WWW.IJRPR.COM

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

Bacteriological Assessment of Bacterial Isolates from Unpasteurized Milk Sold in Selected Markets in Makurdi Metropolis.

[1]Innocent, I.G., [2]Suleiman, M., [3]Doris, T.O., [4]Moris, N

[1]Department of Microbiology, School of Biological Science, Federal University of Technology Owerri, Imo State Nigeria
[2]Federal College of Education Technical Bichi, kano state Nigeria
[3]National Biotechnology Development Agency Abuja, Nigeria
[4]University of Agriculture Makurdi, Benue State Nigeria
giwainnocenti@gmail.com

ABSTRACT

Bacteriological assessment bacterial isolates from unpasteurized milk sold in selected markets in Makurdi metropolis was investigated. A total of 300 samples of unpasteurized milk were examined, out of which 222 (74%) were positive for some bacterial isolates. *Escherichia coli* had the highest percentage of 32.4% (72) followed by *Staphylococcus aureaus* 18.92% (42). *Salmonella species* came third with an occurrence of 13.51% (30), while *Pseudomonas aeruginosa* 7.21% (16) *Bacillus species* 9.91% (22), *Proteus species* 7.21% (16) and *Streptococcus species* 9.01% (20) were isolated in that order. The standard plate counts on plate count Agar ranged from 3.4 x 10^8cfu/ML to 6.8x10^8cfu/ML. Inference from the research showed that government should endeavor to assist the poor local milk producers in buying and getting their products into collection centers where proper equipment for pasteurization are provided before the products gets to the consumers considering dangers inherent in consumption of contaminated milk products. Milk should be pasteurized immediately after collection to reduce bacterial load especially the pathogenic ones. Further study is needed to make the strategies of eliminating the challenges related to unpasteurized milk.

Keywords: Unpasteurized milk and bacteria

INTRODUCTION

Raw milk is milk that has not been pasteurized, a process of heating liquid food to decontaminate them for safe drinking (Beecher and Cookson, 2016), while proponents of raw milk have stated that there are benefits to its consumption, including better flavor, better nutrition, and the building of a healthy immune system, the medical community has warned of the dangers, which include a risk of infection (Smith *et al.,* 2012). The availability and regulation of raw or unpasteurized milk vary around the world. In US, some dairies have adopted low-temperature pasteurization, which they say produces a product similar to raw milk (Smith *et al.,* 2012).

Raw Milk is well recognized as a high quality nutritional food elaborated by nature to foster young ones and to maintain balanced diet by adult. It contains more essential nutrients in significant amount compared to other single foods (Oliver *et al.,* 2005).

Man has consumed milk and milk products from time immemorial and it has been considered nutritionally, as “the most nearly perfect food”. Milk is an outstanding source of calcium and phosphorus and contains vitamin A, B1, B6, and B12 in significant amount which help in bone and teeth development (Oliver *et al.,* 2005).

The color of milk ranges from bluish white to almost golden-yellow, depending on the amount of fat, breed, solids present, and feed consumed by the cow. Milk has no obvious taste but slightly sweet to most individuals. However, any sour taste with a pungent smell is abnormal. Approximately, fresh milk has a hydrogen ion concentration of 6.5 which is a pointer to the acid side of neutrality (Ramesh *et al.,* 2008).

Milk contain approximately 87% water, 3.5-3.7% protein, 4.9% lactose, 0.7% salt/ash, 3.6% fat 4.8% sugar and 12.75 dry matter (Ramesh *et al.,* 2008). With these constituents, milk is described as the most nearly perfect food (Okonkwo, 2011). This complex biochemical composition, nutritional values and high water content render milk an excellent growth medium for both spoilage and pathogenic microorganisms (Bryne, 2004; Parekh and Subhash 2008; Okonkwo, 2011).

Milk must be of high quality because of its role in human nutrition. In underdeveloped areas of the world and in the tropics, milk must have very high quality which most not contaminated (Okonkwo, 2011).

Milk is considered spoilt when it thickens or has color impacted by microorganisms (Olatunji, 1997). When improperly stored, milk enhances rapid proliferation of pathogens since it is an ideal growth medium (Aberra, 2010).

