Bacteriological Quality and the Antibiogram of Isolates from Raw Cow Milk Produced in Ibadan Metropolis Oyo State, Nigeria

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Abstract: This study aimed at evaluating the bacteriological quality as well as the antibiogram (antibiotic sensitivity profile) of organisms isolated from raw cow milk produced in Ibadan metropolis. Raw cow milk samples were collected from University of Ibadan Research farm, Bodija, Sabo and Akinyele cow settlement ibadan. Bacterial counts, isolation and phenotypic identification of bacterial isolated from the assessed raw cow milk were determined using standard procedures. Antibiotic sensitivity tests was carried out by disc diffusion method. The total bacterial counts and total coliform counts ranged between 0.9×10^6 to 4.5×10^6 CFU/ml and 0.4×10^6 to 4.2×10^6 CFU/ml, respectively. Fourty-two (42) bacterial isolates were identiifield as *Staphylococcus aureus* (16), *Escherichia coli* (8), *Salmonella typhimurium* (6), *Shigella dysentriae* (6), *Enterobacter aerogenes* (4) and *Serretia marcescens* (2). Based on the antibiogram, *S. aureus, E. coli , S. typhi* and *S. dysentriae* had 100% resistance to Tetracycline, Gentamycin and Nitrofurantoin while *Serratia marcescens* strains had 100% susceptibility to all the antibiotic except Nitrofurantoin which had 50% susceptibility and Tetracycline which had 100% resistance. All of the *E.coli* isolates were resistance to tetracycline. The raw cow milk samples analysed exceeded the WHO microbiological standard of 1.0×10^5 CFU/mL and 3.0×10^1 CFU/mL for total bacteria count and coliform count for raw milk respectively. The antibiogram studies revealed that some bacteria isolates were resistant to most of the antibiotics used. This may pose a high risk of milk borne illnesses among consumers and put them at risk of being infected with antibiotic resistant strains of pathogenic bacteria. There is utmost need for an awareness program with a follow up mechanism to educate dairy farmers and handlers on hygienic production practices and discourage the indiscriminate use of antibotics to have a wholesome milk.
Keywords: Antibiogram, Bacteriological quality, Cowmilk, Bacterial isolates, Pathogens

INTRODUCTION

Milk is a fresh liquid, clean, and normal of a properly fed and well-kept dairy animal mammary secretion produced by mammals (Rumbold *et al*., 2021). Milk from dairy animals (cattle, sheep, and goats) are an important food item for over 6 billion people all over the globe, and a major contributor to food security as it alleviates poverty and mitigates malnutrition (Rumbold *et al.,* 2021). Raw milk is mlik produced by the secretion of mammary glands of farmed animals which has not been heated to more than 40˚C or has not undergone any effect with equilvalent effects. Raw milk of good hygienic quality meets the nutritional needs of body better than any single food as it contains essential food constituents Once milk is secreted out of the udder of the cow, the retention of milk requires cleanliness, sanitation and cooling (Wallace *et al.,* 2009). The bacteriological quality (Medhammer *et al.,* 2012). However, its composition can vary base on the dairy animal species,age,seasonality, health status of the udder and lactation period etc (Malek *et al.,* 2013). Milk represents an excellent growth medium for many micrrorganisms which can develop and spread in the products causing its spoilage and/or quality decline (Sarkar, 2015). Moreover, It's high nutritional components allow rapid multiplication of bacteria especially under unsanitary production and mishandling of milk during production and storage.. Hence, milk with high quality and safty is not easily accomplished (Worku *et al.,*2012). Raw milk is also a rich source of Lactic acid bacteria and these LAB isolated from the raw milk are capable of synthesizing metabolites (Adediran *et al.,* 2021).

