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Bacteriological quality of raw cow's milk from different dairy farms in Ogbomoso, Nigeria

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Abstract

Cow milk has long been considered a highly nutritious and valuable human food but it is an excellent culture medium for many microorganisms, especially bacterial pathogens. There is a constant challenge in milk production to prevent or minimize the entry and subsequent growth of microorganisms in milk. Production of milk and milk products of superior quality and prolonged shelf-life with the ability to provide a safe and wholesome food for the consumers is needful. This study is therefore aimed at evaluating the microbial quality of raw cow milk from different dairy farms in Ogbomoso, Oyo State, Nigeria. Bacterial pathogens were isolated from the milk samples and the isolates were characterized and identified to be *Salmonella typhii*, *Shigella dysentriae*, *Escherichia coli*, *Enterobacter aerogenes*, *Bacillus cereus*, *Klebsiella pneumoniae*, *Pseudomonas cepacia*, *Aeromonas hydrophilia and Pseudomonas fluorescens*. *Pseudomonas fluorescens* was the most predominant of the isolated bacteria. The total bacterial counts of the milk samples ranged from $0.2x10^6$ CFU/ml to $4.2x10^6$ CFU/ml. Also, the total enterobacteriaceae count ranged from $0.8x10^6$ CFU/ml to $2.6x10^6$ CFU/ml while the total salmonella-shigella count was found to range between $0.5x10^6$ CFU/ml and $1.1x10^6$ CFU/ml. Antibiotic susceptibility profiles of the isolates was determined; 10% resistance and 90% susceptibility to clinically relevant antibiotics was noted amongst the isolated bacteria pathogens. Resistance to more than two antibiotics was found in *Salmonella typhii.* The presence of these bacteria pathogens in the samples analysed is considered to be an indicator of poor hygiene and sanitation during milking and post milking processes. It is therefore recommended that good sanitary measures should be taken by the people handling the cows and it must also be ensured that the cows are always in good health condition.

Keywords: Cow, Milk, Antibiotics, indicator , poor hygiene.

Introduction

Milk is a white liquid produced by the mammalian gland of mammals; it provides the primary source of nutrition for young mammals before they are able to digest other types of food (Michael, 1981). Also, milk is a complex fluid secretion excluding colostrum, with a normal milking (manual or mechanical) of the mammalian gland of a healthy, normally-fed lactating animal (Jensen, 1995). It is a vital type of food for over 6 billion human beings all over the world and a major contributor to food security as it alleviates poverty and mitigates malnutrition (Belewu, 2006). Cow milk has long been considered a highly nutritious and valuable human food and it is consumed by millions daily in a variety of different products

(Bramley and McKinnon, 1990). Raw milk of good hygienic quality meets the nutritional needs of body better than any single food as it contains essential food constituents such as fat, proteins, carbohydrates, minerals and vitamins (Sharm and Joshi, 1992; Medhammar *et al*., 2012). As a result of the presence of these nutritional components, milk is an excellent culture medium for many microorganisms, especially bacterial pathogens (Henry and Newlander, 1997; Saeed *et al*., 2009). Milk is often prone to early contamination and spoilage if not handled properly (Ekici *et al*., 2004).

Milk and milk products are important economic activities in Nigeria, about ninety percent of the dairy cattle belong to the Fulani agro-pasteuralist and their women strictly controls the processing and marketing of their milk (Okeke *et al*., 2014). Raw milk is most perishable, desirable and perfect food for human beings and animals (Bramley and McKinnon, 1990). Due to its high water content, 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). A diversity of nutrients, presence and multiplication of microorganisms cause changes in the quality of milk, thereby limiting its durability and bringing harm to the economy and also to public health (Alves, 2006; Barros *et al*., 2011).

Microorganisms present in milk can be classified into two main groups: pathogenic and spoilage organisms, although some may play a dual role for example *Bacillus cereus*, pathogenic organisms are those capable of inducing food poisoning, thus posing a threat to public health (Logan, 2012). These pathogenic microbial contaminants in milk have been a major factor for public health concern since the early days of dairy industry (Altug and Bayrak, 2003). There is a constant challenge to those involved in milk production to prevent or minimize the entry and subsequent growth of microorganisms in milk (O' Connor, 1994). These is mainly due to the importance of producing milk of good hygienic quality, which is necessary to milk product of superior quality and prolonged shelf-life thereby to provide a safe and wholesome food for the consumers (O' Connor, 1994). Bacterial contamination can generally occur from three main sources; within the udder, outside the udder and from the surface of equipment used for milk handling and storage (Oliver *et al*., 2005).

