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Evaluation of dairy milk and animal feed for the presence of pathogenic bacteria across different Districts of Khyber Pakhtunkhwa, Pakistan

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Abstract

Milk is a perfect biological fluid that provides excellent media for the growth of microorganisms, hence serving as a vehicle for transmission of food borne pathogens in humans. This study was conducted to assess the bacterial contamination of dairy milk and animal feed in different districts of Khyber Pakhtunkhwa (KP), Pakistan. Among 180 raw milk samples; 19.4% *Salmonella*, 41.1% *Escherichia coli,* 29.4% *Staphylococcus aureus*, 19.4% *Pseudomonas aeruginosa* and 16.1% *Klebsiella* species were identified. Tetra pack milk samples showed no growth of any pathogenic bacteria which could be attributed to effective pasteurization methods. Out of 180 animal feed (cottonseed cake 90, Wanda 90) samples; 26.6% *Salmonella* and 18.5% *E. coli* contamination was found. The significant value of raw and tetrapack milk was noted for both ($p < 0.0001$). The bacterial contamination in raw milk at several less developed and less facilitated areas could be attributed to the unsatisfactory and unhygienic conditions during production and processing of raw milk. The presence of pathogenic bacteria in animal feed and especially milk can potentially cause a number of diseases in cattle and humans ranging from short lived temporary vomiting, nausea, diarrhea to life threatening hemorrhagic colitis and Guillen barre syndrome. The study highlights the need of appropriate hygienic and sanitary measures to control and/or minimize the risk of bacterial contamination. Further research is recommended to identify the risk of transmission of food borne pathogens in raw milk at other regions of the Khyber Pakhtunkhwa (KP).

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Introduction

According to Food and Drug Administration (FDA) USA, milk is the whole clean and fresh lacteal secretion of perfectly healthy cows that have been kept and fed properly and practically colostrum's free (Myers. 1987). Milk is the second most nutritionally balance diet that is consumed worldwide by people of every age, especially important for breast feeding mothers (Javed *et al.* 2009, Shunda *et al.* 2013, Weiler *et al.* 2014). Milk is available in raw form and pasteurized forms; which is an example of aseptic packaging system that has been reported and used for beverages and liquids (Gran *et al.* 2003).

Milk due to its intricate nature, provides all the nutrients and environment for the growth of microorganisms, which might cause food borne diseases in humans (Gran *et al.* 2003, Ikwap *et al.* 2014). Pathogenic bacteria that find its way into milk and cause severe human illnesses include; *Salmonella* species, *Campylobacter* species, *S. aureus*, and *Streptococcus* species (Delavenne *et al.* 2011, Motaung *et al.* 2017, Malinowski *et al.* 2001, Ksouri *et al.* 2015, Doyle *et al.* 2015). Milk can be contaminated by pathogenic microorganisms found in environment, sick cow and even on human hands. Pathogenic microorganisms from feed, feces, water and other environmental factors can contaminate cattle milk (Omiccioli *et al.* 2009).

The presence of *S. aureus, E. coli* and other pathogenic microorganisms in milk trigger a pathway for a number of diseases in cattle and humans ranging from temporary short lived nausea, vomiting, diarrhea and abdominal pain to serious complications like hemorrhagic colitis and Guillen barre syndrome (Doyle *et al.* 2015, Marin *et al.* 2013). Apart from contamination in dairy milk, bacteria also contaminate animal feed (cotton seed cake, wanda). Cotton seed cake has been used as an animal feed throughout the subcontinent and serves as an excellent vegetable proteins source (Yunus *et al.* 2015). Besides its importance, in the recent past years' cotton seed cake has been objected for its quality issues (Ilyas. 2011, Pasha. 2012). Animal feed may get contaminated before arrival at and while on the farm with pathogenic bacteria like *Campylobacter* species, *E. coli*, and non-typhoidal serotypes of *salmonella* (Mead *et al.* 1999, Dargatz *et al.* 2005). Food producing animals get infected and colonized by pathogens through consumption of contaminated feed on the farm. Furthermore, these pathogens become part of the food chain and cause serious food borne illnesses in human and animals (Brown *et al.* 2001).

Milk is described as the "utmost" perfect biological fluid nutritionally, which is why it provides the best favorable conditions to microbial propagation and therefore, strict hygienic, sanitary conditions are required to stop and/or minimize the microbial contamination in dairy milk (Kuma *et al.* 2015). The aim of the current study was to evaluate bacterial contamination of dairy milk (domestic milk, farm milk, tetra pack milk) and animal feed (cottonseed cake, wanda) from different regions of KP, Pakistan.

