Isolation and Identification of Bacterial Species Associated with Cow Milk Sold in Some Selected Areas of Bauchi Metropolis, Bauchi State, Nigeria

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Abstract. Milk is well known to be a balanced diet with high nutritional values. Conversely, milk and milk products may serve as potential substrates for the growth and proliferation of a range of bacteria which in turn fatally influences public health concerns. The study was conducted in 50 milk samples collected from apparently healthy cows during the period for isolation, identification of bacterial isolate based on their morphology, staining, cultural, biochemical properties and gram staining to ascertain their identity. The results indicated that *Staphylococcus aureus* (34.58 %) has the highest occurrence, followed by *Bacillus cereus* (19.64 %), followed by *Escherichia coli* (15.89 %), *Salmonella typhi* with (13.08 %), *Pseudomonas aerugnosa* with (7.94 %), *Klebsiella pneumonia* with (5.14 %).

In comparison, *Enterobacter* has the most minor occurrence at (3.74 %). The presence of these organisms could be attributed to dirty and unkempt behaviour of the milking process, handling and storing, which in turn will impose serious health hazards to the immediate community and consumers. To reduce the risk associated with these organisms, all personal hygiene measures and materials involved in the milking process should be sterilised and free from any form of organisms before the final approach.

Keywords: bacteria; Identification; biochemical; hazard.

INTRODUCTION

Milk is an essential part of the human diet, and its consumption has increased in recent years [1]. The essential nutrients in milk, such as vitamins, minerals, protein, lactose and crucial fatty acids, make it a complete food. Raw milk is the product of a cow, sheep or buffalo goat that has not been pasteurised to kill harmful bacteria. This unpasteurised raw milk contains dangerous bacteria such as *Salmonella*, *Escherichia coli*, and many causative *Staphylococcus aureus* and *Listeria genera* food poisoning where damaging effects can come into play for the health of everyone who drinks and eats this raw milk. These bacteria can pose risks to people with weakened immune systems, pregnant women, children and other adult Children and the elderly [2]. Bacteria can get into the milk and contaminate it through various stages such as procurement, processing and distribution. Contamination can also be caused by animal feed, hangers, milk collection materials, Ingredients Added to dairy Products and dairy workers [3, 4]. Microorganisms are always undesirable in cow milk and milk product. These can affect the taste and appearance of milk and the transmission of infectious diseases sick to consumers. Various organisms are introduced into milk through farmers, processors and traders' careless, unsanitary practices. *Salmonella, Staphylococcus, Listeria, Escherichia coli* and coliforms are also detected with milk-borne diseases of cow's milk. These are often isolated from fresh cow milk [5]. The presence of gut bacteria is generally a strong indicator of faecal contamination in water, food, milk, and other dairy products. *Escherichia coli* in food indicates that this possible intestinal microbe and/or toxins may risk public health [6]. Most of these organisms are

free-living, widely distributed in soil, feeds, cows, buffaloes, goats, dairy utensils etc. Contamination of milk and dairy products with pathogens microbes is a global health problem. However, its fatal effects on developing human and animal health countries, including Nigeria, are still solved mainly by research works [7, 8]. Because milk and product ingredients are derived from milk, sufficient to support microbial growth, strict caution is required for imitation and dairy products. Microbiological testing for quality assurance, e.g., consumer safety. The research aimed to isolate and identify bacterial pathogens associated with cow milk sold within Bauchi, Bauchi State.

MATERIALS AND METHOD

Sample Collection. A total of fifty samples of cow milk were collected from restaurants within Bauchi, Bauchi State. The samples were collected using a sterile cotton wool Swab and then taken to the laboratory for immediate analysis as described by [9].

Materials and Sterilisation. All materials used were adequately and appropriately sterilised before and after use. Glass wares, such as test tubes, conical flasks, pipettes, etc., were thoroughly washed with detergents and adequately rinsed with water drained. They were wrapped in aluminium foil and sterilised in a hot air oven at 170 °C for 1 hour. Prepared media and distilled water were autoclaved at 121 °C for 15 minutes. Like the inoculating loop, metal equipment was heated to redness in an open flame before and after use. Before analysis was made, the laboratory bench was always cleaned using 90 % ethanol for disinfection. Every isolation and injection was done near the flame to reduce contamination of the agar plate tubes.

Preparations of Culture Media. The nutrient agar was prepared according to the manufacturer's instructions and Sterilised by autoclaving at 121 °C for 15 minutes.

Identification of Isolated Bacteria. According to standard laboratory methods, the isolated organisms were identified by their colony morphology, microscopic examination, motility study, and relevant biochemical tests. The biochemical characterisations are the indole test, Methyl-Red test, Vogues-Proskauer test Citrate utilisation test, Catalase test, Coagulase Test and Oxidase Test using the Standard method given by [10, 11].

Solution of Organisms. Bacterial colonies developed on the primary plate will be cultured into freshly prepared nutrients and McConkey agar media, respectively, by streaking. The vessel will be incubated for 24 hours, and pure isolates will be stored on an agar slant at a refrigerated temperature for further testing [12].

Cultural Characteristics. The colonies that developed on a pure culture plate will be observed for nature of elevation, pigmentation shape, edge, surface and size [13].

Gram Staining. A drop of saline was placed on a clean glass slide. An inoculating wire drop was sterilised by flaming and allowed to cool. The wire loop was used to lick the colony, and a smear was made in the side, allowing air to dry air. It was quickly passed over a flame to fix the organisms. The streak was flooded with crystal violet for 30 seconds and then washed with water, logos iodine, was drained off and rinsed under tap water. The slide was saturated with iodine, washed with water, and later flooded with safranine for 30 seconds. Finally, the fall was washed with water to allow it to air dry and last viewed under a microscope with an oil immersion objectives lens [14, 15].

