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Comparative evaluation of pathogenic bacterial incidence in raw and pasteurized milk

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ABSTRACT: *Milk is nutrient and essential food for human being and also serves as good medium for microbial growth and contamination. 25 raw milk (Buffalo milk -10, Cow milk-10 and Goat milk - 5) samples and 25 pasteurized milk (Branded milk- 15 and Local milk - 10) samples from different dairy and shops of Allahabad city were analyzed for 88% of pathogenic bacteria isolates were found to be positive from raw milk and 68% of pathogenic were found to be positive from pasteurized milk. Maximum incidence of Raw and Pasteurized was observed. Significant difference in incidence of Escherichia coli were found to occur (43.58%) followed by Staphylococcus aureus (30.76%), Salmonella typhi (17.94%), Pseudomonas aeruginosa (7.69%).*

KEYWORDS: *Pathogenic bacteria, Raw and Pasteurized milk, Microbial load*

I. INTRODUCTION

Milk is an essential part of daily diet for the growing children and expectant mothers. Milk is a major constituent of the diet; its quality assurance is considered essential to the welfare of a community. Milk is nutritious food for human beings, also serves as a good medium for the growth of many microorganisms, especially *Escherichia coli,Salmonella tyhpi, Pseudomonas aeruginosa and Staphylococcus aureus*. Raw or processed milk is a well-known good medium that supports the growth of several microbes with resultant spoilage of the product i.e., infections or intoxications in consumers **(Murinda *et al*., 2004; Oliver *et al*., 2005).** Microbes may gain entry into raw milk directly from dairy cows experiencing sub clinical or clinical mastitis **(Rodojcic-Prodaova and Necev, 1991),** from the farm environment particularly the water source **(Eberhart, 1977)** and utensils used for the storage of milk on farm or during transportation **(Freedman, 1977).**Milk and milk products are excellent high quality foods providing both nutritional and culinary values. However milk is extremely susceptible to spoilage by microorganisms and the microbiologist plays a major role in the dairy industry in quality control of milk. Cow's milk consists of a variety of nutrients such as fats, proteins, minerals vitamins, carbohydrates and water and thus it serves as an excellent medium for bacterial growth. Given the appropriate conditions milk can act as a carrier of disease causing micro-organisms transformation from cow to humans. Bacterial contamination of raw milk can originate from different sources from animals such as air, milking equipment, feed, soil, feces and grass **(Torkar and Teger, 2008).** Milk microflora includes spoilage and pathogenic microorganisms. Much milk borne diseases such as tuberculosis, brucellosis and typhoid fever are known **(Goff and Horst, 1995).** Milk is spoiled by a wide range of microorganisms some of which are pathogenic and are responsible for milk borne diseases. The milk is very easily contaminated if collected unhygeinically and handled carelessly leading to quick spoilage **(Prajapati, 1995, Chatterjee *et al.*, 2006)**and is often contaminated by *Escherichia coli* bacteria under poor sanitary conditions which can affect public health. The coliform group of bacteria is defined as the indicator (faecalcoliform) of suitability of milk for consumption**.**

II. MATERIALS AND METHODS

Place of work

The present study entitled **"Comparative evaluation of pathogenic bacterial incidence in raw and pasteurized milk"**was conducted in the Department of Microbiology and Fermentation Technology, Sam Higginbottom Institute of Agriculture, Technology and Sciences, Deemed–to–be-University, Allahabad.

Study material

25 raw milk samples were (Buffalo-10 samples, Cow-10 samples and Goat-5 samples) and 25 pasteurized milk samples (local milk-15 samples and branded milk-10 samples) were collected from different dairy and shops of Allahabad city.

Collection of samples

The raw milk and pasteurized milk samples were collected from different dairy and shops of Allahabad city, samples were collected in sterile sample bottles. After collection, the samples were transported to the laboratory and stored in sterile condition below 4°C.

Determination of microbial load in raw and pasteurized milk

The samples of raw milk and pasteurized milk were analyzed for the determination of Total Microbial Count. Raw and pasteurized milk samples were serially diluted (from 10^{-1} to 10^{-6}) in ringers' solution on Nutrient Agar medium, MacConkey Agar medium, CLED Agar medium and Mannitol Salt Agar medium and then incubated at 37°C for 24-48 hrs and count the numbers of colony forming units (cfu/ml) were determined. Each assay was performed in duplicates.

