A Study of Bacteriological Quality of Street-Hawked Milk in Ilesha Metroplolis, Osun State, Nigeria

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Summary

INTRODUCTION

Milk, a nutrient-rich liquid food produced in the mammary glands of mammals, contains low bacterial counts when it leaves the udder, but it may get contaminated from the environment, exterior of udder, water, soil, milkers' hands and utensils. Contamination can serve as a source of spread of certain harmful human bacterial diseases like tuberculosis, diphtheria, salmonellosis and food poisoning if consumed in raw form. This study, therefore, was designed to evaluate bacteriological quality of milk samples collected from various localities within Ilesha metropolis.

MATERIALS AND METHODS

Twenty (20) samples of fresh raw milk were collected in sterilized bottles from various nomadic milk hawkers in Ilesha. Methylene blue reductase test, standard plate count on standard plate count agar and isolation of possible pathogens using selective culture media was carried out on the samples.

RESULTS

Of the 20 samples of raw milk collected for bacteriological analysis, 3 samples were found to be of excellent quality, 5 were very good, 4 were good, 5 were fair and 3 were of poor quality. The actual standard plate count for excellent and very good quality ranged between 33-54 and 62 - 80 colony forming units (cfu). The organisms isolated and biochemically characterized from the raw milk samples were found contaminated with *Escherichia coli* (4 strains), *Staphylococcus aureus* (8 strains), *Streptococcus pyogenes* (5 strains), *Streptococcus agalactiae* (3 strains) and *Enterobacter aerogenes* (5 strains).

CONCLUSION

The results obtained from this study showed that the milk sold in raw form could be hazardous to human health if sold without adopting hygienic measures.

Introduction

Milk, from a physiological standpoint, is the secretion of the normally functioning mammary gland of the females of all mammals, which is produced for some time following parturition for the nourishment of the young of the species during the initial period of growth. In terms of physical chemistry, milk is an opaque, whitish fluid of multi disperse phases. It contains all the essential nutritive constituents and is nature perfect food. If it is produced, handled and distributed under thorough hygienic conditions[1].

The lactoglobulin fraction of milk protein contains highly specialized proteins- the immunoglobulins (IgA, IgD, IgE, IgG and IgM). These antibodies protects the individuals particularly children against diseases. Normal milk itself contains low bacterial counts, when it leaves the udder, but it may get contaminated from the environment, exterior of udder, water, soil, milkers' hands and utensils. Contamination can serve as a source of spread of certain harmful human bacterial diseases like tuberculosis, diphtheria, salmonellosis and food poisoning if consumed in raw form. The true solution in milk contains; lactose, vitamins, acids, enzymes, and some inorganic salts. The colloidal phase contains casein, calcium phosphate, and globular proteins [2].

Worldwide, milk of the cow is by far of more commercial importance than milk of any other mammal. In the United States, the term "milk" legally refers to cow's milk. Milk from other species is labeled to indicate the type: sheep's milk, goat's milk, etc. Milk is the whole, clean lacteal secretion of one or more healthy cows properly fed and kept, excluding that obtained within 15 days before calving and three to five days after colostrum, the milk secreted immediately after giving birth, is not considered milk from a legal standpoint. The U.S. Public Health Service's definition of Grade A milk is "the lacteal secretion practically free from colostrums, obtained by complete milking of one or more healthy cows, which contains not less than 8.25% milk solid not-fat (MSNF) and not less than 3.25% milk fat. Milk can be processed in many forms; creams-fat portion of the churned and centrifuge milk, skimmed milk, ghee, curd, butter milk, pasteurized milk etc. But milk in all these processed forms may alter total nutritional composition as a result of heat application capable of altering their temperature and it is a law that any food when heated above blood temperature will start losing its nutritive value. Cow's milk has been reported to produce allergy to many people in the forms of diarrhea, vomiting, colic, eczema, running nose, cough and wheezing [3].

Ilesha is a city located in Osun state, it lies between the Latitude 7^0 37' 40. 40" N and Longitude 4 44^0 29.80"E coordinates in south west Nigeria. The city of Ilesha consisted Ilesha itself and a number of smaller surrounding cities. The state of Ijeshaland was founded in 1300AD by *Ajibogun Ajaka Owa Obokun Onida Raharaha*, a war like grandson of *Emperor Oduduwa* and her population is about 738, 910 peoples[4].

