyemi and Agbonlahor, 1983) and Oludairo *et al*. (2013), also reported 5% prevalence*Salmonella* spp from captive animals in theNational Zoological Garden, Jos.

Two *Salmonella* serotypes, *S.* Typhimurium (3 strains) and *S.* Dublin (5 strains) were isolated from 5, out of the 2000 cattles slaughted for human consumption at Nsukka abattoir in Enugu state (Oboegbulem and Muogbo,1981); there was also *S.* Ochiogu isolated from guinea pigs at Portharcourt (Onyakaba, 1985).

Collard and Sen, (1960) isolated *S.* Bredney, *S.* Edinburgh, *S.* Hadar, *S.* Rubislaw and *S.* Africana from fowls at Ibadan. Six serotypes of *Salmonella*, *S.* Offa, *S.* Glostrup, *S.* Swimborne, *S.* Dublin, *S.* Saint-paul and *S.*Webridge were isolated from captive wild animals in Ibadan, western state of Nigeria (Falade and Durojaiye, 1976).

S. Abaand *S.* Takoradi were isolated from a lizard and dead baby elephant respectively at the university of Ibadan zoo (Falade and Durojaiye, 1976).

Many serotypes and phage types found in humans were also found in cattle, pigs and poultry, suggesting that food producing animals are important sources of food human non-typhoid *Salmonella* infection (Cornell University, 1997).

2.8 Antimicrobial Agents and Drug Resistance in *Salmonella*

The increasing resistance of members of the Enterobacteriaceae to antimicrobial agents has been noted throughout the world (Dulaney and Laskin, 1971; Davis and Rownd, 1972) and particular interest has been focused on *Salmonella* species (Neu *et al*., 1971; Okubadejo *et al*., 1971; Neu *etal*., 1975; Kwaga *et al*., 1984; Ismaeel, 1993; and Nair *et al*., 1995). Despite the large volume of literature on the chemotherapy of salmonellosis, only a relatively small proportion of antimicrobial substances exert action of practical significance againt *salmonellae.* Although therapy using chloramphenicol is indicated in systemically complicated salmonellosis, antibiotic therapy of uncomplicated *Salmonella* gastroenteritis is

often ineffective and therapy may probably lengthen duration of faecal excretion of salmonellae (Gibson, 1961). Several drugs have been found to be effective against *Salmonella* in vitro, but chloramphenicol would probably remain the drug of choice (Neu *et al*., 1971; Jesudasan *et al*., 1990). Timoney (1978) suggested that a more effective control on the veterinary use of chloramphenicol would be needed if resistance to this important drug is to be minimized.

Although chloramphenicol-resistance *Salmonella* have appeared from time to time, the emergence of strains that have acquired simultaneous resistance to all three conventionally used antibiotics namely; chlorampheicol, trimethoprim-sulfamethoxazole and ampicilin (multi-drug resistant) is recent (Anand *et al*., 1990; Jesudasan *et al*., 1990). The prevalence of resistance of penicillin, other β-lactam antibiotics, and tetracycline, has increased during the past two decades (Nair *et al*., 1995). The increase in the incidence of drug resistance among isolates from animals and man is attributed to the development of intensive system of cattle rearing with its attendant large scale indiscriminate use of antibacterial drugs in feeds, prophylaxis, therapy and as growth promoters (Ojo, 1972; Timoney, 1978; Georges-Courbot *et al*., 1990).

When antibiotics were introduced, it was considered that antibiotic resistance was unlikely to develop because, the frequency of mutation of resistance in bacteria was extremely low (Nair *et al*., 1995). However, bacteria can acquire and exchange genetic information with extra-ordinary facility, permitting antibiotic resistance to be acquired and passed on from one microbe to another and creating enormous pressure for selection of antibiotic resistant traits among microbes (Davis, 1994). Salmonellosis caused by resistant organisms has been recognized as a complicating factor during antimicrobial treatment of other infections

(Georges-Courbot *et al*., 1990). These infections are increasing (Baird-Parker, 1990) and have become a cause for great public health concern (Georges-Courbot *et al*., 1990; Lee *et al*., 1993; Lee *et al*., 1994).

The antibiogram of *Salmonella* isolates from animals are generally similar to those encountered in human beings. This is because animal strains have contributed to the pool of resistant salmonellae in human beings (Neu *et al*., 1975; Sojka *et al*., 1975; Timoney, 1978; Nair *et al*., 1995). A similar pattern of resistance to β-lactams and tetracycline has also been noticed among isolates from human beings, foods and environmental sources (Nair *et al*., 1995).

2.9 *Salmonella* in Foods

Salmonella continues to be one of the main causes of food-borne illnesses world-wide. In many countries, salmonellosis is the most frequently reported food-borne disease (Baird-Parker, 1990; Nair *et al*., 1995). Raw foods of animal origin are the major sources of salmonellae in kitchens of restaurants, institution and homes (Kapperud *et al*., 1989; Boyce *et al*., 1996). A number of factors such as improper cooling or heat treatment during food preparation are possible sources of *Salmonella* infection before serving and infected food handlers have been identified as being important in food-borne outbreaks (Bryan, 1980; Zeidler, 1996). In the US, beef, turkey, home made ice cream (containing raw eggs), pork and chicken were identified as vehicles of salmonellosis on several occasion (Bryan, 1981; Bean and Griffin, 1990; Tauxe, 1991). The association between eggs and human salmonellosis due to *Salmonella*Eenteritidis has been widely publicized for several years

and remains an important international public health and economic issue (Boyce *et al*., 1996; Ridley *et al*., 1996).

Studies carried out in the U.K show that the overall level of contamination of retail chicken products in 22.8% (Plummer *et al*., 1995). Oboegbulem (1990) reported a 44% isolation frequency of *Salmonella* from fresh and frozen chicken in Scotland by the whole carcass rinse method. The most frequently occurring serotypes in that study were *S.* Enteritidis (22.7%), *S.* Typhimurium phage 4 type 104 and 49 (16%), *S.* Virchow (8.3%), *S.* Binza (5.7%), *S.* Bredeney (5.2%), and *S.* Hadar (4.6%).

Aquatic foods like raw prawns have also been known to be a source of *Salmonella* infection (Korbscrisate *et al*., 1994). Vegetables and fruits are rarely reported to be vehicles of *Salmonella* species in food-born outbreak (Wei *et al*., 1995). However, salmonellae have been isolated from several types of fresh vegetables, many of which have been linked to human salmonellosis (Beuchat, 1996). Since 1990, four multistate outbreaks of salmonellosis in the United States each involving 100 to 400 confirmed cases have been attributed to fresh fruit and vegetable sources (Hedberg *et al*., 1994).

Salmonellae have been isolated from lettuce and fennel in Italy (Ercolani, 1976), numerous salad vegetable in Spain (Garcia Villanova *et al*., 1987), and bean sprout in Thailand (Jemgklinchan and Sartanu, 1993). *Salmonella* contamination of vegetables could occur at the farm and the sources could include animal excreta, workers, irrigation and rain water, dirt and fertilizer, transportation and vehicles could also be an important source of *Salmonella* contamination (Wei *et al*., 1995). Restaurants and food industries have been vital point sources of *Salmonella* out-break (CDC, 2003, Boyce *et al*., 1996).

In Nigeria, reporting of food-borne disease outbreak is rare; therefore, information on the prevalence and degree of involvement of microorganism in these outbreaks is not available. Studies on food consumed in Nigeria have however, demonstrated that the potential for food borne disease outbreaks due to *Salmonella* exist (Nkanga and Uraih, 1981; Adesiyun, 1984b; Adesiyun *et al*., 1987, Shehu *et al*., 1990; Zaria *et al*., 1992).

2.10 *Salmonella* in Water

Polluted surface water can be a source of *Salmonella* infection to animals and humans (Morse, 1980; Groisman and Ochman, 1997). In areas where typhoid fever is endemic, water from rivers, lakes and dams which is used for public consumption and sometimes contaminated by raw sewage is the main source of infection (Hornick, 1985). It has been clearly demonstrated that the incidence of typhoid fever decreases drastically with the provision of clean water through chlorination and filtration (Hornick, 1985). There has been concern that the use of sewage polluted waters as sources of recreation is a significant route for the dissemination of pathogens from excretors back into the general population (Corliss *et al*., 1981).

In Nigeria, studies on the quality of pipeborne water, water from wells, streams and ponds supplied to some communities have been carried out (Adesiyun *et al*., 1983). These studies suggest that the quality of water supply to most communities is poor. Even though *Salmonella* was not isolated from any of these water sources, there is much evidence to believe that these water sources are contaminated with *Salmonella* which can be detected with more sensitive techniques.

2.11 Situation of *Salmonella* infection in Nigeria

In Nigeria, 43% of tortoises purchased from a market were reported to harbour *Salmonella* in their gastrointestinal tract. Eight of the ten isolates were *S.* Typhi. This indicates that tortoises may be important in the transmission of human salmonellosis (Ogbimi *et al*., 1988). An isolation rate of 1% *Salmonella* in dogs in Zaria has been reported by Kwaga *et al*. (1989). After the examination of rectal swabs from 809 cattle around Zaria, Kwaga *et al*. (1984) reportedan overall carrier rate of *Salmonella* in cattle to be 1.5%. In that study, the strains of *Salmonella* isolated belonged to five serotypes and the most commonly encountered serotype was *S.*Bergen while *S.* Southbank and *S.* Langford were reported for the first time in the bovine in Nigeria. (Kwaga *et al*., 1984).

The results of a serological investigation using 598 sera obtained from chicken in Kaduna and Zaria revealed that 167 (28.0%) were positive for *Salmonella* (Salami *et al*., 1989a). Of the poultry diseases diagnosed in Kaduna for the period 1981 to 1985, fowl typhoid accounted for 8.0%. Also, *Salmonella* Ochiogu was isolated from laboratory guinea pigs during an outbreak of salmonellosis in Port Harcourt has been reported (Onyekaba, 1985).

The first report of *Salmonella* infection was the recovery of *S.* Saintpaul from an aborted goat foetus (Plowright, 1955). In Ibadan, *S.* Agona, *S.* Labadi and *S.* Colindale were isolated from clinical specimens of sheep at post mortem, though the total number of specimens examined were not stated (Ojo, 1972). However, there are reports on the isolation and involvement of *Salmonella* in disease of sheep and goats in Nigeria. In Kaduna and Kano States, examination of 293 goats at 4 locations at slaughter using mesenteric lymph nodes and gall bladder yielded isolation rate of 1.65%.of the 192 goats from Samaru and Zaria, non-yielded *Salmonella*. The serotypes of *Salmonella* obtained were *S.* Give,*S.* Hull and *S.* Kaapsted (Corke *et al*., 1975).

In Nsukka, Nigeria, 27 out of 90 or about 30% household wall lizards (*Gecko gecko* and *Hemidactylus* sp) examined yielded *Salmonella* isolates. In that study, *S.* Weltevredren and *S.* Hvttingfoss were reported in Nigeria for the first time (Oboegbulem and Isegbolimhen, 1985). In another study in the same environment, Oboegbulem and Okoronkwo (1985) reported a 32% isolation rate of *Salmonella* from African great cane rats (*Thryonomys swinderianus*).

2.12 Reservoir and host range of *Salmonella*

Salmonellae are widely distributed in animals, birds, reptiles, fishes, insects, man and the environment (Morse, 1978; Kwaga *et al*., 1989; Henzler and Opitz, 1992; Davis and Wray, 1995). The major reservoirs of *Salmonella* are live animals (Rice and Lowenstein, 1979).

There are two categories of salmonellosis in animals namely: diseases with characteristic clinical manifestations caused by specific *Salmonella* types and diseases whose symptoms only indicate *Salmonella* infection and caused by any serotype (Bruner and Gillespie, 1973). The host adapted serotypes and host animals are *S.* Abortusequi (horse), S. Abortusovis(sheep), *S.* Choleraesuis and *S.* Typhisuis (pig), *S.* Dublin (cattle) and *S.* Pullorum and *S.* Gallinarum (poultry). The other serotypes infect all other animals and can cause disease. The sources of infection for animals are other animals of the same species, farm environment, feed, pasture water, man, other animals (including wildlife), fertilizers and contaminated objects. Congenital and neonatal infections may occur (Richardson, 1973; Petrie *et al*., 1977). The host adapted *Salmonella* serotypes are much more virulent for the species to which they are adapted than for other species. For example, the avian adapted *S.*

Pullorum and *S.* Gallinarum cause severe disease in poultry but very rarely infect man (Baird-Parker, 1990).

2.13 Factors influencing the occurrence of Salmonellosis

Salmonellosis in humans and animals is influenced by several factors. These factors include *Salmonella* serovar, age of host, host immunity, infectious dose, type of foods that are contaminated with *Salmonella* and predisposing diseases (Poppe, 1996).

The rates of reported *Salmonella* isolation are highest in infants 1 to 4 months old and decrease abruptly among childhood age groups, remain relatively constant through the adult years and then increase in the age group over 80 years of age and also the immunologically compromised patients due to infection with human immunodeficiency virus (HIV), leukaemia, and the use of immunosuppressive drugs (Tauxe, 1991; Poppe, 1996). Bacteremia due to *S.* Typhimurium, *S.* Dublin and *S.* Enteritidis appears to be particularly common in AIDS patients. This may be related to the invasiveness of these serotypes and to their frequent occurrence in raw milk, raw eggs or raw beef, which are consumed by patients in the mistaken notion that such food could bolster their immune defenses (Levine *et al*., 1993) and also, the use of anitmirobial agents to which the infecting *Salmonella* is resistant (Ngulo *et al*., 2000).

The infectious dose of *Salmonella* was previously thought to be about 10^6 organisms. However, there is evidence that 100 to 1000 bacteria may cause illness in young children, in the elderly, and in persons who are immuno-compromised, especially if the bacteria are contained in foods with high fat content such as cheese and chocolate (Bryan, 1977; Siliker, 1982; Gill *et al*., 1983; Mintze *et al*., 1994; Poppe, 1996). The pattern of infection also

influences the severity of salmonellosis. The infecting serovar may also determine the severity of the disease. Infection is usually confined to the intestinal tract although; host-specific serovars (*S.*Typhi, *S.* Paratyphi B and *S.* Paratyphi C) cause a much more severe form of salmonellosis known as enteric fever (White, 2010). Serovars that may cause extra intestinal tract disease are *S.* Cholerasuis, a serovar that is host specific for swine; *S.* Dublin, a bovine specific serovar, *S.* Typhimurium, *S.*Heidelberg, *S.* Virchow, *S.* Saintpaul and *S.*Enteritidis, which have a wide host range. The incubation period of *Salmonella* infection in humans is about 6-72 hours (Poppe, 1996).

2.14 The Effect of temperature and acidification on the growth of *Salmonella*

Temperature is a major environmental factor controlling the growth of microorganisms in food (Mitchell, *et al*., 1995). One of the earliest studies on the effect of transient temperature changes on bacterial growth is that of Lark and Maaloe (1954). They imposed temperature changes between 25°C and 37° in 20 seconds on cultures of *Salmonella* Typhimurium by diluting with broth at 3°C and 9°C respectively. They found that for cultures growing in the exponential phase, reducing the temperature from 37°C to cultures growing in the exponential phase, reducing the temperature from 37°C to 25°C resulted in the culture growing at the rate characteristic of 25°C. However, if the exponentially growing culture was raised from 25°C to 37°C, a lag of 20 minutes was induced, followed by a growth rate greater than that associated with the temperature of 37°C and then growth at the rate expected at 37°C.

However, with the rapid advances in development, processing and packaging of foods, there is an increasing need to be able to predict the growth of food poisoning bacteria. Predictive

mathematical models allow processors and manufacturers to estimate shelf-life and safety of product at a given stage. Various models have been proposed which describe the growth response of bacteria to environmental factors (Gibson *et al*., 1996; Zwietering *et al*., 1991; Hills and Wright, 1994; Mitchell *et al*., 1994; Mitchell *et al.*, 1995).

Almonacid-Merino *et al.* (1993), used different sequences of steps between 1-2°C and 3-14°C of a few hours duration applied to a mixture of spoilage organisms in order to analyse temperature abuse in refrigerated foods. Studies have been carried out to investigate whether growth under conditions of time dependent temperatures could be predicted from growth curves obtained isothermally (Mitchell *et al*., 1995).

The dynamic growth model of Baranyi *et al*., (1993) successfully predicted growth in the temperature range 5 - 25°C for suspensions adjusted to pH 7.0 and a concentration of 9.5% (w/v) NaCl, although some deviations were observed after 12 hours of fluctuation when the suspension was adjusted to pH 5.9 and a concentration of 9.5% (w/v) NaCl, although some deviations were observed after 12 hours of fluctuation when suspension was adjusted to pH 5.9 and a concentration of 2.0% (w/v) NaCl. A more significant deviation was observed for temperature fluctuations down to 28°C, especially when the lower pH, higher salt condition was studied.

Suspensions of S. Typhimurium have been subjected to a sinusoidally varying temperature between 12°C and 30°C, that is, within their growth temperature range. Storage at 2°C, particularly in low pH conditions results in loss of viability at a shorter storage time (Mitchell *et al*., 1995).

Different serotypes of *Salmonella* have been found to vary in their heat tolerance or low nutrient environments (Jenkins *et al*., 1988) in contrast, heat sensitivity can be induced by exposure to low temperatures (Humphrey, 1993). Acid tolerance can also be enhanced by exposure to mildly acidic environments (Foster and Hall, 1990; Foster, 1991; Leyer and Johnson, 1992) and by growth at, or shifts to higher temperatures (Humphrey *et al*., 1993). It has been found that human isolates of *S.* Enteritidis phage type 4 are more heat tolerant than chicken isolates (Humphrey *et al*., 1995). Further findings have revealed that, cells which exhibit enhanced tolerance to high temperature are better able to survive in the presence of acid or H_2O_2 or on working surfaces (Humphrey *et al*., 1995). The differing impacts of growth phase and isolate type on the observed tolerances, however, suggest that the cellular attributes which increase tolerance of one damaging environment may not be the same attributes which permit enhanced survival in another environment even when apparent cross-tolerance is observed (Humphrey *et al.*, 1995).

