

## Isolation, Identification and Antibiotic Sensitivity Test of Bacteria in Raw Cow Milk Obtained from Different Sources

Mariam Jamila<sup>1</sup>, Md Zulfekar Ali<sup>2</sup>, Shaharin Sultana<sup>3</sup>, Md Ariful Islam<sup>1</sup> and Mst Minara Khatun<sup>1\*</sup>

<sup>1</sup>Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh, Bangladesh

<sup>2</sup>Animal Health Research Division, Bangladesh Livestock Research Institute (BLRI), Bangladesh

<sup>3</sup>Department of Livestock Services (DLS), Ministry of Fisheries and Livestock, Bangladesh

\*Corresponding Author: Mst Minara Khatun, Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh, Bangladesh.

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### Abstract

The study was conducted to isolate, identify the bacteria, to know the sources of contamination of milk and antibiotic sensitivity of bacteria obtained from various sources. A total of 50 samples were collected from different sources such as hand wash of milkers', udder wash, utensils wash, raw cow milk and environment (air). All these samples were analyzed by culturing in different media such as *Salmonella*-Shigella agar, Eosin Methylene Blue agar, Mannitol Salt Agar, Nutrient agar, Blood Agar, MacConkey agar. Biochemical tests were performed to identify the organism. Among 50 samples, 12 (24%) were *E. coli*. Similarly, 13 (26%) and 36 (72%) were found positive for *Salmonella spp.* and *Staphylococcus spp.* respectively. Results of antibiotic sensitivity test represent that, out of ten antibiotics *Staphylococcus sp.* were sensitive against 9 antibiotics such as Chloramphenicol, Gentamicin, Kanamycin, Sulphamethoxazole, Ampicillin, Amoxicillin, Ciprofloxacin, Tetracycline, Erythromycin and resistant against Nalidixic acid. *Salmonella sp.* was sensitive to 6 antibiotics such as Chloramphenicol, Gentamicin, Kanamycin, Sulphamethoxazole, Ampicillin, Amoxicillin but resistant against Ciprofloxacin, Tetracycline, Erythromycin and Nalidixic acid. Whereas, *E. coli* were sensitive against 7 antibiotics such as Chloramphenicol, Gentamicin, Kanamycin, Sulphamethoxazole, Ciprofloxacin, Tetracycline and Nalidixic acid but resistant against Ampicillin, Erythromycin and intermediate to Ampicillin. In this study Chloramphenicol, Gentamicin, Kanamycin and Sulphamethoxazole were sensitive against the three isolated bacteria. Among all isolates *Salmonella* showing resistance to at least more than three antibiotic classes and *E. coli* were showing multidrug resistant. Data of this study suggested that raw milk contaminated with multidrug resistant bacteria which may cause public health hazard.

**Keywords:** Isolation; Identification; Raw Milk; Bacteria; Antibiotic Sensitivity

### Introduction

Foodborne illnesses are an important challenge to public health and cause significant economic problem in many countries [1]. The crucial goal of all food safety programs is to prevent food products contaminated by potential pathogens from reaching the consumer. Milk is an excellent medium for bacterial growth, which not only spoils the milk and associated products but also can cause infections in consumers. Because of the specific production, it is not possible to fully avoid contamination of milk with microorganisms; therefore, the microbial contamination of milk is an important tool in determining its quality [2]. During the normal milking operation however, milk

is subjected to contamination from many different sources including: (1) the udder and body of cows, (2) dust from the air, (3) litter and floor (4) flies, insects and rodents (5) water supply (6) hands and clothes of the milkier (7) utensils, bottles (8) atmosphere, etc. [3] thus, milk and the dairy products prepared from milk could be an important sources of food borne pathogens [4]. Milk can be contaminated by various types of microorganisms such as *Streptococcus*, *Staphylococcus*, *Micrococcus*, *Escherichia*, *Bacillus*, *Salmonella* and *Pseudomonas sp* [5].

Huge numbers of microbes can get access to milk and various milk products including *E. coli*, which is an indicator of milk contamination, constituting a public health hazard [6]. The diseases transmissible to humans through the consumption of spoiled milk like brucellosis, tuberculosis, salmonellosis, listeriosis, *E. coli* infections and many others were described extensively in 1962 by [7].

Milk production in Bangladesh is characterized by low yield of non-descriptive cows, small producers, production in small lots and under poor hygienic conditions. As a result, milk reaching into market usually contain higher microbial load. The present study was undertaken aiming to isolate and identify the bacteria, to know the sources of contamination of milk and to evaluate the antibiotic sensitivity of bacteria obtained from various sources.

