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Research Article

Assessment of Quality of Raw Milk in References to Antibiotic Residues and Microbial Loads at Farmers and Retailers' Level in Some Selected Areas of Bangladesh

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Abstract

Background: Milk is one of the most nutritious drinks in the world which is suitable for people of all ages. The quality and safety of such drink are of utmost importance for public health issues. Residual antibiotics and microbial quality of milk have thus become as major concerns to consumers. Therefore, present study was conducted to detect antibiotic residues and assess microbial quality of fresh bovine milk samples both at origin and vendors level in Dhaka city and nearby areas of Bangladesh.

Materials and Methods: A total of 150 milk samples were aseptically collected from randomly selected farmers and retailer. Antibiotic residues in milk was detected by streak plate techniques on nutrient agar plate using *Bacillus subtilis* as test organisms. Total bacterial counts and coliform counts were determined following the pour plate technique using nutrients agar and MacConke's agar, respectively.

Results: Antibiotic residues were detected in 10.67% of total milk sample while 18% of farmers' milk and 7% of retailers' milk were positive for the same. The mean standard plate count of raw milk collected from farms and retailer were 7.63 ± 0.14 log10 cfu/ml and 8.46 ± 0.23 log 10 cfu/l of milk, respectively. In contrast, total coliform count in fresh milk samples collected from farms and retailer were 2.64 ± 0.21 log10 cfu/ml and 3.92 ± 0.05 log10 cfu/ml of milk, respectively.

Conclusion: Milk produced and sold in the study areas could be considered as of fair quality in terms of microbial load. However, presence of antibiotic residues in milk could pose human health risk. Therefore, awareness should be created on the sensible use of antibiotic and obedience to drug withdrawal period.

Keywords: Residual Antibiotics in Milk; Microbial Quality of Milk; Bangladeshi Milk; Food Safety of Fresh Milk; Milk Borne Public Health Hazards

Introduction

Nowadays, resistance to antibiotics, especially in disease causing organisms has emerged as a serious public health problem worldwide. It has now been recognized as one of the top health problems encountered both in human and veterinary medicine [1]. The widely accepted causes of antibiotic resistance include over-use and inappropriate use of antibiotics for nonbacterial infections, inadequate or indiscriminate antibiotic usage in the clinical arena and use of antibiotics in veterinary practice [2]. The use of antibiotics in veterinary practice for food producing animals including dairy is thus considered as one of the causes resulting in antibiotic residue in foods of animal origin especially in milk and subsequent resistance in bacteria [2].

Although no comprehensive report on the use of antibiotics in dairy industry of Bangladesh has been documented yet, Islam., *et al*. depicted the antibiotics usage patterns in Bangladeshi broiler farming which reported that 100% of Bangladeshi broiler farms used antibiotics for several reasons including therapy, prophylaxis and growth promotion [3]. Such usages of antibiotics in broiler farming eventually might pose significant health risk to consumers through their residual effects. The similar scenario is also expected in dairy farming in Bangladesh. While farm produces like milk, meat, eggs and their products hold an important place in the nutrition of consumers as well as nutrition and income of producers, there is limited work so far undertaken regarding the detection of antibiotic residues and assessing microbial load of such products in Bangladesh. In a recent work, Islam., *et al*. detected the presence of antibiotic residues in 27% of chicken eggs sold in Bangladeshi market indicating that other farm produces such as milk might contain residual antibiotics [4].

The widespread use of antibiotics in dairy cattle management may result in the presence of antibiotic residue in milk [5]. Consumption of milk with such antibiotic residue by humans predisposes them to serious health effects. Residual antibiotics in milk can encourage the production of antibiotic resistant strain of bacteria [6], potential allergic reactions and technological problems of fermented milk products [2]. Some antibiotics are directly toxic while allergic reactions and toxic side effects of many others may have fatal consequences. Currently, approximately 80% of all food-producing animals receive medication for part or most their lives [2]. Antibiotics given to cows commonly are penicillin, amoxacillin, ciprofloxacin, oxytetracycline, sulfadiadine, metronidazole, cephalosporin, streptomycin, chloramphenicol, etc. Among them the antibiotics which are commonly excreted through milk are amoxycillin, oxytetracycline, chloramphenicol and streptomycin [7]. The major antibiotics used for humans either belong to the same general classes or have the same mode of action as those used for animals [8]. Many of the antibiotics used to treat bacterial infections in humans also have veterinary applications. In fact, the use of antimicrobials for the treatment or prevention of disease in animals closely followed their uses in humans [5].