2.15 Public Health Significance of*Salmonella*

Consumers may suffer food poisoning or acquire infection with *Salmonella* some of which may be antibiotic resistant. It is important to reduce this hazard at all steps in the production and preparation of food. In order to prevent negative consequences of *Salmonella* in contaminated meat, the control of *Salmonella* is necessary. The whole meat production chain should be free from *Salmonella*, including the animals at the farm (Abram, 1990).

Food safety assurance strategies can be implemented at all levels of food production (i.e. pre-harvest, post-harvest, processing and retail) monitoring, prevention and control efforts at the pre-harvest levels are important elements of food safety assurance strategies to

prevent or reduce the transmission at the harvest level of meat production (Mousing*et al*., 1997).

Several studies have shown that the implementation of preventive measures could reduce the prevalence contamination level (NADIS, 2007).

In 1998, the Netherlands was able to control effectively *Salmonella* in pig and pork by codes of good management practices (G M P) from farm to cutting/ retail, which could reduce the levels of *Salmonella*- positive pigs and pork by 50-60%. Moreover, for specific pathogen-free (SPF) pigs, GMP could reduce the *Salmonella* prevalence by 95% (Be rends *etal*., 1998).

In Canada, Public Health Agency of Canada had introduced Center net, a multilateral initiative facilitated by the public health agency of Canada and funded by Agriculture and agric-food Canada through the Agriculture and policy framework initiative for supporting activities that will reduce the burden of enteric (gastrointestinal) diseases, by comprehensive sentinel surveillance implemented though local public health units (Public Health Agency of Canada,2007).

In order to avoid any major risk, many countries now impose regulations that require producers, processors and distributors of food stuffs to set up more frequent and efficient testing plans for the systematic control of at-risk products.

2.16 Use of antibiotics (Letellier *et al.*, 1999)

The prolonged feeding of antibiotics for growth may lead to:

1. Promotion of long use of antibiotics appears not only to influence the resistance pattern of *Salmonella* but also to adversely affect the resistance of the pigs' intestine to colonization.

   In general, the control of *Salmonella* is based upon the implementation of preventive actions throughout the whole production chain. More specifically, measures should be addressed to;

   (i) The prevention of introduction of *Salmonella* into the herd,

   (ii) Theprevention of in-herd transmission and

   (iii) The increase of the resistance to the infection

2. The use of antibiotics for *Salmonella* Enteritidis without septicaemia is controversial. The population of normal intestinal bacterial micro-flora may be altered as well as the possible developmentof antibiotic resistance by *Salmonella* organisms (Randall, 2001).

2.17 Prevention of Salmonellosis

Prevention should involve two avenues, the first being decreasing the chances of exposure to the organism and the second being increasing resistance in cattle.

1. Quarantine and sero-test replacement stock.
2. Avoid wet areas, provide dry loafing areas, clean and disinfect calf pens and maternity areas between calves.
3. Use clean flush water. Use only water from milking parlor.
4. Check feed commodities for *Salmonella*. Store and handle feed properly.
5. Do not allow rendering trucks access to feed or animal areas. Do not use front-end loaders for manure or to have dead animals and then produce feed from them.

6. Avoid introducing potentially infected animals by maintaining closed herd. Quarantine all introduced stocks for at least four weeks.
7. Source new stocks from other farms with high health status and not markets.
8. Avoid shared bulls and communal grazing areas.
9. Clean and disinfect buildings between occupancies. Provide good drainage and waste removal.
10. Protect all feed stores from vermin including birds.
11. Maintain good fences to prevent straying of neighboring stocks.
12. Researchers working with the*Salmonella*organism must perform this in a Biosafety Level 3 containment laboratory. Not only the precautions are important but also the treatments are essential in case of exposure to these agents.
13. Only spread slurry on arable land wherever possible. Leave all grazing land at least three weeks after spreading slurry.
14. Biosecurity measures (insist visitors have clean boots and disinfect before entering and leaving the farms). (NADIS, 2007).

2.18 Control of Salmonellosis

1. Have herd serotested and cull carrier cows.
2. Isolate sick cows. Use only antimicrobials approved by your veterinarian.
3. Control rodents and birds.
4. Isolate sick animals in dedicated isolation boxes and not calving boxes (where healthy calves are kept).

5. Ensure that milk from ill cows (or those cows that have contact with such cows are not fed to calves).
6. Consider herd vaccination where the problems persist, despite the control measures listed above. The risk of salmonellosis in people can be reduced by proper hygiene including thorough hand washing before eating smoking and regular cleaning and disinfection of water proof protective clotting after handling livestock.
7. Young children should be carefully supervised when handling animals especially calves, where cryptosporidiosis is another potential zoonosis (NADIS, 2007).

CHAPTER THREE

3.0MATERIALS AND METHODS

3.1 Materials

3.1.1 Minor Equipments

Petri-dishes, bijou bottles, universal bottles, wire loops, straight wire, Bunsen burner, test-tube holders, measuring cylinder, polythene bags, scissors, conical flasks, test-tubes, pipettes, masking tape, permanent markers and milk samples.

3.1.2 Media and Reagents

3.1.2.1 Enrichment Media

- Rappaport vassiliadis broth (RVB)

3.1.2.2 Isolation Media

- Bismuth sulfite agar

3.1.2.3 Charactarization of Isolates

3.1.2.3.1 Biochemical analysis

1. TSI – Triple Sugar Iron Agar
2. SIM – Medium for motility, indole and H_2S production test
3. Urea base agar + 40% urea solution
4. Simmons citrate
5. Methyl red
6. Voges Proskauer

3.1.2.3.2 Sugar Fermentation tests

- Xylose, mannitol, Rhamnose, Maltose

3.1.2.3.3 Amino acid Decarboxylase test

- Arginine, Ornithine and Lysine

3.1.2.3.4 Microbact 12E kit

- For Testing Salmonella spp with Microbact 12E (Oxoid, 2005)

3.1.2.3.5 Serological test using latex agglutination kit

- *Salmonella* Polyvalent Antiserum (A-S) test kit

3.1.2.3.6 Gram Staining

- Gram staining reagents

    1. Crystal violet
    2. Lugol's iodine
    3. 70% ethanol
    4. 1% safranine

3.1.3 Antibiotic susceptibility testing

- Chloramphenicol (C 30µg), Ciprofloxacin (CIP 5µg), Kanamycin (K 30µg), Nalidixic Acid (NA 30µg) Gentamicin (CN 10µg), Lincomycin (MY 10µg), Tetracycline (TE 10µg), Ampicillin (AMP l0µg), Amoxycillin (AML l0µg), Streptomycin (S 10µg), and Erythromycin (E 15µg).

3.1.4 Minimum inhibitory Concentration Test

- Amoxycillin and Erythromycin M I C Evaluator strips.

3.2 Methods

3.2.1 Study Area

The study was carried out in Zaria and environs, Kaduna State which is located in the Northern region of Nigeria, within latitudes 11^0 07', 11^0 12'N and longitudes 07^0 41'E (Figure 3.1) at an altitude of 550-700 meters above sea level and a total land area of 300km^2. The town is characterized by a tropical climate, a monthly mean temperature ranging from 13.8^{0}C to 36.7^{0}C and an annual rainfall of 1092.8mm (Agbogu *et al*., 2006). It has an estimated population of 408, 198 and a density of 1,360.7 km^2 (NPC, 2006). The main occupation of the people in Zaria is primarily Agriculture. In Kaduna State, cattle population was reported to be 1,144,000 and about 99% of these cattle were being managed/reared under semi-intensive, extensive or pastoral system (Aliyu *et al*., 2014).

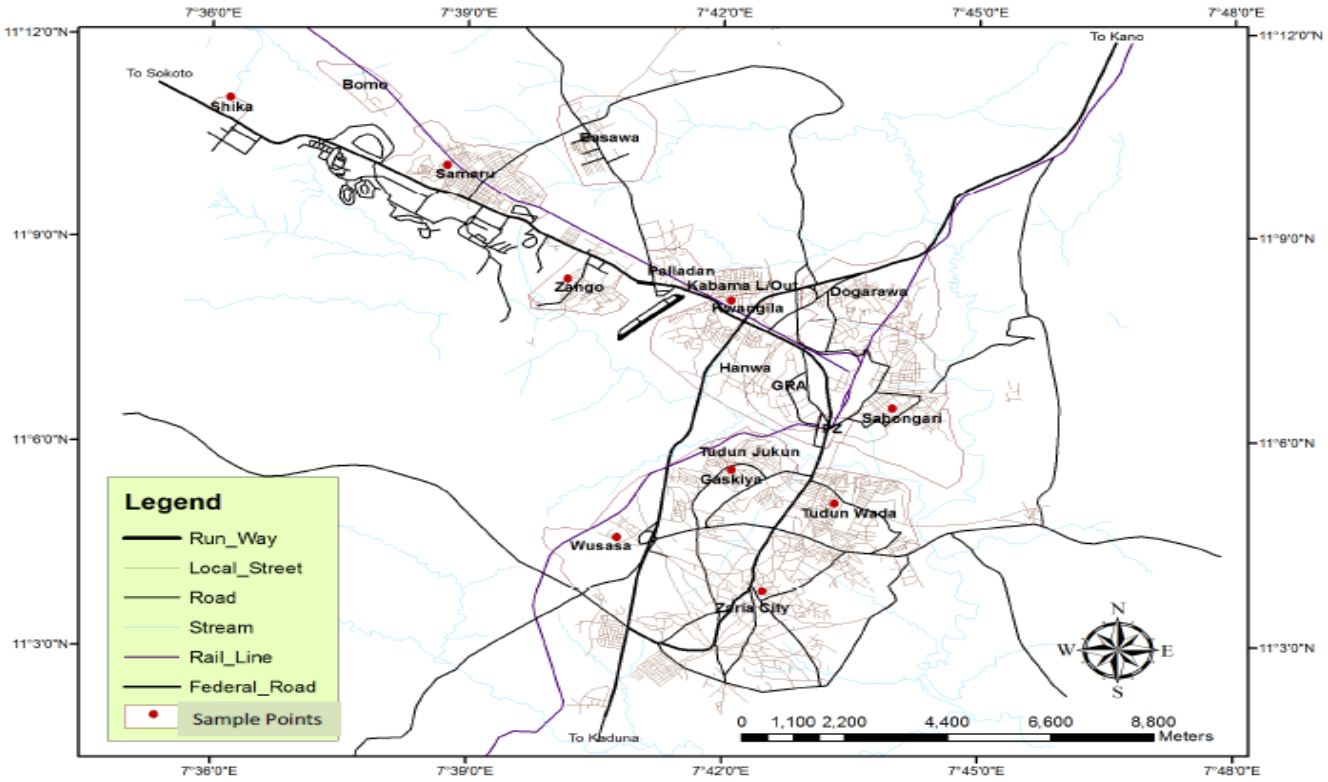


Figure 3.1: Map of Zaria showing sampling points in Zaria and environs (Source: Adopted from Google earth Pro 4.0, 2014).

3.2.2 Study design

A cross-sectional study was conducted. Milk samples were collected from Fulani women at 9 retail points, viz; Shika, Samaru, Zango, Kwangila, Sabon-Gari, Tudun-Wada, Kongo, Zaria-City and Danmagaji.

3.2.3 Sample Size

The sample size was calculated using the formula by Thrusfield (1997) at 95% confidence interval level, 6.4% prevalence for raw milk and 0.8% prevalence for fermented milkby Karshima *et al*., (2013) was used for this study.

$$n = \frac{Z^2Pq}{d^2}$$

Where:

n = sample size

Z appropriate value for the standard normal deviate for the desired confidence = 1.96

P = prevalence (7.2 % overall prevalence for *Salmonella* obtained by Karshima *et al*.,2013).

q = 1-p

d = desired absolute precision = 0.05

Therefore, 6.4% (0.064) prevalence for raw milk (Karshima *et al*., 2013)

$$n = \frac{1.96^2 \times 0.064\ (1-0.064)}{0.04^2}$$

$$n = \frac{3.8416 \times 0.064 \times 0.936}{0.0016}$$

n = 143.83 (10% of 143.8 = 14.383)

n = 14.383+143.83 = 158

This sample size shows the minimum that can be collected.

For, 0.8% (0.008) prevalence for fermented milk (Karshima *et al*., 2013)

$$n = \frac{l.96^2 \times 0.008\ (1-0.008)}{0.02^2}$$

$$n = \frac{3.8416 \times 0.008 \times (1-0.008)}{0.0004}$$

n = 76.22 (10% of 76.22 = 7.622)

n = 7.622 + 76.22 = 83.8

This sample size shows the minimum that can be collected. A total of 350 samples were collected for this study, comprising of 174 raw milk samples and 176 fermented milk samples. The sample sizes (that is, raw milk from 158 to 174; fermented milk from 84 to 176), were increased so as to mimnimize sampling error and to increase the chances of isolating *Salmonella* spp.

3.2.4 Sample collection and transportation

Twenty five samples of raw milk were collected in sterile sample bottles from 2 different cattle herds, weekly. The raw milk samples were collected directly from the cattle (milking cows) from each quarter of the cows' udder. Also, twenty five samples of fermented milk were collected in polyethene bags as they were sold from 25 retailers at different points, weekly. A total of 174 raw milk samples and 176 fermented milk samples were collected from Zaria and environs between April, 2014 and August 2014. About 10mls of raw milk samples were collected into sterile sample bottles using purposive sampling, from different herds between 6.30-7.30am and then immediately transported on ice packs (in Ice-man Cole box), to the Bacterial Zoonoses Laboratory of Veterinary Public Health and Preventive Medicine of Ahmadu Bello University, Zaria, for analysis.

Also 30mls of fermented milk samples were bought in polythene bags using convenience sampling, from different retail points between 1.30-2.30pm and then immediately transported on ice packs in Ice-man Cole box, to the laboratory for analysis.

3.2.5 Total aerobic plate count determination

Duplicate plates were prepared for a Hundred fold serial dilution and the average was taken. The serial dilution was carried out on themilk samples (raw and fermented) by taking 0.1ml into 9.9mls normal saline diluents (10^{-2}) using a Pasteur pipette, then 0.1ml was taken from the 10^{-2} dilution to another 9.9mls normal saline to give 10^{-4} dilution. From the 10^{-4} diluent, 0.1ml was taken to another 9.9mls normal saline to give 10^{-6}dilution and then 0.1ml from 10^{-6}onto nutrient agar platewas inoculated using a surface spread to cover the surface using glass bent rod or hockey stick. From the 9.9mlsnormal saline, it gave a dilution of 10^{-7} on the plate and then incubated at 37^{o}C for 18-24hrs. After 18-24hr incubation the total aerobic count on the nutrient agar plate was carried out. All organisms growing on the agar were considered. The count was calculated in coliform forming unit/per ml (CFU/ml) of the milk samples.

3.2.6 Bacterial culture isolation and characterization

3.2.6.1 Selective enrichment

Enrichment for *Salmonella* spp was carried out on Rappaport Vassiliadis broth (RVB) (SC, Difco USA) on raw and fermented milk samples.1ml of each raw and fermented milk sample was aspirated using a 5ml sterile syringe into 9mls of RVB and then incubated at 37^{o}C for 24hrs.

3.2.6.2 Bacterial Culture enrichment in RVB

Following enrichment in RVB, a loop full of the incubated broth was smeared and streaked onto Bismuth sulfite agar plates using a sterile wire loop and incubated at 37^{o}C for 24 - 48hrs (Hendriksen, 2003). This procedure was carried out for both raw milk and ‘‘Nono’’

3.2.6.3 Gram Reaction

After 24-48hr incubation on BSA, smears from distinct colonies of *Salmonella* were made on sterile glass sldes and flame.Fixed smears were then gram stained using gram staining procedure as described by Cheesebrough, (2002). Stained smears were then viewed under electric microscope at X 100 objective lens for typical *Salmonella* gram reaction and morphology. This procedure was carried out on cultures from both raw and fermented milk product ‘‘Nono’’.

3.2.6.4 Biochemical characterization of isolates

Suspected *Salmonella* isolates on BSA were subjected to biochemical tests on the basis of indole production, H_2S production, motility with SIM medium (Merk, Germany), Citrate utilization with Simmons citrate (Merk, Germany), Methyl Red (MR) and Voges-Proskauer (VP) using MR-VP medium (Merk, Germany) and Urease production (Oxoid Basingstoke,UK).

Presumptive colonies were transferred to tubes of Triple Sugar Iron (TSI) agar, Simmon’s Citrate, Urea, Methyl Red, Voges Proskauer and incubated at 37°C for 18-24hrs. Presumptive isolates was confirmed using Biochemical Identification Kit (Microbact GNB 12E). Confirmed isolates was stored on nutrient agar slants at -4°C for further studies.

Biochemical characterisation was done based on standard techniques (Barrow and Feltham, 1995). All isolates that were typical of *Salmonella* spp were tested and substrates were

considered to belong to the genus *Salmonella*. Typical *Salmonella* reactions such as indole negative, methyl red positive, Voges-Proskauer negative, citrate positive, motile in motility medium and produces H_2S and urease production were considered positive.

3.2.6.4.1 *Triple Sugar iron Agar test* (TSI)

In this test, theTriple Sugar iron Agar was prepared according to the manufacturer's instruction and was inoculated with the isolates by stabbing and streaking respectively. This was followed by incubation at 37^{o}C for 24-48 hours. It was then observed for hydrogen sulfide production (which is indicated by a black precipitate at the butt of the tube) and carbohydrate fermentation indicated by gas production and colour change) (Quinn *et al*., 2002). Also, the test tube showed yellow at the top, leaving the bottom light red, which indicates alkaline over acid which is a typical *Salmonella* reaction on TSI slants (Carter, 1991).