control is essential to identify the degree of contamination, enumeration of selected microorganisms and ensure that milk complies with the regulatory standards (Muehlhoff *et al.,* 2013). Several other factors, such as the health of the herd, the health conditions of the milking place, the excretion from the udder of an infected animal and quality of water used on the farm, may influence the microbiological quality of milk products (Sarkar, 2015). The nature of milk makes it deserves special attention in its production, processing, marketing and consumption. However, despite the fact that presently production is growing using the modern technology, many milk producers still use non-specialized methods, resulting in raw milk of poor quality. When the milk is contaminated by bacteria, it usually becomes unfit for further processing hence will not meet the consumer's expectations in terms of health (nutritional value), safety (hygienic quality) and satisfaction (sensory attributes) (Nanu *et al.,* 2007). The dairy industry has undergone major transformations in an attempt to become more competitive, with benefits to the producer in terms of quality. The production of high-quality milk should therefore be a priority for good quality end products with long shelf life and for marketing of value-added products. It is therefore imperative to create public awareness on the health implication of consuming raw cow milk that does not follow proper hygienic protocols as well as to take preventive measures against cow milk-borne diseases and to also eliminate food spoilage organisms present in cow milk. In addition,some pathogenic bacteria that have become resistant to antibiotic drug therapy have increased the problems of public health, and it is an ever-increasing global health threat (Levy, 2001).Several recent investigations reported the emergence of multidrug-resistant bacterial from origins such as humans,cattles and food which increases the need for routine antimicrobial They were all prepared according to manufacturer specifications. They were susceptibility testing (Makharita *et al.,* 2020).This is currently one of the greatest medical challenges because it is also the cause for low cure rates, loss of human lives,animal lives and milk products.(CDC, 2019). Although, there are few reports on the investigation of the variations of bacterial composition, quality and safety of raw cow milk as well as antibiogram of the bacterial diversity from raw milk produced in Ibadan metropolis. More investigation are necessary in order to ascertain the quality of milk sold in Ibadan metropolis. Hence, this study was aimed at evaluating the bacteriological quality of raw cow milk produced in Ibadan and also to assess the antibiogram (antibiotics susceptibility profile) of the bacteria isolated from the raw cow milk.

MATERIALS AND METHODS

Study area and sample collection

The study was conducted In Ibadan Oyo State Nigeria. The collection points of raw milk samples were University of Ibadan Research farm,Bodija cattle settlement,Akinyele cattle settlement and Sabo cattle settlement Ibadan. A cross sectional sampling of Sixteen (16) raw cow milk samples were randomly collected using sterile container with cover under aseptic condition and immediately placed inside an air tight container containing ice packs and transported to the central laboratory University of Ibadan, Nigeria for further analysis.

Culture Media

Media used includes; Nutrient Agar (NA) for purification of cultures and storage in slants, MacConkey agar for isolation of enterobacteriacea, Eosine Methylene Blue Agar (EMB) for selective isolation of Escherichia coli, Mannitol Salt Agar (MSA) for isolation of Staphylococcus aureus, Salmonella Shigella Agar (SSA) for selective isolation of *Salmonella* and *Shigella* and Muller-Hinton agar for antibiotic sensitivity test. sterilized by autoclaving at 121° C for 15 minutes at 15 Psi and allowed to cool at

about 45° C before been poured unto sterile petri dishes. Materials used imcludes glass, petri dishes,conical flasks, scapel,wire loops and were all sterilized in hot air oven at 160° C and allow to cool before use. The benches were sterilized with 95% ethanol to reduce the microbial load on the work bench described by Cheese brough, (2005).

Enumeration of Total bacteria and Coliform in Raw Milk

This was carried out y pour plate technique. One milliliter(1.0 mL) of raw cow milk samples were taken aseptically and transferred into separate bottles containing 9.0ml of sterile distilled water and serial dilution of the milk samples were made. One mililitre of 10^{-5} dillution of the samples were inoculated into Nutrient agar and MacConkey and then incubated at 37 ℃ for 24 hours. After the expiry of the incubation period, the plates were checked and observed for bacterial growth. The number of colonies in each plate was counted using colony counter. The culture plates with number of colonies less than 300, and its duplicate, for each sample, was selected. To calculate the total bacteria counts and total coliform counts, the count obtained was multiplied by the dilution factor and expressed as colony forming unit (CFU) per milliliter of the original sample.