### Materials and Methods

**Collection of samples:** A total of 50 different samples were collected from Bangladesh Agricultural University (BAU) dairy farm and Bottola village of Mymensingh District of Bangladesh. The samples were hand wash of milker (n = 10), udder wash (n = 10), utensils wash (n = 10). 100 ml of PBS was used for each washing. Raw cow milk (n = 10) were also collected in sterilized test tubes. Environment (air) (n = 10) samples were collected. After collecting the samples aseptically, all the samples were transported immediately to the Bacteriology laboratory of the Department of Microbiology and Hygiene, BAU, Mymensingh. Samples collected from different areas are shown in table 2.

**Isolation of bacteria:** After collection, the samples were grown in the freshly prepared nutrient broth at 37°C for 24 hours. The overnight bacterial broths were streaked on SS agar (for *Salmonella*), EMB (for *E. coli*), Mannitol salt agar (for *Staphylococcus*) which were incubated at 37°C for 24 hours.

**Identification of bacteria:** Identification of bacteria was done on the basis of colony morphology, Gram's staining, motility test, biochemical test- sugar fermentation test (e.g. dextrose, sucrose, lactose, maltose and mannitol), catalase test, coagulase test, indole test and MR-VP test.

**Antibiotic sensitivity test:** Three isolates randomly selected from three genera were tested for antimicrobial drug susceptibility against ten commonly used antibiotics by disc diffusion or Kirby-Bauer method [8]. The results were expressed as resistant, intermediate or sensitive according to the guidelines of Clinical and Laboratory Standards Institute [9]. The table 1 shows antibiotics used in the experiments.

**Data analysis:** Data were analyzed by using MS Excel 2007.

### Results

*E. coli*, *Salmonella sp.* and *Staphylococcus sp.* were isolated from hand wash of milker, udder wash, utensils wash, raw milk and environment (air) samples. The isolation of bacteria from different samples is shown in the table 2.

*Staphylococcus sp* was in the most important position in all the five samples both in BAU dairy farm and Bottola village. In BAU dairy farm 4 *Staphylococcus sp.* were found in hand wash of milker; 3 in udder wash, utensils wash, raw milk and 2 in air. Conversely, 5 *Staphylococcus sp.* were found both in milker hand wash and utensils wash, 4 in both udder wash and air, 3 in raw milk.

Name of the antibiotics	Symbol	Disc concentration (µg/disc)
Ciprofloxacin	CIP	5
Tetracycline	TE	30
Amoxycillin	AML	10
Chloramphenicol	C	30
Kanamycin	K	30
Erythromycin	E	15
Ampicillin	AMP	10
Gentamicin	CN	10
Sulfamethoxazole	SXT	25
Nalidixic Acid	NA	30

Table 1: Antimicrobial agents with their disc concentration; µg= Micro-gram.

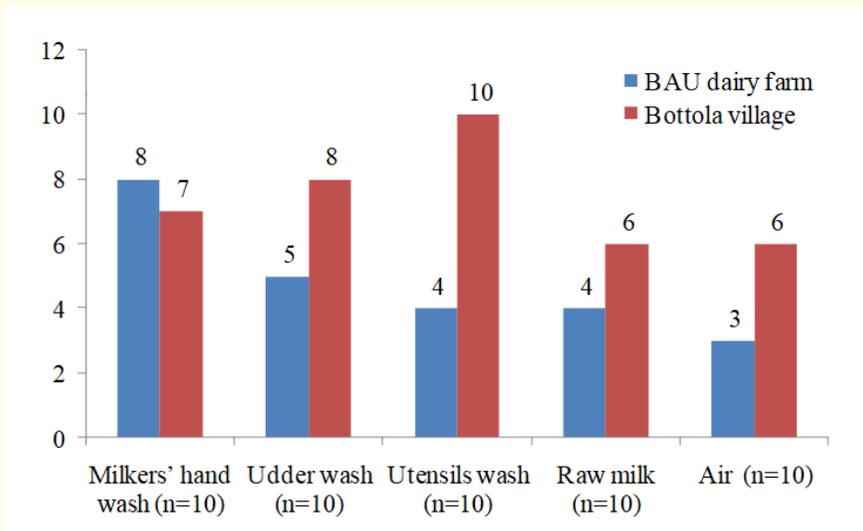
Sample	Location	Number of isolated bacteria		
		<i>E. coli</i>	<i>Salmonella spp.</i>	<i>Staphylococcus spp.</i>
Milkiers’ hand wash (n = 10)	BAU dairy farm	2	2	4
	Bottola village	0	2	5
Udder wash (n = 10)	BAU dairy farm	1	1	3
	Bottola village	2	2	4
Utensils wash (n = 10)	BAU dairy farm	0	1	3
	Bottola village	2	3	5
Raw milk (n = 10)	BAU dairy farm	1	0	3
	Bottola village	2	1	3
Environment (Air) (n = 10)	BAU dairy farm	1	0	2
	Bottola village	1	1	4
Total		12	13	36

