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RESEARCH ARTICLE

QUALITY ASSESSMENT AND OCCURRENCE OF BACTERIAL PATHOGENS IN LOCALLY AVAILABLE  
FULANI NONO (MILK), FERMENTED YOGHURT AND THEIR EFFECT TO HUMANS.  
A CASE STUDY OF SAMARU ZARIA

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ABSTRACT

Laboratory investigations were ascertained to determine the comparative studied of bacteria on fermented yoghurt and locally available fulani nono (Diary product) within Samaru Zaria. Standard plate count (SPC) method was carried out in eight milk samples four from local Fulani nono (A<sub>1</sub>,A<sub>2</sub>,A<sub>3</sub>,A<sub>4</sub>) and four from yoghurt (B<sub>1</sub>,B<sub>2</sub>,B<sub>3</sub>,B<sub>4</sub>), the microbial colonies were found to be high in Fulani nono samples than the yoghurt (B<sub>1</sub>,B<sub>2</sub>,B<sub>3</sub> and B<sub>4</sub>) samples. The methylene blue test performed for the milk sample showed that out of the eight samples two sample were poor, one was fair, four were good and one was excellent. Biochemical characterization was also carried out in-situ. The following pathogens were identified viz; *Bacillus* sp, *Staphylococcus* sp *Streptococcus* sp. *Pseudomonas* sp, *Klebsiella* sp, *Salmonella* sp, *Proteus* sp, *Enterobacter* sp and their occurrence was noted in-situ. Health implication of the foregoing results in their effort of identification of pathogens in local Fulani nono (milk) and the pasteurized yoghurt are discussed.

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INTRODUCTION

Milk is a complex biological fluid and by its nature, a good growth medium for many microorganisms. Because of the specific production it is impossible to avoid contamination of milk with micro-organisms therefore the microbial content of milk is a major feature in determining its quality (Mubarack *et al.*, 2010). Bacterial contamination of raw milk can originate from different sources: air, milking equipment, feed, soil, faeces and grass (Coorevits *et al.*, 2008). The number and types of micro-organisms in milk immediately after milking are affected by factors such as animal and equipment cleanliness, season, feed and animal health (Mubarack *et al.*, 2010). It is hypothesized that differences in feeding and housing strategies of cows may influence the microbial quality of milk (Coorevits *et al.*, 2008). Rinsing water for milking machine and milking equipment washing also involve some of the reasons for the presence of a higher number of micro-organisms including pathogens in raw milk (Bramley, 1990). After milking, milk is cooled, which additionally influence the dynamic of microbial process (Mubarack *et al.*, 2010). The conditions during storage and transport in refrigerated tanks cause the raw milk microbiota to change from predominantly Gram-positive to predominantly Gram-negative organisms as they grow.

Gram-negative bacteria usually account for more than 90% of the microbial population in cold raw milk that has been stored. The Gram-negative flora is composed mainly of psychrotrophic species of *Pseudomonas*, *Achromobacter*, *Aeromonas*, *Serratia*, *Alcaligenes*, *Chromobacterium*, *Flavobacterium* and *Enterobacter* (Martins *et al.*, 2006). Milking is highly aseptic condition is practically free from bacteria flora. Fresh milk may contain varying number of microorganisms depending on the care employed in mailing leaving and handling of utensils the bacteria are associated with milk bone disease are viz; *Bacillus cereus*, *Listeria monocytogenes*, *Yersinia enterocolitica*, *Salmonella* species, *Escherichia coli* and *Campylobacter jejuni* (Alan and Heather, 1990). *Staphylococcus aureus* can be isolated from most samples of raw milk and may be found in unheated or lightly heated dairy product (Rladh, 2005).

The bacteria count of milk is used to measure its sanitary quality and most grading of milk is on the basis of some methods for estimating number of a bacteria such as the standard plate count (SPC) which determines the total number of bacteria in the sample that grow and form countable colonies which incubated aerobically at 32°C for 48 hrs. Generally standard plate count value forms milk that count greater than 105 CFU/ml are the indicative of serious faults in production hygiene. There are a lot of factors that contribute to high bacteria counts and eventually contamination of milk. They are