Table 1: MBRT of Raw Milk from Selected Towns in Ilesha

Identification Number	Sample Site	Reduction time(hrs)	Quality
RM01	Idominasi	8.0	Excellent
RM02	Ilowa	6.0	Very Good
RM03	Idoka	0.5	Poor
RM04	Iponda	4.0	Good
RM05	Ilase	0.5	Poor
RM06	Iregun	2.0	Fair
RM07	Ijeda	4.5	Good
RM08	Isokun	6.0	Very good
RM09	Ibodi	6.0	Very good
RM10	Ijebu-jesa	4.5	Good
RM11	Iperindo	6.0	Very good
RM12	Itaapa	0.4	Poor
RM13	Ikinyinwa	6.0	Very good
RM14	Ibokun	4.2	Good
RM15	Ibala	8.0	Excellent
RM16	Itaore	2.0	Fair
RM17	Itagunmodi	1.8	Fair
RM18	Alakowe-Ijesa	1.6	Fairs
RM19	Iwaraja	1.5	Fair
RM20	Ifewara	8.0	Excellent
RM: Raw Milk			

In Ilesha, milk is mostly transported from villages in calabash gourds, copper and aluminum containers by nomadic children and wives that usually situate their ranches in the jungle and in some cases, time taken in its delivery to cities is 4-6 hours during the hot days of dry season. Milk is a good medium for the growth of microorganisms. Milk that contains large numbers of actively growing bacteria will have a lowered oxidation reduction potential due to the exhaustion of dissolved oxygen by microorganisms. Thus bacterial proliferation in the raw milk is unavoidable. Both the time required to peddle the milk from street to street and climatic conditions of the environment are conducive to rapid microbial multiplication [5].

Bacterial count in milk is the most reliable indication of its sanitary quality. Although human pathogens may not be present in a high count, it may indicate a diseased udder, unsanitary handling of milk, or unsuitable storage temperatures. In general, therefore, a high count means that there is a greater likelihood of disease transmission.

Therefore, milk at its delivery end to the consumer cannot be presumed to be free from bacterial contamination and thus may pose serious health problems to its consumers. Milk is very unique to men, because every mammal's milk is designed and intended for the development of its young ones. Except human beings, no animal drinks the milk of any other animal. It is therefore, important to put human

safety first [6]. This study therefore was designed to evaluate bacteriological quality of milk samples collected from various localities within Ilesha metropolis.

Materials and Methods

Collection of raw milk samples and laboratory analysis

Twenty (20) samples of fresh raw milk were collected in sterilized bottles from various nomadic milk hawkers in Ilesha. The samples were maintained refrigerated at 4°C in chemical ice coolers and brought to the laboratory for immediate analysis.

Methylene blue reductase test (MBRT) of raw milk samples

A volume of 1 mL of methylene blue (1:25,000) was added to 10mL of freshly collected milk sample in each test tube separately, and sealed with a rubber stopper and slowly inverted three times to mix. The preparation was placed in a water bath at 35^0C and examined at intervals of up to 6 hours. The time it takes for the methylene blue to become colorless were recorded as the methylene blue reduction time (MBRT) for each milk sample.

Table 2: Standard Plate Count of Raw Milk from Selected Towns in Ilesha

Identification Number	Undiluted	10^{-1}	10^{-2}	10^{-3}	Actual Plate Count (Per mL)
RM01	54	23	64	14	54
RM02	64	40	23	15	60
RM03	›300	›300	108	20	10520
RM04	100	52	25	18	108
RM05	›300	250	150	90	95 000
RM06	›300	168	28	10	1 580
RM07	220	50	20	4	500
RM08	180	100	56	30	70
RM09	›300	42	8	0	80
RM10	›300	126	10	2	1242
RM11	58	36	11	3	62
RM12	›300	220	162	80	79 100
RM13	169	78	18	6	80
RM14	›300	200	140	65	68 000
RM15	33	13	6	0	33
RM16	›300	212	146	82	76 000
RM17	›300	223	148	69	79 000
RM18	›300	180	134	89	62 000
RM19	›300	205	165	80	79 000
RM20	54	40	20	13	50
RM: Raw Milk					

Table 3: Gram's Reactions and Morphological Characteristics of the Isolates

Name of organisms	No of isolates	Gram reaction	Shape
Escherichia coli	4 :RM(04,07,19,14)	-	Short rod
Staphylococcus aureus	8:RM(03,05,06,12,16,17,18,19)	+	Round or oval
Streptococcus pyogens	5:RM(02,08,09,11,13)	+	Coccus in chains
Streptococcus agalactiae	3: RM(1,14,20)	+	Coccus in chains
Enterobacter aerogenes	5: RM(1, 4, 11,17,19)	-	Cocci to long side rods

Culture of isolates (standard plate count)

A volume of 9 mL of distilled water was pipetted in each tube and sterilized by autoclaving. One (1mL) of undiluted milk sample was pipetted into 9 mL of distilled water to give aliquot of 10^{-1} and serially diluted into various concentrations; 10^{-2} and 10^{-3} for each milk sample and 0.2mL from each dilution factor was seeded into standard plate count agar and poured, respectively . The agar media were allowed to solidify and the culture plates were then incubated at 37 °C for 48 hours.