Material and method

Sample Area and Source

Samples were collected from 9 different districts of KP including Peshawar, Charsadda, Swabi, Mardan, Kohat, Abbotabad, Malakand, Dir and Swat. A total of 450 dairy milk and animal feed samples were collected during the period of November, 2016 to December, 2017. The milk sample was collected in sterilized bottles directly from the udder of the healthy cows in case of raw milk while the feed sample was collected in sterilized zipper bags. The sample after collection was immediately brought in ice packed cooler to the Centre of Biotechnology and Microbiology, University of Peshawar and evaluated for the presence of pathogenic microorganisms. The distribution of sample from different districts is given in table no. 1.

Determination of pH

Using a pH meter (Mettler Delta 340), the pH of domestic, farm and tetra pack milk was determined.

Isolation of Pathogenic Bacteria

Milk and feed samples were inoculated on agar media plates using standard dilution technique with the help of a sterilized glass spreader.

Standard Plate Count (SPC) agar was used for Total Plate Count (TPC) while other media used were; Shigella Salmonella (SS) Agar for *Salmonella*, Mannitol Salt Agar (MSA) for *S. aureus*, Eosin Methylene Blue (EMB) for *E. coli*, MacConkey Agar for *Klebsiella* and Pseudomonas Cetrimide agar (PCA) for *P. aeruginosa*. The plates for TPC were incubated at 35°C for 48 hours while the other plates were kept in incubator at 37°C for 24 hours.

Table 1. Distribution of sample from different districts.

S.No	District	Domestic Milk	Farm Milk	Milk Pack	Cottonseed Cake	Wanda	Total
1	Peshawar	10	10	10	10	10	50
2	Charsadda	10	10	10	10	10	50
3	Sawabi	10	10	10	10	10	50
4	Mardan	10	10	10	10	10	50
5	Kohat	10	10	10	10	10	50
6	Abbotabad	10	10	10	10	10	50
7	Malakand	10	10	10	10	10	50
8	Dir	10	10	10	10	10	50
9	Swat	10	10	10	10	10	50
		90	90	90	90	90	450

Morphological and Biochemical Identification

The pathogenic bacterial isolates were morphologically and biochemically identified using standard techniques as described by Olutiola *et al* 2000. This involved citrate, oxidase, catalase, coagulase and triple sugar ion tests.

Results

Most of the raw milk samples and all tetra pack milk samples fell within the normal pH range which is between 6.4-6.8 and 6.5-6.7 as approved for the cow's milk (Bowen. *et al*).

However, few samples at fourth location had pH lower than the normal range approved. The pH values from different disctricts are given in Table No.2

Table 2. pH values of Dairy milk (Area wise).

Area	Raw milk (pH)	Terta Pack Milk (pH)
Peshawar	6.3-6.8	6.4-6.8
Charsadda	6.2-6.5	6.5-6.6
Sawabi	6.3-6.7	6.4-6.6
Mardan	5.8-6.4	6.4-6.7
Kohat	6.0-6.6	6.5-6.8
Abbotabad	6.1-6.5	6.7-6.8
Malakand	6.3-6.7	6.4-6.7
Dir	6.3-6.8	6.5-6.8
Swat	6.2-6.7	6.4-6.6

Characterization and Identification of Bacterial Isolates

A total of 450 samples of dairy milk (raw milk 180, milk pack 90) and animal feed (cottonseed cake 90, wanda 90) were screened for the presence of pathogenic bacteria. The plates showed significant growth of different bacteria after incubation for 24 hours (Fig. 1).

Fig. 1a

Fig. 1b

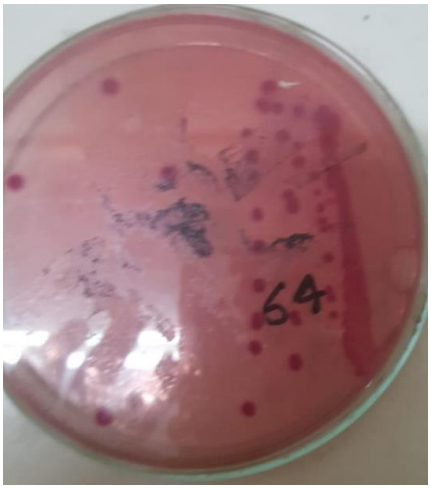

Fig. 1c

Fig. 1d

Fig. 1. Growth of different bacteria on respective agar plates.

Fig. 1a and 1b shows the growth of *Salmonella* and *E. coli* on SS agar and EMB agar respectively. Fig. 1c and 1d shows growth of *Klebseilla* and *P. aeruginosa* on McConkey agar and PCA, respectively.

Bacteria were biochemically identified using API test strips. The biochemical results of bacteria are given in table No. 3 while Fig. 2. shows biochemical results of test pathogens.

Table 3. Biochemical results of bacteria.