Table 2: Summary of isolation of bacteria from various sources of contamination of raw milk. BAU: Bangladesh Agricultural University.

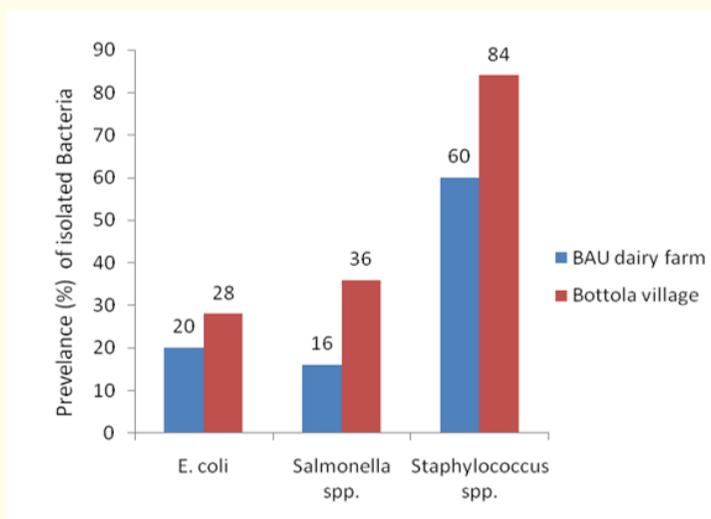
*Salmonella sp.* was the second important isolate in these samples. In milker hand wash, the isolates were 2 and in both udder wash, utensils wash were 1 and no *Salmonella sp.* were found both in raw milk and air in BAU dairy farm. However, the isolate was 3 in utensils wash, 2 in both milker hand wash and udder wash, 1 in both raw milk and environment (air) in ‘Bottola village. The other isolate was *E. coli*. In BAU dairy farm 2 such isolates were found in milker hand wash and 1 in both raw milk and environment (air) and there was no *E. coli* in utensils wash. On the other hand, 2 isolates were found in udder wash, utensils wash and raw milk, 1 in air and no *E. coli* in milker hand wash.

**Isolation rate of bacteria from various sources and locations**

A total of 24 isolates were recovered from BAU dairy farm whereas 37 isolates were recovered from Bottola village. The total number of isolates recovered from various sources and locations are presented in figure 1 and 2.