3.2.6.4.2 *Sulphur, Indole and Motility (SIM) tests*

The ability of isolates to reduce sulphur, produce indole and be motile through the agar (be motile) was examined.SIM is commonly used to differentiate members of enterobacteriaceae. SIM medium was prepared according to the manufacture's instructions. The pure isolates were inoculated into the medium by stabbing and incubated at 37^{o}C for 18-24hrs. They were then observed for hydrogen sulphide (H_2S) production, (indicated as a black coloration in the tube) and motility (indicated by migratory movement along the line of stabbing). Three drops of Kovac's indole reagent were then added and shaken gently. Withinone minute, the reaction is read.

3.2.6.4.3 Methyl red-Voges Proskaeuer Test

Samples were inoculated into 5ml of MR-VP broth and incubated at 37^{o}C for 24hrs. After incubation, 1ml of the broth was transferred to a small serological tube followed by the addition of 2-3 drops of methyl red and the colour on the top of the medium was read immediately. A red coloration on addition of the indicator signified a positive test for *Salmonella* spp. To the rest of the broth in the original tube 5 drops 40% potassium hydroxide (KOH) were added followed by 5 drops of 5% of alcoholic (ethanol) alpha-Naphthol. The cap of the tube was loosened and placed in a sloping position. The development of a red colour starting from the liquid-air interface within 1 hour indicates a positive test. *Salmonella* spp are reported to be Methyl red positive with an orange to red coloration and Voges-proskauer negative with no colouration (Cheesbrough, 2002).

3.2.6.4.4 *Citrate Utilization Test*

In this test a needle was used to pick a single isolated colony which was lightly streaked on the surface of the Simmon's citrate agar slant (prepared according to manufacturer's instruction), which contains pH indicator in a test tube and incubated at 37^{o}C for 18-24hours. A blue coloration of the indicator was observed, thus indicating a positive test. *Salmonella* spp are reported to be distinctively citrate positve (Mac Faddin, 2000).

3.2.6.4.5 *Urease test*

In this test, pure culture was used tostreak the entire surface of the urea slant prepared in a test tube under sterile conditions. The inoculated test tubes were then incubated for 18-24 hours a 37^{o}C. Urease production is indicated by a bright pink colour on the slant which identifies those organisms that are capable of hydrolyzing urea to produce ammonia and

carbon dioxide, *Salmonella* is negative for thetestindicated by the culture medium remaining yellowish in color (Mac Faddin, 2000).

Additional Biochemical Tests

Sugar reduction and some amino acids tests were carried out and the reactions gave the following results; nitrate positive, lysine decarboxylase positive, oxidase negative, ferments glucose, mannitol, dulcitol, and maltose but failed to ferment lactose, sucrose, adonitol and raffinose (Quinn*et al*., 2002).

3.2.6.4.6 *Sugar Fermentation Tests(Mannitol, maltose, Rhamnose, and xylose)*

One gram (1g) of each sugar was weighed as well as1.5g of Andrade peptone waterand mixed in a conical flask and dissolved in 100mls of distilled water anddispensed in 5mls aliquots into sterile test-tubes steamed at 115°C for 5-10 minutes and then allowed to cool. Stored isolates were sub-cultured on to a selective media and then 2 to 3 colonies picked and suspended into the test tubes, after which they were kept in the incubator at 37^{o}C for 24-48hrs and then read. Red colour indicated positive while light pink colour indicated negative

3.2.6.4.7 Amino Acid Decaboxylase Test (Arginine, Ornithine and Lysine).

1g of each amino acid was weighed along with 1.5g of Andrade peptone water and then dissolved in 100ml of distilled water, then dispensed into sterile test-tubes ad then heated for 5-10 minutes then allowed to cool. After which the stored isolates were inoculated into the tubes and then 2 to 3 drops of mineral oil added and incubated for 18-24 hrs. The prepared amino acids are yellow in colour and are expected to change to purple when positive and then left for another 24 hrs after which the purple colour changes to yellow

again meaning that it is positive but if the purple colour does not change back to yellow after 24 hours then the test is negative.

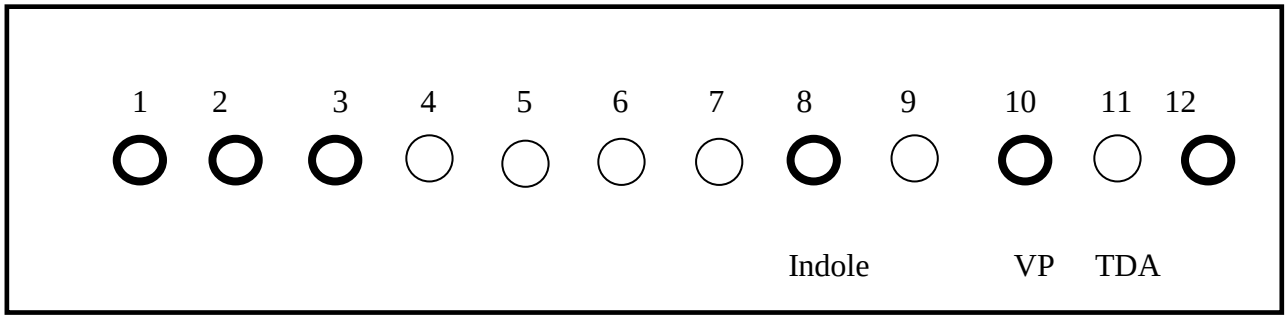
3.2.6.5 Microbact 12E kit

Commercially available biochemical test kit, Microbact GNB 12E(Oxoid) was used according to the manufacturer's instructions to confirm isolates suspected to be *Salmonella* spp.

3.2.6.5.1 Procedure for Testing an Organism with Microbact 12E (Oxoid, 2005)

1. Before use, an oxidase test was performed on the organism to be identified. Oxidase positive organisms cannot be identified using the microbact 12(A) 12(E).

2. The Microbact kit is a plate with 12 wells. The wells (1-12) contain the following substrates/tests: Lysine Ornithine, H_2S, Glucose, Mannitol, Xylose, o-nitrophenyl-β-d-galactopyranoside (ONPG), Indole, Urease, Voges Proskauer (VP), citrate, and Tryptophan Deaminase (TDA) respectively.



3. The isolates to be inoculated were grown on a selective media (BSA). Using a sterile straight wire, 2 to 3 colonies were picked and emulsified in 5.0ml of 0.1% sterilepeptone

waterand incubated at 37^{o}C for 4hours. A sterile pipette was then used to dispense one drop of the peptone water culture into 2.5ml of sterile normal saline solution (0.85%).

4. The wells of the individual substrate sets were exposed by cutting the end tag of the sealing strip and slowly peeling it back. Using a sterile micropipette, 100µl of the bacterial suspension was added to each well in the set. Mineral oil was then used to overlay the substrates in wells 1, 2 and 3, using micropipette. The inoculated rows were then resealed and labeled at the end of the tag with the specimen identification number followed by incubation at 37^{o}C for 24hrs.

5. On 24hours of incubation period, the sealing tape on the test strips were peeled back and evaluated. Results were recorded in a report form as positive or negative by comparing them with a color chart and making reference to the table of reactions provided. However, to well 8 (indole production),two (2) drops of indole (Kovacs) reagent was added and the result evaluated within 2 minutes of addition of the reagent. Well 10 (Voges-Proskauer reaction), 1 drop each of VP1 and VP2 reagent was added and the result evaluated within 15 to 30 minutes after the addition. To well 12 (tryptophan Deaminase), 1 drop of TDA reagent was added and the result evaluated immediately.

6. The reaction colours for negative reaction are; Yellow, Yellow-green, Straw colour, Blue-green, Blue-green, Blue-green, Colourless, Colourless, Straw colour, Straw colour, Green, Straw-colour to the 12 corresponding wells.

7. The reaction colours for positive reactions are; Blue-green, Blue, Black, Yellow, Yellow, Yellow, Yellow, Pink-red, Pink-red, Pink-red, Blue, Cherry red.

Furthermore, reactions in 3 consecutive wells were summed up to give a four digit number, which was fed into the computer software to provide information on the identity of organism, including percentage (%) probability. Values ≥ 75% for each organism was accepted.

3.2.6.5.2Interpretation of Microbact 12 E result

In Microbact 12E, an octal coding system has been adopted in which each group of three reactions produce a single digit of the code. Using the results recorded on the report forms, the indices of the positive reactions are circled and the sum of these indices in each group of three reactions formed a code of four numbers. The code obtained was then entered into the computer aided identification package and the results identified and the organism's probability recorded in percentage.

3.2.6.6 Serological identification

All biochemically positive isolates were confirmed serologically using *Salmonella* polyvalent 'O' group A-S antiserum according to the instructions of the manufacturer (Oxoid Basingstoke, U.K), using slide agglutination test.

3.2.7 Antimicrobial Susceptibity testing of Isolates

This was performed using a panel of 11 commonly used antimicrobial agents by the Agar Disc Diffusion method following Clinical Laboratory Standards Institute (CLSI, 2011) guidelines (Bauer, *et al*., 1966) and cultured on Mueller-Hinton agar (Oxoid Basingstoke, U.K). The following antimicrobial agents and concentrations were used:

Chloramphenicol (C, 30µg), Ciprofloxacin (CIP, 5µg), Kanamycin (K, 30µg), Nalidixic Acid (NA, 30µg) Gentamicin (CN, 10µg), Lincomycin (MY, 10µg), Tetracycline (TE,

10µg), Ampicillin (AMP, l0µg),Amoxycillin (AML, l0µg), Streptomycin (S10,µg), and Erythromycin (E, 15µg) (oxoid Basingstoke, UK).

Colonies (4-5) of the test isolates from overnight cultures on BSA plates were picked and emulsified in sterile normal saline. The turbidity of the suspension was adjusted to match 0.5Macfarland's standard. Ten µl of the suspension was then dispensed and spread on Mueller-Hinton (Oxoid UK) agar plates to create a uniform lawn. The pre-inoculated plates were used for the disc diffusion test. The antibiotic discs were placed on the surface of each of the pre-inoculated Mueller-Hinton plates using a disc dispenser (Oxoid Basingstoke, U.K) and the plates incubated aerobically at 37^0C for 24hours. After incubation, the diameters of the antibiotic inhibition zones were measured to the nearest millimeter (mm) using a meter rule and were classified as susceptible (S), intermediate resistant (I) or resistant (R) according to the CLSI criteria (2011).

3.2.8 Minimum inhibitory concentration evaluation (MICE)

Bismuth Sulfite was used for purification of isolates. 0.5 Mac farland innoculum level was prepared. Several colonies from a pure culture into a suitable suspension medium was emulsified and compared the turbidity to appropriate MacFarland Standard. The suspension was used within 15 minutes of preparation. A sterile cotton swab was dipped into the suspension and the excess moisture removed by pressing against the edge of the tube. The plate was inoculated by swabbing in at least three different directions. The surface of the Agar was allowed to dry completely before applying the M.I.C.Evaluation strips, since excess moisture can cause a distortion of the gradient (Oxoid, 2008).

3.2.8.1 Interpretation of the Minimum inhibitory Concentration Evaluation

There are white and black sections on the M.I.C. Evaluation strips. The MICE strip has values ranging from less than < 0.002µg/ml to > 256µg/ml. If the growth touches the strip on a white section, the M.I.C.E is read as the value in that section and If the growth touches the strip on a black section, the M.I.C.E is read as the value in that section. If there is growth along the entire length of the strip (no zone of inhibition) the M.I.C.E should be read as greater than the highest vaue on the strip. If a large zone of inhibition is obtained and the growth of the organism does not intersect with the strip, the M.I.C.E is read as the lowest value on the strip.

If there is no zone around the strip, then the MICEis read as the highest value e.g. > 256µg/ml, which indicates resistance. If the zone is so large that it does not touch the strip then the MICE is read less than the lowest value on the strip < 0.002µg/ml and if the zone of inhibition touches any line on the strip then that particular point is read.

3.2.9 Data Analyses

The occurrence of *Salmonella* species was calculated using percentages and Chi square to test for association and values of $P < 0.05$ were considered statistically significant and the results were analysed using Statistical Package of Social Sciences (SPSS 20.0).

The prevalence of *Salmonella* in Raw milk and locally fermented milk "Nono" was calculated using the formula:

$$\text{Prevalence} = \frac{\text{Number of positive samples}}{\text{total number of samples collected}} \times 100$$

CHAPTER FOUR

4.0 RESULTS

4.1 Total Aerobic Plate Count

Table 4.1 shows the overall mean total aerobic plate counts of raw milk and fermented milk ‘‘Nono’’.The values for raw milk ranged between 0-9.5 $\log_{10}$ with a mean of 7.42 ± 0.14 standard deviation, while the values for fermented milk ranged between0-7.9 $\log_{10}$ with a mean of 7.22 ± 0.19standard deviation. No significant difference was observed between the means for both raw milk and fermented milk.

4.1.2 Mean Total Aerobic Plate Count of Raw Milk

Table 4.2 shows the mean total aerobic plate count of raw milk based on location. The mean ranged from 0.729 to 1.923 Cfu/ml with significant difference between Sabon Gari (1.923) and Tudun-Wada (0.729) at $P<0.05$ and also between Sabon Gari (1.923) and Samaru (0.922) at < 0.05. Highest mean CFU/ml was recorded in Sabon Gari(1.923) and the lowest in Tudun wada (0.729) (Table 4.2)

4.1.3 Mean Total Aerobic Plate Count of Fermented Milk

The mean total aerobic plate count of fermented milk based on sampling location, ranged between 1.128 and 2.12 Cfu/ml. No significant difference between the locations was observed (Table 4.3).

Table 4.1: Overall Total Aerobic Plate Count (CFU/ml) of sample type

Sample Types	Range (Log_{10}CFU/ml)	Log_{10} CFU/ml (Mean ±SD)
Raw Milk	0 - 9.5	7.42±0.14
Fermented Milk	0 – 7.9	7.22±0.19

X^2 =0.3219; P=0.5705

No significant difference between the sample types

Table 4.2: Mean Total aerobic plate count of raw milk based on sampling Location

S/No	Samling Location	No of samples examined	Mean (Cfu/ml)
1	Sabon Gari (Sg)	25	1.923 ± 0.13^{a}
2	Tudun Wada(Tw)	25	0.729 ± 0.19^{a}
3	Samaru (Sm)	25	0.922 ± 0.19^{a}
4	Shika (Sk)	25	1.625 ± 0.21^{b}
5	ShikaNAPRI(Skn)	25	1.264 ± 0.21^{b}
6	Dan-magaji (Dm)	24	1.326 ± 0.30^{b}
7	Zango (Zg)	25	1.118 ± 0.24^{b}
Total		174	

Mean values with different superscripts (a, b and c) along the group were statistically significant.

At *P = 0.05,

Table 4.3: Mean Total aerobic plate count of fermented milk based on the sampling Location

S/No	Sampling Location	No of samples examined	Mean (Cfu/ml)
1	Samaru	28	1.128±0.29
2	Shika	30	1.921±0.12
3	Kwangila	20	1.231±0.20
4	Sabon Gari	20	2.12±0.29
5	Tudun Wada	20	1.488±0.16
6	Kongo	10	1.764±0.22
7	Zaria City	20	1.948±0.28
8	Dan-magaji	28	1.41±0.20
Total		176	

No significant difference between the locations

4.2Biochemical Characterisation of Isolates.

Table 4.4 shows the biochemical results for raw milk samples based on location, out of 174 samples collected, 58 (33.33%) were *Salmonella* suspects based on conventional biochemical tests while 8 (4.6%) were *Salmonella* suspects based on microbact 12E test.

Table 4.5 shows the biochemical test results for fermented milk samples based on location. Out of 176 samples collected, 22 (12.50%) were *Salmonella* suspects. Based on the conventional biochemical tests while 6 (3.4%) were *Salmonella* suspects based on microbact 12E test. Based on the biochemicals carried out on fermented milk sample, Kwangila had the highest number of *Salmonella* species (3) while Shika, Samaru and Sabon Gari had (1) each and Dan magaji, Kongo and Zaria city had no *Salmonella* (Table 4.5).

Table 4.6 shows the distribution of *Salmonella* spp for raw and fermented milk based on conventional biochemical tests and microbact 12E kit. A total of 350 samples were collected for raw and fermented milk, 80 *Salmonella* suspects were from conventional biochemical tests while 14 suspects were detected using microbact 12E kit

Based on the findings, the study revealed 4.6% prevalence of *Salmonella* in raw and 3.4% prevalence of *Salmonella* in fermented milk giving a mean total of 4.0% prevalence (Table 4.6).

Table 4.4: Biochemical result of *Salmonella* spp for Raw milk samples based on sampling location

S/No	Sampling Location	No Sampled	No (%) positive by Bchm test	No (%) positive by Microbact 12E kit
1	Shika	25	11	1
2	Shika Napri	25	22	1
3	Samaru	25	0	0
4	Sabon Gari	25	18	4
5	Tudun wada	25	3	0
6	Danmagaji	25	0	0
7	Zango	24	4	2
	Total	**174**	**58**	**8**

Key

Bchm = Biochemical

Table 4.5: Biochemical result of *Salmonella* spp for Fermented milk samples based on sampling location

S/No	Location	No Sampled	No (%) positive by Bchm test	No (%) positive by Microbact 12E kit
1	Shika	30	2	1
2	Samaru	28	5	1
3	Kwangila	20	7	3
4	Sabon Gari	20	6	1
5	Tudun wada	20	1	0
6	Danmagaji	28	-	-
7	Kongo	10	-	-
8	Zaria City	20	-	-
	Total	**176**	**22**	**6**

Key

Bchm = Biochemical

Table 4.6: Distribution of *Salmonella* spp from Raw Milk and Fermented milk based on Conventional biochemicals and Microbact tests

Milk type	No. Sampled	No (%) positive by Bchm	No (%) positive by Microbact 12E kit
Raw milk	174	58(33.33)	8(4.6)
Fermented milk	176	22(12.50)	6(3.4)
Total	**350**	**80(22.86)**	**14(4.0)**

X^2= 0.3219; P = 0.5705

Key

Bchm = Biochemical test

4.3Bacteria species isolated from raw milk based on microbact 12E kit.