This study was conducted to identify the bacteriological contaminants of raw milk sold within Makurdi.

Study Population

Local milk handlers (especially Fulani women) who sell cow milk in Makurdi were patronized and unpasteurized milk was collected from Makurdi metropolis. A minimum of two samples of raw cow milk was obtained from each milk seller.

Sample Collection

A total of 300 samples of raw milk were collected among milk handlers in some selected areas within Makurdi (North bank market, Wurukum market, Wadata market, High level Market, Kanshio market, and Fiidi market) in Makurdi metropolis. About 100ML of each raw milk sample bought was aseptically dispensed into sterile sample containers using a sample collector ice box at 4^0C. Samples were well labeled for easy identification and were transported to the Research laboratory of Medical Microbiology and Parasitology at Benue State University Teaching Hospital without delay.

Microbiological analysis

One millilitre of methylene blue is added to ten millilitres of milk for the methylene blue reduction tests (MBRT). To mix, the tube is gently flipped three times and sealed with a rubber stopper. It is kept in a water bath at 35 degrees Celsius and checked every six hours or such. The methylene blue reduction time (MBRT) is the amount of time needed for methylene blue to turn colourless. The milk samples are graded according to the results of the methylene blue reduction test, and the results are shown in Table (Benson, 2002). The methylene blue reduction test relies on the milk's bacteria's capacity to proliferate and absorb dissolved oxygen, which lowers the medium's oxidation reduction potentials.

Determination of Total Viable Count

To make various dilutions of milk samples obtained from Makurdi metropolitan, sterile peptone water was used.Using a sterile pipette, 0.1 ml of each d ilution was inoculated on Nutrient Agar for the purpose of determining the Total Viable Count.Using sterilized glass spreaders, the diluted sample was sprayed as soon as possible onto the plate's surface.Each plate was spread with a single sterile spreader, and the plates were then incubated for 24 to 48 hours at 35°C.Plates displaying 30 to 300 colonies were counted after incubation.To calculate the TVC, the average number of colonies in a given diluti on was multiplied by the dilution factor.

Determination of Total Microbial Counts in Milk

The total colony counts of microorganisms in the raw milk samples were calculated using the standard plate count method (Sanders, 2012).The samples were serially diluted from each of the various locations and plated out using the pour plate technique on Plate Agar.For a whole day, the plates were in cubated at 37 °C.Colony Forming Units per milliliter, or CFU/ml, are the average microbiological loads of each raw milk site as determined by Harriga n and McCance in 1976.

Isolation and identification of bacterial isolates

The bacteria were isolated using standard bacteriological techniques, as advised by Cheesbrough (2006).To start the initial step of identifying the isolates, serially diluted samples were additionally inoculated by pour plate technique in Salmonella Shigella Agar (SSA), Mannitol Salt Agar (MSA), Eosin Methylene Blue (EMB) Agar, MacConkey Agar, and Chocolate Agar. Lactose-fermenting gram negative bacteria were isolated using MacConkey agar; fastidious bacteria were isolated using chocolate agar; enteric coliforms were selectively isolated using Eosin Methylene Blue; salt tolerant bacteria were selectively isolated using Manitol Salt agar; and enteric bacilli were isolated using Salmonella Shigella agar. Following a 24hour incubation period at 37°C, all plates were identified using morphological and cultural criteria.

Statistical Analysis

The Statistical Package for Social Sciences (SPSS) version 20.0 program was used to analyze the data (SPSS, 2012). Calculating averages, proportions (percentages), two-way analysis of variance, and the test for the least significant difference between two means were all part of the analyses.

RESULTS

Out of the 300 samples of raw milk collected, 222 (74%) were positive for some bacterial isolates namely: *Escherichia coli* 72(32.4%), *Staphylococcus aureaus* 42(18.92%), *Salmonella* species 30(13.51%), *Pseudomonas aerogunosa* 16(7.21%), *Bacillus* species 22(9.91%), *Proteus* species 16(7.21%), and *Streptococcus* species 20(9.01%). The percentages of occurrence, frequency of bacterial isolates in unpasteurized milk in table 2. The incidence and prevalence of these bacterial isolates in table 3 and the Methylene Blue Reduction test the biochemical tests and reaction of each bacterial isolate are shown in Table 4 and 5.