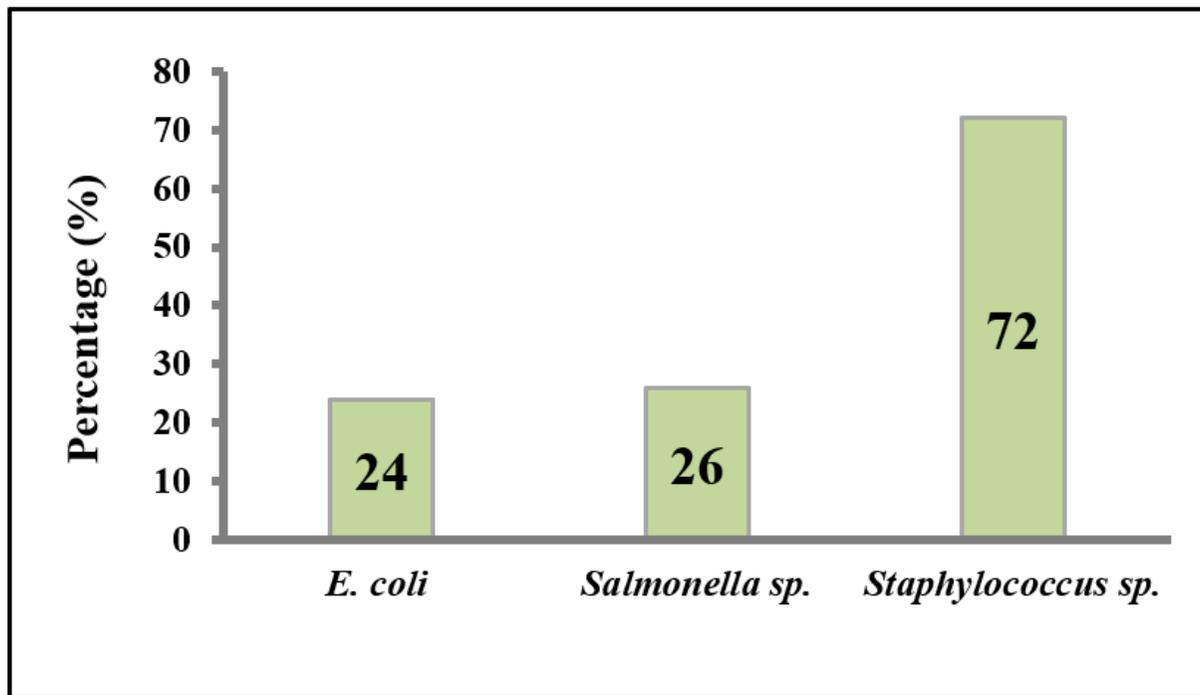
**Figure 1:** Number of isolates recovered from various sources and locations. In case of BAU dairy farm, the highest number of isolated bacteria was recovered from Milkers' hand wash (8 isolates from 5 samples) followed by udder wash (5 isolates from 5 samples), both raw milk and utensils wash (4 isolates from 5 samples) and air (3 isolates from 5 samples). In case of Bottola village, the highest number of bacteria was recovered from utensils wash (10 isolates from 5 samples) followed by udder wash (8 isolates from 5 samples), milkier' hand wash (7 isolates from 5 samples), and both raw milk and air (6 isolates from 5 samples).



**Figure 2:** Prevalence of bacteria isolated from samples of BAU dairy farm (n = 25) and Bottola village (n = 25). In BAU dairy farm prevalence of E. coli, Salmonella sp. and Staphylococcus sp. were 20% (5 of 25), 16% (4 of 25) and 60% (15 of 25) respectively. On the other hand, the prevalence of E. coli, Salmonella sp., Staphylococcus sp. was 28% (7 of 25), 36% (9 of 25) and 84% (21 of 25) respectively at Bottola village.

**Overall prevalence of *E. coli*, *Salmonella sp.* and *Staphylococcus sp.***

Among the 50 samples the total prevalence of *E. coli* found in 12 sample which signify (24%) whereas, *Salmonella sp.* found in 13 (26%), and *Staphylococcus sp.* found in 36 (72%) shown in figure 3.



**Figure 3:** Total prevalence of *E. coli*, *Salmonella sp.*, *Staphylococcus sp.*

**Antibiogram profile**

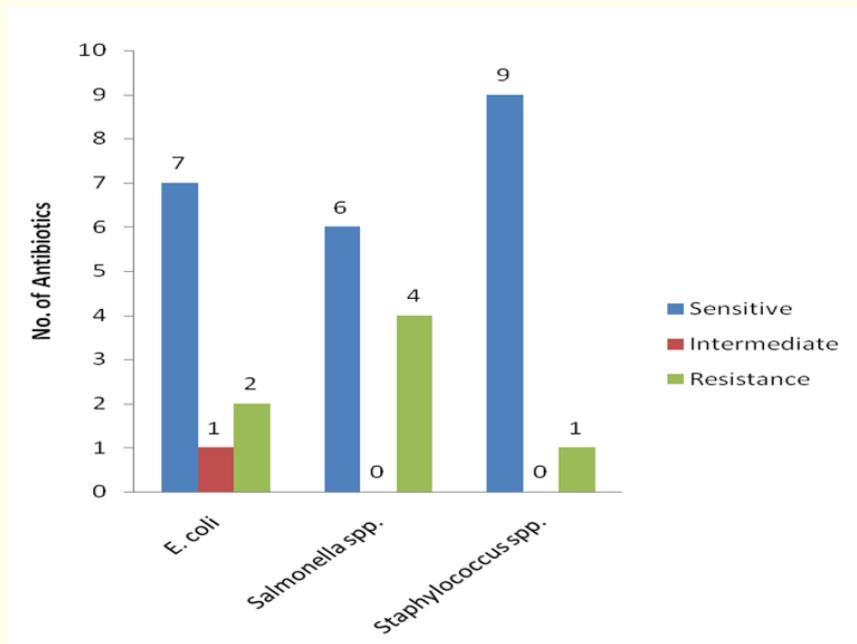
A total of 3 isolates belonging to the 3 genera such as *E. coli*, *Salmonella sp.*, and *Staphylococcus sp.* were subjected to antibiotic sensitivity assay. The results of the antibiotics sensitivity assay are presented in Figure 4.

