

Study of milk and dairy products *Staphylococcus* contamination and antimicrobial susceptibility sold in local markets around Kabul University

Sayed Arif Ahmadi^{1*}, Mohammad Zaher Sakha¹, Sohaila Ebadi¹, Ashok Kumar Panda⁴

^{1,2,3}Department of food technology & hygiene Faculty of Veterinary Science, Kabul University, Jamal mina, Kabul, Afghanistan

⁴Department of Veterinary Public Health and Epidemiology, Dr. G.C. Negi College of Veterinary and Animal Sciences, CSK-Himachal Pradesh Agricultural University, Palampur-176062, Himachal Pradesh, India

*Corresponding author: Sayed Arif Ahmadi (sayedarif.ahmadi@yahoo.com)

Abstract


This research aimed to evaluate *Staphylococcus aureus* contamination from milk and its derivatives available in local dairy sale centers around the Kabul University located in Kabul city. Samples were included raw and pasteurized milk, cheese, ice cream, yogurt, custard, and dough (native product). The mentioned products were collected and then using the conventional method was tested in the laboratory. 70 samples were tested, from tested only 18 samples were presented *Staphylococcus aureus*, as these isolates had a positive reaction for coagulase. The disk diffusion method plus 8 antibiotics which are commonly used in the Veterinary section were used for antimicrobial sensitivity reaction. *Staphylococcus* isolated samples showed resistance against more than 3 antibiotics, maximum resistance was to Nalidixic acid (50%) followed by Ciprofloxacin (44.44%), Norfloxacin (27.77%), Erythromycin and Methicillin (27.77%), and Tetracycline (22.22%). All the isolates susceptibility was high to Polymyxine and resistant to Ampicillin (100%). As we found in our study the biological quality of tested samples sold around Kabul University were no satisfactory as well as resistance against the mentioned antibiotics except two antibiotics. The contaminated products could be a potential risk to humans.

Keywords: Pathogenic, *Staphylococcus Aureus*, Multidrug-resistant, Public Health Concern, Ice Cream.

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Transparency: The authors confirm that the manuscript is an honest, accurate, and transparent account of the study was reported; that no vital features of the study have been omitted; and that any discrepancies from the study as planned have been explained.

Ethical: This study follows all ethical practices during writing.

1. Introduction

Milk is a very valuable and main part of human food and plays a noticeable role in the Afghanistan meal, but raw milk has a variety of micro-organisms and is providing media for bacteria growth [1]. Milk and its products are suspected and imagine being *Staphylococcus aureus* bacteria reservoir for consumers [2]. From the other point of view, beneficial bacteria take part in the production of sour foodstuffs such as cheese, yogurt, beer, wine, and other products. Food as a source of pathogenic organism would transfer disease to a human. Almost 5 percent of Staphylococcal outbreaks occurred due to consumption of milk and milk derivatives in Europe [3]. *Staphylococcus aureus* occurs in men and animals with different illness stages, moderate to severe problems. So, public health concerning milk and milk products and foods for

Staph. Contamination, growth, production of the toxin, and transmission to consumers. Many pathogens such as Staph Spp., E. coli, and Salmonella. Spp. are listed as the top food-borne disease worldwide in research findings. Hence, *Staphylococcus* is a significant considerable pathogen that mostly originated from foods; thus, milk and its products commonly contain these bacteria whenever hygienic circumstances are not observed during, milking, collection, processing, or other handling steps. Milk could be a good source and nutritive media for *Staphylococcus* survival and growth. This bacterium produces a heat-resistance toxin that sustains its chemical structure while exposed to a higher temperature. Many outbreaks of *Staphylococcus aureus* are present in researcher's articles in either raw or pasteurized milk [4-6].

Nonetheless, in Afghanistan information as it appears that dairy systems including all steps are not efficiently handled. Applying hygienic standards restrain food-borne events. On the other hand, bacterial antibiotic resistance arising globally. Among methicillin-resistant *Staphylococcus aureus* could be a serious problem in terms of preventive medicine [7]. Antibiotics generally are used for three purposes, prevention, growth promoters, and treatment. Antibiotic resistance out-bursting from its irregular utilization in both animal and human medicine. Moreover, the reproduction of *Staphylococcus* in dairy products while contaminated with this bacteria, antibiotic-resistant Staph. Against penicillin is another challenge among food chain and health issues [8-10]. In the middle of the 20th century while the 2nd war period, the penicillin antibiotic was used as a broad-spectrum agent for wounded people. Soon penicillin bacterial resistance gradually has been emerged [11]. The phenomenon of this problem was present in Vietnam War then pursued by Afghanistan and Iraq battles and strongly existent in human life [12, 13]. Also, the consumer is contaminated by either zoonosis disease while contacted with animals or taking animal production with poly drug-resistant bacteria. So, the increase of methicillin-resistant *Staphylococcus aureus* MRSA bacteria in the environment is a considerable issue of health care authority to restrain human health from contamination [14, 15].