Table 4.7 shows bacteria species isolated from raw milk based on microbact 12E kit with *Salmonella* spp showing 8 (13.8%), *Escherichiacoli* 8 (13.8%), *Enterobacter* spp 38 (65.5%), *Citrobacter* spp 1 (1.7%), *Serratia* spp 1 (1.7%) and *Klebsiella* spp 2 (3.5%).

Table 4.8 shows bacteria species isolated from fermented milk based on microbact 12E kit. The result showed *Salmonella* spp had 6 (27.3%), *Escherichiacoli* 8 (36.4%), *Enterobacter* spp 6 (27.3%), *Citrobacter* spp 1 (4.5%) and *Acinotobacter* spp 1 (4.5%).

Table 4.9 shows the microbact result of bacteria spp isolated from raw milk and fermented milk. *Salmonella* spp had 14 (17.5), *Eschericiacoli* 16 (20.0%) *Enterobacter* spp 44(55.0%),*Citrobacter* spp 2(2.5%), *Serratia* spp 2 (1.3%), *Acinotobacter* spp 1(1.3%) and *Klebsiella* spp 2 (2.5%).

4.4 Serological Test

Out of the 80 islotes stored on nutrient agar slants from raw and fermented milk, based on typical colonial morphology of *Salmonella* spp were subjected to biochemical analyses. 3(0.86%) *Salmonella* spps were identified by conventional biochemical test and confirmed serologically using *Salmonella* polyvalent antiserum (A-S), while Microbact result identified additional 11 *Salmonella* isolates besides the 3 isolates identified conventionally, giving a total of 14 (4%) *Salmonella* sppand then the isolates were also confirmed using *Salmonella* polyvalent antiserum (A-S), (see Appendix VI).

Table 4.7: Bacteria isolated from raw Cow milk based on Microbact 12E kit.

S/n	Organisms Isolated	Number of organisms	Number in Percentage (%)
1	*Salmonella* spp	8	13.8
2	*Escherichia coli*	8	13.8
3	*Enterobacter* spp	38	65.5
4	*Citrobacter* spp	1	1.7
5	*Serratia* spp	1	1.7
6	*Klebsiella* spp	2	3.5
Total		58	100.0

Table 4.8Bacteria Isolated from fermented milk based on Microbact 12E kit

S/n	Organisms Isolated	Number of organisms	Number in Percentage (%)
1	*Salmonella* spp	6	27.3
2	*Escherichia coli*	8	36.4
3	*Enterobacter* spp	6	27.3
4	*Citrobacter* spp	1	4.5
5	*Acinotobacter* spp	1	4.5
	Total	22	100.0

Table 4.9: Bacteria Isolated fromRaw and Fermented milk isolates based on Microbact 12E kit

S/n	Organisms Isolated	Number of organisms	Number in Percentage (%)
1	*Salmonella* spp	14	17.5
2	*Escherichia coli*	16	20.0
3	*Enterobacter* spp	44	55.0
4	*Citrobacter* spp	2	2.5
5	*Serratia* spp	2	1.3
6	*Acinotobacter* spp	1	1.3
7	*Klebsiella* spp	2	2.5
Total		80	100.0

4.5 Antibiotic Susceptibility

Table 4.10 shows the eleven (11) antimicrobial agents used for the susceptibility testing of the 14 *Salmonella* isolates. All 14 (100%) isolates were susceptible to ciprofloxacin and gentamicin(100% susceptible) while13 (93%) isolates were susceptible to chloramphenicol, kanamycin, and nalidixic acid. Nine (64.3%) isolates were susceptible to streptomycin and tetracycline,3 (21.4%) isolates were susceptible toAmoxycillin,2 (14.3%) isolates were susceptible to Erythromycin,Ampicillin and Erythromycinand no isolate (0%) was susceptible to Lincomycin. Based on the antibiotic susceptibility test, the isolates were 100% resistance to lincomycin followed by erythromycin (85.71%) then ampicillin (85.71%) and amoxicillin (78.57%). Ciprofloxacin and Gentamycin were 100% susceptible to all the isolates (that is 0% resistant to all isolates). More so, based on the resistant isolates MIC was carried out on Amoxycillin and Erythromycin.

Table 4.11 shows the antibiotic resistance patterns of 14 *Salmonella* isolates from raw and fermented milk indicating 9 different resistance patterns of the11 antimicrobial agents used; AMP, AML, S, MY, E with 1 (7.14%), AMP, AML, S, MY with 1 (7.14%) AMP, S, MY, E with 1 (7.14%), AMP, AML, S, MY, E, TE with 2 (14.30%), MY with 1 (7.14%), AMP, AML, MY, E with 3 (21.43%), K, AMP, AML, MY, E with 1 (7.14%) and C, NA, MY, E with 1 (7.14%).

Table 4.10: Susceptibility of *Salmonella* isolates from raw and fermented milk samples to 11 antimicrobial agents

S/No	Antimicrobial Agent	Concentration	No. of *Salmonella* isolates susceptible	% of *Salmonella*isolates susceptible
1	Chloramphenicol	C 30µg	13	93.0
2	Ciprofloxacin	CIP 5µg	14	100.0
3	Kanamycin	K 30µg	13	93.0
4	Nalidixic Acid	NA 30µg	13	93.0
5	Gentamicin	GN 10µg	14	100.0
6	Ampicillin	AMP 10µg	2	14.3
7	Amoxicillin	AML 10µg	3	21.4
8	Streptomycin	S 10µg	9	64.3
9	Lincomycin	MY 10µg	0	0.0
10	Erythromycin	E 15µg	2	14.3
11	Tetracycline	TE 10µg	9	64.3
	Total			

KEY

Chloramphenicol (C 30µg), Ciprofloxacin (CIP 5µg), Kanamycin (K 30µg), Nalidixic Acid (NA 30µg) Gentamicin (CN 10µg), Lincomycin (MY 10µg), Tetracycline (TE 10µg), Ampicillin (AMP l0µg), Amoxycillin (AML l0µg), Streptomycin (S 10µg), and Erythromycin (E 15 µg).

Table 4.11: Antibiotic Resistance Patterns of *Salmonella* isolates from milk

S/N	Resistance Pattern	Frequency	Percentage (%)
1.	AMP, AML, S, MY, E	1	7.14
2.	AMP, AML, S, MY	1	7.14
3.	AMP, S, MY, E	1	7.14
4.	AMP, AML, S, MY, E, TE	2	14.30
5.	MY	1	7.14
6.	AMP, AML, MY, E	3	21.43
7.	K, AMP, AML, MY, E	1	7.14
8.	C, NA, MY, E	1	7.14
9.	AMP, AML, MY, E,TE	3	21.43
	TOTAL	14	100

KEY

AMP l0µg-Ampicillin	E 15µg-Erythromycin	NA 30µg-Nalidixic Acid
AML l0µg-Amoxycillin	TE 10µg-Tetracycline	
S 10-Streptomycin,	K 30µg-Kanamycin	
MY 10µg-Lincomycin	C 30µg-Chloramphenicol	

4.6Minimum Inhibitory Concentration Evaluation (MICE)

Table 4.12 shows that for the MICE test carried out, all isolates 9 (90%) were resistant to the two antibiotics (amoxycillin and erythromycin) with values >256,except for 1(10%) isolate that was susceptible to amoxicillin, with a value of <0.12µg/ml (table 4.12).

Table 4.12: Minimum Inhibitory Concentrationof Amoxycillin and Erythromycinon *Salmonella* Species

S/N	Sample No.	Antibiotics	
		AML	E
1.	RSk 41	S	R
2.	RSkn 57	R	R
3.	RSm 76	R	R
4.	RSg77	R	R
5.	RSg 79	R	R
6.	RZg 110	R	R
7.	RZg 121	R	R
8.	FSm 1	R	R
9.	Fkw 50	R	R
10.	Fkw5	R	R

Key
Sm = Samaru, Sk = Shika, Skn = Shika Napri, Sg = Sabon Gari, Zg = Zango, Kw = Kwangila, R = Raw milk, F = Fermented milk

Antibioticstrips: AML = Amoxycilin and E = Erythromycin
S = sensitive, R = resistance

CHAPTER FIVE

5.0 DISCUSSION

One hundred and twenty three of the raw milk samples (71%) had Total Aerobic Plate Counts (TAPC) higher than the permissible level recommended by World Health Organisation (W. H. O), while 145 (82%) of the fermented milk samples had TAPC higher than the W H O standard. The raw milk samples had values from 0-9.5 $\log_{10}$ and fermented milk samples had values that ranged between 0- 7.9 Log_{10}. This could be due to poor hygiene and lack of pasteurization.This agrees with the work of Okolocha *et al*. (2008)who reported higher values of 8 -11 $\log_{10}$ before pasteurization and lower values after pasteurization (7-8.9 $\log_{10}$). However, it is not in agreement with Lawan *et al*.(2012) who reported lower total aerobic plate counts before pasteurization between (5.7 - 6.04) and after pasteurization (3.7-4.20). Furthermore, the WHO maximum permissible value for TAPC in milk is 6.0 $\log_{10}$ CFU/ml (Ajogi *et al*., 2005).

This study, established the presence of *Salmonella* species (*Salmonella arizonae*) with overall prevalence of 4.0%. Subspecies II, IIIa, IIIb, IV, VI and S.*bongori* are usually isolated from cold blooded animals and the environment (rarely from humans) (Pui *et al*., 2011). In this study, members of subspecies IIIa (*S. arizonae*), were isolated. The prevalence of *Salmonella* in raw milk was 4.6% while fermented milk had 3.4% prevalence. The difference in prevalence between raw and fermented milk could be due to low pH and effect of fermentation in fermented milk because fermented milk is not a suitable environment for the majority of spoilage bacteria (FAO, 2013).

The overall prevalence of 4.0% (Raw milk 4.6% while fermented milk 3.4%) in this study is lower than Karshima *et al*.(2013)who also found a prevalence of 6.4% from raw milk and 0.8% from fermented milk, but higher than that reported by Mhone *et al*. (2012), who carried out a study in Zimbabwe from selected farms on raw and processed cow milk and showed no*Salmonella* spp. However, Karshima *et al*. (2013) and Mhone *et al*. (2012) used the same method of isolation (ISO, 2003 by Hendriksen).

The 4% prevalence established in this work is of public health importance, since the presence of one *Salmonella* species can lead to recall of food items from the market following the WHO standard (Codex Alimentarius Commission). The prevalence found in this study is probably due to the poor sanitary conditions of the milkers' hands, clothings and the environment.The presence of flies in the environment where fermented milk was sold and also the addition of baoba seeds, river or stream water, ice block could be potential sources of the *Salmonella* organism (verbal communication, B. Mohammed).The higher prevalenceof*Salmonella* spp in milk from Sabon Gari LGA could be due to the poor sanitary environment where the milk was sold.

The antibiotic susceptibility of *Salmonella* isolates showed that *Salmonella* isolates from raw and locally fermented milk were resistant to commonly used antibiotics. There was also resistance to multiple antimicrobials, including lincomycin, ampicillin, erythromycin, amoxycillin and tetracycline but all the isolates were sensitive to gentamicin and ciprofloxacin. This finding is similar to that described by Chen *et al*. (2004) who carried outantibiotic sensitivity on *Salmonella* isolates in China and reported that all isolates were sensitive to gentamycin and ciprofloxacin. The probable reason of multidrug resistance with 100% resistance on lincomycin, erythromycin, ampicillin and amoxicillin maybe due to the inappropriate use of antibiotics by farmers and animal feed producersin preventing or

treating certain diseases of their animals (FVE, 2012). This could lead to mutations from susceptible bacteria to new resistant bacteria through gene transfer (that is emergence of antimicrobial resistance). It could also lead to prolonged treatment and additional cost of diagnostic testing on animals and calls for concern (Acar, 1997; Addis, 2015).

This study also revealed 9 antibiotic patterns from the 11 antimicrobial agents used for the antibiotic susceptibility testing on14 *Salmonella* isolates, which is very alarming. This shows multiple drug resistance,and 13 (93%) of the isolates showed resistance to at least 4 antibiotics except 1 (7%) of the isolateswhich was resistant to only one antibiotic (lincomycin).

The minimum inhibitory concentrations (MIC), of Amoxicillin and Erythromycin strips were determined. Amoxicillinhad an M.I.C of 0.12µg/ml for one of the isolates while 9 isolates had zones of inhibition > 256µg/ml on both antibiotics used.Moreover, there was high frequency of resistance to β-lactams while all isolates were sensitive to floroquinolones and aminoglycosides, which agrees with the work of Tafida *et al*. (2013) who also determined the susceptibility of*Salmonella* isolates from retail beef and related meat products in Zaria and reported low prevalence of resistance to floroquinolones (Ciprofloxacin) and aminoglycoside (Gentamycin) but high prevalence of resistance to macrolide (Erythromycin) and amantadine (Amoxycillin).

The findings of this study ascertain that these isolates have developed resistance for commonly prescribed antimicrobials. The MIC antibiotic resistance shown by 90% of the *Salmonellaarizonae* in raw and fermented milk pose considerable health hazard to

consumers and this indicates indiscriminate use of antibiotics by both the Veterinarians and the animal owners and calls for prudent control measures.

However, the presence of *Salmonellaarizonae* is of serious public health significance because even though it mostly affects cold blooded animals (reptiles, amphibians, fish and the environment), serious infections like watery diarrhoea, weight loss and gastroenteritis have been reported in hosts with immunosuppressive therapy, organ transplantation, human immunodefficicency virus infection and young children less than 7 years old, though serious infection has not been documented in healthy adults (Jeffrey and Curtis, 2005).

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

In conclusion, this study has established high values of aerobic plate count in milk samples which is above the permissible values of the WHO standard. This indicates poor hygienic levels among the nomads and therefore calls for correctivemeasures through public health enlightenment.

This research has established the presence of *Salmonella* specieswith an overall prevalence of 4.0% in the milk samples, with raw milk recording 4.6% prevalence and locally fermented milk (Nono)recording 3.4% prevalence in Zaria and environs.Furthermore, this study also established the presence of other bacteria of the enterobacteriaceae family apart from *Salmonella* spp like *Escherichiacoli*, *Enterobacter* spp, *Citrobacter* spp, *Acinotobacter* spp, *Serratia* spp and*Klebseilla* spp in raw and locally fermented milk in Zaria.Codex Alimentarius Commission of the World Health Organization (WHO) states that milk for human consumption must be free of *Salmonella*. Thus, theobserved presence of *Salmonella* species in the sampled milk is contrary to the standard of the Codex Alimentarius Commission.

With the above findings, the milk sold in Samaru and Sabon Gari, Zaria therefore, constitutedhealth danger to the publicand could cause food-borne diseases to individuals consuming raw or partially pasteurized milk.

Also, the work has established that there were*Salmonella* spp resistant to commonly used antibiotics and pose considerable health hazards to the consumers unless prudent control measures are instituted. This could be due to indiscriminate use of antibiotics in those areas or the use of substandard antibiotics or improper storage of antibiotics (as this could affect the potency of the drugs) by the farm owners. However, the study showed that the *Salmonella* spp isolated were 100% sensitive to ciprofloxacin and gentamycin.

6.2 Recommendations

Based on the finding in the study, the following recommendations are provided:

1. There should be active surveillance of salmonellosis in cattle herds to screen and treat those with the infection so as to reduce the prevalence of salmonellosis in the environment.
2. Raw milk should be pasteurized before consumption andbecause it also forms the basis for other milk products like locally fermented milk (*Nono*).
3. The milkers should practice personal hygiene by wearing clean clothing and washing their hands regularly with soap and water before milking the animals.
4. There should be public health education on the need to keep the environment where the animals are kept clean and also to wipe the udder of the cow before milking with a clean cloth with warm water and edible disinfectant.
5. Government agencies like National Agency for Food and Drug Administration and Control (NAFDAC) to assess the indiscriminate use of antibiotics by animal owners.
6. The minimum inhibitory concentration evaluation should be carried out on the resistant isolates so as to determine the break point of the resistant organisms isolated.

7. Further studies should be carried out so as to identify the *Salmonella* spp in foods using more sensitive and faster techniques in order to reveal the true prevalence of food-borne diseases particularly salmonellosis in Nigeria.

