Bacteriological Quality and Safety Evaluation of Raw Cow Milk in Ilorin, North Central Nigeria.

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Abstract: Microbial contamination of raw milk has become a global health problem. Large number of people in Nigeria consume raw cow milk due to its cheap status. These study was undertaken to investigate the bacterial quality of of raw cow milk in Ilorin, and surrounding villages, Nigeria and the effect of pasteurization on the samples. Raw milk samples (n=12) were aseptically collected from the milking bowls from different locations. The samples were analyzed within three hours of procurement. Isolation, enumeration and identification of the prevailing bacteria were carried out following the standard procedure. The pH of the samples ranges between 6.3-6.8 while the TTA is from 0.87-1.98. Analysis of the Milk sample revealed high load of bacterial pathogens such as, *Listeria monocytogenes, Staphylococcus aureus, Salmonella spp., Escherichia coli, Klebsiella spp., Pseudomonas spp., Proteus spp.* and *Bacillus cereus*. Total viable count ranges from 1.16 x 10^6 to 2.60 x 10^6 while the pasteurization count ranges between 0.8 x 10^2 to 1.2 x 10^2. Results indicate the potential health risk of consuming raw cow milk under the current production and collection condition.

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Introduction:

Raw milk has been a known vehicle for pathogens for more than 100years (Gillespie *et al*.,2003). Milk has been part of the human diet for thousands of years and it is a compulsory part of daily diet for expectant mothers as well as growing children. It contains lipids, proteins (casein, whey), carbohydrates (lactose), amino acids, vitamins and minerals (calcium), essential for growth(Haug *et al*., 2007; Javaid *et al*., 2009). It is a vital type of food for over 6 billion human zbeings all over the world and a major contributor to food security as it alleviates poverty and mitigates malnutrition (Belewu, 2006). Milk is predominantly (80–87%) water and protein in milk is predominantly (82–86%) casein with smaller amounts of globulins. Raw milk pH ranges from 6.4 to 6.8, with an average pH of 6.6 making it slightly acidic (William *et al.,* 2005). It 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 (Karmen and Slavia, 2008). It is hypothesized that differences in feeding and housing strategies of cows may influence the microbial quality of milk (Coorevits *et al*., 2008). 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 (Karmen and Slavia, 2008).

According to U.S. Food and Drug Administration (ICMSF, 2006), EEC directive 92/46(EC 2001), Beuvier and Buchin,2004 the principal pathogens of concern associated with milk and processed milk products are *Salmonella* spp., *Listeria monocytogenes*, *Staphylococcus aureus*, pathogenic *E. coli*. Many of the common enteric pathogens such as *Salmonella*, *Escherichia coli* O157: H7 and *Campylobacter* are carried in the intestinal tract of ruminants, including domestic animals used in milk production, e.g. cows, sheep and goats(Baylis, 2009). Effective cleaning procedures, including removing faecal material from udders prior to milking and good manufacturing practices during cheese making process can reduce the risk (Baylis, 2009). Microbes may gain entry into raw milk directly from dairy cows experiencing sub clinical or clinical mastitis (Rodojcic-Prodaova *et al.,*1991). A number of bacteria including *S. aureus, Escherichia coli* and *Salmonella* have been recovered from raw milk (De Buyser *et al*., 2001) and some of these have been determined to be pathogenic and toxicogenic, and implicated in milk- borne gastroenteritis (Maguire *et al*., 1992). In recent year's *E. coli* 0157: H7 strain has become very important milk-borne pathogen and cattle are considered its main reservoir (Betts, 2000 ; Karmali, 1989).