Table1: Enumeration of Microorganisms from the different Unpasteurized Milk

Samples by Standard plate count method from Selected Markets (cfu/ML).

Markets	**SPC**
North Bank MKT	6.8×10^8
High Level MKT	4.6×10^8
Wadata MKT	3.7×10^8
Kanshio MKT	5.2×10^8
Fiidi MKT	6.7×10^8
Wurukum MKT	3.4×10^8

Key: SPC- Standard Plate count

Table 2: Occurrence Frequency of Bacterial Isolates from Unpasteurized Milk purchased from Selected Markets in Makurdi metropolis in percentage (%).

Markets	Sample size	No. of sample positive for bacteria isolation in unpasteurized milk
North Bank MKT	50	42 (84%)
High Level MKT	50	38 (76%)
Wadata MKT	50	44 (88%)
Kanshio MKT	50	30 (60%)
Fidi MKT	50	32 (64%)
Wurukum MKT	50	36 (72%)

Key: MKT- Market

Table 3: Prevalence of different bacterial isolated from unpasteurized Milk samples with respect to Markets in Makurdi metropolis in percentage.

				Bacterial	Pathogens			
Markets	No. of isolates	*E.coli*	*S. aureus*	*Salm. spp*	*p. aeruginosa*	*Bacillus cereus*	*Proteus spp*	*Srept. spp*
NBMKT	42	18(42.9%)	10(23.8%)	4(9.5%)	2(4.8%)	4(9.5%)	2(4.8%)	2(4.8%)
HLMKT	38	10(26.3%)	8(21.1%)	4(10.5%)	4(10.5%)	4(10.5%)	4(10.5%)	4(10.5%)
WAMKT	44	16(36.4%)	10(22.7%)	6(13.6%)	2(4.5%)	4(9.1%)	2(4.5%)	4(9.1%)
KMKT	30	10(33.3%)	4(13.3%)	6(20.0%)	2(6.7%)	4(13.33%)	2(6.7%)	2(6.7%)
FMKT	32	8(25.0%)	4(12.5%)	8(25.0%)	2(6.25%)	2(6.25%)	4(12.5%)	4(12.5%)
WUMKT	36	10(27.8%)	6(16.7%)	6(16.7%)	4(11.11%)	4(11.11%)	2(5.6%)	4(11.11%)

Key: NBMKT- North bank Market, HLMKT- High Level Market, WAMKT- Wadata Market, KMKT – Kanshio Market, FMKT- Fidi Market, WUMKT- Wurukum Market and *spp*- species.

Table 4: The Methylene Blue Reduction Test and Total Bacterial Count Showing the Quality of Unpasteurized Milk in Makurdi Metropolis

No of Samples	MBRT(minutes)	TBC/ml	Raw milk quality
1	420	35,000	Good
2	300	350,000	
3	240	600,000	Fair
4	180	850,000	
5	120	1,500,000	
6	60	3,450,000	Poor
7	30	10,000,000	
8	15	15,000,000	Very Poor

Key: MBRT- Methylene Blue Reduction Time

TBC-Total Bacterial Count

Table 5: Biochemical tests for the identifications of isolates obtained from Unpasteurized Milk in Makurdi Metropolis.

	Isolates						
Biochemical Tests	*Escherichia Coli*	*Staphylococcus aureus*	*Salmonella typhi*	*Pseudomunas aeruginosa*	*Bacillus cereus*	*Proteus spp*	*Streptococcus pyogenes*
Catalase test	+	+	+	+	+	+	─
Coagulasetest	Nil	+	Nil	─	Nil	Nil	Nil
Citrate utilization test	─	+	─	+	+	+	Nil
MR test	+	+	+	─	─	+─	─
VP test	─	+	─	─	+		
Indole test	+	─	─	─	─	─	Nil
Oxidase test	─	─	─	+	─	─	Nill
TSI A test Glu	+	+	+	─	+	+	+
Lact	+	+	─	─	─	─	+
Suc	vary	+	─	─	Vary	─	+
Urease test	─	+	─	─	Nil	+	─

Key: ─ Negative, + Positive, MR- Methyl Red, VP-voges-Proskauer, TSIA- Triple sugar iron Agar

DISCUSSION

Pathogenic bacteria have been a major concern to the public all over the world. The fact that milk contains a lot of nutrients has made it possible for growth and development of most 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.