Bacteria	Citrate	Oxidase	Catalase	Coagulase	TSI
Salmonella	+	-	+	-	+
Staphylococcus aureus	+	-	+	+	+
E.coli	-	-	+	-	+
Klebseilla	+	-	+	-	+
P. aeruginosa	+	+	+	-	+

Fig. 2. API strips showing positive and negative results of test pathogens.

The percentage of different disease causing bacteria found in dairy milk and animal feed is given in Fig. 3 and 4 respectively.

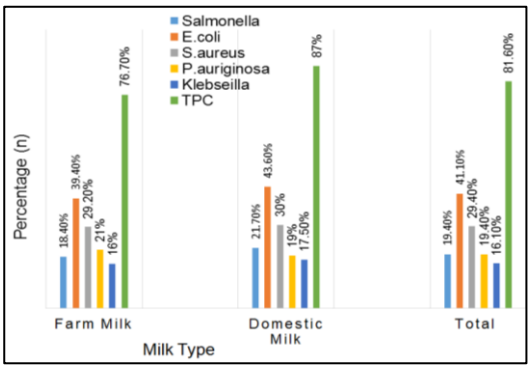

Fig. 3. Percentage of pathogenic bacteria in raw milk.

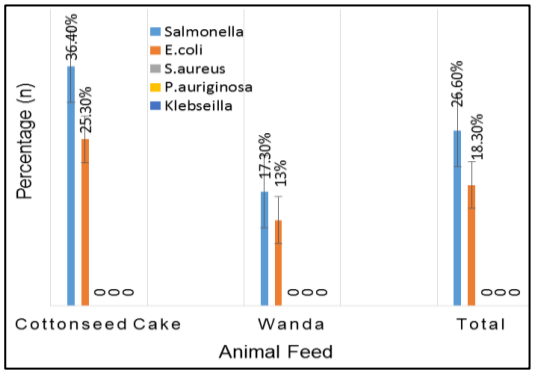

Fig. 4. Percentage of pathogenic bacteria in animal feed

A total of 180 raw milk samples were collected from 9 different districts across KP, with 20 samples from each location. The percentage of bacteria in 20 samples from each district is given in Fig. 5.

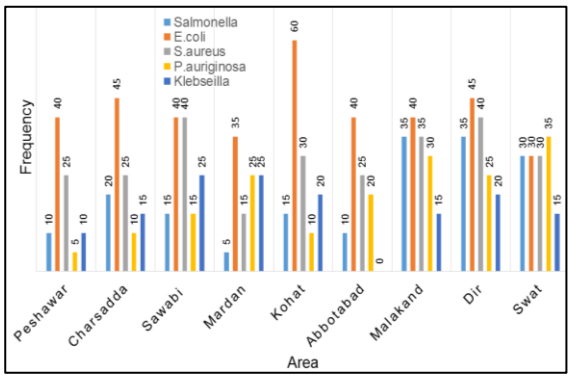

Fig. 5. District wise prevalence of pathogenic bacteria in raw milk (by percentage).

Similarly, 180 samples of animal feed (20 samples from each location) were collected and screened for the presence of pathogenic bacteria.

The percentage of different bacteria out of twenty samples from each district is given in Fig. 6.

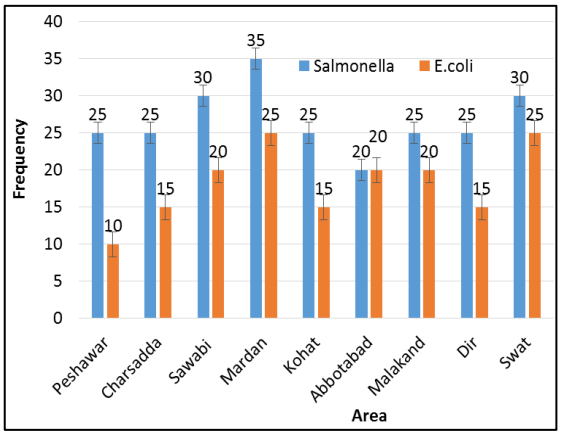

Fig. 6. District wise prevalence of pathogenic bacteria in animal feed (by percentage).