Raw milk advocates claim that raw milk is healthier and has higher nutritional value than pasteurized milk, however research has shown no significant difference in the nutritional value of pasteurized and unpasteurized milk (Centers for Disease Control and Prevention, 1999; Hegarty et al., 2002). The coliform group of bacteria is defined as the indicator (faecal coliform) of suitability of milk for drinking (Chatterjee *et al*.,2006). Some isolates of S. aureus produce staphylococcal enterotoxins (SEs) that may cause food poisoning if food containing sufficient preformed SE is ingested. Symptoms typically have a rapid onset (1–6 h) and often include nausea, vomiting, diarrhoea and abdominal pain (Jablonski and Bohach 1997). Usually the condition is self-limiting and recovery is rapid. Minor outbreaks of staphylococcal food poisoning (SFP) are therefore likely to go unreported, and the true incidence of SFP is probably underestimated (Jorgensen *et al*., 2005). Outbreaks of foodborne illnesses following consumption of raw milk and products made from raw milk caused by Shiga toxin-producing *Escherichia coli* (**STEC),** (Proctor and Davis, 2000), *Salmonella* spp. (Mazurek *et al*., 2004), and *Listeria monocytogenes* (Centers for Disease Control and Prevention, 2001) have been reported. Gillespie *et al*. (2003) reported that between the years of 1992 and 2000, 52% of foodborne outbreaks in England and Wales were attributed to raw milk. Raw milk and products made from raw milk have been implicated in similar numbers of documented cases of foodborne illness in France (De Buyser *et al*.,2001). An estimated 1.4 million cases of salmonellosis occur annually in the United States (Mead *et al*., 1999). Sales of raw milk directly to the public have resulted in foodborne outbreaks of multidrug-resistant salmonellosis in California and Washington (Reed and Grivetti, 2000),

In Nigeria, raw milk is traditionally consumed at the small farms and in town where it is taken in addition with other food materials or process into soft cheese. The risk of contaminated and pathogen containing products could therefore be even greater than when the milk is processed at household level (FAO and WHO 1997). The importance of various etiological agents in milk borne disease has changed dramatically over time. The presence of these pathogenic bacteria in milk emerged as major public health concerns, especially for those individuals who still drink raw milk (Riser, E.T. 1998). *E. coli* 0157: H7 has become serious threat to the dairy industries ranging from mild diarrhoea to potentially fatal hemolytic uremic syndrome (HUS), hemorrhagic colitis and thrombotic thrombocytopenic purpura (Coia, *et al* 2001).

The intent of pasteurization of milk is to eliminate pathogenic microbes, also to lowers microbial numbers, which prolongs milk's good quality and shelf life under refrigeration. However, spores are not affected by pasteurization **(**Montville and Matthew, 2005). This study investigate the bacteriological quality, safety and effects of Pasteurization on raw milk samples.

Materials and Methods

Sample Collection

Twelve raw milk samples were collected from the milking bowl from Fulani cattle settlement in sterile bottles in Ilorin and surrounding villages, Nigeria. The samples were collected in the morning and transported to the laboratory on ice maintaining sterile condition and analyzed within 2 hours of sampling.

Microbiological Analysis.

Isolation and enumeration of microbes were performed using serial dilution of samples carried out up to 10^{-6} in peptone water(OXOID, Unipath Ltd., Basingstoke, Hampshire, England). Samples were plated in duplicate using pour plate technique. 0.5ml of diluted samples was delivered by pipette into 19.5ml of enriched agar. The plates were incubated at 37^0C for 24-48 hours. Total viable count were carried out on plate count agar. The number of colony forming unit(CFU/ml) were recorded after the incubation period. The presence of specific microorganism were done through pour plate technique on selective media. De Mann Rogosa and Sharpe(MRS-agar)(OXOID, Unipath Ltd., Basingstoke, Hampshire, England). for Lactic acid bacteria, Salmonella Shigella agar(SS-agar) for salmonella colonies, Coliform count was carried out with MacConkey Agar and Eosine Methylene Blue agar(EMB-agar).

Identification and characterization of microbial isolates.

Following incubation, the isolated colonies were pure cultured and Gram stained. Biochemical characterization of the isolated colonies was carried out using standard protocols (Kannan, 2002). Identification was carried out according to Bergey's Manual.

Laboratory Pasteurization Count(LPC).

10ml of the raw cow milk samples were dispensed into sterile MacCartney bottles and heated at a temperature of 62.9^0C for 30 minutes using the water bath. The samples were then inoculated on agar plates and incubated at 37^0C for 24 hours after which the plates were observed

RESULTS

Table 1 shows the physico-chemical analysis of the raw milk samples. The colour of the samples are white, light yellow and yellowish white. The pH of the samples ranges between 6.3 to 6.8 whose average is 6.6. The titratable acidity(TTA) of ranges from 0.87 to 1.98. As the pH reduces, the TTA increases.