The unpasteurized milk samples has microbial load due to probably the sanitary 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 set down the hygienic limit ≤ 100,000 CFU/ML milk for the total bacterial count (TBC) in cow's raw milk. TBC is one of the main indicators of hygienic quality of cow's milk that is also used to set the purchase price of milk (Cempirkora, 2007).

The results obtained from this study indicated that the samples were heavily contaminated by bacteria. This agreed with Syed *et al*. (2014) who recorded similar high results in unpasteurized milk in Beed City. The results of unpasteurized milk is consistent with that reported in Keffi by Makwin *et al.* (2014) and contrary to the one reported by Laba and Udonsek (2013) in Ilorin.

In this study, the incidence of bacterial isolates obtained from different Markets in Makurdi metropolis, 50 samples were collected in markets namely, North Bank market, High Level market, Wadata market, Kanshio market, Fiidi market and Wurukum market, and the percentage of positive bacterial isolates were 42 (84%), 38 (76%). 44 (88%), 30 (60%), 32 (64%) and 36 (72%) respectively.

The results reported in this study are likewise high when compared to those documented, by Alian *et al.* (2012), Shitanidi and Sternesjo (2004), Gundogen *et al.* (2006) and that improper hygiene and poor farm management practices contributed to the presence of high microbial growth in the milk, consistent with that reported by Jyoti *et al*. (2014) who reported the microbial content of unpasteurized milk from different dairy shops of Allahabad city.

This study investigated the prevalence of different bacterial pathogen in raw milk within Makurdi city. Laboratory results indicated that, in the raw unpasteurized milk, *Echerichia coli* in North bank market had the highest prevalence of (42.9%), followed by Wadata market (36.4%) while Fiidi market had the least (25.0%) as the most prevalent bacteria similar to the finding of Jyoti *et al.* (2014) and Kewler *et al*. (1992) and contrary to that of Makwin *et al*. (2014) in Keffi and higher than that of Olatunji *et al.* (2013). The isolation of *Staphylococcus aureus* was higher in North bank market (23.8%), followed by wadata market (22.7%) and Fiidi market had the least (12.5%) in this study which agrees with that of Makwin *et al*. (2014), Olatunji *et al.* (2013), Jyoti *et al.* (2014) and Anklo and Sterneojo (2006) who isolated 30%, 26.7%, 33.33%, 20.4% and 15% respectively from unpasteurized milk. The implication of the presence of *Salmonella species* is that it causes tyhoid, which is a serious health problem to the consumers. The prevalence of *Salmonella species* (25.0%) in Fiidi market was closely followed in Kanshio market (20.0%) and North bank market had the least (9.5%) which is in agreement with the findings of Jyoti *et al.* (2014) and Makwin *et al.* (2014). The lower percentages were obtained among *P. aerugunosa* (11.11%), *Bacillus spp* (13.33%), *Proteus spp* (12.50%) and *Streptococcus spp* (12.5%) which agreed with Olatunji *et al.* (2013), Donkor *et al.* (2007), Laba and Udonsek (2013) and Makwin *et al.* (2014). On analyzing the data, the isolates were found to be statistically non-significant ($p>0.5$).

CONCLUSION

Higher incidences of different pathogenic microorganisms were observed in unpasteurized milk sold in different markets in Makurdi metropolis. Bacterial isolates observed in this study are suspected to contaminate the samples from various sources, which could be due to poor handling and storage of milk collection. The environment, utensils used the state of hygiene of the animal from which the milk was collected and the sanitary conditions of the milk collectors are all possible source of contamination. It is a great matter of concern for human health also because a good percentage of the people consume this milk.

REFERENCES

Benson, J.H. (2002). Microbiological Application. Laboratory manual in general Microbial. 8th Edt. 1-478.

Cheesbrough, M. (2006). District Laboratory Practice in Tropical Countries, Part II. Cambridge University Press, Cambridge. Pp442.