Isolation and identification of bacteria

One (1) ml of each samples were dispensed in sterile test tubes containing sterile de-ionized water and serially diluted. 1ml of the solution was plated by pour plate method on Nutrient agar, mannitol salt, MaCconkey and Salmonella-shigella agar and incubated at 37ºC for 24hrs. After incubation, colonies having different morphology, shape and size were sub cultures until pure culture were confirmed. Colonies were picked for gram's staining by standard methods. Preliminary tests were carried out according to Dykes *et al.* (1994) and identification was carried out using morphological and biochemical characterization according to the method descried by Sharpe, 1981.

Antibiotic sensitivity test

This was determined by disc diffusion method as described by cheesebrough, 2000. The plates were incubated for 24 hours at 37 ℃ and zone of inhibition were measured and recorded. Standard inoculum of 18 hours broth was spread on Muller-Hinton agar using sterile swab. The plates were dried before placing the antibiotic disc at equidistance and incubated at 37℃ for 24 hours. The diameter of zone of inhibition were measured and recorded. The antibiotic used were Streptomycin (10µg), Amoxicillin (30µg), Ofloxacin (5µg), Cefriazone (30µg), Gentamycin (10µg), Pefloxacin (5µg), Co-cotrimoxazole (25µg), Ciprofloxacin (10µg) Augumentin (30µg) Nitrofurantoin (20µg), Tetracycline (30µg), erythromycin (5µg) and chloramphenicol (30µg).

RESULTS

Total bacterial and coliform counts of raw milk samples from different dairy farm and cow settlement in Ibadan Oyo State was evaluated (Table 1). The total bacterial counts and total coliform counts ranged from 0.9×10^6 to 4.5×10^6 CFU/mL, and 0.4×10^6 to 4.2×10^6 CFU/mL respectively.The highest Total bacterial counts was recorded in sample BO1 and was not significantly different from sample AY3 but significantly different from other samples.

Table 1: Enumeration of Total bacteria and Coliform counts in raw cow milk

Samples	Total bacteria counts (10^6 CFU/mL)	Total coliform counts (10^6 CFU/mL)
UI_1	1.0 ± 0.20^{d}	0.5 ± 0.10^{cd}
UI_2	0.9 ± 0.30^{d}	0.4 ± 0.00^{d}
UI_3	1.8 ± 0.20^{cd}	0.9 ± 0.10^{bcd}
UI_4	2.3 ± 0.70^{bcd}	1.6 ± 0.60^{bcd}
BO_1	4.5 ± 1.10^{a}	4.2 ± 0.20^{a}
BO_2	3.6 ± 0.40^{ab}	2.8 ± 0.20^{abc}
BO_3	3.9 ± 0.60^{a}	2.9 ± 0.20^{ab}
BO_4	3.5 ± 0.00^{ab}	2.5 ± 0.00^{abcd}
AY_1	3.5 ± 0.20^{ab}	2.6 ± 0.40^{abcd}
AY_2	3.1 ± 0.10^{abc}	2.9 ± 1.10^{ab}
AY_3	4.3 ± 0.30^{a}	4.0 ± 1.00^{a}
AY_4	3.7 ± 0.10^{ab}	2.9 ± 1.10^{ab}
SO_1	3.5 ± 0.00^{ab}	2.5 ± 0.00^{abcd}
$S0_2$	3.2 ± 0.05^{abc}	2.6 ± 1.30^{abcd}
SO_3	3.4 ± 0.00^{ab}	2.8 ± 0.20^{abc}
SO_4	3.1 ± 0.90^{abc}	2.7 ± 1.30^{abcd}
WHO standard	(1.0×10^5 CFU/mL)	3.0×10^1 CFU/mL)

The results in the table above were presented in means± standard deviation. The superscripts indicate the ranking of the post hoc test using Duncan Multiple Range Test. The means with different superscripts were significantly different from one another down the column while those with the same superscripts were similar ($P\leq0.05$).

