Research Article

Archives of Clinical and Medical Microbiology

Prevalence of Microbiological Contaminants in Milk Samples in Kathmandu, Nepal

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Submitted: 23 Jan 2023; **Accepted:** 10 mar 2023; **Published:** 30 Mar 2023

Citation: Rizal, S., Amatya, N., Dhungana, G., Saud, B., Chand, K., et. al. (2023). Prevalence of Microbiological Contaminants in Milk Samples in Kathmandu, Nepal. *Archives Clin Med Microbiol, 2* (1), 07-10.

Abstract

Contaminated milk is responsible for mild to life threatening disease in humans. This study was aimed to access the microbiological contaminants present in milk samples collected from different sites in Kathmandu. A cross-sectional study was conducted between January and July 2019. A total of 90 milk samples were collected (30 farm milk, 30 dairy milk and 30 pasteurized packaged milk) from local farms and outlets. All the collected samples were processed for bacterial and fungal growth in standard in-vitro conditions. Identification was done via colony characteristics, biochemical tests and staining property. Identified bacterial isolates were tested for antibiotic susceptibility. Out of 90 samples, 41.1% were contaminated with bacteria and 46.6% showed fungal growth. Overall prevalence of S.aureus and E.coli was 26.6% and 14.4% respectively. 25.0% of the S.aureus isolates were methicillin-resistant. In addition, the prevalence of Aspergillus spp. was 26.6% and Penicillium spp. was 20.0%.The highest mean bacterial (279.13 x10 cfu/ml) and coliform count (175.53x10 cfu/ml) was recorded in dairy milk. Also, six samples showed polymicrobal growth. In conclusion, dairy milk samples are highly contaminated than farm and pasteurized packaged milk.

Keywords: Milk; Pasteurized Packaged Milk; MRSA; Mean Bacterial Count; Mean Coliform Count

Introduction

Milk is an excellent source of nutritional supplements such as protein, lactose, fat, minerals and vitamins. According to survey of the Food and Agriculture Organization of the United Nations in 2020/21, annually 75 litres of milk is consumed by a single person in Nepal [1, 2]. Generally, people consume either unpasteurized milk directly from farm and dairy shops or pasteurized packaged milk from different outlets. Naturally milk contains beneficial lactic acid bacteria such as *Streptococcus, Lactococcus, Lactobacilli, Bifidobacteria, Enterococcus*, and *Pediococci* that produce several secondary metabolites which are advantageous to human. In addition to these useful bacteria, milk serves as a rich source of nutrients for growth of pathogenic bacteria, which is influenced by several other factors such as temperature, pH, humidity, etc. [3]. It was observed that dairy products were found to be contaminated with pathogens such as *Mycobacterium bovis, Campylobacter spp.,* non-typhoidal *Salmonella enterica* and *Brucella spp*., *Cryptosporidium spp*., Shiga-toxin producing *Escherichia coli and Toxoplasma gondii*.

In developing countries, food-borne diseases were associated with disability adjusted life years that was 20 per 100,000 individuals [4]. A study from Iran revealed that *E. coli, S. aureus, L. monocytogenes,* and *Salmonella spp.* isolated from raw milk were resistant to drugs. Simultaneously, poor sanitation and hygiene, lack of advance facilities in farming industry, and inadequate training are responsible for the microbiological contamination of milk. Furthermore, irrational use of antibiotics in dairy industry is escalating drug resistant microorganisms which are responsible for public health burden. Therefore, this study is designed to investigate the prevalence of microbial contaminants in the milk samples collected from different sites in Kathmandu.

Materials and Methods

A cross-sectional study was conducted between January and July 2019 on 90 milk samples (30 farm milk, 30 dairy milk and 30 pasteurized packaged milk) collected from different farms, dairy shops and local stores in Kathmandu. Exactly, 5ml of milk samples were collected in a sterile leak-proof screw-capped container and transported to Department of Medical Laboratory Technology, Janamaitri Foundation Institute of Health Sciences, Lalitpur in an icebox. Collected samples were subjected to ten-fold serial dilution in sterile peptone water. Then, pour plate culture was performed in Violet Red Bile Lactose Agar (VRBA) and Plate Count Agar (PCA) and incubated for 24 hours at 37°C to estimate total coliform count and total bacteria count respectively. Also, undiluted milk samples were streaked on Mannitol Salt Agar (Hi-Media), MacConkeyAgar (Hi-Media) and Sabouraud Dextrose Agar with Chloramphenicol (Hi-Media) for bacterial and fungal isolation at standard in-vitro conditions. Further,