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APPENDICES

Appendix I: TOTAL AEROBIC PLATE COUNT FOR RAW MILK SAMPLES

S/NO	SAMPLE NO	TAPC ON NA (CFU/ml) 10^7	GROWTH ON BSA
1	Sg 1	2.89	-
2	Sg 2	1.80	-
3	Sg 3	2.10	-
4	Sg 4	2.95	-
5	Sg 5	1.75	-
6	Sg 6	2.00	-
7	Sg 7	2.45	-
8	Sg 8	2.75	-
9	Sg 9	2.82	-
10	Sg 10	1.35	-
11	Sg 11	2.26	-
12	Sg 12	1.43	-
13	Sg 13	1.35	-
14	Sg 14	1.62	-
15	Sg 15	2.26	-
16	Sg 16	1.78	-
17	Sg 17	1.47	-
18	Sg 18	1.23	-
19	Sg 19	1.54	-
20	Sg 20	2.65	-
21	Sg 21	1.32	-
22	Sg 22	2.00	-
23	Sg 23	2.00	-

TOTAL AEROBIC PLATE COUNT FOR RAW MILK SAMPLES			
S/NO	**SAMPLE NO**	**TAPC ON NA (CFU/ml) 10^7**	**GROWTH ON BSA**
24	Sg 24	2.30	-
25	Sg 25	0	-
26	Tw 26	1.00	-
27	Tw 27	0	-
28	Sg 28	0	-
29	Tw29	1.20	+
30	Tw30	2.00	+
31	Tw 31	0	+
32	Tw 32	0	+
33	Tw 33	0	+
34	Tw34	0	+
35	Tw 35	0	+
36	Tw 36	0	+
37	Tw 37	0	+
38	Tw 38	1.14	+
39	Tw 39	3.00	+
40	Tw 40	1.00	+
41	Tw 41	0	+
42.	Tw 42	0	+
43.	Tw 43	2.10	+
44.	Tw 44	0	+
45	Tw45	2.40	+
46	Tw46	1.26	-
47	Tw47	1.13	+

TOTAL AEROBIC PLATE COUNT FOR RAW MILK SAMPLES			
S/NO	**SAMPLE NO**	**TAPC ON NA (CFU/ml) 10^7**	**GROWTH ON BSA**
48	Tw48	2.00	+
49	Tw49	0	-
50	Tw 50	0	+
51	Sm 51	1.00	+
52	Sm52	1.20	+
53	Sm53	1.76	+
54	Sm54	2.41	+
55	Sm55	0	+
56	Sm56	0	+
57	Sm57	TNTC	+
58	Sm58	2.44	+
59	Sm59	1.10	+
60	Sm60	TNTC	+
61	Sm61	TNTC	+
62	Sm62	1.50	+
63	Sm63	1.30	+
64	Sm64	0	+
65	Sm65	0	+
66	Sm66	0	+
67	Sm67	TNTC	+
68	Sm68	TNTC	+
69	Sm69	2.13	+
70	Sm70	1.40	+
71	Sm71	0	+

TOTAL AEROBIC PLATE COUNT FOR RAW MILK SAMPLES			
S/NO	**SAMPLE NO**	**TAPC ON NA (CFU/ml) 10^7**	**GROWTH ON BSA**
72	Sm72	0	+
73	Sm73	1.00	+
74	Sm74	1.20	+
75	Sm75	0	+
76	Sk76	TNTC	+
77	Sk77	1.90	+
78	Sk78	2.00	+
79	Sk79	2.60	+
80	Sk80	0	+
81	Sk81	0	+
82	Sk82	1.80	+
83	Sk83	2.90	+
84	Sk84	TNTC	+
85	Sk85	TNTC	+
86	Sk86	2.13	+
87	Sk87	2.19	+
88	Sk88	TNTC	+
89	Sk89	TNTC	+
90	Sk90	1.50	+
91	Sk91	TNTC	+
92	Sk92	TNTC	+
93	Sk93	1.14	+
94	Sk94	2.20	+
95	Sk95	1.23	+

TOTAL AEROBIC PLATE COUNT FOR RAW MILK SAMPLES			
S/NO	**SAMPLE NO**	**TAPC ON NA (CFU/ml) 10^7**	**GROWTH ON BSA**
96	Sk96	1.44	+
97	Sk97	TNTC	+
98	Sk98	TNTC	+
99	Sk99	1.35	+
100	Sk100	TNTC	+
101	Skn101	4.00	-
102	Skn 102	0	-
103	Skn 103	2.10	-
104	Skn 104	2.00	-
105	Skn 105	1.00	-
106	Skn 106	1.21	+
107	Skn 107	0	-
108	Skn 108	2.30	-
109	Skn 109	0	-
110	Skn 110	2.00	+
111	Skn 111	1.00	-
112	Skn 112	1.39	-
113	Skn 113	2.10	-
114	Skn 114	2.00	+
115	Skn 115	2.20	-
116	Skn 116	0	-
117	Skn 117	1.00	-
118	Skn 118	2.30	-
119	Skn 119	1.00	-

TOTAL AEROBIC PLATE COUNT FOR RAW MILK SAMPLES			
S/NO	**SAMPLE NO**	**TAPC ON NA (CFU/ml) 10^7**	**GROWTH ON BSA**
120	Skn 120	0	-
121	Skn 121	0	+
122	Skn 122	0	-
123	Skn 123	2.00	-
124	Skn 124	0	-
125	Skn 125	2.00	-
126	Dm126	7.00	+
127	Dm 127	0	+
128.	Dm 128	2.00	+
129.	Dm 129	2.10	+
130	Dm 130	0	+
131	Dm 131	1.12	+
132	Dm 132	0	+
133	Dm 133	2.20	-
134	Dm 134	1.00	+
135	Dm 135	2.00	-
136	Dm 136	0	+
137	Dm 137	1.32	+
138	Dm 138	2.00	+
139	Dm 139	2.00	+
140	Dm 140	2.30	+
141	Dm 141	0	+
142	Dm 142	1.00	+
143	Dm 143	2.10	+

TOTAL AEROBIC PLATE COUNT FOR RAW MILK SAMPLES			
S/NO	**SAMPLE NO**	**TAPC ON NA (CFU/ml) 10^7**	**GROWTH ON BSA**
144	Dm 144	1.00	+
145	Dm 145	0	+
146	Dm 146	0	+
147	Dm 147	0	+
148	Dm 148	2.00	+
149	Dm 149	0	-
150	Dm 150	2.00	+
151	Zg 151	0	+
152	Zg 152	0	-
153	Zg 153	0	+
154	Zg 154	1.10	+
155	Zg 155	1.50	-
156	Zg 156	2.10	+
157	Zg 157	TNTC	+
158	Zg 158	1.00	-
159	Zg 159	TNTC	-
160	Zg 160	1.30	+
161	Zg 161	2.80	+
162	Zg 162	1.90	+
163	Zg 163	4.00	-
164	Zg 164	1.99	-
165	Zg 165	0	+
166	Zg 166	0	+
167	Zg 167	0	+

TOTAL AEROBIC PLATE COUNT FOR RAW MILK SAMPLES			
S/NO	**SAMPLE NO**	**TAPC ON NA (CFU/ml) 10^7**	**GROWTH ON BSA**
168	Zg 168	0	+
169	Zg 169	0	+
170	Zg 170	0	+
171	Zg 171	2.00	+
172	Zg 172	1.0	+
173	Zg 173	1.6	+
174	Zg 174	2.3	+

KEY

TAPC – Total Aerobic Plate Count; Cfu/g = Coliform forming unit/milligram; NA = Nutrient Agar, Bismuth Sulfite Agar = BSA; + = growth, - = no growth, TNTC = Too numerous to count

Sm = Samaru, Sk = Shika, Zg = Zango, Sg = Sabon Gari, Tw = Tudun wada

Dm = Dan-Magaji, Kn = Kongo, Zc = Zaria city

Appendix II: TOTAL AEROBIC PLATE COUNT FOR FERMENTED MILK SAMPLES

S/NO	SAMPLE NO	TAPC ON NA (CFU/ml) 10^7	GROWTH ON BSA
1	Sm 1	1.10	+
2	Sm 2	2.00	+
3	Sm 3	0	-
4	Sm 4	0	-
5	Sm 5	0	-
6	Sm 6	0	-
7	Sm 7	0	-
8	Sm 8	7.00	+
9	Sm 9	3.00	-
10	Sm 10	1.10	-
11	Sm 11	0	-
12	Sm 12	0	+
13	Sm 13	0	-
14	Sm 14	1.00	+
15	Sm 15	2.30	+
16	Sm 16	1.42	-
17	Sm 17	TNTC	-
18	Sm 18	1.50	-
19	Sm 19	2.00	-
20	Sm 20	1.74	-
21	Sk 21	2.20	+
22	Sk 22	TNTC	+
23	Sk 23	1.19	+

24	Sk 24	TNTC	+
TOTAL AEROBIC PLATE COUNT FOR FERMENTED MILK SAMPLES			
S/NO	**SAMPLE NO**	**TAPC ON NA (CFU/ml) 10^7**	**GROWTH ON BSA**
25	Sk 25	2.36	-
26	Sk 26	TNTC	+
27	Sk 27	2.10	+
28	Sk 28	TNTC	+
29	Sk 29	1.78	+
30	Sk 30	TNTC	+
31	Sk 31	1.00	+
32	Sk 32	1.31	+
33	Sk 33	TNTC	+
34	Sk 34	TNTC	-
35	Sk 35	2.00	-
36	Sk 36	1.67	-
37	Sk 37	1.90	-
38	Sk 38	2.30	-
39	Sk 39	TNTC	-
40	Sk 40	1.45	-
41	Kw 41	2.20	-
42	Kw 42	1.60	-
43	Kw 43	1.00	-
44.	Kw 44	2.00	-
45.	Kw 45	0	-
46.	Kw 46	1.32	+
47.	Kw 47	2.00	+

TOTAL AEROBIC PLATE COUNT FOR FERMENTED MILK SAMPLES			
S/NO	**SAMPLE NO**	**TAPC ON NA (CFU/ml) 10^7**	**GROWTH ON BSA**
48.	Kw 48	2.00	+
49.	Kw 49	2.30	+
50.	Kw 50	0	+
51.	Kw 51	1.00	-
52.	Kw 52	2.10	+
53.	Kw 53	1.00	-
54.	Kw 54	0	-
55.	Kw 55	0	-
56.	Kw 56	0	+
57.	Kw 57	2.00	+
58.	Kw 58	0	-
59.	Kw 59	2.00	-
60.	Kw 60	2.10	-
61.	Sg 61	TNTC	+
62.	Sg 62	1.98	+
63.	Sg 63	200	+
64.	Sg 64	1.46	+
65.	Sg 65	TNTC	+
66.	Sg 66	TNTC	+
67.	Sg 67	1.67	-
68.	Sg 68	1.12	+
69.	Sg 69	1.32	-
70.	Sg 70	2.14	-

TOTAL AEROBIC PLATE COUNT FOR FERMENTED MILK SAMPLES			
S/NO	**SAMPLE NO**	**TAPC ON NA (CFU/ml) 10^7**	**GROWTH ON BSA**
71.	Sg 71	4.00	-
72.	Sg 72	TNTC	-
73.	Sg 73	TNTC	-
74.	Sg 74	4.00	-
75.	Sg 75	TNTC	-
76.	Sg 76	1.33	-
77.	Sg 77	1.50	-
78.	Sg 78	TNTC	-
79.	Sg 79	TNTC	-
80.	Sg 80	2.89	-
81.	Tw 81	2.00	-
82.	Tw 82	1..32	-
83.	Tw 83	1.14	-
84.	Tw 84	1.22	-
85.	Tw 85	1.79	-
86.	Tw 86	1.34	+
87.	Tw 87.	1.00	+
88.	Tw 88	1.40	-
89.	Tw 89	2.89	-
90	Tw90	2.19	-
91.	Tw91	1.76	-
92.	Tw92	1.11	-
93.	Tw93	1.46	-

TOTAL AEROBIC PLATE COUNT FOR FERMENTED MILK SAMPLES			
S/NO	**SAMPLE NO**	**TAPC ON NA (CFU/ml) 10^7**	**GROWTH ON BSA**
94.	Tw 94	2.00	-
95.	Tw95	2.08	-
96.	Tw96	1.49	-
97.	Tw97	1.5	-
98.	Tw 98	0	-
99.	Tw 99	0	-
100.	Tw 100	1.9	-
101	Kg 101	1.30	-
102.	Kg 102	1.70	-
103.	Kg 103	2.00	-
104.	Kg 104	2.60	-
105.	Kg 105	TNTC	-
106.	Kg 106	1.00	-
107.	Kg 107	1.45	-
108.	Kg 108	2.30	-
109.	Kg 109	TNTC	-
110.	Kg 110	TNTC	-
111.	Zc 111	2.56	-
112	Zc 112	1.90	-
113	Zc 113	2.00	-
114	Zc 114	TNTC	-
115	Zc 115	2.10	-
116	Zc 116	TNTC	-

TOTAL AEROBIC PLATE COUNT FOR FERMENTED MILK SAMPLES			
S/NO	**SAMPLE NO**	**TAPC ON NA (CFU/ml) 10^7**	**GROWTH ON BSA**
117	Zc 117	1.20	-
118	Zc 118	2.30	-
119.	Zc 119	2.14	-
120	Zc 120	1.00	-
121	Zc 121	1.19	-
122	Zc 122	2.00	-
123	Zc 123	2.20	-
124	Zc 124	1.50	-
125	Zc 125	1.67	-
126	Zc 126	6.00	-
127	Zc 127	1.00	-
128.	Zc 128	2.20	-
129	Zc 129	2.10	-
130	Zc 130	0	-
131	Dm 131	1.12	-
132	Dm 132	0	-
133	Dm 133	2.20	-
134	Dm 134	1.00	-
135	Dm 135	2.00	-
136	Dm 136	0	-
137	Dm 137	2.20	-
138	Dm 138	2.00	-
139	Dm 139	1.00	-

TOTAL AEROBIC PLATE COUNT FOR FERMENTED MILK SAMPLES			
S/NO	**SAMPLE NO**	**TAPC ON NA (CFU/ml) 10^7**	**GROWTH ON BSA**
140	Dm 140	2.30	-
141	Dm 141	0	-
142	Dm 142	1.20	-
143	Dm 143	1.32	-
144	Dm 144	1.00	-
145	Dm 145	0	-
146	Dm 146	0	-
147	Dm 147	0	-
148	Dm 148	2.00	-
149	Dm 149	0	-
150	Dm 150	2.00	-
151	Dm 151	1.20	-
152	Dm 152	1.50	-
153	Dm 153	0	-
154	Dm 154	4.00	-
155	Dm 155	1.80	-
156	Sm 156	0	-
157	Sm 157	0	-
158	Sm 158	2.10	-
159	Sm 159	2.14	-
160	Sm 160	1.16	-
161	Sm 161	0	-
162	Sm 162	1.00	-

TOTAL AEROBIC PLATE COUNT FOR FERMENTED MILK SAMPLES			
S/NO	**SAMPLE NO**	**TAPC ON NA (CFU/ml) 10^7**	**GROWTH ON BSA**
163	Sm 163	2.00	-
164	Sm 164	0	-
165	Sm 165	0	-
166	Sm 166	0	-
167	Sk 167	TNTC	-
168	Sk 168	2.43	-
169	Sk 169	TNTC	-
170	Sk 170	TNTC	-
171	Sk 171	TNTC	-
172	Sk 172	2.49	-
173	Sk173	2.00	-
174	Sk 174	2.56	-
175	Sk 175	TNTC	-
176	Sk 176	TNTC	-

KEY

TAPC – Total Aerobic Plate Count; Cfu/g = Coliform forming unit/milligram; NA = Nutrient Agar, Bismuth Sulfite Agar = BSA; + = growth, - = no growth, TNTC = Too numerous to count

Sm = Samaru, Sk = Shika, Zg = Zango, Sg = Sabon Gari, Tw = Tudun wada

Dm = Dan-Magaji, Kn = Kongo, Zc = Zaria city

Appendix III: MICROBACT 12E AND GRAM REACTION RESULT

S/NO	SAMPLE NO	ORGANISM	GRAM REACTION
1.	Sk 30	*Serratia liquifaciens*	Negative Short rods
2.	Sk 31	*Enterobacter gergoviae*	Negative Short rods
3.	Sk 32	*Enterobacter gergoviae*	Negative Short rods
4.	Sk 33	*Escherichia coli*	Negative Short rods
5.	Sk 34	*Enterobacter gergoviae*	Negative Short rods
6.	Sk 35	*Enterobacter aerogenes*	Negative Short rods
7.	Sk 36	*Escherichia coli*	Negative Short rods
8.	Sk 38	*Escherichia coli*	Negative Short rods
9.	Sk 39	*Escherichia coli*	Negative Short rods
10.	Sk 41	*Salmonella arizonae*	Negative Short rods
11.	Sk 45	*Enterobacter gergoviae*	Negative Short rods
12.	Skn51	*Enterobacter gergoviae*	Negative Short rods
13.	Skn 52	*Enterobacter gergoviae*	Negative Short rods
14.	Skn 53	*Enterobacter gergoviae*	Negative Short rods
15.	Skn 54	*Enterobacter gergoviae*	Negative Short rods
16.	Skn 55	*Enterobacter gergoviae*	Negative Short rods
17.	Skn 56	*Enterobacter gergoviae*	Negative Short rods
18.	Skn 57	*Salmonella arizonae*	Negative Short rods
19.	Skn 58	*Escherichia coli*	Negative Short rods
20	Skn 59	*Enterobacter gergoviae*	Negative Short rods
21.	Skn 60	*Enterobacter gergoviae*	Negative Short rods
22.	Skn 61	*Enterobacter gergoviae*	Negative Short rods
23.	Skn62	*Enterobacter gergoviae*	Negative Short rods
24.	Skn 63	*Enterobacter gergoviae*	Negative Short rods