Table 1: **Physico-chemical analysis of samples.**

Sample	Colour	pH	TTA
M-1	Light yellowish	6.8	0.87
M-2	White	6.6	1.30
M-3	Yellowish White	6.5	1.55
m-4	Yellowish white	6.6	1.36
M-5	White	6.5	1.56
M-6	Yellowish white	6.3	1.98
M-7	White	6.7	0.99
M-8	Yellowish white	6.6	1.37
M-9	Light yellowish	6.4	1.87
M-10	Yellowish White	6.5	1.59
m-11	Yellowish white	6.6	1.33
M-12	Light yellowish	6.4	1.86

Key: TTA – Titratable Acidity.

Table 2 shows the enumeration of microorganism of the different milk samples using the standard method. The results shows that the standard plate count on plate count agar ranges from 1.16 x 10^6 to 2.60 x 10^6 while the laboratory pasteurization count reveals that the count ranges from 0.7 x 10^2 to 1.2 x 10^2.

Table 2. Enumeration of microorganism in different milk sample by standard plate count method.

Sample	SPC	LPC
M-1	2.60 x 10^6	1.2 x 10^2
M-2	2.49 x 10^6	1.2 x 10^2
M-3	1.16 x 10^6	1.5 x 10^2
M-4	1.94 x 10^6	0.8 x 10^2
M-5	2.34 x 10^6	0.9 x 10^2
M-6	1.76 x 10^6	0.8 x 10^2
M-7	2.59 x 10^6	1.1 x 10^2
M-8	2.50 x 10^6	1.2 x 10^2
M-9	1.18 x 10^6	0.7 x 10^2
M-10	1.92 x 10^6	0.8 x 10^2
M-11	2.31 x 10^6	1.0 x 10^2
M-12	1.76 x 10^6	0.9 x 10^2

Key;
SPC - Standard Plate Count
LPC - Laboratory plate Count

Figure 1 shows the frequency of occurrence of bacterial isolate in the samples. E. coli and S. aureus was discovered in all the sample making them the highest while the lowest occurrence is recorded in *Salmonella spp.* and *Klebsiella Spp.*

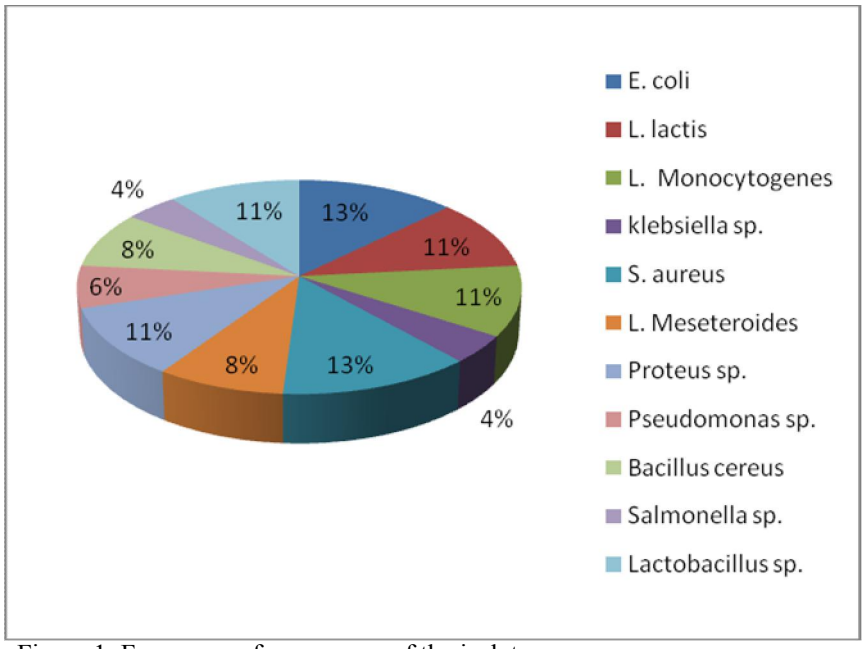

Figure 1. Frequency of occurrence of the isolates.