gram staining, biochemical tests and Lactophenol cotton blue staining were performed for identification of the isolates. Antimicrobial sensitivity test was then performed via Kirby-Bauer method using the following antibiotic discs: Ampicillin(10µg), Amikacin(30µg), Gentamicin(10µg), Chloramphenicol(30µg), Cotrimoxazole(2µg), Levofloxacin(5µg),Imipenem(10µg),Piperacillin-Tazobactam(100/10µg), Cefotaxime(30µg), Cefoxitin(30µg), Erythromycin(15µg). Data were entered in Statistical Package for Social Sciences version 20 for further analysis and interpretation. Ethical approval was taken from Nepal Health Research Council, Kathmandu, Nepal with reference number 3339.

Results

In overall, out of 90 milk samples, microbiological prevalence was found to be 79 (87.7%), where 37 (41.1%) samples were positive for bacterial growth and 42 (46.6%) samples were positive for fungal growth. S.aureus was the most commonly found bacteria in farm milk (50.0%) followed by dairy milk (37.5%). However, *E. coli* was mainly prevalent in pasteurized packaged milk (46.1%). *Aspergillus spp*. and *Penicillium spp.* were mainly present in dairy milk samples, details are shown in Table 1.

Table 1: Prevalence of selected microbial contaminants

Isolates	FM (n,%)	DM (n,%)	PPM (n,%)
S. aureus (n=24)	12 (50.0)	9 (37.5)	3 (12.5)
E. coli (n=13)	3 (23.1)	4 (30.8)	6 (46.2)
Aspergillus spp. (n=24)	5 (20.8)	12 (50.0)	7 (29.2)
Penicillium spp. (n=18)	6 (33.3)	8 (44.4)	4 (22.2)

Keys: FM- Farm milk; DM-Dairy milk; PPM-Pasteurized packaged milk.

The mean bacterial and mean coliform count significantly reduced with dilution in pasteurized packaged milk samples compared to farm and dairy milk samples. Also, polymicrobial growth was observed in six samples, abundantly in pasteurized packaged milk sample. The mean bacterial count was highest in dairy milk ($70.13 \times 10^6 \pm 118.08$) followed by farm milk ($39.83 \times 10^6 \pm 77.39$) in contrast to pasteurized packaged milk ($3.30 \times 10^6 \pm 10.84$). Also, the mean total coliform count was comparatively higher in dairy milk ($13.13 \times 10^6 \pm 54.90$) than pasteurized packaged milk ($2.67\times 10^6 \pm 10.87$) while farm milk had $2.20\times 10^6\pm 5.26$, details are shown in table 2.

Table 2: Mean bacterial and coliform count in milk samples

Milk dilution	Mean Bacterial Count (cfu/ml) in PCA			Mean Coliform Count (cfu/ml) in VRBA		
	FM	DM	PPM	FM	DM	PPM
$\times 10^1$	238.27	279.13	220.93	151.53	175.53	104.77
$\times 10^2$	197.40	249.27	163.17	105.03	115.17	42.50
$\times 10^3$	156.03	211.03	86.47	45.10	63.43	17.37
$\times 10^4$	123.67	132.93	34.50	17.47	45.23	7.93
$\times 10^5$	61.73	94.67	10.03	6.30	20.50	4.97
$\times 10^6$	39.83	70.13	3.30	2.20	13.13	2.67

Notes: FM: Farm milk, DM: Dairy milk, PPM: Pasteurized packaged milk.

Out of 24 *S. aureus* isolates, 25.0% isolates were methicillin-resistant *Staphylococcus aureus* (MRSA). Also, *S.aureus* isolates from farm milk and dairy milk were mainly resistant to Ampicillin and Chloramphenicol. Interestingly, out of three *S.aureus* isolates from packaged pasteurized packaged milk, one was MRSA. Besides, *E.coli* was mainly resistant to Cefotaxime and Ampicillin in pasteurized packaged milk as shown in table 3.