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among others, microbial contamination from the exterior of the udder, influences of equipment cleaning and sanitizing procedure and milk storage temperature time. Microbial contamination occurs from within the udder because as raw milk leaves the udder of healthy cow, it contains very low number of microorganisms (Ali and Abdelgadir, 2011). However, cow with mastitis have the potential to shed large number of microorganisms into the milk (Wallace, 2009). Pasteurization is the method of heat treatment of milk to make it safe for human consumption by removal of pathogenic bacteria such as *Tuberclebaccli*, *Streptococcus pyogenes* or *Brucella* which are frequently carried in milk, it is not a method of sterilization, but organisms which remain after pasteurization are said to be harmless when ingested (Shojaei and Yadollahi, 2008). Fermentation is the catabolism break during of substances by the cells in which the organic substrate acts as electron donor, and an organic molecules derived from the subtracts act as an it is a metabolic process by which microbes produce energy in the absence of oxygen and other terminal electron acceptors in the electrons transport chain such as nitrate and fume rate. (Frederickson et al., 2003).

Yoghurt is produced with a yoghurt starter, which is a mixed culture of *Streptococcus thermophilus*, *Lactobacillus acidophiles* and *Lactobacillus* which are the lactic acid bacteria in a 1:1 ratio. The *coccus* grows faster than *bulgaricus* the rod and primarily responsible for and production which the rod acids flavor and aroma. The associative growth of the two organisms result in lactic acid production at a rate greater than that produce by either when growing along, and were acetaldehyde (the chief volatile flavor component of yoghurt) is produce by the *Lactobacillus bulgaricus* when growing association with *Staphylococcus thermophiles* (Frederickson et al., 2003). In Nigeria the traditional method of processing and selling milk exposes it to danger of microbial contamination from spoilage and pathogenic microbes. The milk produced by the local cattle herders, the Fulani's is mostly boiled before sale to the public, thus, it may be considered pasteurized. However, the milk may be re contaminated due to post pasteurization contamination from handler, utensils and other external sources (Makinde, 1980). Thus, the aim of the present study was to evaluate the bacteriological quality of locally processed milk in comparisons with the pasteurization fermented yoghurt so as to highlight regular consumers about the danger in taken locally pasteurized Fulani nono which is prone to weakened and persistently causing trace of acute typhoid to many innocent and citizen of Nigerian.

## MATERIALS AND METHODS

### Collection of samples

A total of eight processed commercial milk samples were purchased randomly from various areas within Zaria metropolis. One sample was duly screamed in each of the four areas viz; Hayin Dogo market (A<sub>1</sub>), Daraka market (A<sub>2</sub>), Mangorori market (A<sub>3</sub>) and community market (A<sub>4</sub>) and also for the pasteurization fermented yoghurt samples from Sanyoghurt(B<sub>1</sub>), Nappri yoghurt (B<sub>2</sub>), Habib yoghurt( B<sub>3</sub>) and Basako yoghurt (B<sub>4</sub>) respectively. Twenty to twenty-five ml of each Nono and yoghurt were collected in sterile universal

bottles aseptically, labeled and transported immediately to the laboratory in an ice cold box. The pH of each fermented milk and local Nono was determined and recorded using a pH meter (Corning 450, NY, Y.S.A).

### Bacteriological test

Standard plate count (SPC): This method was followed as proposed by Andrew (Kanika, 2009) to determine the total colony count to the samples. It is agar plate method for estimating bacteria population. It consists essentially of the following steps. Serial dilution of the samples ( $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$  and  $10^{-5}$ ) were conducted in the laboratory. One ml of each dilution was mixed with aerobic plate agar in petri dishes. The sample was mixed with molten, cooled batch at 47-50°C of growth medium (15-20ml). Each dilution growth medium mixture was pour into sterile petri dishes and incubated at 35-37°C after solidification on medium. The number of colonies within the solid medium was counted with regard to pour plate techniques.

### Microbiological test of milk

#### The breed count

0.01ml of the milk sample was spread over 1cm<sup>2</sup> of a microscopic slide and the film was allowed to dry. The dried fix smear was then steam boiled in water bath for 5mins before the addition of xylene reagent to remove the fat content for 1 minute. Xylene free was done by the addition alcohol reagent. Methylene blue (MB) was used as staining reagent on the sample for 15 sec and finally scanned under microscope for further enumeration. The slide was examined microscopically and the number of bacteria per microscopic field was determined. The reciprocal of this number, is known as the microscopic factor (MF) is multiplied by the average count per field to obtain the number of bacteria per milliliter.