MICROBACT 12E AND GRAM REACTION RESULT			
S/NO	**SAMPLE NO**	**ORGANISM**	**GRAM REACTION**
25.	Skn 64	*Enterobacter gergoviae*	Negative Short rods
26.	Skn 65	*Enterobacter gergoviae*	Negative Short rods
27.	Skn 66	*Enterobacter gergoviae*	Negative Short rods
28.	Skn 67	*Enterobacter gergoviae*	Negative Short rods
29.	Skn 68	*Enterobacter gergoviae*	Negative Short rods
30.	Skn 70	*Enterobacter gergoviae*	Negative Short rods
31.	Skn 72	*Enterobacter gergoviae*	Negative Short rods
32.	Skn74	*Enterobacter gergoviae*	Negative Short rods
33.	Skn 75	*Klebsiella oxytoca*	Negative Short rods
34.	Sg 76	*Salmonella arizonae*	Negative Short rods
35.	Sg 77	*Salmonella arizonae*	Negative Short rods
36.	Sg 78	*Escherichia coli*	Negative Short rods
37.	Sg 79	*Salmonella arizonae*	Negative Short rods
38.	Sg 81	*Enterobacter gergoviae*	Negative Short rods
39.	Sg 83	*Enterobacter gergoviae*	Negative Short rods
40.	Sg 84	*Enterobacter gergoviae*	Negative Short rods
41.	Sg 85	*Enterobacter gergoviae*	Negative Short rods
42.	Sg 86	*Enterobacter gergoviae*	Negative Short rods
43.	Sg 87	*Salmonella arizonae*	Negative Short rods
44.	Sg 88	*Enterobacter gergoviae*	Negative Short rods
45.	Sg 91	*Enterobacter gergoviae*	Negative Short rods
46.	Sg 92	*Enterobacter gergoviae*	Negative Short rods
47.	Sg 93	*Enterobacter gergoviae*	Negative Short rods
48.	Sg 96	*Serretia liquefaciens*	Negative Short rods

MICROBACT 12E AND GRAM REACTION RESULT			
S/NO	**SAMPLE NO**	**ORGANISM**	**GRAM REACTION**
49.	Sg 97	*Enterobacter gergoviae*	Negative Short rods
50.	Sg 98	*Enterobacter gergoviae*	Negative Short rods
51.	Sg 99	*Enterobacter gergoviae*	Negative Short rods
52.	Zg 106	*Escherichia coli*	Negative Short rods
53.	Zg 110	*Salmonella arizonae*	Negative Short rods
54.	Zg 114	*Escherichia coli*	Negative Short rods
55.	Zg 121	*Salmonella arizonae*	Negative Short rods
56.	Tw 127	*Klebsiellapnemonaee*	Negative Short rods
57.	Tw128	*Enterobacter gergoviae*	Negative Short rods
58.	Tw131	*Citrbacter diversus*	Negative Pleumorphic rods
59.	FSm 1	*Salmonella arizonae*	Negative Short rods
60.	FSm 12	*Escherichia coli*	Negative Short rods
61.	FSk 24	*Escherichia coli*	Negative Short rods
62	FSk 26	*Acinetobacter iwoffii*	Negative Short rods
63.	FSk 28	*Salmonella arizonae*	Negative Short rods
64	FSk 31	*Citrbacter diversus*	Negative Pleumorphic rods
65.	FSk 32	*Enterobacter gergoviae*	Negative Short rods
66.	FKw 46	*Escherichia coli*	Negative Short rods
67.	FKw 47	*Escherichia coli*	Negative Short rods
68.	FKw 48	*Salmonella arizonae*	Negative Short rods
69.	FKw 49	*Enterobacter gergoviae*	Negative Short rods
70.	FKw 50	*Salmonella arizonae*	Negative Short rods
71.	FKw 52	*Enterobacter gergoviae*	Negative Short rods

MICROBACT 12E AND GRAM REACTION RESULT			
S/NO	**SAMPLE NO**	**ORGANISM**	**GRAM REACTION**
72.	FKw 57	*Salmonella arizonae*	Negative Short rods
73.	FSg 62	*Escherichia coli*	Negative Short rods
74.	FSg 63	*Enterobacter gergoviae*	Negative Short rods
75.	FSg 64	*Enterobacter gergoviae*	Negative Short rods
76.	FSg 65	*Enterobacter gergoviae*	Negative Short rods
77.	FSg 66	*Salmonella arizonae*	Negative Short rods
78.	FSg 68	*Escherichia coli*	Negative Short rods
79.	FTw 86	*Escherichia coli*	Negative Short rods
80.	FTw 87	*Escherichia coli*	Negative Short rods

KEY

Sampling Areas; Sm = Samaru, Sk = Shika, Zg = Zango, Sg = Sabon Gari, Tw = Tudun wada

Dm = Dan-Magaji, Kn = Kongo, Zc = Zaria city, F = Fermented milk

Appendix IV: **Biochemicals result for Raw milk Isolates**

S/No	Sample No	TSI	Simon's citrate	Urea	Indole	Motility	H_2S	M.R	V.P	Organism
1.	Sk 29	A/AG	+	+	-	-	-	+	+	*Klebsiella* spp
2.	Sk 30	A/AG	+	+	-	+	-	-	+	*Enterobacter* spp
3.	Sk 31	A/A	+	+	+	-	-	+	-	*Citrobacter* spp
4.	Sk 32	A/AG	+	+	-	+	-	-	+	*Enterobacter* spp
5.	Sk 33	A/AG	+	+	+	+	-	+	-	*Citrobacter* spp
6.	Sk 34	A/AG	+	+	+	+	-	+	-	*Citrobacter* spp
7.	Sk 35	A/AG	+	+	+	+	-	+	-	*Citrobacter* spp
8.	Sk 36	A/AG	+	+	+	+	-	+	-	*Citrobacter* spp
9.	Sk 37	A/AG	-	-	+	+	-	+	-	*Escherichiacoli*
10	Sk 38	A/AG	+	+	+	+	-	+	-	*Citrobacter* spp
11	Sk 39	A/AG	+	+	+	+	-	+	-	*Citrobacter* spp
12	Sk 40	A/AG	+	+	+	-	-	-	+	*Klebsiella* spp
13	Sk 41	A/AG	+	+	-	+	-	+	+	*Proteus* spp
14	Sk 42	A/AG	-	-	+	+	-	+	-	*Escherichia coli*
15	Sk 43	A/AG	-	-	+	+	-	+	-	*Escherichia coli*
16	Sk 44	A/AG	-	-	+	+	-	+	-	*Escherichia coli*
17	Sk 45	A/AG	+	+	-	+	-	+	+	*Proteus* spp
18	Sk 47	A/AG	+	+	-	-	-	+	+	*Klebsiella* spp
19	Sk 48	A/AG	+	+	-	-	-	+	+	*Klebsiella* spp
20	Sk 50	A/AG	-	-	+	+	-	+	-	*Escherichia coli*
21	Skn 51	A/AG	+	-	-	+	-	-	+	*Enterobacter* spp
22	Skn 52	A/AG	+	+	-	+	-	-	+	*Enterobacter* spp
23	Skn 53	A/AG	+	+	-	+	-	-	+	*Enterobacter* spp
24	Skn 54	A/AG	+	+	-	+	-	-	+	*Enterobacter* spp
Biochemicals result for Raw milk Isolates										

S/No	Sample No	TSI	Simon's citrate	Urea	Indole	Motility	H_2S	M.R	V.P	Organism
25	Skn 55	A/AG	+	+	-	+	-	+	+	*Enterobacter* spp
26	Skn 56	A/AG	+	+	+	+	-	+	-	*Citrobacter* spp
27	Skn 57	A/AG	+	+	+	+	-	+	-	*Citrobacter* spp
28	Skn 58	A/AG	+	+	+	+	-	+	-	*Citrobacter* spp
29	Skn 59	A/A	+	+	+	+	-	+	-	*Citrobacter* spp
30	Skn 60	A/AG	+	+	+	+	-	+	-	*Citrobacter* spp
31	Skn 61	A/A	+	+	-	+	-	-	+	*Enterobacter* spp
32	Skn 62	A/AG	+	+	-	+	-	-	+	*Enterobacter* spp
33	Skn 63	A/AG	+	+	-	+	-	-	+	*Enterobacter* spp
34	Skn 64	A/A	+	+	+	+	-	+	-	*Citrobacter* spp
35	Skn 65	A/A	+	+	+	+	-	+	-	*Citrobacter* spp
36	Skn 66	A/A	+	+	-	+	-	-	+	*Enterobacter* spp
37	Skn 67	A/AG + H_2S	+	+	-	-	+	+	-	*Proteus* spp
38	Skn 68	A/A	+	+	+	+	-	+	-	*Citrobacter* spp
39	Skn 69	A/AG	+	+	+	-	-	-	+	*Klebseilla* spp
40	Skn 70	A/A	+	+	-	+	-	-	+	*Enterobacter* spp
41	Skn 71	A/AG	+	+	-	+	-	-	+	*Serratia* spp
42	Skn 72	A/A	+	+	-	+	-	-	+	*Enterobacter* spp
43	Skn 73	A/A	-	-	+	+	-	+	-	*Escherichia coli*
44	Skn 74	A/AG	+	+	+	+	-	+	-	*Citrobacter* spp
45	Skn 75	A/AG	+	+	-	+	-	-	+	*Enterobacter* spp
46	Sg 76	A/AG	+	+	+	+	-	-	+	*Citrobacter* spp
47	Sg 77	A/AG	+	+	+	+	-	+	+	*Citrobacter*spp
Biochemicals result for Raw milk Isolates										

S/No	Sample No	TSI	Simon's citrate	Urea	Indole	Motility	H_2S	M.R	V.P	Organism
48	Sg 78	A/AG	+	+	+	+	-	+	+	*Citrobacter* spp
49	Sg 79	Alk/AG+ H_2S	+	-	-	+	+	+	-	*Salmonella* spp
50	Sg 80	A/AG	+	+	-	+	-	-	+	*Serratia* spp
51	Sg 81	A/AG+ H_2S	+	+	-	+	-	-	+	*Proteus* spp
52	Sg 82	A/AG	+	+	-	+	-	-	+	*Serratia* spp
53	Sg 83	A/AG	+	+	+	+	-	-	+	*Citrobacter* spp
54	Sg 84	A/AG	+	+	+	+	-	+	+	*Citrobacter* spp
55	Sg 85	A/AG	+	+	-	+	-	-	-	*Enterobacter* spp
56	Sg 86	A/AG	+	+	+	+	-	-	-	*Citrobacter* spp
57	Sg 87	Alk/AG	+	-	-	+	-	+	-	*Salmonella* spp
58	Sg 88	Alk/AG	+	-	-	+	-	-	+	*Enterobacter* spp
59	Sg 89	A/AG	+	+	-	+	-	-	+	*Serratia* spp
60	Sg 90	A/AG	+	+	+	+	-	-	+	*Aerobacter* spp
61	Sg 91	A/AG	+	+	-	+	-	-	-	*Enterobacter* spp
62	Sg 92	Alk/AG	+	+	-	+	-	-	+	*Proteus* spp
63	Sg 93	A/AG	+	+	-	+	-	-	-	*Enterobacter* spp
64	Sg 94	A/AG	+	+	+	+	-	-	+	*Aerobacter* spp
65	Sg 95	A/AG	+	+	+	+	-	-	+	*Aerobacter* spp
66	Sg 96	A/AG	+	+	+	+	+	+	-	*Citobacter* spp
67	Sg 97	A/AG	+	+	-	+	-	-	+	*Enterobacter* spp
68	Sg 98	A/AG	+	+	-	+	-	-	+	*Enterobacter* spp
69	Sg 99	A/AG	+	+	+	+	-	+	+	*Citrobacter* spp
70	Sg 100	A/AG	+	+	+	+	-	-	+	*Aerobacter* spp

Biochemicals result for Raw milk Isolates										
S/No	Sample No	TSI	Simon's citrate	Urea	Indole	Motility	H_2S	M.R	V.P	Organism
71	Zg 106	A/AG	+	+	+	+	-	+	-	*Citrobacter* spp
72	Zg 110	A/AG	+	+	+	+	-	+	-	*Citrobacte*r spp
73	Zg 114	A/AG	+	+	+	+	-	+	-	*Enterobacter*

										spp
74	Zg 121	Alk/AG	+	-	-	+	-	+	-	*Salmonella* spp
75	Tw126	Alk/AG	+	+	-	+	-	-	+	*Aerobacter* spp
76	Tw127	A/AG	+	+	-	+	-	-	+	*Enterobacter* spp
77	Tw128	Alk/A + H_2S	+	+	-	+	+	+	-	*Proteus* spp
78	Tw129	A/AG	+	+	-	+	-	-	+	*Enterobacter* spp
79	Tw130	A/AG	+	+	-	+	-	-	+	*Enterobacter* spp
80	Tw131	A/AG	+	+	-	+	-	-	+	*Enterobacter* spp
81	Tw132	A/AG	+	+	+	+	-	-	+	*Enterobacter* spp
82	Tw134	A/AG	+	+	+	+	-	+	-	*Citrobacter* spp
83	Tw136	Alk/AG	+	+	-	+	-	-	+	*Aerobacter* spp
84	Tw137	A/AG	+	+	-	+	-	-	+	*Enterobacter* spp
85	Tw138	A/AG	+	+	-	+	-	-	+	*Enterobacter* spp
86	Tw139	Alk/AG	+	+	-	+	-	-	+	*Aerobacter* spp
87	Tw140	A/AG	+	+	-	+	-	-	+	*Enterobacter* spp
88	Tw141	Alk/AG	+	+	-	+	-	-	+	*Aerobacter* spp
89	Tw142	A/AG	+	+	+	+	-	-	+	*Enterobacter* spp
90	Tw143	A/AG	+	+	-	+	-	-	+	*Enterobacter* spp
91	Tw144	Alk/AG	+	+	-	+	-	-	+	*Aerobacter* spp
92	Tw145	A/AG	+	+	-	+	-	-	+	*Enterobacter*spp
Biochemicals result for Raw milk Isolates										
S/No	**Sample No**	**TSI**	**Simon's citrate**	**Urea**	**Indole**	**Motility**	**H_2S**	**M.R**	**V.P**	**Organism**
93	Tw146	Alk/AG	+	+	-	+	-	-	+	*Aerobacter* spp
94	Tw147	A/AG	+	+	-	+	-	-	+	*Enterobacter*spp
95	Tw148	A/AG	+	+	+	+	-	+	-	*Citrobacter* spp
96	Tw150	Alk/AG+ H_2S	+	+	-	+	+	-	+	*Proteus* spp
97	Dm 151	A/AG	+	+	+	+	+	+	-	*Citrobacter* spp
98	Dm 153	A/AG	+	+	-	+	-	+	+	*Citrobacter* spp
99	Dm154	A/AG	+	+	-	+	-	+	-	*Citrobacter* spp
100	Dm	A/AG	+	+	+	+	-	-	+	*Enterobacter*

	156									spp
101	Dm 157	Alk/AG+ H_2S	+	+	-	+	+	+	-	*Proteus* spp
102	Dm 160	A/AG	+	+	+	+	-	-	+	*Citrobacter* spp
103	Dm 161	A/AG	+	+	+	-	-	+	+	*Citrobacter* spp
104	Dm 162	A/AG	+	+	+	+	-	+	-	*Citrobacter* spp
105	Dm 165	Alk/AG	+	+	+	+	+	-	+	*Proteus* spp
106	Dm 166	Alk/AG	+	+	-	+	+	+	+	*Enterobacter* spp
107	Dm 167	Alk/AG	+	+	+	+	-	+	-	*Proteus* spp
108	Dm 168	A/AG	+	+	+	+	-	+	+	*Citrobacter* spp
109	Dm 169	Alk/AG	+	+	+	+	-	-	+	*Enterobacter* spp
110	Dm 170	A/AG	+	+	+	+	+	+	-	*Citrobacter* spp
111	Dm 171	A/A	+	+	-	+	-	+	+	*Citrobacter* spp
112	Dm 172	Alk/A	+	+	-	+	+	+	+	*Citrobacter* spp
113	Dm 173	A/AG+ H_2S	+	+	+	+	+	+	-	*Proteus spp*
114	Dm 174	A/AG	+	+	+	+	+	+	+	*Citrobacter* spp

Appendix V: Biochemicals result for Fermented milk Isolates

S/No	Sample No	TSI	Simon's citrate	Urea	Indole	Motility	H_2S	M.R	V.P	Organism
1.	Sm 1	Alk/AG	+	-	-	+	-	+	-	*Escherichia coli*
2.	Sm 2	A/A	+	+	-	+	-	+	+	*Serratia* spp
3.	Sm 8	Alk/A	+	+	-	+	-	-	+	*Aerobacter* spp
4.	Sm 12	A/A	+	+	+	+	-	+	-	*Citrobacter* spp
5.	Sm 14	Alk/A	+	+	-	+	-	-	+	*Aerobacter* spp
6.	Sm 15	Alk/A	+	+	-	+	-	-	+	*Aerobacter* spp
7.	Sm 21	Alk/A	+	+	-	+	-	-	+	*Aerobacter* spp
8.	Sm 22	Alk/A	+	+	-	+	-	-	+	*Aerobacter* spp
9.	Sk 23	A/AG	+	+	-	-	-	-	+	*Klebsiella* spp
10.	Sk 24	A/AG	+	+	+	+	-	+	-	*Citrobacter* spp
11.	Sk 26	A/AG	+	+	+	+	-	+	-	*Citrobacter* spp
12.	Sk 27	Alk/AG	+	+	-	+	-	-	+	*Aerobacter* spp