Key: UI= Univerisy of ibadan research farm; BO= Bodija cow settlement; AY= Akinyele cow settlement; SO= Sabo cattle settlement

Table 2 shows the identification of bacteria isolates from raw cowmilk samples using morphological and biochemical identification. A total of 42 isolates were isolated from 16 raw cowmilk samples. They were identified as *Staphylococcus aureus* (16), *Escherichia coli* (8), *Salmonella typhimurium* (6), *Shigella dysentriae* (6), *Enterobacter aerogenes* (4) and *Serretia marcescens*(2).

Table 2: Morphological and biochemical identification of bacterial isolates from raw cowmilk samples.

I	G	M	S	Ca	Co	U	O	In	M	V	Gl	La	Su	Fr	Ma	Ga	Probable organisms
1	+	_	C	+	+	_	_	+	+	_	B	A	A	A	A	A	*Staphylococcus aureus*
2	+	_	R	+	_	_	_	+	_	+	B	A	A	A	A	A	*Escherichia coli*
3	_	+	R	_	_	_	_	_	+	+	B	A	A	A	A	A	*Salmonella typhimurium*
4	_	_	R	_	+	_	_	+	+	+	_	_	B	B	B	_	*Shigella dysentriae*
5	_	+	R	_	_	+	_	+	+	+	B	A	A	A	A	A	*Enterobacter aerogenes*
6	_	+	CB	_	_	+	+	+	+	+	B	B	B	B	B	B	*Serratia marscescens*

Key: += Positive; -= Negative; G= gram's reaction; CB= Coccobacilli; M= Motility; S= Shape; A=Acid production; B= Acid and gas production; C=cocci; R= Rod; I= Isolate; O= Oxidase; In= Indole; Ca= Catalase; Co= Coagulase U= Urease; M= Methyl-red; V= Voges-proskeur; GL= Glucose; La= Lactose; Fr= Fructose; Su= Sucrose; Ma= Maltose; Ga=Galactose

Distribution of bacteria isolates in the raw milk samples as calculated (Table 3) shows that BO_3 and AY_4 are the most contaminated milk samples,having the presence of all the pathogens except *S. marscescens*. *S.aureus* has the highest occurrence present in all the 16 milk samples (100%), *E. coli* was present in 8 out of 16 samples (50%) while *Serratia marcescens* has the least occurrence, occurring in 2 out of the 16 samples (12.5%).

Table 3: Distribution of bacterial isolates in raw cowmilk samples.

Samples	*S.aureus*	*E.coli*	*S. typhimurium*	*S. dysentriae*	*E. aerogenes*	*Serratia marscescens*	Total (%)
UI_1	+	_	+	+	+	_	4(66.6)
UI_2	+	_	+	+	+	_	4(66.6)
UI_3	+	_	_	_	_	_	1(16.6)
UI_4	+	+	+	+	_	_	4(66.6)
BO_1	+	+	_	_	_	+	3(50)
BO_2	+	_	_	_	_	_	1(16.6)
BO_3	+	+	+	+	+	_	5(83.3)
BO_4	+	+	_	_	_	_	2(33.3)
AY_1	+	+	+	_	_	_	3(50)
AY_2	+	_	_	_	_	_	1(16.6)
AY_3	+	_	_	_	_	+	2(33.3)
AY_4	+	+	+	+	+	_	5(83.3)
SO_1	+	_	_	_	_	_	1(16.6)
SO_2	+	_	_	_	_	_	1(16.6)
SO_3	+	+	_	+	_	_	3(50)
SO_4	+	+	_	_	_	_	2(33.3)
Total	16(100%)	8(50%)	6(37.5%)	6(37.5%)	4(25%)	2(12.50)	

Key: += Detectable; -= Not detectable; UI= Univerisy of ibadan research farm; BO= Bodija cow settlement; AY= Akinyele cow settlement; SO= Sabo cattle settlement

The percentage susceptibility(antibiogram) of bacterial isolates from raw cow milk to some antibiotics shows that *S. aureus, E. coli, S. typhi* and *Shigella dysentriae* were resistant to tetracycline,gentamycin and Nitrofurantoin and Nitrofurantoin. For *Serratia marcescens*, all the isolates were 100% susceptible to all the antibiotics except Nitrofurantoin which had 50% susceptibility and Tetracycline which had 100% resistance. It was revealed that all of the *E.coli* isolates were resistance to tetracycline (no single susceptible isolate were found) but susceptible to Ciprofloxacin.