### Screening the quality of milk

#### 1. Dye- reduction test

- a. **Methylene blue Reduction test (Reductase test):** One ml of methylene blue was added to 10ml milk sample in a test tube. The tube was then inverted three times after plugging with stopper and place in water bath for 35° C. Good quality milk methylene blue was not recorded within 6 hours and poor quality milk methylene blue was recorded within 2 hours. (Kanika, 2009)
- b. **Resazurin test:** One ml of Resazurin was added to 10ml milk sample. The solution was then place in water bath for some time before it was incubated at 37°C Resazurin (Slate blue) = Resorufin (Pink) = Dihydroresorufin (Colouless) (pH 6.6 accepted) (pH 5.3 moderate) (pH 4.8 poor)

#### 2. Phosphatase test

One ml of milk sample (phosphatase) was added to 5 ml Disodium P-nitrophenyl phosphate (0.5N) in a test tube. The solution was then place in a water bath and incubated for 2 hours at 37°C. It was recorded that perfect

pasteurization showed colourless and imperfect pasteurization showed yellow. (Kanika, 2009).

### Turbidity

Twenty ml of sterilized milk was added to 40gm of Ammonium sulphate (NH<sub>4</sub>)<sub>2</sub> SO<sub>4</sub> and was thoroughly shaken before it was allowed to stand for 5 minute. The solution was filter to obtain the filtrate which was later place in water bath for 5 minute. It was observed that adequate sterilization does not show turbid (clean). While inadequate sterilization showed turbid (cloudy) (Kanika, 2009).

### Enumeration and identifications pathogens in milk

The enumeration and identifications of pathogens presence in Fulani Nono and pasteurized fermented yoghurt and biochemical test such as, citrate test, indole test, MRVP test, catalase test, coagulase test, oxidase test, gelatinase test, etc were conducted according to (Fawole and Oso, 2004) and (Salman and Hammad, 2011) respectively.

## RESULTS AND DISCUSSION

The unprecedented consumption of milk as human diet also supports the growth of pathogenic organisms. Early in this century, it was discovered that milk can transmit tuberculosis, brucellosis, diphtheria, scarlet fever, and Q fever to humans. Fortunately, over the decades, the threat of these diseases and the incidence of outbreaks involving milk and milk products have been greatly reduced due to improved sanitary of the milk production practices and pasteurization technique. However, a variety of microorganisms still contribute to illnesses and disease outbreaks. Raw (unpasteurized) milk has been found to participate in spreading out of illnesses caused by *Listeria*, *Campylobacter*, *Yersinia*, *Salmonella*, *Staphylococci* species, and *E. coli* (Kirma and Grazyna (2006). In this current investigation, the determining results of the quality and grading of bacterial load in local Fulani nono and pasteurized fermented yoghurt obtain from different locations within zaria metropolis. Methylene blue and phosphatase reagents are used as an indicator to detect the contaminated milk and grading the milk into different categories are shown in Table 1, 2 and 3.

**Table 1. Grading of milk samples on the basis of methylene – blue test in different milk samples**

Quality of milk	Decolourizing time
Excellent	More than 8 hours
Good	Between 6 hours
Fair	Between 2 to 6 hours
Poor	Less than 2 hours

**Table 2. Decolorizing time and grading of Fulani Nono samples collected from different part of Samaru – Zaria**

Quality of Fulani Nono milk sample	Methylene decolourization time, hours	Grade
A1	1.33h	Poor
A2	1.48h	Poor
A3	5.88h	Good
A4	5.28h	Fair

**Table 3. Decolorizing time and grading of fermented milk collected from different part of Samaru – Zaria**

Quality of pasteurized milk sample	Methylene decolourization time, hours	Grade
B <sub>1</sub>	8.33h	Excellent
B <sub>2</sub>	7.40 h	Excellent
B <sub>3</sub>	6.55h	Good
B <sub>4</sub>	6.22h	Good

Both were categorized into poor, fair, good and excellent. The fulani nono was categorized as being poor and fair in most occasions as well as good and excellent for the pasteurized fermented yoghurt (low in bacteria loading). This study agreed with the report of (Nandy and Ventatech, 2010). A possible explanation could be that the greater the number of microorganisms the more the oxygen demand and lesser the oxygen concentration in the medium, resulting in the faster disappearance of the colour. In Table 4 and 5, as shown the locally available fulani nono is highly contaminated with truck load of pathogens in comparison to the pasteurized fermented yoghurt.