13.	Sk 28	Alk/A	+	-	-	+	-	+	-	*Salmonella* spp
14.	Sk 29	Alk/A	+	+	-	+	-	-	+	*Aerobacter* spp
15.	Sk 30	Alk/A	+	+	-	+	-	-	+	*Aerobacter* spp
16.	Sk 31	A/AG	+	+	+	+	-	+	-	*Citrobacter* spp
17.	Sk 32	Alk/AG	-	+	+	+	-	+	-	*Proteus* spp
18.	Sk 33	Alk/AG	+	+	-	+	-	-	+	*Aerobacter* spp
19.	Kw 46	A/AG	+	+	+	+	-	+	-	*Citrobacter* spp
20.	Kw 47	A/AG	+	+	-	+	-	-	+	*Enterobacter* spp
21.	Kw 48	A/AG	+	+	+	+	-	+	-	*Citrobacter* spp
22.	Kw 49	A/AG	+	+	-	+	-	-	+	*Enterobacter* spp
23.	Kw 50	A/AG	+	+	+	+	-	+	-	*Citrobacter* spp
24.	Kw 52	A/AG	+	+	-	+	-	-	+	*Enterobacter* spp
25.	Kw 56	A/AG	+	+	-	+	-	+	+	*Serratia* spp
26.	Kw 57	A/AG	+	+	+	+	-	+	-	*Citrobacter* spp
27.	Sg 61	A/AG	+	+	-	+	-	+	+	*Serratia* spp
28.	Sg 62	A/AG	+	+	+	+	-	+	-	*Citrobacter* spp
29.	Sg 63	A/AG	+	+	-	+	-	-	+	*Enterobacter* spp
30.	Sg 64	A/AG	+	+	+	+	-	+	-	*Citirobacter* spp
31.	Sg 65	A/AG	+	+	-	+	-	-	+	*Enterobacter* spp
32.	Sg 66	A/AG	+	+	+	+	-	+	-	*Citrobacter* spp
Biochemicals result for Fermented milk Isolates										
S/No	Sample No	TSI	Simon's citrate	Urea	Indole	Motility	H_2S	M.R	V.P	Organism
33.	Sg 68	A/AG	+	+	+	+	-	+	-	*Citrobacter* spp
34.	Tw 86	A/AG	+	+	+	+	-	+	-	*Citrobacter* spp
35.	Tw 87	A/AG	+	+	+	+	-	+	-	*Citrobacter* spp

Key

Biochemical interpretations:

A=Acid, Alk = Alkaline, G=Gas, H_2S=Hydrogen Sulphide, + = positive, − = negative

Sm=Samaru, Sk = Shika, Zg = Zango, Sg= Sabon Gari, Tw=Tudun wada

Dm = Dan-Magaji, Kn=Kongo, Zc=Zaria city

Appendix VI: Conventional and Microbact result for *Salmonella* Spp

S/No	Sample No	Conventional Bchm for *Salmonella*	Microbact 12E	Serological test
1.	RSk 41	_	+	+
2.	RSkn 57	_	+	+
3.	RSg 76	_	+	+
4.	RSg 77	_	+	+
5.	RSg 79	+	+	+
6.	RSg 87	+	+	+
7.	RZg 110	_	+	+
8.	RZg 121	_	+	+
9.	FSm 1	_	+	+
10.	FSk 28	+	+	+
11.	FKw 48	_	+	+

12.	FKw 50	_	+	+
13.	FKw 57	_	+	+
14.	FSg 66	_	+	+

Key

Bchm = Biochemical tests

Sm=Samaru, Sk = Shika, Zg = Zango, Sg= Sabon Gari, Tw=Tudun wada

Dm = Dan-Magaji, Kw = Kwangila, R = Raw milk, F = fermented milk

+ = positive, − =negative

Appendix VII: ANTIBIOTIC SENSITIVITY TEST FOR *SALMONELLA*

S/NO	SAMPLE NO	C 30	CIP 5	K 30	NA 30	CN 10	AMP10	AML 10	S 10	MY 10	E 15	TE 10
1.	RSk 41	S	S	S	S	S	R	R	R	R	R	S
2.	RSkn 57	S	S	S	S	S	R	R	R	R	S	S
3.	RSg 76	S	S	S	S	S	R	S	R	R	R	S
4.	RSg 77	S	S	S	S	S	R	R	R	R	R	R
5.	RSg 79	S	S	S	S	S	S	S	S	R	S	S
6.	RSg 87	S	S	S	S	S	R	R	S	R	R	S
7.	RZg 110	S	S	S	S	S	R	R	S	R	R	S
8.	RZg 121	S	S	R	S	S	R	R	S	R	R	S
9.	FSm 1	S	S	S	S	S	R	R	S	R	R	S
10.	FSk 28	R	S	S	R	S	S	S	S	R	R	S
11.	FKw 48	S	S	S	S	S	R	R	S	R	R	R
12.	FKw 50	S	S	S	S	S	R	R	S	R	R	R
13.	FKw 57	S	S	S	S	S	R	R	R	R	R	R
14.	FSg 66	S	S	S	S	S	R	R	S	R	R	R

KEY:

Chloramphenico (C 30µg), Ciprofloxacin (CIP 5µg), Kanamycin (K 30µg), Nalidixic Acid (NA 30µg), Gentamicin (CN 10µg), Ampicillin (AMP 10µg), Amoxyxillin (AML 10µg), Streptomycin(S 10µg), Lincomycin (MY 10µg), Erythromycin(E 15µg), Tetracycline (TE 10µg).

INTERPRETATION≤ 13 is resistant, > 13 is sensitive (Zone of inhibition standard by clinical laboratory standard institute, 2011).

Sampling Areas; Sk = Shika, Skn = Shika NAPRI, Sg = Sabon Gari, Zg = Zango, Sm = Samaru, Tw = Tudun wada, Kw = Kwangila

R = Raw milk, F = Fermented milk

Appendix VIII: Results of Conventional and Microbact 12E tests of *Salmonella* spp from Raw Milk Based on Local Government Areas (LGA)

LGA	No. Sampled	Bchm in %	Microbact in %
S/Gari	74	22 (37.93)	6 (75)
Zaria	50	3 (5.17)	0 (0)
Giwa	50	33 (56.90))	2 (25)
Total	174	22	8

Key

LGA = Local Government Areas

Bchm = Biochemical

Appendix IX: Results of Conventional and Microbact 12E tests of *Salmonella* spp from Fermented Milk Based on Local Government Areas (LGA).

LGA	No. Sampled	Bchm in %	Microbact in %
S/Gari	68	15 (68.18)	5 (83.33)
Zaria	78	2 (9.09)	0 (0)
Giwa	30	5 (22.73)	1(16.67)
Total	176	22	6

Key

LGA = Local Government Areas

Bchm = Biochemical

Additional biochemical results

The 3 *Salmonella* suspects from conventional biochemical test were further subjected to sugar fermentation test using (Xylose, Mannitol, Rhamnose and Maltose). There was colour change from light pink to deep pink which indicates positive for *Salmonella* species (Appendix X).

Also on the same 3 *Salmonella* suspects from conventional biochemical test, Amino acid test was carried out using (Lysine, Ornithine and Arginine). There was colour change from light yellow to purple which indicates positive for *Salmonella* species (Appendix XI)

Appendix X: Sugar test fermentation result on *Salmonella* suspects

S/n	Sample no	Xylose	Mannitol	Rhamnose	Maltose
1.	Sg87	+	+	+	+
2.	Zg121	+	+	+	+
3.	FSk28	+	+	+	+

Key

Sg = Sabon Gari, Zg = Zango, FSk = Fermented milk (Shika)

Appendix XI: Amino acid test result on *Salmonella* suspects

S/n	Sample no	Lysine	Ornithine	Arginine
1.	Sg87	+	+	+
2.	Zg121	+	+	-
3.	FSk 28	+	+	+

Key

Sg = Sabon Gari, Zg = Zango, FSk = Fermented milk (Shika)

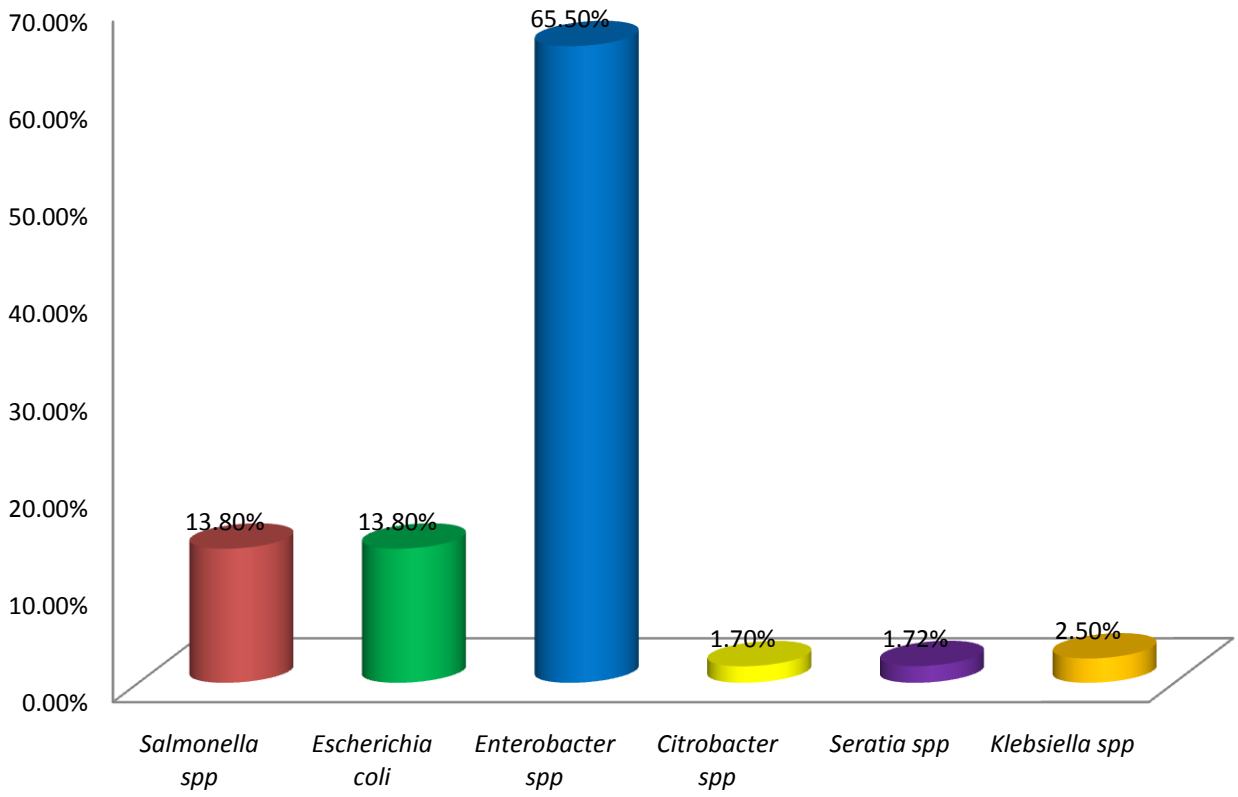
Appendix XII: Minimum Inhibitory Concentration Evaluation on *Salmonella* Species

S/N	SAMPLE NO.	ANTIBIOTICS	
		AML	E
1.	RSk 41	0.12	> 256
2.	RSkn 57	> 256	> 256
3.	RSg 76	> 256	> 256
4.	RSg77	> 256	> 256
5.	RSg 79	> 256	> 256
6.	RZg 110	> 256	> 256
7.	RZg 121	> 256	> 256
8.	FSm 1	> 256	> 256
9.	FKw 50	> 256	> 256
10.	FKw5	> 256	> 256

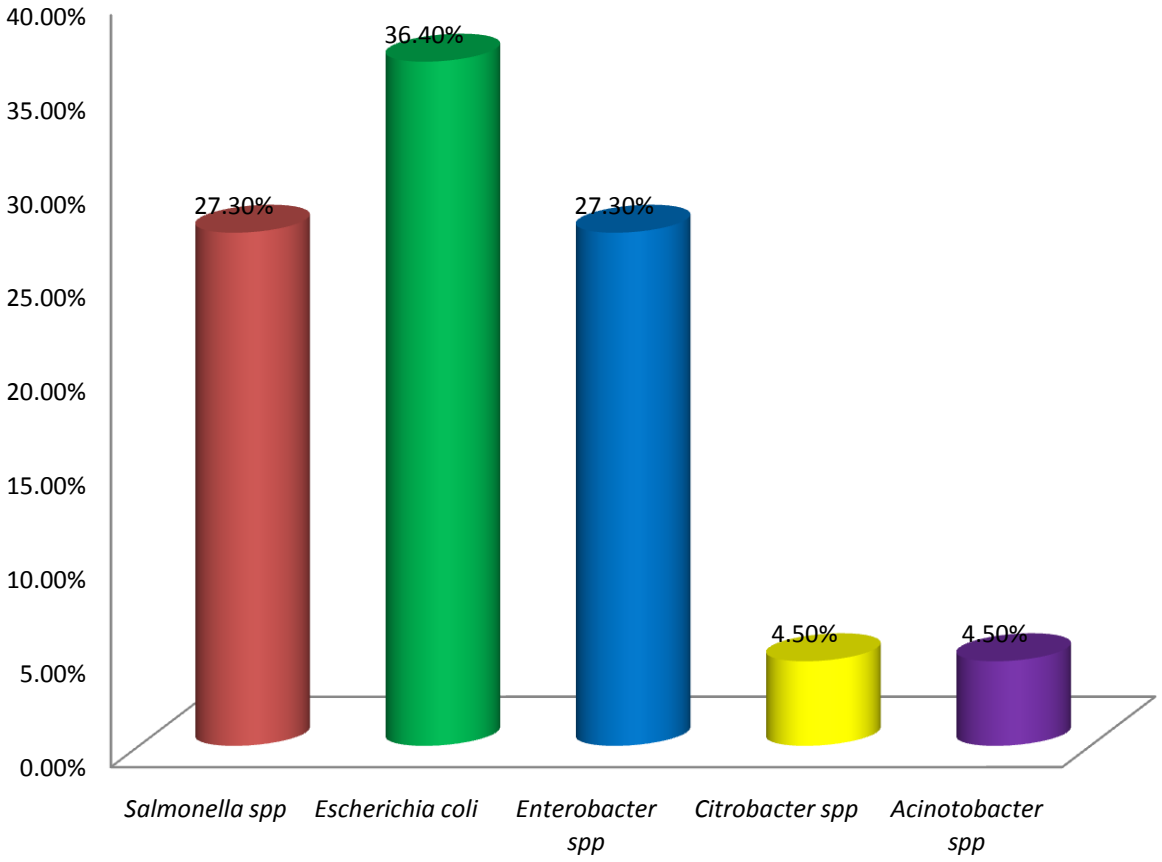
Key

Sm = Samaru, Sk = Shika, Skn = Shika Napri, Sg = Sabon Gari, Zg = Zango, Kw = Kwangila, R = Raw milk, F = Fermented milk
Antibioticstrips: AML = Amoxycillin and E = Erythromycin

Appendix XIII: Distribution of *Salmonella* and other enterobacteriaceae in Raw milk based on Microbact 12-E kit.



Appendix XIV: Distribution of *Salmonella* and other enterobacteriaceae in Fermented milk based on Microbact 12-E kit.



Appendix XV: Distribution of *Salmonella* and other enterobacteriaceae in raw and locally fermented milk based on Microbact 12-E kit

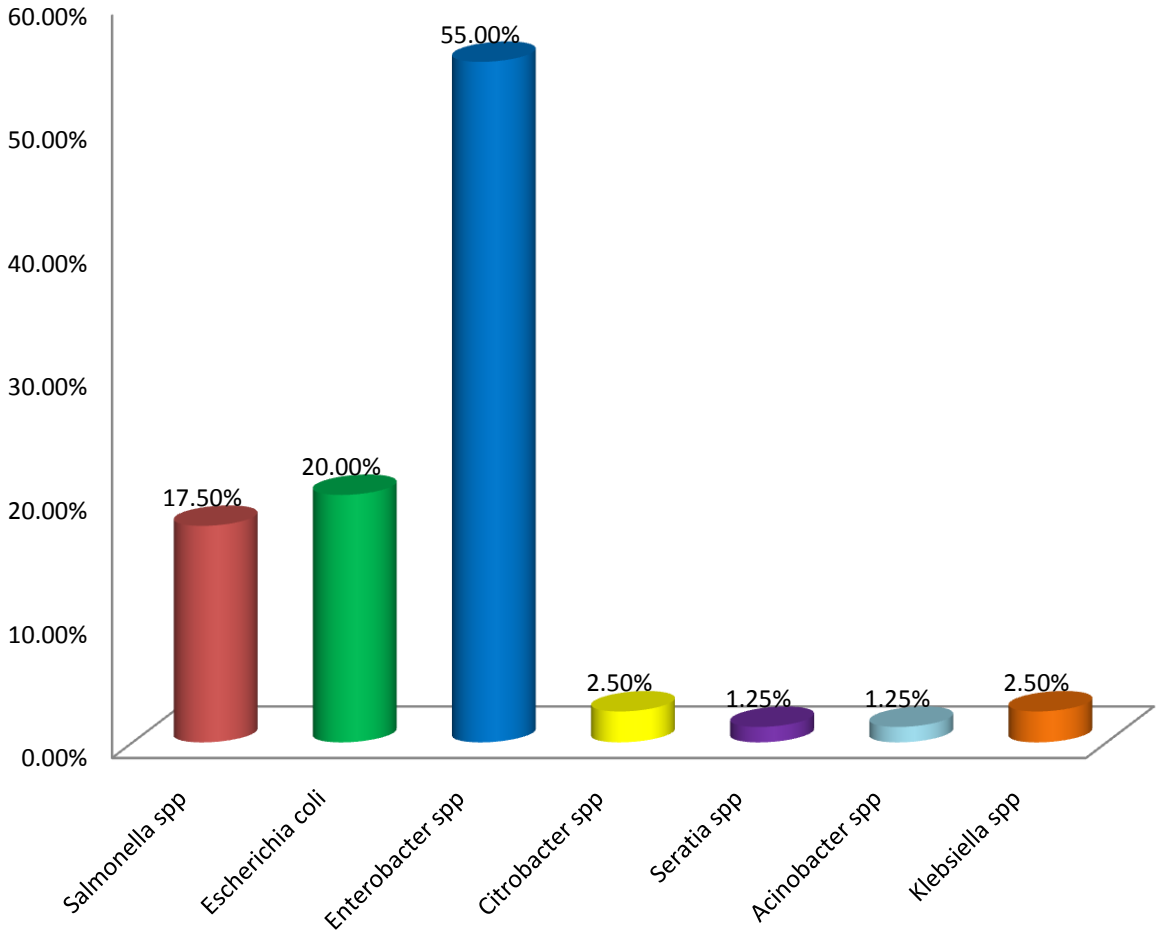
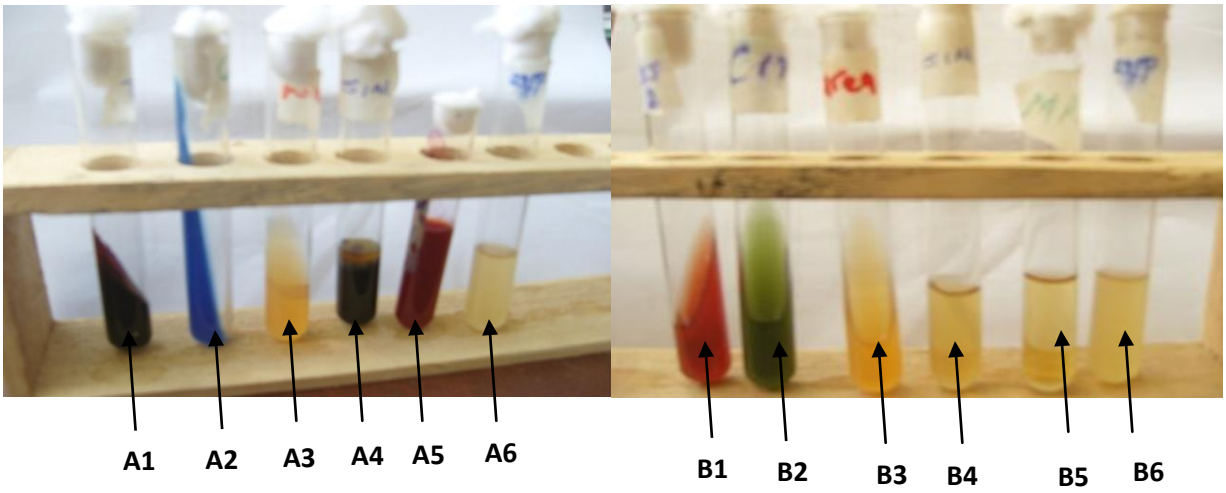


