International Journal of Food Microbiology 278 (2018) 11–19

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Microbiological quality assessment of milk at different stages of the dairy value chain in a developing country setting

Mohammad Aminul Islam[a,*], Subarna Roy[a], Ashikun Nabi[a,1], Sultana Solaiman[a], Mahdia Rahman[a], Mohsina Huq[a,2], Nurul Amin Siddiquee[b], Niyaz Ahmed[c]

[a] Enteric and Food Microbiology Laboratory, Laboratory Sciences and Services Division, International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Mohakhali, Dhaka 1212, Bangladesh
[b] CARE, Bangladesh
[c] Laboratory Sciences and Services Division, International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Mohakhali, Dhaka 1212, Bangladesh

ARTICLE INFO

Keywords:
Bacteriological quality
Raw milk
Processed milk
Pathogens

ABSTRACT

The main objective of the study was to assess the microbiological quality of milk at different stages of the dairy value chain from farm to the factory in Bangladesh. A total of 438 raw milk samples (387 from primary producers, 32 from collectors, 15 from chilling plants, 4 from local restaurants) and 95 commercially processed milk samples were collected from northern part of Bangladesh. Almost 72% (n = 280) of samples at producer level and 100% from both collectors (n = 32) and chilling plants (n = 15) were contaminated with coliforms while 57% (n = 220) of samples from producers, 91% (n = 29) of samples from collectors and 100% (n = 15) from chilling plants were contaminated with fecal coliforms. Around 31% (n = 119) of samples from producers were positive for *E. coli* whereas > 60% (n = 20) and 100% (n = 15) samples from collectors and chilling plants, respectively were positive for *E. coli*. One quarter of samples from collectors were positive for *B. cereus* and coagulase positive staphylococci and 33% (n = 5) of samples from chilling plants were positive for both of these microorganisms. In case of commercially processed milk, 77% (n = 46) and 37% (n = 22) of pasteurized milk samples had a high aerobic plate count (APC) (10^4 CFU/ml) and coliform count (> 10 CFU/ml), respectively. None of the samples was positive for *Shigella* spp., *Salmonella* spp., and *Campylobacter* spp. Among 158 *E. coli* positive raw milk samples, 9% (n = 14) contained pathogenic *E. coli*, and enteroaggregative *E. coli* (EAEC) and Shiga-toxin producing *E. coli* (STEC) were found to be the predominant pathotypes. Of the 23 pathogenic *E. coli* identified from 14 samples based on their gene contents, > 95% (n = 22) were resistant to at least one antibiotic and 13% (n = 3) of isolates were resistant to ≥3 classes of antibiotics. Several factors including the time of milking, hygiene practices of the producers, cow breed and amount of milk produced by the cow were found to be significantly associated with high APC of milk samples. In conclusion, both raw and commercially pasteurized milk are highly contaminated with fecal organisms. For intervention, more emphasis should be given at producer's level as microorganisms introduced to milk at this stage get the longest time for survival and multiplication.

1. Introduction

Milk is a complex biological fluid which by nature serves as an excellent growth medium for many microorganisms (Godič Torkar and Golc Teger, 2008). It contains a wide range of nutrients including vitamins, proteins, fats and carbohydrates and therefore, nutritionally supports many different microorganisms under suitable growth conditions (Godefay and Molla, 2000; Parekh and Subhash, 2008). Milk contaminated with high level of spoilage bacteria often becomes unsuitable for further processing (Mhone et al., 2011). Microbial contamination can generally occur from various sources during the milking procedure: from within the udder, from the exterior of the udder, from the surface of milk handling and storage equipment and from milkmen if milking is done manually (Bramley and McKinnon, 1990; Oliver et al., 2005).

Microbiologically qualified milk means that the milk is free from

* Corresponding author at: Enteric and Food Microbiology Laboratory, Laboratory Sciences and Services Division, icddr,b, Bangladesh.
E-mail address: maislam@icddrb.org (M.A. Islam).
[1] Present address: Ashikun Nabi, Department of Biology, University of Vermont, Burlington, VT 05405, USA.
[2] Present address: Mohsina Huq, School of Science, RMIT University, Australia.

https://doi.org/10.1016/j.ijfoodmicro.2018.04.028
Received 20 July 2017; Received in revised form 24 February 2018; Accepted 15 April 2018
Available online 17 April 2018
0168-1605/

pathogenic bacteria and harmful and toxic products of bacteria and that it is low in total bacterial counts. Total viable bacterial count is considered as one of the acceptance criteria for categorizing milk for human consumption and processing for dairy products (PMO, 2015). In most countries, the legal limits for total viable bacterial count in pasteurized milk range from 5×10^3 to 5×10^5 CFU/ml (Shojaei and Yadollahi, 2008). In Bangladesh, the acceptance limit for viable bacteria in pasteurized milk is $\leq 2 \times 10^4$ CFU/ml (BSTI, 2002). Among the coliforms, *Escherichia coli* is the most common contaminant of raw and processed milk and is a reliable indicator of fecal contamination (Kumar and Prasad, 2010). *E. coli* is a commensal microorganism inhabiting the intestine of animals and humans but its recovery from food may be of public health concern due to the possible presence of enteropathogenic and/or toxigenic bacteria, for instance, Shiga toxin-producing *E. coli*. *Staphylococcus aureus*, another important causative agent of food-borne diseases in humans is commonly associated with intoxications of food through its capacity to produce different kinds of potent enterotoxins (Balaban and Rasooly, 2000; Le Loir et al., 2003). Although heat may kill *S. aureus* cells, the enterotoxin may persist in food because it is more heat stable than the bacteria (Banwart, 1998). *Bacillus cereus* is a ubiquitous Gram-positive, spore-forming, motile rod, which is also responsible for spoilage of raw milk. It is also frequently found in pasteurized milk, causing deterioration of milk quality by producing lipases and proteases (Cromie et al., 1989; Meer et al., 1991).