A negative control was made up of plate count agar only. The plates were then placed on a Quebec colony counter and the number of bacterial colonies was recorded. Different selective media; eosin methylene blue agar, mannitol salt agar, Sheep blood agar base and chocolate agar were used for the isolation of suspected pathogens; *Escherichia coli, Staphylococcus aureus, Streptococcus pyogens, Streptococcus agalactiae Streptococcus agalactiae* and *Enterobacter aerogenes* and sugar fermentation tests were also carried out on the isolates obtained from the samples.

Results

Twenty (20) raw milk samples were tested to for quality by methylene blue reductase test. Of the twenty samples tested, three were found to be of excellent quality, five were very good while the remaining samples were found to be good, fair and poor in ratio 4, 5 and 3 respectively as shows (Table 1). Standard plate count of the raw milk samples shows varied proportions of microbial loads, both the undiluted and dilutions of $10^{-1} 10^{-2}$ 10^{-3} and actual plate count colony forming units were estimated by Quebec colony counter (Table 2).

Table 4: Biochemical Characteristics of the Isolated Pathogens

Name of organisms	Glucose	Maltose	Lactose	mannitol	Indole	Nitrate	Methyl red	H$_2$S	Voges prokaeur
Esch. coli	+ gas	+ gas	+ gas	+ gas	+	+	+	-	-
Staph. aureus	+ acid	+ acid	+ acid	+ acid	-	+	+	-	+
Strept. pyogenes	+ acid	+ acid	+acid	-	-	-	-	-	-
Strept. agalactiae	+acid	+acid	+acid	-	-	-	-	-	-
Enterobacteraerogenes	+acid/gas	+acid/gas	+/gas	-	-	-	-	-	-

Discussion

A total of twenty (20) samples of raw milk from 20 different localities in Ilesha were examined physically and processed for the methylene blue reductase test. Methylene blue reductase was recommended by National Dairy Council as accepted method for evaluation of milk quality. The greater the numbers of bacteria in milk, the quicker the oxygen that will be consumed, and in turn the sooner will the colour disappear. Thus the time of reduction is taken as a measure of the number of organism in milk [7].

Of the 20 samples examined, 3(15%) from Idominasi, Ibala, and Ifewara were of excellent quality and 5 (25%) from Ilowa, Isokun, Ibodi, Iperindo and Ikinyinwa were very good, 4(20%) from Iponda, Ijeda, Ijebu-jesa and Ibokun were good, 5(25%) from Iregun, Itaore, Itagunmodi, Alakowe-Ijesa and Iwaraja were fair and 3(15%) from Idoka, Ilase and Itaapa were of poor quality as shows in Table 1. The variation in the proportion of quality obtained from the raw milk in this study could be attributed to the health status of the cow milked, the mode of transport vessels for hawking, the proximity of ranches in each locality of the consumers that determined the hawkers' timing and the inadequate knowledge of the milkers on possibilities of fermentation of milk. This was similar with the study of Subha Ganguly.*et.al.,* (2017) on bacteriological examination of cow milk samples suspected of clinical mastitis [8].

Standard plate counts were determined for the undiluted, and three separate dilutions. The colony forming units of the undiluted, 3 separate dilutions and actual plate counts were recorded. The actual standard plate count numbers of colonies on the sample plates adjudged to be excellent in quality ranged between 33 and 54 while samples that were accepted to be very good ranged from 62 and 80 as shows in Table 2. This agrees with the report of National Dairy Council (1993) on the knowledge of milk and other fluid dairy products [9].

The morphological and biochemical characterization of the raw milk sample showed varied potential pathogens which can initiate food poisoning when present in high dose. The organisms were*; Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes, Streptococcus agalatiae* and *Enterobacter aerogenes* in varied proportion as elicited in Tables 3 and 4, which could be attributed to the utensils, milker's hand, udder of animals, milking environment and other related factors [10].

Therefore, regular sterilization of dairy equipment such as washing of utensils, milkers' hand, udder of animals, eradication of diseased animals, proper transportation and if possible within the local environment and pasteurization of milk should be carried out before its distribution for human consumption.

Conclusion

The results obtained from this study showed that the milk sold in raw form could be hazardous to human health if sold without adopting hygienic measures.

Recommendation

There is a need for strict preventive measures for hygienic and wholesome supply of milk to the market

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