**Table 4. Total Viable Bacteria Count in local Nono sample**

Samples	Colony forming 10 <sup>-3</sup>	Local Nono 10 <sup>-5</sup>
A1	167	58
A2	54	33
A3	87	20
A4	102	45

A1= Hayin Doyo market A2 = Daranka market A3= Magorori Market and A4 = community market.

**Table 5. Total Viable Bacteria Count in yoghurt (pasteurized)**

Samples	Colony forming 10 <sup>-3</sup>	Local Nono 10 <sup>-5</sup>
B1	3	2
B 2	2	None
B 3	8	3
B 4	8	3

B1 = San Yoghurt  
B2 = Nappri yoghurt  
B3 = Habib yoghurt  
B4 = Basako yoghurt

This could also be attributable to the fact that, the Fulani nono contained higher number of micro flora probably due to contamination from animals in manure soil and water may enter milk due to dairy utensils and milk contact surfaces. If the milk contact surfaces are inadequately cleaned, bacteria may develop in large number. The predominant species of bacteria found in Table 6, were viz; *Bacillus* sp, *Pseudomona* sp, *Proteus* sp, *Staphylococcus* sp, *Streptococcus* sp, *Klebsiella* sp, *Enterobacter* sp and trace of some *Salmonella* sp. Occurrence of pathogens in their percentage grading in fulani nono and fermented yoghurt are shown in Table 7 and 8 reveal that the Fulani nono has higher percentage occurrence of pathogens compared to fermented yoghurt. The exceptionally observed spore formers and the capsulated bacteria (*Bacillus* sp and *Klebsiella* sp) respectively could with stand adverse temperature. Resistance by *Staphylococcus* sp however is not surprising despite not being a spore former, it may be heat tolerant to some degree.

Table 6. Morphological and biochemical tests for identification of the isolates

Characters	<i>Pseudomonas</i> sp	<i>Enterobacter</i>	<i>Proteus</i> sp	<i>Klebsiella</i> sp	<i>Bacillus</i> sp	<i>Streptococcus</i>	<i>Salmonella</i> sp	<i>Staphylococcus</i> sp
Umbonate colony	+	-	-	-	-	-	-	+
Trimethylamine odour	+	-	-	-	-	-	-	+
Gram reaction	-	-	-	-	+	+	-	<i>Cocci</i>
Rods/cocci	<i>Rods</i>	<i>Rods</i>	<i>Rods</i>	Rods	Rods	Cocci	Rod	+
Pigment-production	-	-	-	-	-	-	-	-
Motility	+	+	+	-	+	+	+	-
Swarming	-	+	+	-	-	-	-	A
Spore formation	-	-	-	-	+	-	-	+
Aerobic(A)facultative anaerobic(F.A)	A	F.A	F.A	F.A	A/F.A	A	A/F	-
Catalase activity	+	+	+	+	+	+	+	-
Oxidase activity	+	+	+	+	+	+	+	+
Gelatin liquifation	+/-	+	-	-	-	-	-	-
Lipase production	-	ND	ND	ND	-	ND	ND	+
Indole production	ND	-	+/-	+/-	ND	-	+	-
Citrate Utilization	-	+	+/-	+/-	-	+	-	+
Urease Activity	ND	+/-	+	+	ND	-	ND	ND
Denitrication	-	+	+	+	+	+	ND	ND
H <sub>2</sub> S-Production	ND	-	-	-	ND	-	+	-
Growth in 12%NACL	ND	ND	ND	ND	+	-	-	+
Methyl- Red	ND	+	+	+/-	ND	+	+	+
Voges-Production	+	-	+/-	-	ND	+	-	+
Capsule test	-	-	-	+	-	-	-	-
Fermentation								
Glucose	+/-	<i>Acid/gas</i>	(Acid/gas	+	+	+	-	+
Sucrose		+	+	+	+	+	-	+
Maltose		+	+	+	-	-	-	-
Lactose		+	-	+	-	-	-	-
		+	-	+	-	+	-	-

+ = Positive; - = Negative; A = Acid; AG = Acid and Gas, F/A = Facultative Anaerobic, A = Aerobic, NA= not done

Table 7. The occurrence of the isolated organisms (bacteria) in Fulani Nono analyzed from different source