In industrial countries, milk is produced at a commercial scale in dairy farms and collected through automated systems and further processing is done by maintaining good hygienic practices. However, in Bangladesh, milk is produced mostly in unorganized and informal ways. Even though very small proportion of milk is going to commercial processors, one of the biggest hurdles is to ensure safety and quality of milk all the way from the producers to the processing factory and to consumers. In this study, we aimed to assess the microbiological quality of raw milk through different stages of formal supply chain starting from the primary producer to the milk chilling stations of different commercial milk processing industries located in the northeast part of Bangladesh. Same assessment was done for commercially processed milk samples collected from retail outlets. In addition, factors associated with contamination of milk at the primary producer's level were investigated.

2. Materials and methods

2.1. Sampling site and sample collection

This study was conducted in 18 upazillas of 7 districts located in northern part of Bangladesh, including Bogra, Gaibandha, Nilphamari, Dinajpur, Joypurhat, Rangpur and Sirajganj. We collected a total of 438 milk samples of which 387 were from the primary producers (farmer), 32 from collectors (pooling points), 15 from chilling plants and 4 from local restaurants. The district and upazilla-wise distribution of milk samples are illustrated in Fig. 1. We determined the number of samples collected from each level of the value chain based on the study population enrolled in "Strengthening Dairy Value Chain (SDVC) project". The SDVC project was carried out by an international Non-Governmental Organization (NGO) called CARE Bangladesh (http://www.carebangladesh.org). During the time of our study, the total number of producers/farmers in the SDVC area was 15,309 and they were clustered in 519 small groups, distributed in nine districts.

For this study, we randomly selected a proportionate number of groups from each district with 6 producers per group. Of these 6 producers, we collected samples from 4 producers based on their availability and convenience. Accordingly, we selected 95 groups from which 570 (95 × 6) producers were initially included in the study, and finally samples were collected from 387 producers. We sampled one cow per producer and accordingly collected milk samples from 387 cows. We collected approximately 200 ml of milk in a sterile plastic bag (Fisher Scientific, China) by pouring milk out of a bucket immediately after milking the cow. We did not interrupt the normal timing and practice of milking which was in maximum cases the early morning and manual expression through hands.

In case of collection points, we followed the distribution chain from producers to collectors. We selected all collection points within the SDVC project area that collect milk from producers enrolled in our study (producers/collection point: minimum 4; maximum 29; median 13). Accordingly, we selected 32 collection points for sampling.

In case of chilling plants, we included all plants that collected milk samples from producers/collectors (producers/chilling plant: minimum 5; maximum 68; median 19 and collection points/chilling plant: minimum 1; maximum 4; median 1) enrolled in our study. A total of 15 chilling plants were included for sample collection.

As we selected primary producers within the SDVC project, majority of them deliver milk to collectors who supply the bulk to chilling plants belonged to different milk processing industries (formal chain). Only a few producers were found who sell milk to the local restaurants (informal chain). A total of 4 restaurants that used to receive milk from the producer groups enrolled in our study were identified, 3 of these were located in Bogra and one in Joypurhat district (Fig. 1). We collected milk samples from these restaurants at the point of reception.

We collected milk samples by maintaining an aseptic condition. Like at the primary producers, we collected at least 200 ml of milk from each of the collection points as well as the chilling plants in a sterile sampling bag (Fisher Scientific, China) and immediately placed them in a cool box having a temperature of +4 to +8 °C. Unlike primary producers, samples collected at collection points and chilling plants comprised milk originating from different producers. All samples were transported to the lab maintaining the cool chain for 6–8 h after collection and stored at +4 °C until analyzing of the samples. All samples were tested within 18–24 h of sample collection.

In addition to raw milk samples, we collected 95 commercially processed pasteurized milk (n = 60) and UHT milk (n = 35) from retail shops mostly located in Dhaka and Bogra. We collected products of those industries which have their chilling plants in the sampling area, including all medium and large size dairy industries in Bangladesh. For each product, we purchased 1–2 sealed packets of milk depending on the pack size so that we got at least 200 ml of product for analysis. We ensured that all products had different batch numbers and were within their shelf-life as labeled by the industry. All samples were placed in a cool box and transported to the laboratory by maintaining a cold chain for 6–8 h after collection.

2.2. Questionnaire-based survey

A questionnaire-based survey among all primary producers was carried out to determine factors associated with microbiological contamination of milk at primary producer level. Data were collected mainly related to the breeding type of cow, volume of milk produced by the cow, environmental condition surrounding the cattle house, construction materials of cattle house floor, type of food supplied to the cattle, source of drinking water for cattle, educational status of the farmer, time of milking, hygienic condition during milking and type of milk pot used for milking.

2.3. Microbiological analysis

All milk samples including both raw and commercially processed ones were tested for aerobic plate count (APC), lab pasteurized count (LPC), total coliform count (TC), thermotolerant coliform (fecal coliforms) count (FC), and enumeration of β-glucuronidase positive *E. coli*, *Bacillus cereus* and coagulase positive staphylococci by using standard methods described in the following. In addition, all samples were tested for foodborne pathogens including *Shigella* spp., *Salmonella* spp. and *Campylobacter* spp. using standard method as described in the