Therefore, the concerns over drug residues have become an important for public health issue. Residues of animal drugs in human food chain threaten human health by being acutely or cumulatively allergenic, organotoxic, mutagenic, teratogenic or carcinogenic [9]. For example, residues of penicillin cause allergic reaction in persons who consume animal products containing residues [1]. Many other drugs including tetracyclines, sulphonamides and aminoglycosides can also cause allergic reaction [10]. Aminoglycosides (e.g. streptomycin) can cause varying degree of nephrotoxicity and ototoxicity, a potential to cause muscular paralysis, a tubacurare like activity on respiratory muscles and death [11]. Drug resistance appears to be the most important hazard of drug residues all over the world [6]. The resistant bacteria could then cause disease that is difficult to treat in humans and may also transfer the resistant gene to some other human pathogens [6,9,10].

On the other hand, although milk is sterile during secretion from healthy mammals, it may get contaminated with various contaminants during and after milking. Once, the milk comes outside the udder, microbial contamination may occur due to normal handling procedures. The presence of bacteria in raw milk reduces the quality of milk and certain bacteria with their associated enzymes and toxins may even survive pasteurization and heating that may result in health hazards [12]. High bacterial counts in raw milk are indicator of poor production hygiene [13]. Accordingly, the safety of milk with respect to food-borne diseases has become as a great public health concern globally. This is especially true in developing countries where production of milk and various milk products takes place under unsanitary conditions and poor production practices [14]. Among the microorganisms contaminating milk, *Staphylococcus aureus* and *Escherichia coli* are most common who are responsible for food-borne illness [15]. Thus, understanding the microbial load of raw milk is needed to measure the hygienic quality of milk. High population of bacteria in fresh milk samples and detection of antibiotic residues are important indications of the public health and food safety issues of milk [16].

Therefore, understanding the microbial quality and detection of the residues in milk intended for human consumption are essential for the safety of consumers. But surprisingly enough, very little work has been documented till to date on microbial quality and residual antibiotics in fresh milk in Bangladesh. Therefore, the study was undertaken to explore the prevalence of residual antibiotics as well as the microbial quality of fresh milk at origin and retailer level.

Materials and Methods

Study area and period

The study was conducted in Dhaka city and nearby areas of Bangladesh for a period of 6 months starting from January 205 to June 2015. Especial emphasis was given to the areas well known for commercial milk production.

Collection and preservation of sample

A total of 150 fresh milk samples were collected from different farms (n=50) and retailers (n=100) in and around Dhaka city, Bangladesh. For each sample, an aliquot of 200 ml of fresh milk sample was collected in 250 ml sterilized container. The samples were taken from bulk cans with necessary precautionary measures to avoid external contamination to the samples. Ice- boxes were used to carry the samples from the collection points to the laboratory of the Department of Medicine and Public Health, Sher-e-Bangla Agricultural University maintaining standard procedure. Samples were subjected to bacteriological examination within 3 to 4 hours of collection.

Detection of antibiotic residues

The presence of antibiotic residues in milk was detected following the methods as described previously [17]. *Bacillus subtilis* was used as test organism on nutrient agar plates. Spore suspension of the organism in nutrient broth was used to streak on nutrient agar plates. A sterile cotton swab dipped in spore suspension was rubbed gently on the agar plates so as to cover the entire agar surface. Sterile discs (HiMedia, India) were used to prepare the test discs. For each sample, a separate disc was prepared by dipping the disc in small amount of milk sample for a little while and then shaking off the excess milk. Freshly prepared and still wet discs were then placed on agar surface previous streaked with test organism. Four discs were placed on single plate so that the zone of inhibition is easily readable. Plates were then incubated overnight at 37^{o}C. The plates were observed next day for bacterial growth and zone of growth inhibition, if any. Discs surrounded by a distinct zone of growth inhibition around them were regarded as positive for residual antibiotics in respective milk sample.

Assessment of Microbial load

Total Bacterial Counts (TBC) and coliform counts were determined in the samples following the pour plate technique as described previously [18]. Briefly, samples were shaken for at least 25 times to get a homogenous mixture. A serial ten-fold dilution was made for each homogenized sample using normal saline solution. An aliquot of 0.1 ml from each dilution of the sample was inoculated on sterile petri plates in duplicate. Care was taken to transfer the last drop of sample into the petri plates. Nutrient agar media prepared previously following manufacturer's recommendation was cooled to 45º C, and poured into each pairs of petri dishes for total bacterial count. On the other hand, MacConke's agar was used to determine coliform counts. The inoculum and the media were thoroughly mixed by rotating and tilting. Petri dishes were kept on a plain surface to cool down. Control samples were prepared in following the same methods where sterile water was used instead of market milk samples to ascertain sterility of apparatus and the medium used.

Petri dishes were incubated overnight in inverted position at 37º C. Plates with more than 30 but less than 300 separated colonies of bacteria were counted with the help of bacteriological colony counter. Total number of viable bacteria and *E. coli* were counted from the mean colony counts of two plates multiplied with the specific dilution factor and finally multiplication by ten to obtain total number of viable bacteria per ml of milk sample.

