

## ORIGINAL ARTICLE

# Isolation and Identification of Microorganisms from Raw Milk

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### ABSTRACT

Milk is normally sterile as secreted by the lactating glands of healthy animals. But there may be the chance of contamination. It mostly occurs from two major sources that are the normal flora of the mammary ducts and flora of the external environment. Milk is an excellent growth medium for microorganisms. Therefore, this study was performed in this context to check the pathogenic microorganisms in raw milk by using presumptive tests. Fifty samples of raw cow and buffalo's milk were collected during this study from various parts of district Abbottabad Khyber Pakhtunkhwa. Samples were collected from different milk shops as well as from various dairy farms. Tetra packs of different companies were also examined. In all milk samples 40 were buffalo's milk, 5 were cow's milk and 5 were Tetra packs. A number of pathogenic bacteria were found in almost all milk samples. The identified bacteria and their isolation rate were *E. coli* (92%), *Salmonella sp.* (12%), *Shigella sp.* (0%), *Staphylococcus* (36%) and *Streptococcus* (2%). The most prevalent organism overall was *E. coli*, while the least prevalent was *Streptococcus*. These results shows that milk become contaminated by milk handlers, utensils and environment.

**Key words:** Milk, cow, buffalo, tetra packs, bacteria, KPK

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## INTRODUCTION

Milk is a white fluid created by the mammary organs of all vertebrates including humans. Milk is a finished equalization diet on earth because of its incredible blend of supplements, including calcium, phosphorus, potassium, protein, fat, starch and nutrient (energy, carbon, nitrogen sources, mineral and vitamin) [1]. Due to its complex biochemical composition and high water and nutrient activity milk serves as an excellent culture medium for the development and multiplication of numerous kinds of microorganisms [2]. Milk is for all intents and purposes a clean liquid when emitted into alveoli of udder but contamination may occur through environmental factors and human factors. There are two main factors of contaminations, first are inside by mastitis udder and fore milk [3]. Secondly contamination may happen from various sources; outside the udder, from the storage equipments, feed, water, utensils and cooling of milk. However milk is additionally tainted via air, feed, soil, dust particles, milker hand and it is vaccinated by the skin normal flora [4].

Microbial load and incidence of the bacterial pathogens in milk are indicators of food quality. The quality and safety of milk and milk products are determined by the presence of indicator bacteria and other bacteria in lesser number [5]. Milk delivered under clean conditions from healthy animals not contains more than  $1 \times 10^5$  CFU/ml [6]. Two groups of microbes present in milk which cause spoilage include mold and yeasts [7]. Other groups are pathogens include *Streptococcus*, *Staphylococcus*, *Escherichia*, *Clostridium sp.*, *Brucella*, *Mycobacterium*, *Salmonella*, *Listeria*, *Candida*, *Anthrax bacillus*, *Corynebacterium*, *Cryptococcus*, *Coxiella*, *Nocardia* and *Rabies virus* [8]. The lactic acid bacteria are the dominant microbial population in bovine, goat, sheep and buffalo milk (*Lactococcus*, *Lactobacillus*, *Leuconostoc*, *Streptococcus* and *Enterococcus*) [9]. The presence of such type of bacteria in milk can cause public health problems especially for those individuals who use contaminated milk.

Milk is a productive vehicle for transmission of sickness to people. Around 90% of dairy related ailments in person emerge from unhygienic milk. Milk borne diseases caused by pathogenic organisms are Salmonellosis, TB, Gastroenteritis, Brucellosis and Staphylococcal food poisoning [10]. Chronic gastritis, Enterocolitis and Septicemia caused by *Campylobacter*. Shiga toxins include non-bloody diarrhea, diarrhea-associated hemorrhagic colitis, hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura [11]. *L. monocytogenes* can cause neurological disease, abortion or asymptomatic infections. Zoonotic milk borne pathogens present in milk are *Brucella abortus* and *Mycobacterium bovis*. In order to produce milk of good hygienic quality, it is therefore important to have clean healthy cows and clean utensils for milking and storage of milk.

The objective of this work was to evaluate the level of microbiological contamination of raw milk samples & pasteurized milk sample taken from Abbottabad city local market. This study was carried out for isolation and identification of microorganisms from raw milk & pasteurized milk sold in Abbottabad city.

## MATERIAL AND METHODS

The study was carried out in the microbiology laboratory of Veterinary Disease and Research Investigation center (VD & RIC) Abbottabad.