Plate XVIa: Biochemical test on *Salmonella* isolate (positive) Plate XVIb: Biochemical test on *Salmonella* isolate (control)



KEY

A1, TSI = $^{Alkaline}/_{Acid\ G + H_2S}$ i.e. orange slant and yellow butt plus G (gas)

A2, Citrate = positive i.e. blue colour

A3,Urea = negative i.e. yellow colour

A4, SIM-: Motility = positive with diffuse growth

H_2S = positive with blackening

Indole = negative with no pink ring colouron the surface.

A5, MR = positive with red colour

A6, VP = negative with no reddish brown ring colouron the surface.

B1=TSI, B2=Citrate, B3=SIM, B4= MR – UP, B6=Urea are the control.

AppendixXVII: An un-inoculated plate of BSA



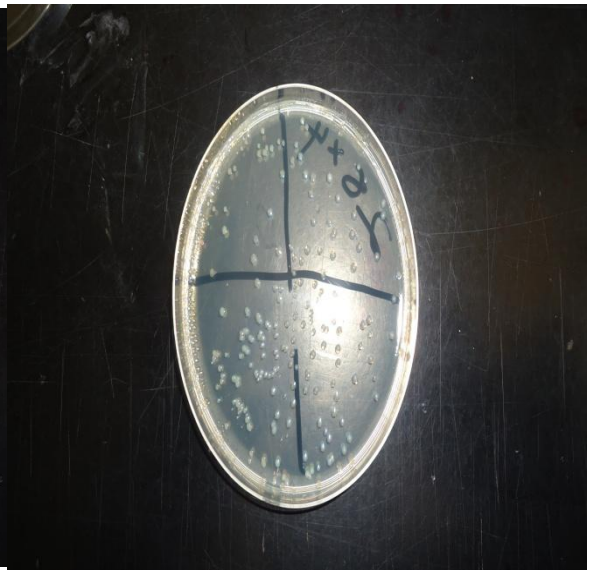
AppendixXVIII: An inoculated plate of BSA



AppendixXIX: An uninoculated plate of NA TAPC



Appendix XX: An inoculated plate of NA for



KEY

BSA =Bismuth Sulfite Agar, NA = Nutrient Agar,TAPC = Total Aerobic Plate Count

AppendixXXI: A Fulani herd at Hayin Mallam



AppendixXXII: A Fulani herd at Hayin Mallam with the adults separated from the calves



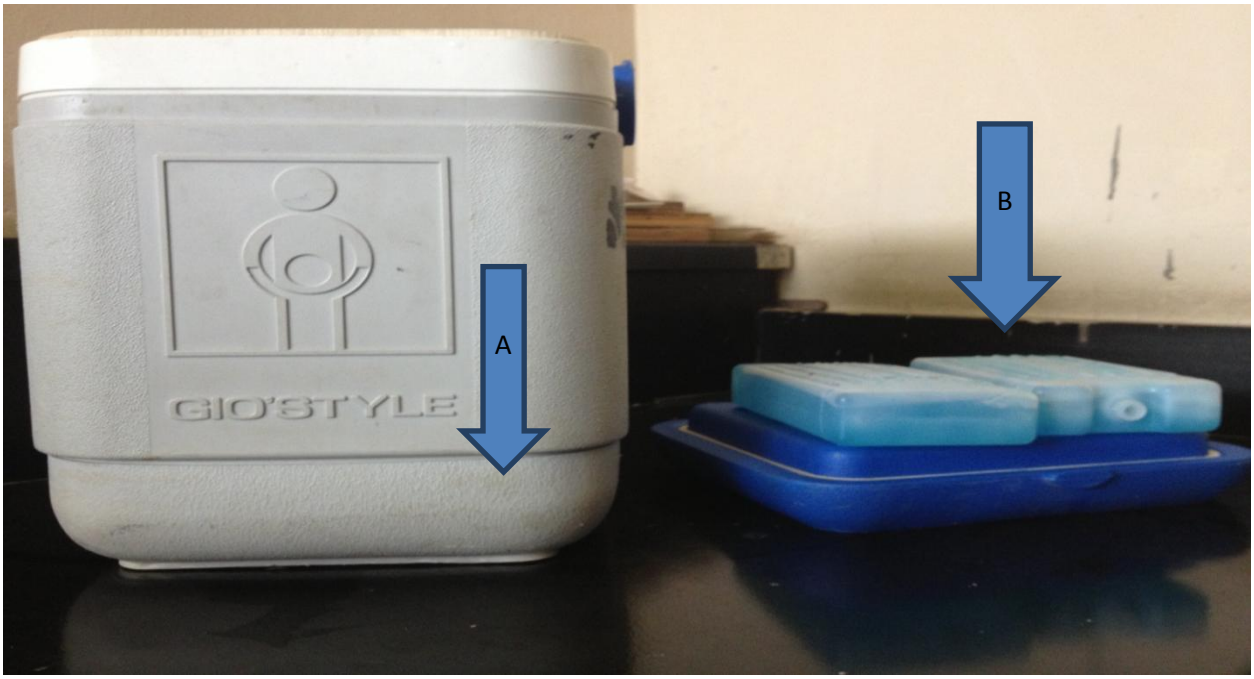
AppendixXXIII: A Fulani herd at Zango during milking



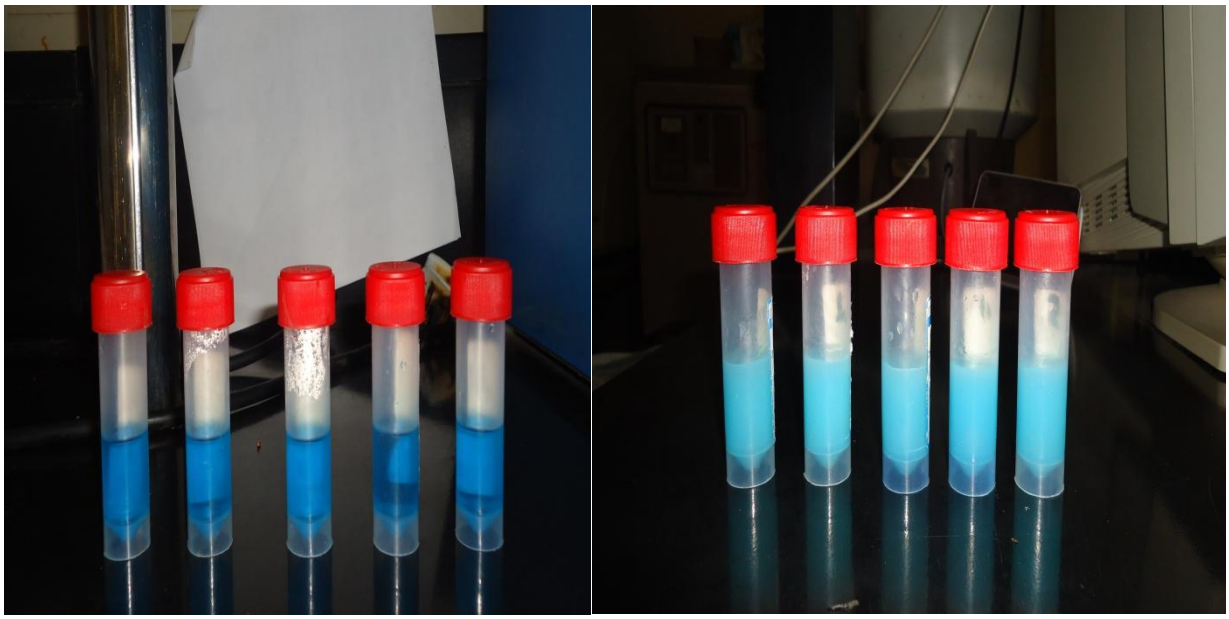
Appendix XXIV: The researcher analyzing milk samples (indicated by ablack arrow) immediately after transporting them to the laboratory in the safety hood.



AppendixXXV: An Ice-man Cole box (A) and ice packs (B) used for transportation of milk samples



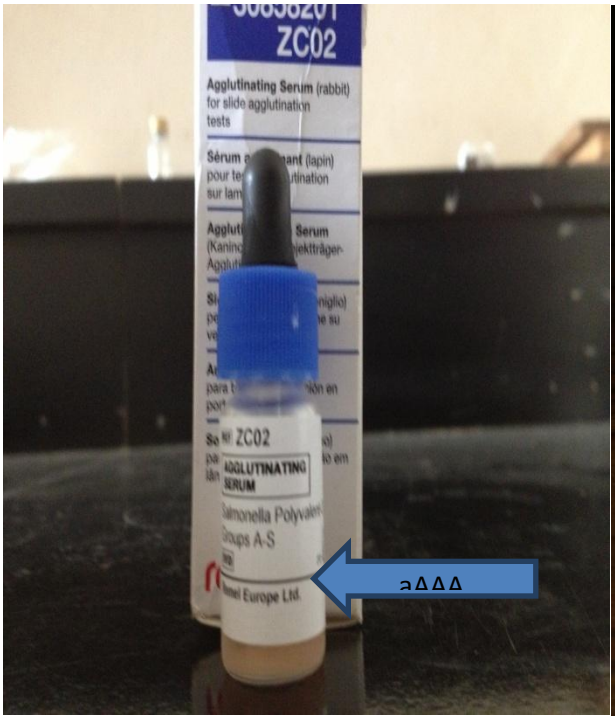
Appendix XXVI: RVB in sterile bottles before innoculation Appendix XXVII: RVB in sterile bottles after inoculation with milk samples



KEY

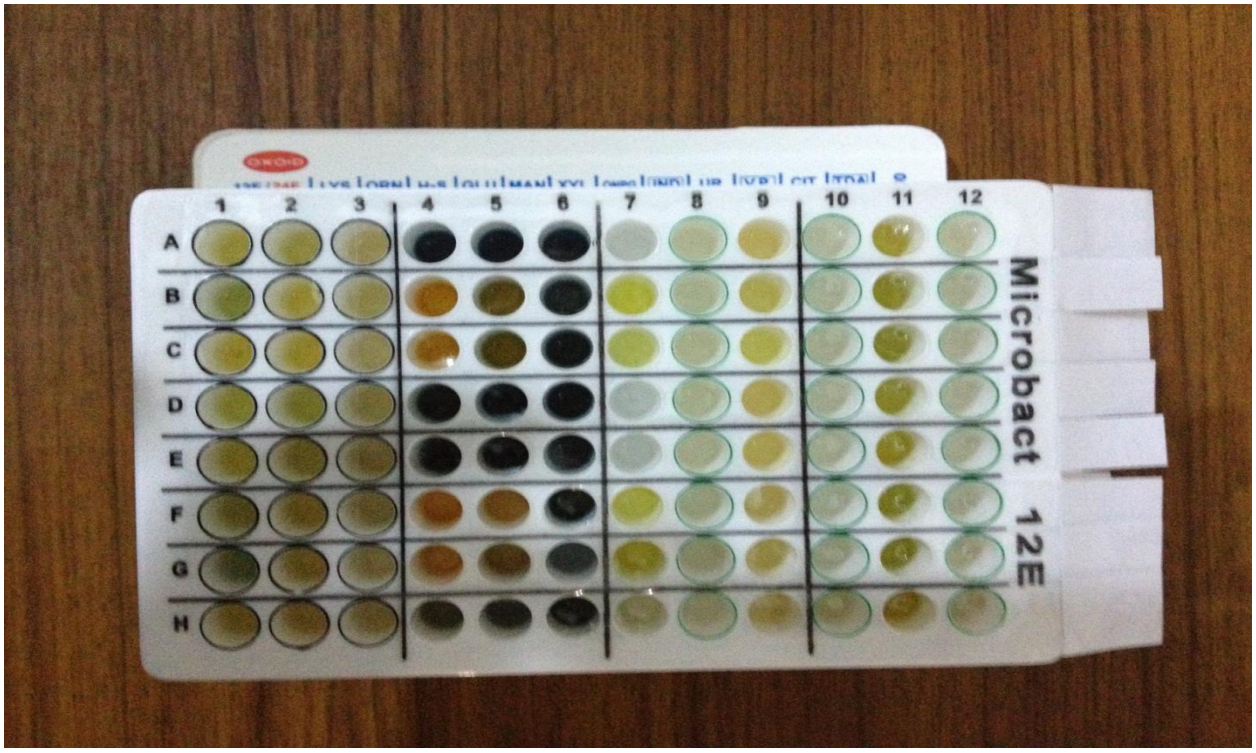
RVB = Rapapport Vassiliadis broth

Appendix XXVIII: *Salmonella* Polyvalent antiserum Appendix XXIX: Microbact Reagents for microbact Test kit(Kovac's, VPI, VPII, TDA)





Appendix XXX:Microbact 12E plate before adding reagents



AppendixXXXI :Microbact 12E plate after adding reagents

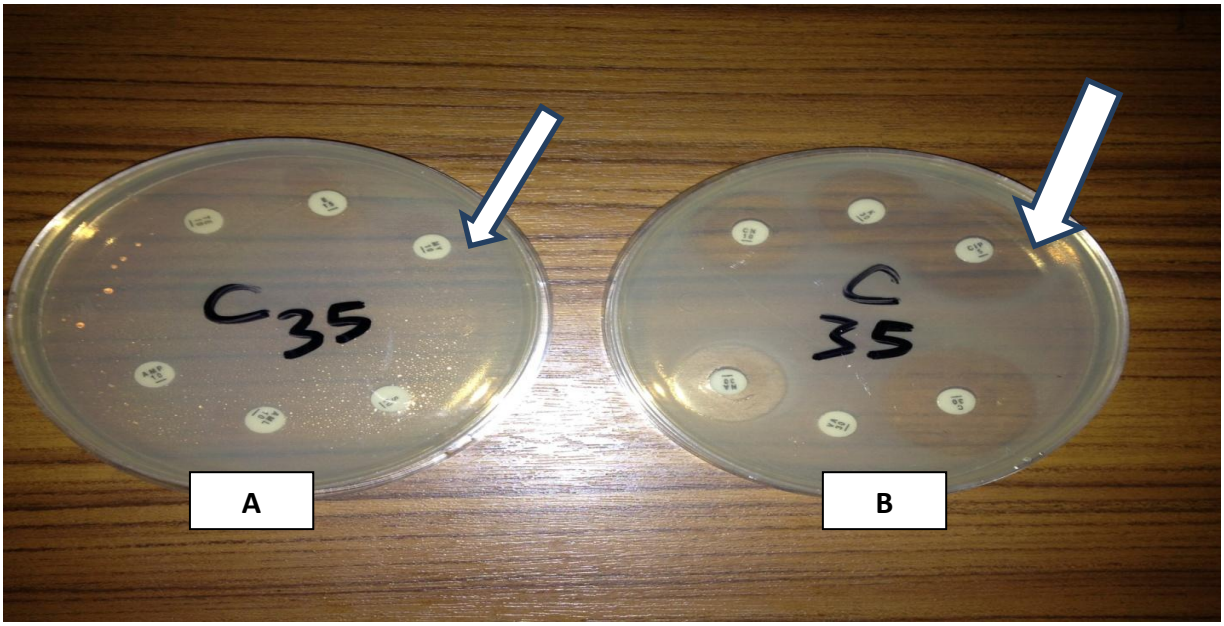


Appendix XXXII: Disc dispenser Appendix XXXIII: Disc dispenser with antibiotics inserted





Appendix XXXIV: Antibiotic Disc without zone of inhibitionA and Antibiotic Disc indicating zone of inhibition B



Appendix XXXV: MIC Plates without zone of inhibiton A and MIC Plates with the zone of inhibiton B