Isolation of pathogenic organisms

Raw milk and pasteurized milk samples were collected in sterile sample bottles. Samples were streaked on the surface of the MacConkey agar plates, CLED agar plates, Nutrient agar plates and Mannitol salt agar plates (*E.coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, and *S. aureus*). The plates were incubated at 37°C for 24-48 hrs, pure bacterial culture were isolated from each milk samples.

Identification of isolates from milk samples

The bacterial isolates were identified by cultural and physiological, morphological and biochemical tests according to Bergey's manual of determinative bacteriology **(Holt *et al.*, 1984).**

Pigment Test

Nutrient agar was prepared, autoclaved and pours in a sterile Petri plate. Then streak the plates with the culture and one plate was kept as control. Incubate the streaked plates at 37°C for 24 h. Then isolates were observed on the basis of pigment characteristics. The appearance of bacterial growth and pigments on the surface of media and growth were observed.

Biochemical characterization

The biochemical identification of the isolates was performed as per the procedure given by **Bergey's Manual.**

	Characteristics	*S. aureus*	*E. coli*	*P. aeruginosa*	*S. typhi*
Cultural characteristics	Colour in N.A Medium	Shiny yellow	Grayish white	Greyish	White
	Margin	Entire	Irregular	Entire	Irregular
	Elevation	Convex	Convex	Flat	Mucoid
	Opacity	Opaque	Opaque	Opaque	Opaque
	Pigmentation	Golden yellow	No	Green	No
Morphological characteristics	Gram stain Reaction	.+ve	-ve	-ve	-ve
	Shape	Spherical	Rods	Rods	Rods
Bio-chemical Characteristics	Motility test	-ve	+ve	+ve	+ve
	Gelatinase test	+ve	-ve	+ve	-ve
	Indole test	-ve	+ve	-ve	-ve
	M.R	+ve	+ve	-ve	+ve
	V.P	-ve	-ve	-ve	-ve
	Citrate utilization test	-ve	-ve	+ve	+ve
	TSI	A/A	A/A	K/K, H_2S	A/A
	Nitrate reduction test	+ve	+ve	+ve	-ve
	Urease	+ve	-ve	+ve	-ve
	Phenylanaline deaminase	-ve	-ve	-ve	-ve
	Lysine decarboxylase	-ve	+ve	-ve	+ve
	Aesculine hydrolysis	-ve	-ve	+ve	-ve
	ONPG test	-ve	-ve	-ve	-ve
	Catalase	+ve	+ve	+ve	-ve
	Oxidase	-ve	-ve	+ve	-ve
	Effect of salt concentration	+ve	-ve	-ve	-ve
	Lipase	-ve	-ve	-ve	-ve
	Starch hydrolysis	-ve	-ve	-ve	-ve
	Arginine hydrolysis	+ve	-ve	+ve	+ve
	Ornithine decorboxylase	-ve	+ve	-ve	+ve

Carbohydrate fermentation	**D-Glucose**	**+ve**	**+ve(G)**	**+ve**	**+ve(G)**
	Sucrose	+ve	+ve	-ve	-ve
	D-Mannitol	+ve	+ve	-ve	+ve
	Lactose	+ve	+ve	-ve	-ve
	Maltose	+ve	+ve	-ve	+ve
	Dulcitol	-ve	-ve	-ve	+ve
	Salicin	-ve	-ve	-ve	+ve
	D-Sorbitol	+ve	+ve	-ve	+ve
	L-Arabinose	-ve	+ve	-ve	+ve
	Raffinose	-ve	-ve	-ve	-ve
	D-Xylose	-ve	+ve	-ve	+ve
	Trehalose	+ve	+ve	-ve	+ve
	Cellobiose	-ve	-ve	-ve	-ve
	D-Arabitol	-ve	-ve	-ve	-ve
	Inositol	-ve	-ve	-ve	+ve
	Glycerol	-ve	+ve	+ve	-ve
	D-Mannose	-ve	+ve	-ve	+ve

Carbon Source (+ve)= Acid positive; (G) = Gas positive; -ve= Acid negative); TSI (A=acid production; K=alkaline reaction; G=gas production; H_2S=sulfur reduction