Statistical analysis

Data were entered into Microsoft Excel. Descriptive statistics was used to analyze the data for antibiotic residues as percentage of samples tested positive for the presence of antibiotic residues. Total bacterial counts and coliform counts of the different collection sources were transformed in to log values then data were analyzed.

Result and Discussion

A total of 150 fresh milk samples were studied in the laboratory for the detection of residual antibiotics as well as microbial quality of the same. Inhibition zone of growth about 2 mm around a disc was considered as positive for the presence of residual antibiotics. As many as 16 (10.7%) of the total study samples were found positive for the residual antibiotics (Figure 1).

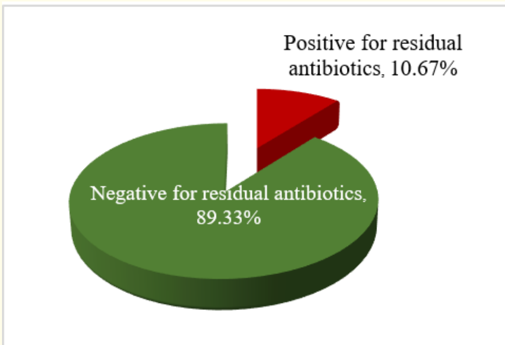

Figure 1: Overall prevalence of antibiotic residues in fresh milk samples.

However, 18% (9 out of 50) of the samples collected from farms were found positive for residual antibiotics in comparison to that of 7% (7 out of 100) of the samples collected from retailers (Figure 2). The higher prevalence of antibiotics residues in farm samples may be attributed to the fact that samples were collected immediately after milking and no dilution was made. Consequently, the concentration of residual antibiotics in farm sample remained high. On the other hand, retailers' samples might have been adulterated (diluted) with water, which is a common practice in Bangladesh that in turn diluted the residual antibiotics in the respective samples. However, our findings are in good agreement with Movassagh and Karami, 2010 who reported 5% of raw milk samples to be positive for antibiotic residues [19]. Present study findings also correspond well with the findings of Ondieki., *et al*. 2015 who conducted also evaluated the prevalence of residual antibiotics in farmers and vendors level and reported that 15.5% samples from farmers 18.4% of the samples from vendors tested positive for antimicrobial residues [20]. However, disagreement was found with the findings of Amonsin., *et al*. 1996 who found 27% fresh milk samples positive for residual antibiotics [21]. In contrast, residual antibiotics were detected in as high as 46 % of milk samples by Psomas., *et al*. 1994. [22]. The variation might be due to the variation in the drug dose and dosage form used, duration of treatment, duration of drug withdrawal period followed for the antibiotics used in the study areas. However, the higher prevalence of antimicrobial residues observed in our study could be due to unnecessary and imprudent use of antimicrobials, prophylactic or therapeutic treatment of dairy animals which led to the excretion of the residues of antibiotics in milk. Moreover, lower levels of awareness of withdrawal periods amongst farmers might have contributed to the occurrence of higher level of antibiotic residues in fresh milk.

The average standard plate counts (Total Viable Counts) for milk samples collected from farms and retailer were 7.63 ± 0.14 log10 cfu/ml and 8.46 ± 0.23 log 10 cfu per ml of milk, respectively (Figure 3). Similar findings were also reported by Khan., *et al*. 2008 and Hossain., *et al*. 2017 who studied microbial quality of Bangladeshi milk samples in some selected areas and reported total plate count as 5.92 to 6.08 log10 cfu/ml and 7.47 to 7.59 log 10 cfu per ml, respectively [23,24]. For a satisfactory grade of milk hygiene, total viable count of bacteria should be less than 20,000 CFU per ml. Total viable count more than 20,000 CFU per ml milk indicates that the production system needs improvement in hygiene. On the other hand, counts over 100,000 CFU per ml milk indicates a serious hygiene problem and potential contamination [25]. In this study, total viable count in fresh milk were more than 20,000 CFU per ml milk indicating the public health concerns associated with the bacteria. Comparatively higher bacterial counts per ml of milk in retailers' samples might be due to the improper handling, use of unclean utensils, and unhygienic condition market, long holding period of milk due to long distance of transportation from the source to the marketing place, etc.

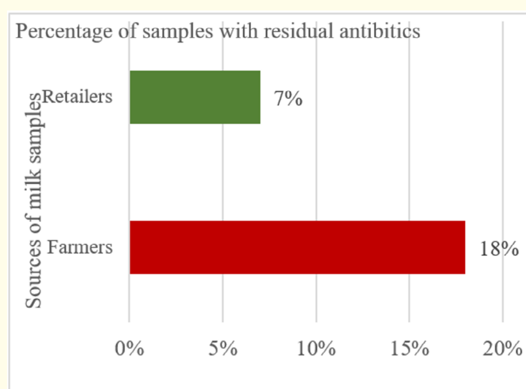

Figure 2: Prevalence of antibiotic residues in fresh milk samples according to origin.