Bacteria Isolates	A1	A2	A3	A4	Total	%
<i>Pseudomonads</i> sp	2	2	2	2	8	11.59
<i>Enterobacter</i> sp	1	2	2	1	6	8.6
<i>Proteus</i> sp	-	-	-	-	-	-
<i>Klebsiella</i> sp	2	2	4	4	12	17.39
<i>Bacillus</i> sp	3	4	5	5	17	24.63
<i>Streptococcus</i> sp	1	1	1	1	4	5.79
<i>Salmonella</i> sp	2	2	4	2	10	14.49
<i>Staphylococcus</i> sp	2	2	4	4	12	17.39

**Table 8. The occurrence of the isolated organisms (bacteria) in Fermented Milk (Yoghurt) analyzed from different source**

Bacteria Isolates	B1	B2	B3	B4	Total	%
<i>Pseudomonads</i> sp	1	0	1	0	2	5.88
<i>Enterobacter</i> sp	0	0	1	0	1	2.94
<i>Proteus</i> sp	-	-	-	-	-	-
<i>Klebsiella</i> sp	2	2	4	3	11	32.35
<i>Bacillus</i> sp	2	4	5	3	14	41.17
<i>Streptococcus</i> sp	0	0	1	0	1	2.94
<i>Salmonella</i> sp	0	0	1	2	3	8.83
<i>Staphylococcus</i> sp	0	0	1	1	2	5.88

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Some *Bacillus* sp. (Lingathurai and Vellathurai, 2010) which generally do not survive pasteurization but the undesirable effects of their activity (proteins and fat degradation through proteases and lipases) remain. The result is reduced yield of milk products, shortened shelf life, off- flavors, rancidity etc. These types of organisms occur in raw milk due to poor udder preparation, inadequate equipment cleaning and sanitizing procedures and possibly from contaminated water sources. In this study and it should be worthwhile to know their pathogenicity (disease forming potency), the source of the milk, the occurrence as well as incubation period if necessary.

*Bacillus* sp. is large (4–10mm). Gram-positive, strictly aerobic or facultatively anaerobic encapsulated bacilli. They have the important feature of producing spores that are exceptionally resistant to unfavourable conditions. *Bacillus* sp are classified into the subgroups *B. polymyxa*, *B. subtilis* (which includes *B. cereus* and *B. licheniformis*), *B. brevis* and *B. anthracis*. *Bacillus* sp can be saprophytes in water, soil and vegetation. They are aerobic organisms which does not form heat – resistant spores (Cruickshank *et al.*, 1990), hence they can survive in air or in soil or harsh condition for a long period of time. The milk collected from Fulani nono probably had the bacteria transmitted on to the local milk from the products of the Fulani. They use their hands to get products cause the transfer of the organism into their hands which they eventually used in handling the local nono. Among the pathogenic *Bacillus* sp, anthracis which cause anthrax from the literature of the 12 strain tested for anthrax resistance spore only 6 strains were destroyed in the autoclave at 121°C. The incubation period was 37° C and pH was slightly alkaline between 7.5 and 7.8 (Lingathurai and Vellathurai, 2010). Another member which can be pathogenic is *Bacillus cereus* which causes food poisoning in man (Turk *et al.*, 1983). The condition can be acquired by man when the organisms are ingested in food if the handler of the local nono (milk) does not wash hand with soap before tapping.

*Staphylococcus aureus* is an aerobic or anaerobic, non-motile, non-spore-forming, catalase- and coagulase-positive, Gram-positive coccus, usually arranged in grapelike irregular clusters. The genus *Staphylococcus* contains at least 15 different species. Apart from *S. aureus*, the species *S. epidermidis* and *S. saprophyticus* are also associated with disease in humans. Although *Staphylococcus aureus* is a common member of the human microflora, it can produce disease through two different mechanisms. One is based on the ability of the organisms to multiply and spread widely in tissues, and the other is based on the ability of the organisms to produce extracellular enzymes and toxins. Infections based on