III. RESULTS AND DISCUSSION

Microbial load in different sources of raw milk sample

The twenty five raw milk samples were examined in present study. Out of twenty five samples, in Buffalo milk 71×10^3 cfu/ml, in Cow milk 62×10^3 cfu/ml and in Goat milk 52.2×10^3 cfu/ml microbial were observed in Nutrient agar medium. Similarly in CLED agar in Buffalo milk 62.5×10^3 cfu/ml, in Cow milk 42.2×10^3 cfu/ml and Goat milk 36.8×10^3 cfu/ml microbial load were observed. While in case of MacConkey agar medium, in case of Buffalo milk 52.5×10^3 cfu/ml, in case of Cow milk 37.2×10^3 cfu/ml and in Goat milk 31.8×10^3 cfu/ml microbial load were determined. But in M.S.A medium 1.2×10^3 cfu/ml microbial load were observed in Buffalo milk, 0.9×10^3 cfu/ml in Cowmilk and 0.4×10^3 cfu/ml in Goat milk. On analyzing the data, the microbial load was found to be statistic significant in Nutrient agar medium, CLED agar medium and MacConkey agar medium. But it found non significant in M.S.A.medium (Table 1, figure 1). In comparison to present study **Perko (2011)** reported 164×10^5 cfu/ml was determined on PCA medium. Similarly **Devi and Sowmy (2012)** study recorded similar count of microbial load in high medium from the statically analysis of data it was found that similar studies made by **Dan *et al*. (2008)** determined 10^6cfu/ml bacterial load.

Table 1 Microbial load in different sources of raw milk sample

TYPES OF MILK	Average bacteria count (10^3cfu/ml)			
	Nutrient Agar	**CLED Agar**	**MacConkey Agar**	**M.S.Agar**
BUFFALO	**71**	**62.5**	**52.5**	**1.2**
COW	**62**	**42.2**	**37.2**	**0.9**
GOAT	**50.2**	**36.8**	**31.8**	**0.4**

Due to N.A F_{cal}=261.15 >F_{tab}=4.46; S=Significant, C.D=1.94.
Due to CLED F_{cal}=275.43 >F_{tab}=4.46; S=Significant, C.D=2.46.
Due to MacConkeyF_{cal}=345.87 >F_{tab}=4.46; S=Significant, C.D=1.39.
Due to MSA F_{cal}=1.96 <F_{tab}=4.46; NS=Non significant, C.D=0.087