Discussion

Milk secreted by healthy cattle is usually bacteria-free but contamination can occur from environment, utensils, cleaning water and udder surface. (Bramley. 1982). Milk secreted from cattle suffering from mastitis is reported to be highly contaminated with *E. coli*, *staphylococcus*, *streptococcus* species and other pathogenic bacteria (Bramley. 1982, Leigh. 1999). In the present study, out of 180 raw milk samples, 19.4% *Salmonella* (non typhi), 41.1% *E. coli,* 29.4% *S. aures*, 19.4% *P. aeruginosa* and 16.1% of *Klebseilla* species were identified through biochemical identification using API strips. A similar study conducted in 2012 on the prevalence of gram negative bacteria reported the occurrence of *E. coli* (30%), *P. aeruginosa* (18.9%) and *Klebseilla* (17%) in raw milk (Garedew *et al.* 2012). Similarly, another study conducted on the hygienic status of raw milk in three countries shows the presence of *E. coli* (49%), *S. aureus* (33.3%) and *Salmonella* (1.8%) (Hempen *et al.* 2004). A relatively higher frequency of *E. coli Salmonella, S. aureus, P. aeruginosa* and *Klebseilla* has been reported in this study than the studies conducted by Garedew, Hempen and Bramley while all the tetra pack milk samples collected from different superstores and shops at different shelf life showed no growth of gram staining bacteria on the plates. This could be explained by the effective pasteurization method that reduced the chances of post-pasteurization contamination.

A higher *E. coli* contamination of milk does not necessarilly mean that it is a public health risk but it could be an indication for a potential health risk. Gram negative bacteria like *E. coli* and *Pseudomonas* species are commonly found on dairy sheds. Furthermore, mastitis causing coliforms are abundantly found in herd environment, animal bedding and bovine feces. Also, the pathogens are abundantly found in cleaning water for equipment, collection and storage of milk (Hogan *et al.* 1989). The higher frequency of *E. coli, Klebseilla* and *P. aeruginosa* could be attributed to the lack of hygienic bedding conditions on the farm, absence of teat dipping disinfection practices, mastitis udder and contaminated water used for milking systems (Galten. 1986, Bramley. 1990).

Likewise, *S. aureus* is the most common bacterial pathogen that contaminates raw milk. *S. aureus* causes toxic shock syndrome, life threatening endocarditis and other common types of chronic mastitis (Lowry. 2008). *Staphylococcus aureus* contamination in milk is most often originated from the udder of the cow, however milk may also get contaminated after handling in non-hygienic unsanitary conditions. On the contrary to *S. aureus* infection or food poisoning, Salmonellosis is caused by the ingestion of viable *salmonella* species. *Salmonella* group species in raw milk can come from feaces, polluted water, farmer or his family and dust etc. (Hockin. 1989).

In other part of the study, animal feed including cottonseed cake and wanda was evaluated for the presence of pathogenic bacteria. Animal feed is consequently contaminated by microorganisms present on the seed, leaves and roots of plants that serve as protein source in animal feed. The contamination mainly comes from environment, human handlers, storage conditions and equipment (Preston. 1986, Bell. 2010). The current study was designed to assess the bacteriological quality of locally available animal feed. A total of 180 feed samples including; 90 samples of cottonseed cake and 90 samples of wanda, were evaluated for presence of pathogenic bacteria.

Among 180 animal feed samples, 26.6% *Salmonella* and 18.3% of *E.coli* contamination was detected. No other pathogenic bacteria were identified.

Maciorowski *et al.* (2006) investigated that salmonella in animal feed can come during the feed production processes. However, the specific feed ingredients in animal feed, originating from both animal and plant sources, is a potential source of *salmonella* species contamination (Coma 2003, Davis *et al.* 2003). Afterwards, *Salmonella* contamination in feed can occur if the feed is disrobed by wild birds, insects or animal harboring *Salmonella* species (Maciorowski *et al.* 2006).

In a similar study conducted on the analysis of *Salmonella* and *E. coli* species in animal feed by Beilei *et al.* (2013), concluded 22.9% *Salmonella* and 39.3% *E. coli* contamination in animal feed ingredients from plant and animal sources. Similar studies conducted by Dragatz *et al.* (2005) and Lynn *et al.* (1998) shows the presence of *Salmonella* (24%) and *E. coli* (30.1%) in cattle feed samples collected from different regions. *E. coli* has been detected in animal feed ingredients with different percentage rates ranging from absolutely none to 48.2% (Lynn *et al.* 1998, Da Costa *et al.* 2007, Kinley *et al.* 2010). Different studies have concluded that the application of spreading cattle slurry on pastures provides a potentially significant source of contamination through feaces from infected animals (Jeffrey *et al.* 1998).

Conclusion

The current study has concluded that a considerable number of samples from raw milk and animal feed is contaminated with potentially pathogenic bacteria. These pathogenic bacteria find its way into milk from the cow's udder (infected), milker's hand, milking procedure and other environment factors. Similarly, the practice of washing hands before milking and cleaning the udder is also not very common in domestic cattle. Therefore, educating dairy farm owners and local farmers about safety procedures, sanitary udder preparation and hygienic practices at milk collection centers is recommended to minimize the microbial contamination and improve hygienic quality of milk. Similarly feed ingredients should be properly stored and kept away from insects and wild birds.

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Conflict of interest

No potential conflict of interest

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