Once milk is secreted out of the udder of the cow, the retention of milk requires cleanliness, sanitation and cooling (Wallace *et al*., 2009). Fresh milk drawn from a healthy cow normally contains a low microbial load of less than 10^3Cfu/milliliter (Lingathurai *et al*., 2009; Wallace *et al*., 2009). But may increase up to 100 fold or more if stored for sometimes at ambient (30 to 35^0c) temperature (Lingathurai *et al*., 2009). Milk produced under hygienic conditions from healthy animals should not contain more than 1 x 10^5 Cfu/ml (O'Connor, 1994).. Therefore, this study is aimed at isolation and identification of bacterial pathogens from raw cows' milk and determination of the antibiotic susceptibility profile of the isolates.

Materials and Methods

Collection of samples

The raw cow milk samples were collected from the five different locations in Ogbomosho, Oyo state. The samples were collected into sterile bottles and immediately placed inside an air tight container containing ice packs and transported to the laboratory for analysis.

Culture media

The culture media used include nutrient agar, MacConkey agar and salmonella/ shigella agar. The medium were prepared according to the manufacture specification. These medium were sterilized in an autoclave at 121^0C for 15minutes.

Total colony count

One milliliter of each sample was dispensed in sterile test tubes containing sterile de-ionized water and serially diluted. One milliliter of appropriate dilutions was seeded on plate count agar using spread plate method, and the medium was then incubated at 37°C for 24 h. The plate count agar was examined and colonies present were counted and recorded after incubation at 37°C for 24 h, to get the total colony count in CFU/mL.

Isolation of micro-organisms

One milliliter of each sample was serially diluted, 1mL of an appropriate dilution was inoculated on the MacConkey, nutrient and salmonella/shigella agar. The plates were incubated for 24hours at 37^0C and after 24 hours sterile wire loop was used to pick the isolate from the plate and was streaked on a freshly prepared nutrient agar then incubate for 24hours at 37°C in order to get pure cultures. Pure cultures were then stored in a refrigerator at 4^0C. The routine laboratory method of Cruickshank *et al*. (1975) was used to characterize different isolates. The isolates were identified using their macroscopic, cultural, physiological and biochemical characteristics.

Antibiotic sensitivity test

Mueller-Hinton agar was evenly seeded throughout the plate with the isolate which had been previously diluted at a standard concentration (approximately 1 to 2 x 10^8 colony forming units per ml). Commercially prepared disks, each of which was pre-impregnated with a standard concentration of a particular antibiotic, were lightly pressed onto the agar surface; the plates were incubated for 24 hours at 37^0C. The antibiotics used included ofloxacin (OFL: 5µg), ciprofloxacin (CPR: 5µg), augumetrin (AUG: 30µg), gentamicin (GEN: 10µg), cefuroxime (CRX: 30µg), ceftazidinie (CAZ: 30µg), nitrofurantoin (NIT: 30µg). After an overnight incubation, the bacterial growth around each disc was observed.

Results and Discussion

A total of sixteen organisms were isolated from raw milk samples, the isolates were subjected to biochemical tests such as Gram staining, oxidase test, starch hydrolysis, methyl red growth at different pH and so on. They were identified to be *Salmonella typhii*, *Shigella dysentriae*, *Escherichia coli* (2), *Enterobacter aerogenes*, *Bacillus cereus* (2), *Klebsiella pneumoniae* (3), *Pseudomonas cepacia*, *Aeromonas hydrophilia and Pseudomonas fluorescens* (4). The distribution of the bacteria isolate in the samples is shown in Table 2 with *Pseudomonas fluorescens* being the most predominant and this shows that the raw milk produced in the study area could be harmful especially to immune compromised consumers. The presence of these bacteria pathogens in the samples analysed is considered to be an indicator of poor hygiene and sanitation during milking and post milking processes. The presence of some of these bacteria in the milk samples can also be linked to contamination by cows' excrement, land and water used (Chye *et al*., 2004).

Table 1: Distribution of bacterial isolates in different samples

Organisms	OSLG	ONLG	OLG	ALG	SLG
Salmonella typii	+	-	-	-	-
Escherichia coli	-	-	+	-	+
Klebsiella pneumonia	+	+	-	-	+
Pseudomonas flourescens	+	+	+	+	-
Pseudomonas cepacia	-	-	-	-	+
Aeromonas hydrophilia	-	-	-	+	-
Shigella dysenteriae	-	-	-	+	-
Enterobacter aerogenes	-	-	-	-	+
Bacillus cereus	-	+	-	-	+

+ = detected, - = not detected

Table 2: Microbial load of the raw milk samples

Sample	TBC (CFU/ml)	TEC (CFU/ml)	TSSC (CFU/ml)
SLG	3.0 x 10^6	1.4 x 10^6	1.1 x 10^6
ONLG	4.2 x 10^6	1.2 x 10^6	1.0 x 10^6
OSLG	2.0 x 10^6	2.6 x 10^6	1.1 x 10^6
ALG	0.2 x 10^6	0.8 x 10^6	0.5 x 10^6
OLG	2.5 x 10^6	1.2 x 10^6	0.8 x 10^6