RESULTS AND DISCUSSION

Upon cultural, morphological and biochemical examination of bacterial species such as *Escherichia coli*, *Enterobacter*, *Staphylococcus aureus*, *Bacillus Cereus*, *Pseudomonas*, *Klebsiella* and *Salmonella* were isolated.

The present research aimed to isolate and identify bacteria associated with cow milk sold within Bauchi, Bauchi State. A total of Forty Bacterial isolates were recovered. The bacterial isolates were identified and characterised based on their colonial morphology and gram-staining characteristics (Table 1–4).

Of the isolates recovered, 33 were gram-positive bacteria, while seven were gram-negative. The four bacterial isolates were characterised based on biochemical tests rather than the morphological and colonial features alone.

Table 1 – Cultural Characteristics of Bacteria Isolates from the Cow Milk Samples

Shape form	Colour	Morphological Appearance	Gram Staining	Suspected Organisms
Circular	Metallic Green	Entire	Raised	*Escherichia coli*
Irregular	Lack of Sheen	Entire	Convex	*Enterobacter*
Spherical	Golden Green	Entire	Raised	*Staphylococcus aureus*
Spherical	Swampy Growth	Undulate	Convex	*Bacillus cereus*
Circular	White Media or Light Green	Entire	Flat	*Pseudomonas aeruginosa*
Irregular round	Shiny Mucoid Colony	Undulated	Flat	*Klebsiella pneumonia*
Circular	Colourless Colony	Entire	Flat	*Salmonella typhi*

Table 2 – Morphological Characteristics of Bacterial Isolates

Name of Bacteria	Gram Staining	Shapes	Arrangement
Escherichia coli	Negative	Rod	Single
Staphylococcus aureus	Positive	Cocci	Grape-like cluster
Pseudomonas aeruginosa	Positive	Cocci	Pair short chain
Klebsiella pneumonia	Negative	Rod	Single/pair
Salmonella typhi	Negative	Rod	Single
Enterobacter	Negative	Rod	Single
Bacillus cereus	Positive	Rod	Single

Table 3 – Biochemical Characteristics of the Bacterial Isolates

IN	MR	VP	CI	CA	OX	CO	MF	LF	Suspected Organisms
-	+	+	-	+	-	+	+	-	*Staphylococcus aureus*
-	-	-	-	+	+	-	+	-	*Bacillus cereus*
-	+	-	+	+	-	-	+	-	*Salmonella typhi*
+	+	-	-	+	-	-	+	-	*Klebsiella pneumonia*
+	+	-	-	+	+	-	+	+	*Escherichia coli*
-	-	-	+	+	+	-	-	-	*Pseudomonas aeruginosa*
-	+	+	-	+	+	+	-	-	*Enterobacter*

Table 4 – Number of Percentage Occurrence of the Bacterial Isolates

Isolates	Number of occurrences	% Occurrence
Staphylococcus aureus	74	34.58
Bacillus cereus	42	19.63
Salmonella typhi	28	13.08
Pseudomonas aeruginosa	17	7.94
Escherichia coli	34	15.89
Klebsiella pneumonia	11	5.14
Enterobacter	8	3.74

The results of the occurrence distribution of different bacterial isolates are presented in Table 4. A total of 50 milk samples were examined for the isolation of bacteria. Results are: 42(19.63%) were positive for *Bacillus cereus*, 74(34.58 %) isolates were *Staphylococcus aureus*, 34(15.89 %) for *Escherichia coli,* 28(13.08 %) were *Salmonella typhi*, 17(7.94%) were for *Pseudomonas aerugnosa*, 11(5.14 %) were for *Klebsiella pneumonia* and 8(3.74 %) were for *Enterobactrer*.

The incidence of different types of bacteria isolated from milk sample correlate with the findings of [16, 17] with slight variation. A high occurrence of *Staphylococcus aureus* in cow milk is a significant bacterial foodborne disease. Its

presence in food samples could result from the dirty and unkempt behaviour of the food handlers. Symptoms could include vomiting of the bacterial toxin [18]. The results of this study showed a close link with a previous study conducted by [19]. According to the current study results, the presence of Bacteria in the studied samples is sufficiently indicative of severe health risk upon consumption of the dairy products tested unless appropriate bacteriological measures are not taken. Standard legislative actions are thus of major clinical significance.

CONCLUSIONS

It was carried out in line with the previous study findings on the Bacteriological analysis of cow milk and milk products. The present study revealed the presence of a range of pathogenic bacteria which were of public health concern. Maintaining proper hygiene while handling and processing cow milk and adequate application of sterilisation procedures such as pasteurisation and UHT could ensure food quality and, most importantly, consumers' safety.

Finally, it can be concluded that most examined cow milk collected from local markets in Bauchi, Bauchi State, were contaminated with pathogenic bacteria, which indicates poor health measures adopted during milking, manufacturing, handling and distribution of cow milk. The following guidelines should be taken into consideration to improve milk quality; raw milk must be produced from healthy animals, proper cleaning of animal's udder before milking, cleaning and disinfection of all tools and dairy equipment and pasteurisation/boiling milk required and refrigeration of milk, to about 7 to 10 °C and milk products.

Conflict of interest

The authors declare no conflict of interest.

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