Lake of research facilities effects on the applying and research results. So, in Afghanistan systematic and many reports have not been conducted in this area. One side is fewer data of antimicrobial-resistant researches and another side is public health concerns. Therefore, this research achieved to identify and separate Staph. aureus in dairy products sold in Kabul University of Kabul city.

2. Materials and Methods

2.1. Sample Collection

Different dairy products which are stated in the Table 1 from many dairy sellers around Kabul University were collected. Samples collection and tests performed from March through August 2019. Seventy samples were aseptically collected in sterilized tubes and bags and then transferred to an icebox to restrain and stop bacterial production. Samples were immediately carried to the laboratory for further process.

2.2. Bacteria Isolation and Identification

For enrichment of bacteria into nutrient broth appropriate quantity of sample was transferred into tubes and incubated for 24 hours. After 24 hours of incubation, samples were cultured onto *Staphylococcus* medium 110 agar (Oxoid, England) and Mannitol salt agar (HiMedia, India) which are selective media for Staph. Growth, then incubated at 37°C for 2 days. The grown bacteria to get pure colonies then subculture on the Mannitol salt agar. For morphological confirmation gram stain and subculture on the blood, agar was performed. Biochemical tests like catalase and coagulase tests also were done for additional identification Table 2.

2.3. Antimicrobial Sensibility Test

The disk diffusion method introduced by Dutta, et al. [16]. S. Dutta et al. was followed for all positive coagulase colonies to determine the sensitivity of isolated samples. These antibiotics were included in the tests: norfloxacin (30 µg), methicillin (5µg) ciprofloxacin (5µg), nalidixic acid (5µg) tetracycline (30µg), polymyxin (10µg), erythromycin (15µg), polymaxine (30µg), Streptomycin (30µg) and ampicillin (10µg) a production of Oxoid and Himedia [16]. Inhibition of observed zones and their diameter was accounted for and interpreted according to the given standards CLSI, clinical laboratory standards institute [17].

3. Results and Discussion

The all tested samples such as 18 milk and milk products were positive for *Staphylococcus* species. Using isolation and identification methods Table 1. Raw milk showed the highest percentage (7.14%) of *staphylococcus* contamination followed by ice-cream (5.71%), Cheese and custard (4.28%), Yoghurt (2.85%), Doogh (1.42%) and pasteurized milk Nil. The accuracy of Staph. colony confirmed either morphologically and or colony's appearance on the selective media. *Staphylococcus* spp. when grown on the 110 agar produces orange color that was confirmed based on bacteriological analytical manual 5th edition AOAC, Washington DC, report [18]. Staph. aureus in Mannitol salt agar developed media color from red to yellow the yellow pigment colony production in this media is another characteristic of Staph. aureus during the media fermentation. On gram stain, the colonies look like violet round-shaped and similar grape form while seen under a normal microscope [19]. According to the planned schedule, all positive samples biochemically were tested for catalase and coagulase reactions. Then coagulase-positive samples were subjected to culture on the blood agar Table 3. Staph. aureus in catalase test produced bubble in the presence of hydrogen peroxide and in coagulase test the colony changed to curd like clot/mass [18].

Hummerjohann, et al. [20]. stated in their findings that the 5(7.14%) *Staphylococcus aureus* during culture and biochemical tests, positive samples revealed β -hemolysis when cultured on the blood agar which contained 5% of sheep blood [20]. Likewise, in the present study, all 18 samples showed β -hemolysis. Eight antibiotics were used for antibiotic sensitivity reaction for those were coagulase positive. Among eight antibiotics coagulase + samples showed more sensitivity against ampicillin and weaker reaction for polymyxin. The reaction for erythromycin was in the middle stage. In raw milk and ice cream samples, the *Staphylococcus aureus* showed stability versus antibiotics which are commonly used in the Veterinary section. Staph. aureus coagulase positive was more stable against methicillin (27.77%) According to the [10] and [20] findings, as their result was remarkably higher than our test. 16% of Staph. aureus samples were multidrug-resistant according to the antibiotic sensitivity reaction. The phenomenon was that 100% of isolates were resistant to ampicillin as the Table 4 reports were almost near to the present study [10, 20].