TBC – Total Bacteria Count, TEC – Total Enterobacteriaceae Count, TSSC – Total Salmonellas-Shigella Count

Table 3 shows the total bacterial counts of the raw cows' milk samples ranged from $0.2x10^6$ CFU/ml to $4.2x10^6$ CFU/ml. Also, the total Enterobacteriaceae count ranged from $0.8x10^6$ CFU/ml to $2.6x10^6$ CFU/ml. The total salmonella-shigella count was found to range between $0.5x10^6$ CFU/ml and $1.1x10^6$ CFU/ml. The total bacterial count obtained in this study was generally high as compared to the acceptable level of $1.0x10^5$ bacteria per ml of raw cow's milk (O'Connor, 1994). This study shows that the quality of milk produced in the study areas were poor. This was evident from the high values of total bacteria count (TBC) and there is the need for adequate sanitary measures at different stages of production to consumption. Most microorganisms found in the raw milk are contaminants on the outer surface of the udder, milking utensils and milkers (Chye *et al*., 2004). The quality of water use for washing utensils could also be part of the reasons for obtaining a poor microbiological quality in this milk samples.

Table 3: Antibiotic Susceptibility pattern of the isolate

Organisms	CAZ	CRX	GEN	CPR	OFL	AUG	NIT	AMP
Salmonella typhi	R	R	18.5	12.0	10.0	23.5	21.0	R
Shigella dysenteriae	18.5	21.5	20.0	23.5	25.5	23.5	22.0	R
Escherichia coli	30.0	20.0	21.0	14.0	13.5	7.0	22.0	8.5
Enterobacter aerogenes	32.0	26.5	24.0	26.5	24.5	25.5	21.5	16.0
Klebsiella pneumoniae	28.0	26.5	22.0	20.0	12.0	23.0	28.0	8.5
Pseudomonas cepacia	22.5	18.0	21.0	21.5	16.0	31.0	13.5	9.0
Pseudomonas fluorescens	22.5	13.0	25.0	20.0	17.5	8.5	14.5	8.0
Aeromonas hydrophilia	31.0	19.0	21.0	11.5	10.5	6.0	23.0	R
Bacillus cereus	22.5	24.0	8.0	22.0	R	R	20.0	26.0

CAZ – ceftazidime (30µg), CRX – cefuroxime (30µg), GEN – gentamicin (10µg), NIT –nitrofurantoin (300µg), CPR – ciprofloxacin (5µg), OFL - ofloxacine (5µg), AUG- augumentin (30µg), AMP – ampicillin (30µg), R - Resistant

Antibiotic susceptibility profile of the isolates was determined and it was discovered that *Salmonella typhii* was resistant to ceftazidime (CAZ), cefuroxime (CRX) and ampicillin (AMP) but sensitive to other clinically relevant antibiotics used. *Shigella dysenteriae* and *Aeromonas hydrophilia* were resistant to only ampicillin (AMP) but sensitive to other antibiotics. *Bacillus cereus* was found to be resistant to ofloxacine (OFL) and augumentin (AUG) while *Escherichia coli, Enterobacter aerogenes, Klebsiella pneumoniae*, *Pseudomonas cepacia* and *Pseudomonas fluorescens* were susceptible to ceftazidime, cefuroxime, gentamicine, ciprofloxacine, ofloxacin, augumentin, nitrofurantoin and ampicillin as shown in Table 3. The zones of inhibition ranged between 8.0 and 32.0mm. According to Oladipo *et al*. (2009; 2010a,b; 2011, 2014) resistance to antimicrobial agents in bacterial pathogens is a major hindrance to successful therapy and bacterial strains have been reported that are resistant to most available antimicrobial treatments. An extremely serious public health problem associated with the outbreak of major epidemics is multiple drug resistance (Canton *et al*., 2003). Measures that can be taken to ensure that currently available antibiotics remain effective as long as possible includes greater awareness among the public, health care professionals and the food- and agriculture sector regarding the importance of rational use of these medicines as well as ways to prevent infections and spread of antibiotic resistant bacteria (Freire-Moran *et al*., 2011).

In conclusion, it is therefore recommended that good sanitary measures should be taken by the people handling the cows, these measures should include proper handling of the cow, personal hygiene, treatment of udder infection of the cow, use of hygienic milking and processing equipment, improving milk and milk handling environment among others. It must therefore be ensured that the cows are always in good health condition, and this should be ensured by a veterinary doctor.

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