the multiplication of the organisms are significant problem in hospitals and other health care facilities. Multiplication in tissues can result in manifestations such as boils, skin sepsis, post-operative wound infections, enteric infections, septicaemia, endocarditis, osteomyelitis and pneumonia. The onset of clinical symptoms for these infections is relatively long, usually several days. Gastrointestinal disease (enterocolitis or food poisoning) is caused by a heat-stable staphylococcal enterotoxin and characterized by projectile vomiting, diarrhoea, fever, abdominal cramps, electrolyte imbalance and loss of fluids. Onset of disease in this case has a characteristic short incubation period of 1–8 h. The same applies to the toxic shock syndrome caused by toxic shock syndrome toxin-1. *Staphylococcus aureus* is an organism which is a part of man's normal commensal, and can be found on the exterior nasal mucosa of healthy adults, throats, faeces and on the skin. They are also capable of multiplying in these areas (Merchant *et al.*, 1983). Furthermore, they are spread from droplets, from nose and throats into the air as well as by shedding of the skin scales so that they are widely distributed in dust, air clothing and bedding. Most of its infectively involves the skin pustules, furuncles, boils impetigo, styles, Cutaneous and subcutaneous infections. They are also usually responsible for *Staphylococcal enteritis*. Transmission can be direct contact or through contaminated fomites (Turk *et al.*, 1983) transmission through local Fulani nono (milk) therefore could take cognizance of the hands the utensils used and nibble of the cow. *Staphylococcus* sp are the most resistance of the cocci (even though they are non spore formers). Varying reports are given concerning its resistance to heat, due to the different condition under which the tests are conducted usually they are carried out at 60°C for 1½ hours while some are done at 80°C. Pathogenic *Staph aureus* is associated with most of the cases of suppurative wounds in animal and man. Species may be localized in any tissue of the body with incubation at 37°C and pH of 7.2 (Merchant *et al.*, 1967).

*Klebsiella* sp are Gram-negative, non-motile bacilli that belong to the family *Enterobacteriaceae*. The genus *Klebsiella* consists of a number of species, including *K. pneumoniae*, *K. oxytoca*, *K. planticola* and *K. terrigena*. The outermost layer of *Klebsiella* sp consists of a large polysaccharide capsule that distinguishes the organisms from other members of the family. Approximately 60–80% of all *Klebsiella* sp isolated from faeces and clinical specimens are *K. pneumoniae* and are positive in the thermotolerant coliform test. *Klebsiella oxytoca* has also been identified as pathogen and often indicated in pneumonia, notably of the form characterized by multiple cavitations of lungs. They can also cause meningitis, Otitis and sinusitis (Cruickshank *et al.*, 1980). They are present in

sputum, thus are air borne and can be spread by contact hence transmission through used of utensils. The organism is destroyed at 60°C in 20 minutes and by common disinfectant. Incubation is also at 37°C at a pH range of 6.8 to 7.2 (Mohammed et al., 2009).

*Pseudomonas* sp is a member of the family *Pseudomonadaceae* and is a polarly flagellated, aerobic, Gram-negative rod. When grown in suitable media, it produces the non-fluorescent bluish pigment pyocyanin. Many strains also produce the fluorescent green pigment pyoverdine. *Pseudomonas* sp, like other fluorescent *Pseudomonads*, produces catalase, oxidase and ammonia from arginine and can grow on citrate as the sole source of carbon. *Pseudomonas* sp is a common environmental organism and can be found in soil, water and in milk. Many species of the bacteria found in dairy products. The bacteria gain entrance into dairy product from the bovine udder, through exposure of the milk by the Fulani women and in the farm land. It can multiply in water environments and also on medium surface of suitable organic materials in contact with water. *Pseudomonas* sp can cause a range of infections but rarely causes serious illness in healthy individuals without some predisposing factor. It predominantly colonizes damaged sites such as burn and surgical wounds, the respiratory tract of people with underlying disease and physically damaged eyes. From these sites, it may invade the body, causing destructive lesions or septicaemia and meningitis. Cystic fibrosis and immunocompromised patients are prone to colonization with *Pseudomonas*.sp, which may lead to serious progressive pulmonary infections. Water-related folliculitis and ear infections are associated with warm, moist environments such as swimming pools and spas. Many strains are resistant to a range of antimicrobial agents, which can increase the significance of the organism in hospital settings (Bartram 2003).

*Salmonella* sp belongs to the family *Enterobacteriaceae*. They are motile, Gram negative bacilli that do not ferment lactose, but most produce hydrogen sulfide or gas from carbohydrate fermentation. Originally, they were grouped into more than 2000 species (serotypes) according to their somatic (O) and flagellar (H) antigens (Kauffmann-White classification). It is now considered that this classification is below species level and that there are actually no more than 2–3 species (*Salmonella enterica* or *Salmonella choleraesuis*, *Salmonella bongori* and *Salmonella typhi*), with the serovars being subspecies. All of the enteric pathogens except *S. typhi* are members of the species *S. enterica*. Convention has dictated that sub species are abbreviated, so that *S. enterica* Serovar *paratyphi* A. *Salmonella* sp are widely distributed in the environment, but some species or serovars show host specificity. Notably, *S. typhi* and generally *S. paratyphi* are restricted to humans, although livestock can occasionally be a source of *S. Paratyphi*. A large number of serovars, including *S. typhimurium* and *S. enteritidis*, infect humans and also a wide range of animals, including poultry, cows, pigs, sheep, birds and even reptiles. The pathogens typically gain entry into water systems through faecal contamination from sewage discharges, livestock and wild animals. Contamination has been detected in a wide variety of foods and milk. *Salmonella* infections typically cause four clinical manifestations:

gastroenteritis (ranging from mild to fulminant diarrhoea, nausea and vomiting), bacteraemia or septicaemia (high spiking fever with positive blood cultures), typhoid fever / enteric fever (sustained fever with or without diarrhoea) and a carrier state in persons with previous infections. In regard to enteric illness, *Salmonella* sp can be divided into two fairly distinct groups: the typhoidal species/serovars (*Salmonella typhi* and *S. paratyphi*) and the remaining non-typhoidal species/serovars. Symptoms of non-typhoidal gastroenteritis appear from 6 to 72 h after ingestion of contaminated food or water. Diarrhoea lasts 3–5 days and is accompanied by fever and abdominal pain. Usually the disease is self-limiting. The incubation period for typhoid fever can be 1–14 days but is usually 3–5 days. Typhoid fever is a more severe illness and can be fatal. Although typhoid is uncommon in areas with good sanitary systems, it is still prevalent elsewhere, and there are many millions of cases each year (Escartin, 2002).

*Enterobacter* sp is a motile, Gram-negative, non-spore-forming, rod-shaped bacterium that has been found in infant formulas as a contaminant. *Enterobacter* species are biochemically similar to *Klebsiella*; unlike *Klebsiella*, however, *Enterobacter* is ornithine positive. *Enterobacter* sp has been found to be more resistant to osmotic and dry stress than other members of *Enterobacteriaceae* family. The reservoir for *Enterobacter* sp is unknown. Various environmental samples (surface water, soil and cow milk) have tested negative. It has been identified in the guts of certain flies. The organism has been frequently identified in factories that produce milk powder and other food substances and in households. Commercially produced *Enterobacter* sp has been associated with sporadic cases or small outbreaks of sepsis, meningitis, cerebritis and necrotizing enterocolitis. Most of the infections are seen in low-birth-weight infants (i.e., less than 2 kg) or infants born prematurely (i.e., less than 37 weeks of gestation). Mortality has been reported to be as high as 50% but has decreased to less than 20% in recent years.

*Streptococcus* sp is widely distributed in nature largely as parasite of man and animals. They are heavy among the normal flora of the human respiratory tracts. They exist in the throat and nostril. Some are mostly harmless e.g. *Streptococcus pyogenes* and *Streptococcus pneumoniae*. However some can cause sore throat, scarlet fever, impetigo, mastitis and some skin infection. (Mohammed et al., 2009) In this current investigation, during milking operation, however milk may be exposed to contamination from the animal handler utensils especially the exterior of the udder and adjacent areas. Bacteria found in manure, soil and water may enter from this source such contamination can be reduced by clipping the cow and washing the udder with water or a germicidal solution before milking. Contamination of cow with manure, soil water may also be reduced by paving and draining barnyards, keeping cow from stagnant pools and cleaning manure from the barns or milking parlors. Pasteurization kills pathogens that may enter the milk and improve the keeping quality of milk. Quality assessment of fermented yoghurt was found to be in this decreasing order Basako-yoghurt > Habib- yoghurt >> San - yoghurt >>> Nappri-yoghurt.

## Conclusion

In this investigation, the samples of yoghurt and locally Fulani Nono (Diary milk) analyzed met the requirement for the total aerobic Mesophilic counts standard but the sample showed evidence of contamination by *Staphylococcus aureus*, *Bacillus* sp, *Streptococcus* sp, *Klebsiella* sp and other likely bacteria species isolated. The comparative study of the two different samples (yoghurt and locally available Fulani diary Nono (milk) showed that it contained relatively high count of *S. aureus*, *Bacillus* sp and *Salmonella* species and may constitute a health hazard to consumers. Therefore, Nono (milk) if produced under refrigerator temperature (below 5°C) and pasteurized will reduce the microbial load to be within acceptable limit. Likewise yoghurt samples should be subjected to proper fermentation among yoghurt producer in order to get rid of the possibility of food borne pathogen in the finished products.