### Collection of milk samples

50 samples of raw cow and buffalo's milk were collected during this study from various parts of district Abbottabad Khyber Pakhtunkhwa. The location include Mandain, College road, Missile chowk, Main bazar, Kunj Kehal, Jhugain, Jhangi, Sarbon colony, University road, Mirpur, Fawarachowk. Samples were collected from different milk shops as well as from various dairy farms and the tetra packs of different companies were also examined.

### Preparation of presumptive test

Lactose broth and nutrient broth were used for the detection of coliforms in milk.

#### Lactose broth

13grams of lactose broth was suspended in 250ml distilled water. The medium was heated to dissolve completely. It was dispensed in tubes containing inverted fermentation vial (Durham's tube) as desired. Then it was sterilized by autoclaving at 121°C, 15 lbs pressure for 15 minutes.

#### Preparation of Nutrient broth

8grams of the medium was suspended in 250ml of distilled water. It was mixed well and dissolved by heating with frequent agitation. Then dispensed it into appropriate containers and sterilized in autoclave at 121°C, 15 lbs pressure for 15 minutes.

#### Presumptive test

Milk samples were inoculated into the tubes of lactose and nutrients broth with Durham tubes. In lactose broth we also added two drops of phenolphthalein red as indicator which turns red to yellow. The tubes were incubated at 37°C for 24-48 hours for total coliform and the gas formation was observed.

### Isolation of different pathogens from different samples

#### Coliform test

To determine the coliform bacteria 1ml of sample from lactose broth was taken and streaked on MacConkey's agar and nutrient agar and were incubated at 37°C for 24-48 hours. If plates showed positive results then the coliform were subjected to the confirmatory test. For isolation and identification of *E. coli* the enriched sample was cultured on selective medium Levin Eosin Methylene Blue agar and incubated at 37°C. Green metallic colonies on EMB indicated presence of *E. coli*.

#### Isolation of Salmonella

*Salmonella Shigella* (SS) Agar is moderately selective and differential medium for the isolation, cultivation and differentiation of *Salmonella spp.* and some strains of *Shigella spp.*

A wire loop was sterilized by heating it red hot and cooled off a little then dipped it in lactose broth in which presumptive test was positive and streaking was done on SS agar, Nutrient and MacConkey's agar petri dishes. The plates were incubated for 24 hours at 37°C. The appearance of pale or yellow colonies and few black center colonies on MacConkey and SS agar indicated the presence of *Salmonella* in milk.

#### Isolation of Staphylococcus

The streaking from nutrient broth was done on nutrient media with the help of sterilized wire loop. The plates were incubated for 24 hours at 37°C, presence of yellow colonies indicated *Staphylococcus* colonies which were further confirmed by performing catalase test.

#### Biochemical tests

For the identification and confirmation of bacterial species biochemical tests were performed. These tests included Indole, Simon citrate test, Vogues-Proskauer (VP), Methyl Red (MR), Triple sugar test (TSI) and catalase test.

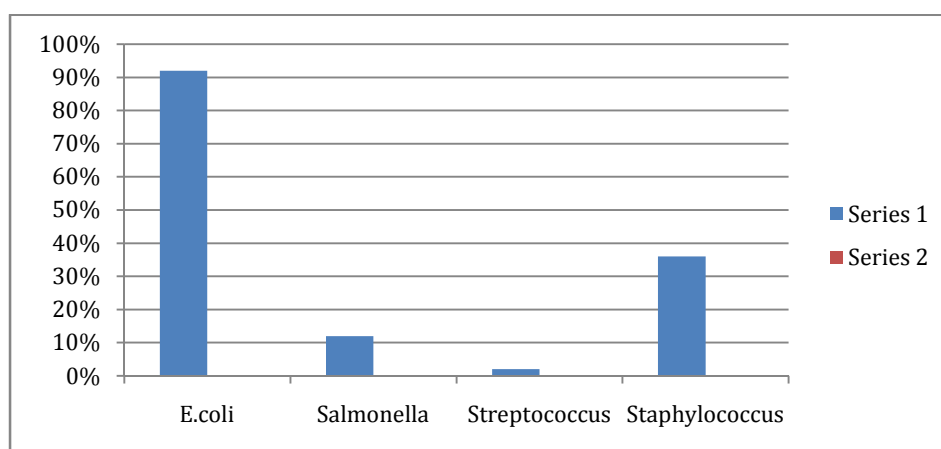
## RESULTS AND DISCUSSION

### Bacteriological analysis

As shown in the Table 1 the identified bacteria and their isolation rate were *E.coli* (92%), *Salmonella sp.* (12%), *Shigella sp.* (0%), *Staphylococcus* (36%) and *Streptococcus* (2%). These are indicative of significant contamination of milk and important human pathogens. The most prevalent organism overall was *E.coli*, while the least prevalent was *Streptococcus sp* which can be seen in Figure 1.