DISCUSSION

Pathogenic bacteria have been a major concern to the public all over the world. The fact that milk contain a lot of nutrients made it havens for growth and development of host of microorganisms including the pathogenic ones(Saeed *et al*., 2009). Pareke and Subhash,(2008) asserted that, animal health, milking utensil and the environment are contributors to contamination of fresh raw milk.

Also, the unclean or unsterilized teat can introduce a lot of microorganism into the raw milk sample. Table 1 shows the physico-chemical analysis of the raw milk sample collected in diverse location in Ilorin, Nigeria. From all the raw milk sampled, 50% were yellowish white in colour, 25% white and 25% light yellow which agrees with Judkins and Mach (1995) who reported normal milk are yellowish white in colour due to the presence of casein, fat colouring matters. Also, Khan et al.,2008 stated that the difference in colour of milk may be due to nature of feeds or breed of the cow. The physicochemical properties of the milk were also noted to have significantly favored the microbial growth i.e. adequate temperature within mesophilic range and pH close to neutrality (Oladiji *et al.,* 2004). Most bacteria especially in milk normally grow within the neutrophilic pH. The pH of raw milk therefore plays an important role as it has an effect on the distribution and growth rate of micro organisms in the milk. The pH ranges between 6.4 and 6.8 in which the average was 6.6, this agrees with William *et al*., 2005. Also the titratable acidity ranges from 0.80 to 1.76 which shows that as the pH of the samples reduces the titratable acidity increases. From this study, eleven microbial flora were isolated, they include *Lactococcus lactis, Psuedomonas spp, Bacillus cereus, Salmonella spp., Staphylococcus aureus and Esherichia coli.* Others are *Proteus spp., Lactobacillus spp., Leuconostoc meseteroides, Klebsiella spp. and Listeria monocytogenes*. The presence of these large number of microflora suggests the extent to which the milk is contaminated by the animal, environment and the milking utensils(Murphy and Boer, 2002).

The Fulani herdsmen do not disinfect the teats and udders prior to milking despite the fact that the cow lie in muddy barnyard and dirty environment which inevitably contaminate the milk and increase the microbial load. Bramley and McKinnon(1990) reported that organism associated with the beddings materials which contaminate the surface of teats and udders includes Staphylocci, Spore formers, coliforms, Streptococci and other Gram negative bacteria.

The sampled raw milk has high microbial load due probably to the insanitary condition of the environment and the season (raining).The bacterial count far exceed the EC Regulation(No. 853, 2004) of the European Parliament and of the Council (EC) which sets down the hygienic limit ≤ 100,000 CFU/ml milk for the total bacteria count (TBC) in cow's raw milk. TBC is one of the main indicators of hygienic quality of cow's raw milk that is also used to set the purchase price of milk(Cempirkova, 2007). Jayarao and Henning, 2001 reported that operational conditions mainly a failure to observe the hygienic rules of milking process contributes to the impairment of microbial quality of bulk samples of cow's raw milk.

From the study, the potential pathogenic bacteria isolated includes *Staphylococcus aureus, Listeria monocytogens, Salmonella spp., Escherichia coli, Proteus spp., Pseudomonas spp. Klebsiella spp. and Bacillus cereus.* The incidence of Salmonella spp. was in the raw milk sample was high which pose health risk for the consumers if taken without pasteurization. De- Buyser (2001)reported that Salmonella is one of the most etiological agent response for several outbreaks associated with the consumption of raw milk and milk products. All salmonellae are of public health concern having the ability to produce infection ranging from a mild self-limiting form of gastroenteritis to septicemia and life threatening typhoid fever (Oliver *et al.,* 2005). Thus, their presence in the raw milk sample pose a health risk to consumer that consumed it without any heat treatment. 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 -Bayser 2001). The presence of Listeria monocytogenes which was known to cause listeriosis, that is, a major cause septicemia, meningitis and encephalitis in the infected person. In case of pregnant women, it may cause intrauterine or cervical infections which may result in an abortion or still birth (Oliver *et al*., 2005 ;Cheesebrough, 2007)). Hence, the presence and consumption of this pathogen in raw milk is highly dangerous. *Staphylococcus aureus* and *Escherichia coli* was discovered in all the collected samples(Figure 1). An overview of annual reports of food borne diseases from several country indicated that S. aureus was far the most frequent pathogen associated with these fooborne outbreak followed by Salmonella. S. aureus is considered the third most important cause of disease in the world amongst the reported food-borne illnesses (Zhang *et al*., 1998). S. aureus poisoning is a mild, generally self-limiting disease, with symptoms that include vomiting with or without diarrhea (Dinges *et al.,* 2000), hospitalization is required in approximately 10% of the cases (Holmberg and