Summary of antibiogram profile of *E. coli*, *Salmonella sp.* and *Staphylococcus sp.* against 10 antibiotics is presented in Figure 5.

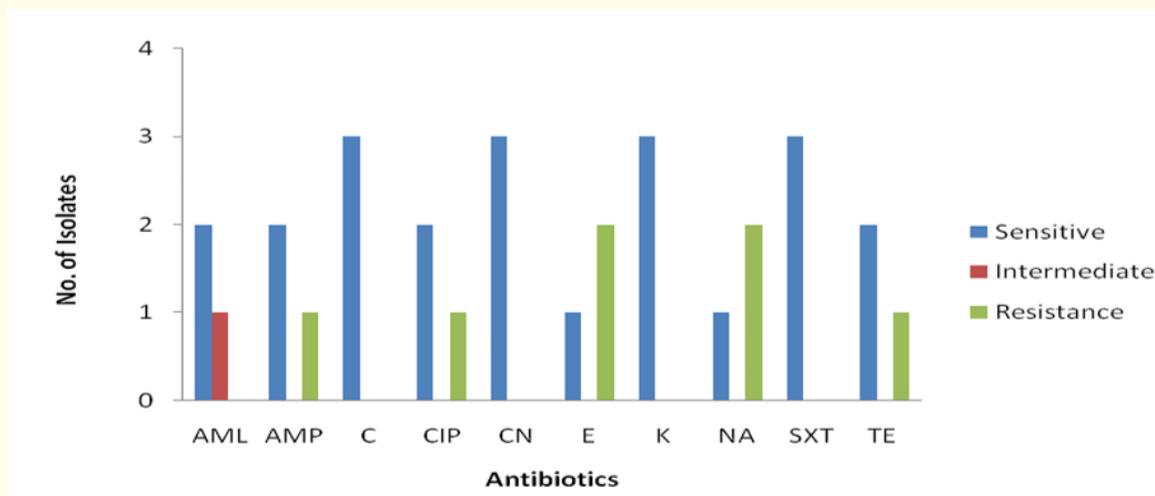
**Discussion**

In the present study, *E. coli*, *Salmonella sp.*, and *Staphylococcus sp.* were found in the collected samples (such as milker hand wash, udder wash, utensils wash, raw milk, air). Similar results were also reported by other investigators such as [10]. The high numbers of the isolated microorganisms not only contaminate the milk but also multiply and grow in it. This might be because milk is a good nutritive medium for the growth of microorganisms, especially with poor sanitary procedures and lack of the cooling facilities [11].

In this study, colony characteristics of *E. coli* observed in EMB, MC and SS agar were similar to the findings of [12]. In Gram's staining, the morphology of the isolated bacteria exhibited Gram negative character with short rod arranged in single or paired and motile which was correlated with the findings of several authors namely [13]. The colony characteristics of *Salmonella sp.* observed in NA, SS agar, MC agar;



**Figure 4:** Summary of antibiogram profile of *E. coli*, *Salmonella sp.*, and *Staphylococcus sp.* against 10 antibiotics. *E. coli* showed sensitivity towards 7 antibiotics, intermediately against 1 and resistance against 2 antibiotics. *Salmonella sp.* showed sensitivity towards 6 and resistance against 4 antibiotics. *Staphylococcus sp.* showed sensitivity towards 9 and resistance against 1 antibiotic.



**Figure 5:** Antibigram studies against the isolated bacteria. This figure represents that out of ten antibiotics Chloramphenicol, Gentamicin, Kanamycin and Sulphamethoxazole were sensitive against the three isolated bacteria, followed by Ampicillin, Amoxicillin, Ciprofloxacin and Tetracycline, which were sensitive against two isolates, whereas, Erythromycin and Nalidaxic Acid were sensitive against one isolated bacteria. On the other hand, Erythromycin and Nalidaxic Acid were resistant against two bacteria while Ampicillin, Ciprofloxacin and Tetracycline were resistant against one bacterium. Only Amoxicillin was intermediately sensitive against one bacterium.

Blood Agar, EMB agar were similar to the findings of [14]. In Gram's staining, the morphology of the isolated bacteria exhibited Gram negative appearance along with small rod arranged in single or paired and motile which was in agreement with the findings of the author [15].