The total coliform count (TCC) in fresh milk samples collected from farms and retailer were 2.64 ± 0.21 log10 cfu/ml and 3.92 ± 0.05 log10 cfu/ml of milk, respectively. The results of this study are in agreement with the findings of Khan., *et al*. 2008 and Hossain., *et al*. 2017 who recorded total coliform counts in fresh milk ranging between 2.51 and 3.62 log10 cfu/ml of milk [23,24]. However, the Coliforms counts of fresh milk should be less than 100 cfu/ml [25]. Therefore, it is obvious that the study samples had comparatively higher coliform counts which are the indications of contamination of milk samples. There might be different explanation for the contamination. As udder and anus are anatomically closely located, milk may get Coliform since they are normal flora of the intestine. Again, udder and the teat may get contaminated or dirtied by animal dung when they lie down on it. Thus, eventually milk get contaminated too with coliforms, if the udder and nearby areas are not cleaned properly before milking.

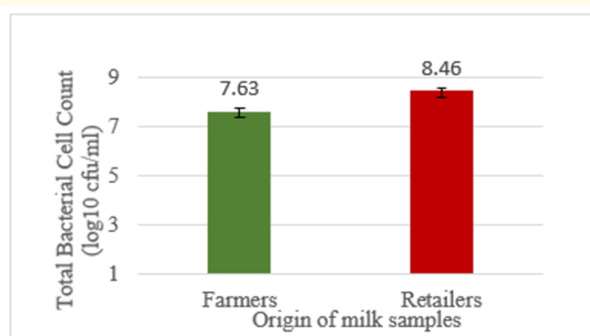

Figure 3: Total viable bacterial counts in fresh milk samples according to origin.

The present finding revealed that the mean coliform counts of both farmers and retailers milk samples collected from different places of Dhaka and nearby areas were higher than the standard of milk [25]. This is indicative that the milk might be adulterated with water which was contaminated with feces as well as the unhygienic practices in some farms. Interestingly, total coliform counts were higher in retailers' milk sample than that of farmers/farm milk samples like total viable counts reported in this study. This again indicate the possible adulteration of retailer milk with unclean or unhygienic water which is a very common practice in Bangladesh.

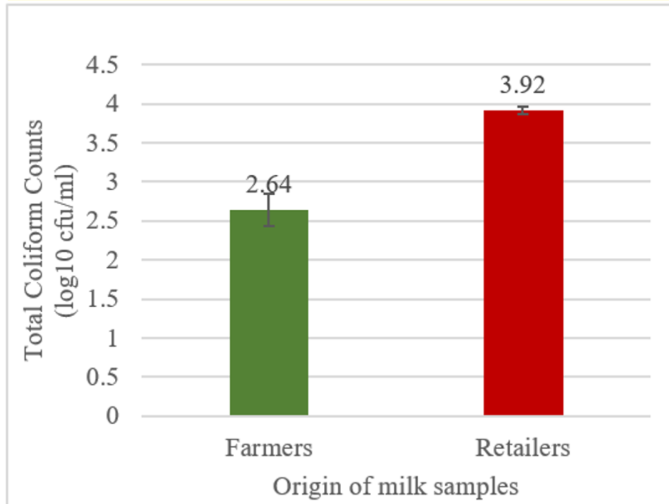

Figure 4: Total coliform counts in fresh milk samples according to origin.

Conclusion

The observed prevalence of antibiotics residues and higher total bacterial as well as coliform counts in our study indicate a need to commence addressing the problem both at the farm and market levels. The findings of the present study clearly indicate the lacks of public health controls as well as the evidence of the careless use of antibiotics in the livestock industry, which both form a risk to public health. This indication should be confirmed by a nationwide study with in-depth analyses of antibiotics' presence in food chain originated from animals. Our study findings contribute to the generation of a baseline data on the current presence of antimicrobial residues and microbial quality of locally produced milk at both farmers and retailers' level. The outcome of this study will provide valuable baseline information for local governmental authorities for the development of effective monitoring system for the use and misuse of veterinary antimicrobial drugs.

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Authors' Contributions

K. B. M. S. I. was the project leader and principal investigator. K. B. M. S. I. planned, designed and monitored the whole experiment. K. B. M. S. I. and S. K. S. collected sample, conducted experiments and kept record. K. B. M. S. I., S. K. S. and S. S. U. M. analyzed data and prepared manuscript. All authors have read and approved the manuscript.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this article.

Ethical Approval

The present research work does not contain any studies performed on animals/humans subjects by any of the authors.

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