**Table 1. Isolated organisms from milk samples**

Bacteria Isolated	Total Samples	No of positive samples	Percentage
<i>E.coli</i>	50	46	92%
<i>Salmonella</i>	50	06	12%
<i>Shigella</i>	50	00	00
<i>Streptococcus</i>	50	01	02%
<i>Staphylococcus</i>	50	18	36%



**Figure 1. Percentage of bacteria isolated from milk**

### Bacterial Colony Morphology

Bacterial colonies were identified on the basis of morphology and color. Cultural characteristics of bacteria on different media are shown in Table 2 along with colonies on Nutrient, SS and MacConkey agar in figure 2 and 3.

**Table 2. Cultural characteristic of *E.coli* and *Staphylococcus* on different media**

Media used	Culture character of <i>E.coli</i>	Culture character of <i>Staphylococcus</i>	Culture character of <i>Salmonella</i>
Nutrient Agar	White colonies	Yellow colonies	White and few Yellow colonies
MacConkey Agar	Pink colonies with few Yellow	Yellow colonies	-
SS agar	Pink colonies	Yellow colonies	Black center colonies with few pink colonies
Eosin Methylene Blue Media	Greenish colonies	-	-



**Figure 2. Bacterial colonies on Nutrient agar are *E.coli* and *Salmonella* and *E.coli* colony on SS agar**

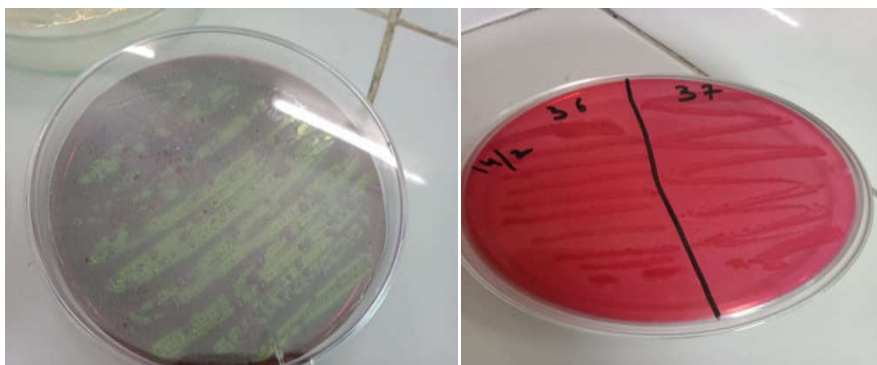


Figure 3. *E. coli* colonies on MacConkey Agar and EMB

### Biochemical tests

Besides gram staining, various Biochemical tests were also performed for identification. On the basis of these tests bacteria were identified.

Table 3. Biochemical Test Results

Biochemical test	Reaction	Identified bacteria
Simmon's citrate	+	-
TSI	+	-
VogesProskauer	-	<i>E. coli</i>
Methyl red	+	<i>E. coli</i>
Indole	+	<i>E. coli</i>



Figure 4. Indole Test for *E. coli*



Figure 5. TSI Test for *E. coli*



Figure 6. MR & VP Test for *E. coli*



Figure 7. Biochemical Test for *E. coli*

Due to the presence of various types of microorganisms undesirable changes occur in the milk's appearance, smell and taste. Many microorganisms present in milk, may endanger the consumer's health. Various bacterial species like *Salmonella* are the major and important pathogenic bacteria found in milk. In the study conducted by Abbas *et al*[9] a total of 60 cows' raw milk samples were processed for bacteriological and biochemical analyses, collected from different locations of District Peshawar, Pakistan. Coliforms were detected in 35% milk samples in which *E.coli* were detected in 31.67% milk. *Salmonella* was detected in lowest percentage with 6.67%. These results are in agreement to our study in which we examined 50 samples of raw cow and buffalo's milk collected from different milk shops as well as from various dairy farms of district Abbottabad Khyber Pakhtunkhwa. *E.coli* was positive for 46 samples (92%), *Salmonella* 12%, *Streptococcus* 2% and *Staphylococcus* (36%).

## CONCLUSION

Many diseases arise from unhygienic condition of milk like typhoid, Salmonellosis and many other enteric diseases. These can be avoided if milk handlers take care of their hygiene and the vats in which the milk is stored. There should be proper checking of all milk shops and samples should be taken for microbiological examination.

Secondly the milk samples which were collected during this study should be screened whether it is milk or something else. Government should take actions to analyze this fluid which is sold in the name of milk.

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