Beecher, N. R and Cookson, G. F (2016). "Raw Milks explosive growth comes with costs of the state" Food safety News.

Bryne, R. (2004). Microbiological Hazards that need to manage during and after processing: An Overview; Processing of the IDF/FAO international Symposium on Dairy Safety and Hygiene Cape Town, South Africa

Donkor, O.N., Henrikson, A., Vasiljerk, T. and Shah, N.P, (2007). Effect of acidification on the activity of probiotics in Yoghurt during Cold Storage, *International Dairy Journal* 16: 1180-1189.

Jyoti, Y., Saurav, P., Jyotsna, K. P., Yashab, K., Ajay, K.S., Florin, M. and Harison, M. (2004). Comparative evaluation of pathogenic bacterial incidence in raw and pasteurized milk *International Journal of Engineering Science Invention ISSN (Online): 2319 – 6734.*

Jyoti, Y., Saurav, P., Jyotsna, K. P., Yashab, K., Ajay, K.S., Florin, M. and Harison, M. (2014). "Comparative evaluation of pathogenic bacterial incidence in raw and pasteurized milk" *International Journal of Engineering Science Invention. 3, 5: 11-20*

Laba, S.A and Udosek, C.E. (2013) "Bacteriological Quality of Raw Cow Milk in Ilorin, North Central Nigeria". *Natural Science* 11(10):73-79.

Makwin, D. M., Nyam, M. A., Tarfena, Y. A and Abbul-Mutalib, A. (2014). Antibiogram of Bacteria Isolated from Locally Processed Cow Milk Products Sold in Keffi Metropolis, Nasarawa State, Nigeria. *Journal of Biology, Agriculture and Healthcare* 2224-3208

Mngutyo, I. D and Ogwuche, J. (2013). "Comparative Analysis of Effects of Annual Flooding on the Maternal Health of Women Floodplain and Non Floodplain Dwellers in Makurdi Urban Area, Benue State, Nigeria", *Wudpecker Journal of Geography and Regional Planning, 1 (1)*: 57-89.

National Population commission (2009). Federal republic of Nigeria, Abuja, and ICF Macro Calverton Mayland USA, 630.

Okonko. O. I (2011). Microbiological Analyses and Safety Evaluation of Nono: A Fermented Milk Product Consumed in most parts of Northern Nigeria. *International Journal of Dairy Science,* 6: 181-189.

Olatunji, E.A. (1997). Effect of Different Methods on keeping quality Of Cheese. Proceeding of 10th Annual Conference of The Biotechnology Society of Nigeria. 54-63.

Olatunji, A. E., Ahmed, R and Njidda, A. A. (2013). Bacterial assessment and keeping quality of milk obtained from savanna brown doe; *Academic African Journal of Agriculture Research*, 8(27): 3604-3607.

Olayinka, D. N., Nwilo, P.C and Adzandeh, A. E. (2013)**.** From Catchment to Reach: Predictive Modeling of Flood in Nigeria, *Environment for Sustainablility,* 2013.

Olive,r S. P., Boor, K. J., Murphy, S. C. and Murinda, S. E. (2009). Food Safety Hazards Associated with Consumption of Raw Milk. *Food borne pathogens and disease* 19**(6):**6534-6558.

Ramesh, C.C., Kilara, A and Shah, N. P. (2008). "Dairy Processing and Quality Assurance. *International Journal of Dairy Science*, 7: 103-108.
Sanders, E.R. (2012). Aseptic Laboratory Techniques: Plating Methods. *Journal of Visual Ex*periments, *63*: e3064.

Smith, S. C. M; Brandeau, G. E; Hunter, J., Clay-Bavinger, M., Pearson P. J., Eschbach, V., Sundarah, H, Leu. P., Schirmor. C., Stave I., Olkin, B and Dravata, B.M. (2012). Are Organic Food Safer or Healthier than conventional alternative. Systemic review. *Annual International Journal of Medicine* 157: 348-366.

Syed, Z. H., Shaker, M., Gulve, R.M. and Asef Iqbal, M. (2014). Bacterial Analysis of Raw and Packed Milk of Beed City, *Journal of Advances in Applied Sciences and Technology. 1(1): 53-58*