Blake, 1984). As a consequence food products may originally become contaminated during the milking processes or after due to the fact that is can be found on the body surfaces of animals and man. Tamarapu *et al*., (2001) reported that *S. aureus* has been isolated from several foods such as Chicken, meat, milk and dairy products, fermented food items, etc

The standard set maximum amount of coliform bacteria in raw milk is not more than 10 bacteria per milliliter of raw milk. This level is consistent with both national and international public health and food safety requirements (FAO/WHO, 2002). This theory however, was not in correlation with the count shown in the result as the total coliform count was too high for all the samples. This may signify contamination from the grazing environment. Thus, poor herd hygiene, contaminated water, unsanitary milking practices and improperly washed milking bowl mayl have led to elevated coliform counts in raw milk. The presence of *Escherichia coli* in the milk is an indicator of fecal contamination and could be dangerous as the strain isolated may be either toxigenic or enteropathogenic, causing major public health hazard (FAO/WHO, 2002). *E. coli* O157:H7 strain has been associated with a number of food-borne outbreaks and is the cause of bloody diarrhea, frequently associated with dairy cattle, microbial contamination of raw milk and soft cheeses can result in disease. Drinking of raw milk in rural areas could be of health concern due to the presence of *E. coli* 0157:H7 *species* in the raw milk (Fook *et al.,* 2004).

Different measures can also be taken to reduce microbial contamination of raw milk, these includes the animal health which may be a vehicle for infecting the consumer. The environment should also be worked on to reduce contamination of the animals. The sanitary state of the milk handler is of paramount importance. The milking bowl should be washed with detergent and disinfected after use. Before milking, the teat and other breast area should be disinfected.

The occurrence of both *Staphylococcus spp*. in all the milk samples can be attributed to the fact that they are both normal flora of the human body as well as animals. They have been implicated as agents of nosocomial infection in hospitals (Okpalugo *et al.,* 2008). *Bacillus cereus* isolated from the raw milk samples, are aerobic, rod shape bacteria that are ubiquitous in nature especially in the soil. These characteristics accounted for their resistance to heat and presence in the milk after the raw milk was pasteurized (Goff and Griffiths, 2006).

The presence of *Pseudomonas Spp*. and its ability to survive laboratory pasteurization is probably due to the fact that Some strains of this organism particularly, dominate the micro flora of refrigerated raw milk and secrete heat-stable extracellular enzymes (proteases and lipases), which survive pasteurization and even ultra-heat treatments (UHT) and degrade the casein and fat components of raw milk causing a reduction in cheese yield, gelation of UHT milk and off flavors in many dairy products (Dunstall *et al*., 2005).

The result of pasteurization is shown in Table 2. After the raw milk was pasteurized, the microbial load was drastically reduced although some bacteria were still found present resisting the thermal application of heat on the raw milk samples. Organisms found includes *Bacillus cereus, Pseudomonas spp.* and *Lactobacillus spp* as reported by Jay, (1996). According to Jay, (2000), this may be due to several factors such as product water activity, pH, quantity of protein and number of physiological status of organisms in the total population. From the result of pasteurization, it is pertinent that pasteurization of raw milk is the antidote to preventing and reducing food borne infection as a result of consuming raw milk. This research along with previous work on consumption of raw cow milk indicated that raw milk consumer stand a high risk of exposure to foodborne pathogen. The consumption of this raw milk is a preventable cause of foodborne illness which support the call for drinking pasteurized milk in the interest of public health. All the sample tested were exposed to differs microbial contamination and they were handled under unhygienic conditions which pose health risk to consumers. Based on these findings, it is strongly recommended that people should desist from taken raw milk and their products. Also, the Fulani herdsmen should be educated on proper personal and environmental hygiene. Disinfection of the teat and mammary gland area closer to the where milking is done should be encouraged. Corresponding Author

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