Table 3: Antimicrobial resistance prevalence

Antimicrobials	*Staphylococcus aureus (n=24)*			*Escherichia coli (n=13)*		
	FM (n,%)	DM(n,%)	PPM(n,%)	FM(n,%)	DM(n,%)	PPM(n,%)
Ampicillin	5(20.8)	3 (12.5)	1(4.1)	0	0	1 (7.6)
Amikacin	0	0	0	0	1 (7.6)	0
Gentamicin	0	0	0	0	0	0
Chloramphenicol	1 (4.1)	1 (4.1)	0	0	0	0
Levofloxacin	0	0	0	1 (7.6)	0	0
Cefoxitin	3(12.5)	2 (8.3)	1(4.16)	NT	NT	NT
Erythromycin	0	0	1(4.16)	NT	NT	NT

Clindamycin	0	0	0	NT	NT	NT
Cotrimoxazole	NT	NT	NT	1 (7.6)	0	0
Imipenem	NT	NT	NT	0	0	0
Piperacillin-Tazobactam	NT	NT	NT	0	1 (7.6)	0
Cefotaxime	NT	NT	NT	0	1 (7.6)	2 (15.3)

Keys: NT- Not tested

Discussion

In this study, 87.7% milk samples were contaminated with microorganisms where 41.1% samples were positive for bacterial growth. Similarly, a study from Northern Ethiopia showed 52% prevalence of microbial contaminants in milk samples [5]. Milk nutrients act as an enriched medium for microbial growth. Usually milk is free from pathogenic bacteria. However, during handling, processing, transportation and storage as well as non-compliance to the hygiene practices lead to microbial deterioration of milk. S.aureus was the most commonly isolated species in our study with a prevalence rate of 26.6% (24/90), which was similar to a study done in Eastern Ethiopia where the prevalence was 24.2%. However, E.coli was the most commonly isolated species 58.0% which is in contrast to our study (14.4%) [6]. In an effort to measure the level of microbiological quality and practices associated with handling, coliform counts are considered to be important indicators. It has been indicated in many studies that coliform and Staphylococcus are most commonly isolated species because of their sufficient attainability in the animal body as well as the environment. Since S.aureus is a habitual bacteria which can arise from various parts of warm blooded animals, as well as from faeces, soil and fresh water, this might have contributed to higher count in our study. In Greece, a study revealed 47.8% prevalence of S. aureus of which 4.1% was MRSA and had ability to produce enterotoxin and biofilm [7]. In our study MRSA prevalence was 25.0%. Likewise, the high coliform count can be attributed to the use of substandard water quality at their source, detrimental milking practice, unsanitary utensils or milking equipment as well as poor conditions of storage. In this study, Aspergillus spp. (26.6%) was most commonly isolated followed by Penicillium spp.(20.0%). A similar study from Egypt has found the opposite result, where Penicillium spp. were most commonly isolated [8]. Also, Cladosporium spp. were isolated from milk products of China [9]. Besides this, Mucor spp. was also found in milk products [10]. It was also noted that the presence of pathogenic fungus was found mostly in the raw milk of cow, ewe and goat [11]. Hence, the fungal contamination in milk and its products may be due to inadequate cleaning practices, improper sanitation and lack of proper storage, transportation and packaging techniques.

In a similar study conducted in Kathmandu valley, the mean total bacterial count of pasteurized, unpasteurized and raw milk was 1.2X106cfu/ml, 2.3 X 107 cfu/ml and 2.0 X 107 cfu/ml respectively which was similar to our study [12]. Similarly, the mean total coliform count of pasteurized, unpasteurized and raw milk was higher in our study. A study conducted in farm milk and its products in Poland found that the isolated bacteria were mainly resistant to penicillin (50.8%), chloramphenicol (5.7%) and tetracycline (4.1%) [13]. In our study, bacterial isolates were mainly resistant to ampicillin and chloramphenicol. A study from Connecticut has found that raw milk bacterial isolates were resistant to streptomycin, penicillin, ampicillin, neomycin, chloramphenicol, polymyxin, and tetracycline. In addition to this, bacterial isolates from pasteurized dairy products were resistant to polymyxin, penicillin and ampicillin [14]. However, in our study, isolates from pasteurized packaged milk were resistant to ampicillin, cefoxitin, erythromycin and cefotaxime. In our study 25.0% S. aureus were MRSA. A similar study have found that 33.2% of S. aureus isolates were MRSA [12]. The high numbers of drug resistant bacterial isolates were due to the irrational use of antibiotics in livestocks [15]. Furthermore, milk contains many beneficial probiotic bacteria. They produce beneficial secondary metabolites which exhibit antimicrobial activity against several human pathogens [16]. In addition, molecules from probiotic bacteria have shown anti-tumorigenic activity [17, 18]. The limitations of this study are: only selected bacteria were processed, anti-fungal sensitivity test was not performed due to limited laboratory facilities and genomic analysis was not done.

Conclusion

Microbiological quality of milk samples does not meet the requirements of the standard guidelines by Food and Drug Administration. Dairy milk samples are mainly contaminated with bacteria and fungi rather than farm milk and pasteurized packaged milk samples.