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## REFERENCES

- Alan, H.L and Healther, M.D. 1990. Torley and Wilson's principle of bacteriology virology and immunity (1) 51 – 68.
- Ali AA, Abdelgadir WS 2011. Incidence of *Escherichia coli* in raw cow's milk in Khartoum state. *Br. J. Dairy. Sci.* 2(1):23-26.
- Bartram J. 2003. Heterotrophic plate counts and drinking-water safety: the significance of HPCs for water quality and human health. WHO Emerging issues in Water and Infectious Disease Series. London, IWA Publishing.
- Bramley AJ, McKinnon CH 1990. The Microbiology of Raw Milk. In: Dairy Microbiology, I, (Ed.: Robinson, R.K.). London, New York, Elsevier Appl Sci, 171.
- Coorevits A, De Jonghe V, Vandroemme J, Reekmans R, Heyrman J, Messens W, De Vos P, Heyndrickx M. 2008. Comparative analysis of the diversity of aerobic-spore-forming bacteria in raw milk from organic and conventional dairy farms. *System. Appl. Microbiol. in press.*
- Cruickshank R, Duiguich TP, Marimon BP R 1990. Medical Microbiology. The practice of medical microbiology Edinburgh, Churchill living stones 449.
- Escartin EF 2002. Potential *Salmonella* transmission from ornamental fountains. *J. Environ Health*, 65:9–12.
- Fawole MO, Oso BA 2004. Laboratory manual of microbiology spectrum book Ltd ring road Ibadan Pp 45-4.
- Frederickson A M Hokoraela B 2003. Low occurrence of pathogenic *Yersinia enterocolitica* in clinical food and environmental samples: A methodological problem. *Clinical microbial review* 16: 220 – 229.
- Kanika S 2009. Manual of Microbiology Tools and Techniques 2<sup>nd</sup> Ed. Ane Books PVT Ltd. Darya Ganj. New Delhi Indian.
- Kirma GAH, Grazyna S 2006. Public health hazard due to mastitis in dairy cow. *Animal Sci Paper and Report*, 25(2):73-85.
- Lingathurai S, Vellathurai P 2010. Bacteriological quality and safety of raw cow milk in Madurai, South India. *Webmed Central. Microbiol.* 1(10):1-10.
- Makinde MO 1980. The effect of addition microbial loads of milk, *J. Food and Agricultural* 1:31 – 34.
- Martins ML, Pinto CLO, Rocha RB, Araujo EF, Vanetti MCD, Nelson FE 2006. The Genetic diversity of Gram-negative proteolytic, psychrotrophic bacteria isolated from refrigerated raw milk. *Int. J. Food Microbiol.*, 111: 144–148.
- Merchant JA, Packer RA 1967. Veterinary Microbiology and preventive medicine. IOWA State university Press Ames Owa USA 386 – 387.
- Mubarack MH, Doss A, Dhanabalan R, Balachander S 2010. Microbial quality of raw milk samples collected from different villages of Coimbatore district, Tamilnadu, South India. *Ind. J. Sci. Technol.* 3(1):61-63.
- Muhammad K, Altaf I, Hanif A, Anjum AA, Tipu MY 2009. Monitoring of hygienic status of raw milk marketed in Lahore city, Pakistan. *J. Anim. Plant Sci.* 19(2):74-77.
- Nandy SK, Venkatesh KV 2010. Application of methylene blue dye reduction test (MBRT) to determine growth and death rates of microorganisms. *Afr. J. Microbiol. Res.* 4(1):61-70.
- Rladh A 2005. A comparison on microbial conditions between traditional dairy products sold in karak and some products produced by modern dairies Pakis *J. Nutrition* 4(5) 345 – 348.
- Salman MA, Hamad IM 2011. Enumeration and identification of coliform bacteria from raw milk in Khartoum state, Sudan. *J. Cell Anim. Biol.* 5(7):121-128.
- Shojaei ZA, Yadollahi A 2008. Physicochemical and Microbiological Quality of raw milk, Pasteurized and UHT milks in Shops. *Asian J.Sci. Res.*, 1(5): 532
- Turk DC, Porter IA, Deurden BI, Reid TM 1983. A short text book of medical microbiology Great Britain Hodder and Stoughton 101:188 – 191.
- Wallace RL 2009. Bacteria count in raw milk. Dairy Cattle. Management pp. 1- 4.

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