Table 4. Percentage susceptibility (antibiogram) of bacterial isolates from raw cowmilk to antibiotics (%).

Isolate	n	ST	AM	OF	CE	GE	PE	CO	CP	AU	NI	TE	ER	CH
A	16	87.5	87.5	100	75	0	100	81.3	75	NT	NT	0	81.25	50
B	8	100	87.5	100	75	0	81.5	0	100	0	0	0	100	100
C	6	NT	100	100	0	0	100	100	100	0	0	0	100	100
D	6	NT	83.3	100	0	0	100	100	100	0	0	0	100	100
E	4	NT	83.3	100	100	100	100	100	100	0	75	100	100	100
F	2	100	100	100	100	100	100	100	100	100	50	0	100	100

Keys: A-*S.aureus* B-*E.coli* C-*S.typhimurium* D-*S.dysendriae* E-*E. aerogenes* F- *S.marscescens* ST-Streptomycin (10µg), AM-Amoxillin (30µg), OF- Ofloxacin (5µg), CE-Ceftriazone (30µg), GE-Gentamycin (10µg), PE-Pefloxacin (5µg), CO-Cotrimoxazole (25µg), CP-Ciprofloxacin (10µg),AU-Augumentin (30µg), NI-Nitrofurantoin (20µg), TE-Tetracycline (30µg), ER-Erythromycin (5µg), CH-Chloramphenicol (30µg).n- Number of tested bacteria isolate NT- antibiotics are absent in selected disc for gram + ve or gram – ve isolate 0- Resistance (100%).

DISCUSSION

The total bacterial count and coliform count ranged between 0.9×10^6 to 4.5×10^6 CFU/mL and 0.4×10^6 to 4.2×10^6 CFU/mL respectively. All the samples were high compared to the WHO acceptable level of $1.0x10^5$ml and $3.0x10^1$ml respectively. This findings also agree with the high coliform mean average value of $98.88 \pm 7.68 \times 10^8$ CFU/ML obtained in raw cow milk in Zaria Metropolis, Nigeria as reported by Chagwa *et al.,* 2021.This could be attributed to dirty udder conditions, unhygienic milking procedures and location of the cow settlement in side a busy market. In this study, the presence of high bacteria in the milk samples is an indicator of gross contamination indicating low level hygiene maintained during the handling and processing of the milk. Although, a large percentage of the Fulanis' who are the handlers of these animals are illiterate and are not mindful of the possibility of contamination of milk from the kind of water and utensil used during milking and processing. The highest Total bacterial counts recorded in sample BO1 was not significantly different from sample AY3 but significantly different from other samples.The differences could be attributed to differences in location of the sampling and the possibility of contaminants from the surface of the udder, milking utensils, air and milk processors. It was observed that 2 out of the 4 samples collected from the university of Ibadan Research Farm had the least total bacteria and coliform count (0.9±0.03 and 1.0±0.20 CFU/mL respectivly). This could be attributed to a fair level of hygiene practices by milk handlers, use of clean equipment, including washing of udder as compared to other dairy farms.

The results in Table 2 conformed with the work done by Oladipo *et al.* (2016) who reported similar enteric bacteria in milk and milk products. The work differed slightly from the results of Attah *et al.,* 2021 who observed *Bacillus spp* and *Staphyloccocus epidermidis* in their work. The differences could be due to environmental factors, climatic conditions, pH of the milk, health of the aimals or milk handlers.