The morphology of the *Staphylococcus* sp. in Gram's staining exhibited Gram-positive cocci arranged in grape like cluster which showed similarity with the findings of [16].

The *E. coli* isolates revealed a complete fermentation of 5 basic sugars by producing both acid and gas which was in agreement with [15]. The isolates also revealed positive reaction in MR test and Indole test but negative reaction in VP test.

Isolates of *Staphylococcus* sp. revealed complete fermentation of 5 basic sugars and production of acid which correlated with the results of OIE (2008).

Of the 50 samples tested, the prevalence of *E. coli* in BAU dairy farm and Bottola village were 20% and 28% respectively. This variation could be due to the hygienic management during milking in BAU dairy farm. The overall prevalence of *E. coli* was 24% in various sources of contamination associated with milk. Such finding was in consistent with the prevalence of 24.39% *E. coli* found by [17]. However, a significant variation in prevalence of *E. coli* was described in several findings, for example 31.6% by [18]. It might be due to the variation of sample size and geographical locations.

The prevalence of *Salmonella* sp. was recorded as 16% and 36% respectively in BAU dairy farm and Bottola village. The overall prevalence of *Salmonella* sp. was found 26%, which was more or less similar to [19].

The prevalence of *Staphylococcus* sp. in BAU dairy farm and Bottola village was 60% and 84% respectively. In this study, the overall prevalence of *Staphylococcus* sp. 72% was in agreement with the findings of [20], although, a major variation in prevalence of *Staphylococcus* sp. was described in several findings, for example, 54% by [21].

Conditions for contamination of raw milk at different critical points are due to less hygienic practices in pre-milking udder preparation, sub-optimal hygiene of milk handlers, and poor sanitation practices associated with milking and storage equipment's, higher environmental contamination during transportation or contamination during waiting along the roadside [22]. In this study, the highest number of isolates were found in milker hand wash in BAU dairy farm whereas the most prevalent isolate was found in utensils wash in Bottola village. It was also found that the isolates of *E. coli* were sensitive to ciprofloxacin, chloramphenicol, gentamicin, kanamycin, sulfamethoxazole, tetracycline, nalidixic acid; resistant to erythromycin and ampicillin; intermediately sensitive to amoxicillin. These findings were more or less similar with the findings of other researchers (Ali, *et al.* 2017). Reports from other researchers had also indicated *E. coli* isolates' resistance to ampicillin and cephalothin (84.6%), tetracycline (88.9%), and gentamicin (65.9%) [23].

Furthermore, it was revealed that *Salmonella* sp. were sensitive to amoxicillin, ampicillin, chloramphenicol, gentamycin, kanamycin, sulfamethoxazole. This result closely correlated with the results of Ali and Sultana, [24]. The isolates were, however, resistant to ciprofloxacin, erythromycin, tetracycline, nalidixic acid, which was in agreement with Ali, [25]. As regards *Staphylococcus* sp., it was found that the organisms were sensitive to all the antibiotics tested except nalidixic acid. Similar observations were also noted by Xuemei, *et al.* 2009. Antimicrobial resistance emerges from the use of antimicrobials in animals and human and the subsequent transfer of resistance genes and bacteria among animals, humans, animal products, and the environment. In Ethiopia, there have been reports on the drug resistance of *E. coli* isolates from animal-derived food products [26,27].

The study showed that the development of antibiotic resistance against *bacteria* could pose serious threat for consumers in the study area. Information obtained by the results concluding that strict hygienic measures should be applied during production; processing and distribution of milk and its products to avoid contamination. Periodical inspection must be carried out by specialists on the dairy farms to minimize milk contamination with different types of microorganism [28-30].

## Conclusion

Considering the findings of this piece of research work, it may be concluded that *Staphylococcus sp.* and *Salmonella sp.* were the most prevalent bacteria in various sources of contamination of milk. In BAU dairy farm and "Bottola" village, the maximum number of organisms was found in samples of milker hand wash and utensils wash respectively. Chloramphenicol, gentamicin, kanamycin and sulfamethoxazole would be the choice of drug against *E. coli*, *Staphylococcus sp.* and *Salmonella sp.* The study revealed that the development of antibiotic resistance against *bacteria* could pose serious threat for consumers in the study area. Therefore, attention should be given for strict monitoring and the implementation of effective hygienic and biosecurity measures in the whole food chain as well as proper handling and using recent antibiotics in the treatment of diseases both in humans and in animals.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

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