Table-1.

Total samples positive for *Staphylococcus* in different milk and milk products around Kabul University.

No.	Types of food item	No of samples collected	No of samples positive for <i>Staphylococcus</i>	No of samples positive for <i>Staphylococcus</i> in (%)
1	Raw milk	10	5	(7.14%)
2	Pasteurized milk	10	Nil	Nil
3	Ice Cream	10	4	(5.71%)
4	Cheese	10	3	(4.28%)
5	Yoghurt	10	2	(2.85)
6	Doogh	10	1	(1.42%)
7	Custard	10	3	(4.28%)
	Total	70	18	25.68%

Out of a total sample of 70, 18(25.71%) samples of milk and dairy milk revealed the presence of *Staphylococcus* Table 1. Raw milk showed the highest percentage of (7.14%) of *Staphylococcus* contamination followed by Ice Cream (5.71%) and cheese Custard (4.28%). A similar study designed to isolate specification of *Staphylococcus aureus* from Milk and Dairy derivatives observed *Staphylococcus aureus* contamination in raw milk, yogurt, roshmalai, Burhani, and cheese. Haque, et al. [21]. detected 8 isolates (57%) were sold in some local Markets in Bangladesh [21]. The other study also reported by in Bangladesh shows the presence in raw milk sample 12 (25.53%) samples were confirmed as *Staphylococcus aureus* [22].

Table-2.

Biochemical specifications of the insulted *Staphylococcus aureus* from raw milk and dairy product.

Total No. of specimen	No. of <i>S. aureus</i> positive	No. of catalase positive samples	No. of coagulase positive samples	No. of blood agar hemolysis isolate
70	18	18	18	15

Table-3.

Morphological, cultural, and gram staining specifications of the isolated *Staphylococcus aureus*.

Cultural characteristics				Morphological and Staining characteristics
Nutrient broth	<i>Staphylococcus</i> Medium No. 110	Mannitol salt agar	Blood agar	were purple cocci like appearance and grape shape form.
Turbidity was appeared during one night incubation.	Colony showed round shape with orange color	The media color changed with fermentation; the circular colony was yellow to orange.	Beta hemolysis presented with small circular colony	

Table-4.Results of antibiotic susceptibility test of isolated *Staphylococcus aureus*.

Antimicrobial medicines	No. of samples with their antibiotic sensitivity outline (%)		
	Resistant	Intermediate	Sensitive
Ampicillin	18 (100%)	Nil	Nil
Erythromycin	5 (27.77%)	4 (22.22%)	10 (55.55%)
Tetracycline	4 (22.22%)	2 (11.11%)	12 (66.66%)
Methicillin	5 (27.77%)	3 (16.60%)	9 (50%)
Nalidixic Acid	9 (50%)	Nil	9 (50%)
Ciprofloxacin	8 (44.44%)	(11.11%)	8 (44.44%)
Norfloxacin	7 (38.88%)	3 (16.60%)	8 (44.44%)
Polymyxine	Nil	Nil	18 (100%)

5. Conclusion

The biological quality of unpasteurized milk and dairy product marketed around Kabul University at the time of this study does not satisfy the requirements set by the Afghanistan National Standards Authority (ANSA) for the quality of milk. So far, many strains are isolated from animal and animal products such as milk and milk derivatives worldwide. Our study focused on different types of Staph. Strains, those contaminated milk and milk derivatives sold in Kabul city of Afghanistan. While the hygienic standards are not observed properly and completely on dairy plants or milk collection centers, hence pathogens like Staph. aureus transferred through itself and its products to the final yield. The findings of this study were a window to know the dairy section, however further studies are required to understand dairy and dairy products status, in lightening of current outcome a strategy for better and safe handling of milk need to be developed. We concluded that the present study emphasizes the necessity for applying and keeping good hygienic practices in the shop and markets to ensure public health safety. It also underlines the need for some kind of regulatory mechanism and periodic screening of food channels for potential foodborne pathogens. The indiscriminating use of antibiotics in animal and human medicine may have underwritten the antibiotic-resistant strains of *staphylococcus* available in dairy samples sold around Kabul University which is a cause of public health apprehension.

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