The high occurrence of *S.aureus* could be as a resuilt of its ubiquitous characteristics such as been present everywhere. It is a normal flora of the human skin, and may colonize the nasopharyngeal region. The presence of this species of bacteria in the milk products could have came from the human handlers and it provide evidence of hygienic compromise because milk is virtually a sterile fluid secreted from alveoli of udder. The presence of *E. coli* and *Salmonella typhi* in these raw milk showed poor hygienic state of the milk products and presents a potential hazard to the consumers. *E. coli* can find its route into milk through faeces, manure and soil (Mosu *et al*., 2013). However, the evidence of fecal contamination was indicated by presence of the coliforms bacteria. Infected udders, contaminated water, poor sanitation practices, contaminated containers and milk handlers themselves may be the source of Salmonella in the raw cow's milk, Since the milk is transported at an ambient temperature.

The resistance of *S. aureus, E. coli, S. typhi* and *Shigella dysentriae* to tetracycline,gentamycin and Nitrofurantoin is in agreement with the findings of Van *et al.* (2007) who reported that *Salmonella* and *E.coli* were resistant to most of the antibiotic used and Lubna *et al.,* 2023 who reported high resistance (72.72%) of staphylococcus aureus isolated from raw milk of lactating dairy cattle to tetracycline.This may be due to persistent and pervasive usage of tetracycline . The treatment of *S. aureus* infection in cattle is widespread. In various countries, farmers routinely use penicillin and tetracycline to treat infection in cattle and this increase their resistant (Jamali *et al.,* 2015).

For Serratia marcescens, all the isolates were 100% susceptible to all the antibiotics except Nitrofurantoin which had 50% susceptibility and Tetracycline which had 100% resistance. *S. aureus, E. coli , S. typhi* and *Shigella dysentriae* were resistant to tetracycline and gentamycin. It was revealed that all of the *E.coli* isolates were resistance to tetracycline (no single susceptible isolate were found) but susceptible to Ciprofloxacin. This is in agreement with the report of Nigatu *et al.* (2017). Nigatu *et al.* (2017) also reported that 44.44% of the *E. coli* isolated from cow milk samples in Modjo town, Ethopia were resistance to Gentamycin which was lower than the current finding. This variation could probably be attributed to the expression of resistant gene by the pathogen which is associated with emerging and re-emerging of the isolates with regards to different agro-ecology (Reuben and Owuna, 2013). .

The sensitivity of *Staphylococcus aureus* to erythromycin, streptomycin and ofloxacin (81.25%, 87.5% and 100% respectively) is in agreement with the report of Doss and Vijayasanthi, (2016). *Serratia marcescens* were resistant 100% to Tetracyclin. This report is in agreement with the report of Asmaa *et al.* (2017). The resistance of most of the bacteria isolate to Gentamycin, Augumentin, Nitrofurantoin and Tetracycline is in agreement to the findings of Abike *et al.,* 2015 who reported resistance of *E.coli* to tetracycline (56.80%) and gentamicin (68.1%). This could be a reflection of the use and misuse of antibiotics. This is not surprising because there is indiscriminate use of these antibiotics in animal production by the Nigerian public (Oloso *et al.,* 2018). The public health implication of this investigation is that antimicrobial resistant strains of pathogenic bacteria may colonize the human population through consumption of contaminated cow milk sold locally and this would lead to failures of chemotherapy among consumers of any of these products.

CONCLUSION

Conclusively, the total bacteria and coliform count of samples exceeded the FAO/WHO acceptable level of $1.0x10^5$ml and $3.0 x10^1$ml respectively. There is occurence of foodborne pathogens in most of the milk samples. Moreover, the antibiogram (antibiotics susceptibility profile) of the bacterial population indicates that most isolates were resistant to Gentamycin, Augumentin, Nitrofurantoin and Tetracycline. This is indicative of poor handling of milk as well as abuse of the antibiotics. It is therefore imperative to educate the milkers and the handlers of the implications of poor hygiene and ensure that microbial safety procedures are taken. Ultimately, the milk testing programs should become components of the quality process, not only at the preservation and supply level, but also at the production level. Again, there should be control of indiscriminate use of antibiotics to prevent antibiotic resistance strain of cow milk pathogens.

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