Funding: None.

Conflict of Interest: No conflict of interest.

References

1. Nepal short of 13 liter per capita per year to be self-reliant in milk - myRepublica - The New York Times.
2. Peters, B. M., Jabra-Rizk, M. A., O'May, G. A., Costerton, J. W., & Shirtliff, M. E. (2012). Polymicrobial interactions: impact on pathogenesis and human disease. Clinical microbiology reviews, 25(1), 193-213.
3. Hickey, C. D., Sheehan, J. J., Wilkinson, M. G., & Auty, M. A. (2015). Growth and location of bacterial colonies within dairy foods using microscopy techniques: a review. Frontiers in microbiology, 6, 99.
4. Grace, D., Wu, F., & Havelaar, A. H. (2020). MILK Symposium review: Foodborne diseases from milk and milk products in developing countries—Review of causes and health and economic implications. Journal of dairy science, 103(11), 9715-9729.
5. Berhe, G., Wasihun, A. G., Kassaye, E., & Gebreselasie, K. (2020). Milk-borne bacterial health hazards in milk pro-

duced for commercial purpose in Tigray, northern Ethiopia. BMC Public Health, 20, 1-8.
6. Reta, M. A., Bereda, T. W., & Alemu, A. N. (2016). Bacterial contaminations of raw cow's milk consumed at Jigjiga City of Somali Regional State, Eastern Ethiopia. International Journal of Food Contamination, 3(1), 1-9.
7. Papadopoulos, P., Angelidis, A. S., Papadopoulos, T., Kotzamanidis, C., Zdragas, A., Papa, A., ... & Sergelidis, D. (2019). Staphylococcus aureus and methicillin-resistant S. aureus (MRSA) in bulk tank milk, livestock and dairy-farm personnel in north-central and north-eastern Greece: Prevalence, characterization and genetic relatedness. Food microbiology, 84, 103249.
8. El-Shafe, H. M., Abo-Zaid, K. F., & Madkour, A. M. (2017). Effect of microbial contamination with fungi, aflatoxin m1 and enteric gram negative bacteria on milk and some dairy products. Egyptian Journal of Agricultural Research, 95(4), 1871-1889.
9. Pei, X., Tekliye, M., & Dong, M. (2021). Isolation and identification of fungi found in contaminated fermented milk and antifungal activity of vanillin. Food Science and Human Wellness, 10(2), 214-220.
10. Moubasher, A. A., Abdel-Sater, M. A., & Soliman, Z. S. M. (2018). Yeasts and filamentous fungi associated with some dairy products in Egypt. Journal de mycologie medicale, 28(1), 76-86.
11. Delavenne, E., Mounier, J., Asmani, K., Jany, J. L., Barbier, G., & Le Blay, G. (2011). Fungal diversity in cow, goat and ewe milk. International journal of food microbiology, 151(2), 247-251.
12. Acharya, S., Bimali, N. K., Shrestha, S., & Lekhak, B. (2017). Bacterial analysis of different types of milk (pasteurized, unpasteurized and raw milk) consumed in Kathmandu Valley. Tribhuvan University Journal of Microbiology, 4, 32-38.
13. Rola, J. G., Czubkowska, A., Korpysa-Dzirba, W., & Osek, J. (2016). Occurrence of Staphylococcus aureus on farms with small scale production of raw milk cheeses in Poland. Toxins, 8(3), 62.
14. Hankin, L., Lacy, G. H., Stephens, G. R., & Dillman, W. F. (1979). Antibiotic-resistant bacteria in raw milk and ability of some to transfer antibiotic resistance to Escherichia coli. Journal of food protection, 42(12), 950-953.
15. Adhikari, S., Saud, B., Paudel, G., & Bajracharya, D. (2019). Emergence of antimicrobial drug resistant bacteria in Nepal: a current scenario. Proteomics Bioinformatics Current Res, 1(1), 31-33.
16. Saud, B., Pandey, P., Paudel, G., Dhungana, G., & Shrestha, V. (2020). In-vitro antibacterial activity of probiotic against human multidrug resistant pathogens. Archives of Veterinary Science and Medicine, 3(1), 31-39.
17. Saud, B., & Adhikari, S. (2018). Probiotics: A promising anti-cancer agent. assessment, 88(11), 1744-1748.
18. Shamekhi, S., Lotfi, H., Abdolalizadeh, J., Bonabi, E., & Zarghami, N. (2020). An overview of yeast probiotics as cancer biotherapeutics: possible clinical application in colorectal cancer. Clinical and Translational Oncology, 22, 1227-1239.