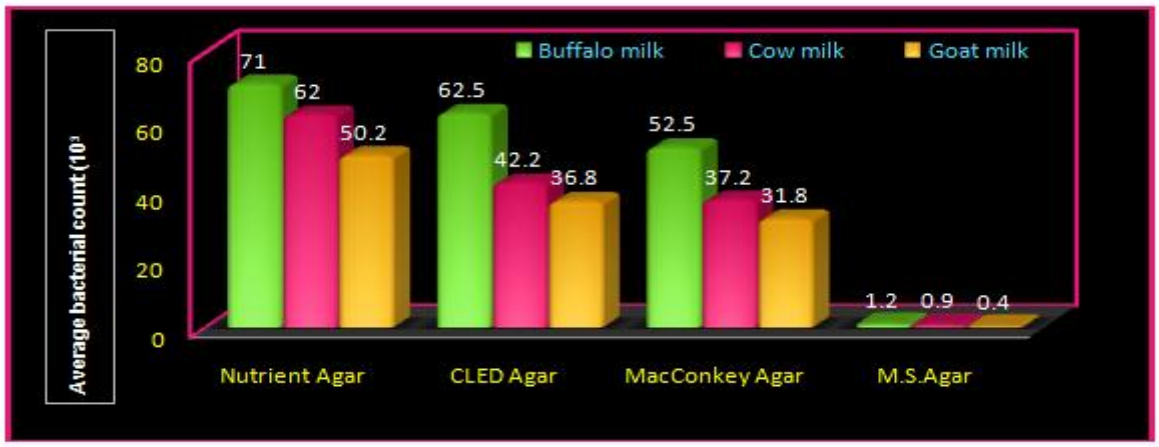

Figure 1: Microbial load in different sources of raw milk samples

Microbial load in different sources of pasteurized milk samples

Out of twenty five pasteurized milk samples tested, 25.53×10^3cfu/ml was found in Local milk. While 17.6 × 103cfu/ml microbial loads were found in Branded milk, in CLED agar medium 15.53 × 103cfu/ml microbial loads were determined in local milk and 13×10^3cfu/ml were determined in Branded milk. Similarly 13.13×10^3cfu/ml and 9.6×10^3cfu/ml were reported in Local and Branded milk. Simultaneously while 0.9×10^3cfu/ml and 0.6×10^3cfu/ml microbial load were observed in Local and Branded milk samples on MSA medium. On analyzing the data the microbial load were found to be statically significant (Table 2, figure 2). **Rizwan *et al.* (2011)** determined 101×10^4cfu/ml in Branded milk. But it showed 105×10^4cfu/ml in Local milk. **Anderson *et al.* (2011)** determined the 500cfu/ml microbial load in pasteurized milk samples.

Table 2 Microbial load in different sources of pasteurized milk samples

	Average bacteria count (10^3cfu/ml)			
	Nutrient agar	**CLED agar**	**MacConkey agar**	**M.S. agar**
Local milk	**20.53**	15.53	13.13	0.9
Branded milk	17.6	13	9.6	0.6

Due to N.A $F_{cal}=178.85 > F_{tab}=6.61$; S=significant.
Due to CLED $F_{cal}=12.00 > F_{tab}=6.61$; S=significant.
Due to MacConkey $F_{cal}=276.49 > F_{tab}=6.61$; S=significant.
Due to MSA $F_{cal}=33.75 > F_{tab}=6.61$; S=significant

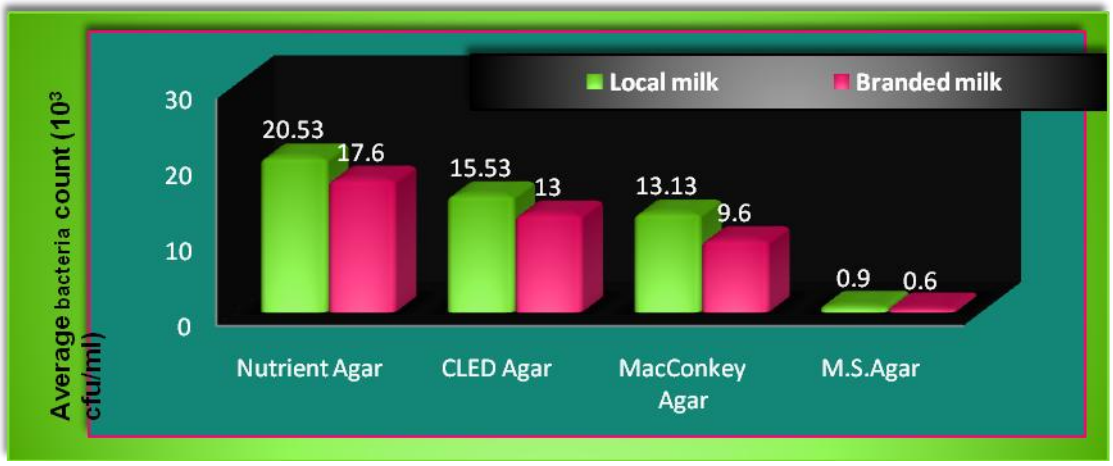

Figure 2:Microbial load in different sources of pasteurized milk samples

Incidence of bacterial isolates from raw milk with respect to types of milk samples

In this study, the incidence of bacterial isolates from Buffalo milk, Cow milk and Goat milk were obtained from different provinces of Allahabad city. Ten samples (22.5%) were positive for Buffalo milk. Ten samples (22.5%) were positive for Cow milk. Similarly (20%) samples were positive for Goat milk (Table 3; Fig: 3). The results reported in study are likewise high when compare to those documented **Alian *et al.* (2012)**, **Shitandi and Sternesjo (2004), Gundogan *et al.* (2006)** and reported that improper high gene and poor farm management practices unattributed to the presence of high microbial growth in the milk.

Table3. Incidence of bacterial isolates from raw milk with respect to types of milk samples

Types of milk	Sample size	No. of samples +ve for bacterial isolation in different raw milk
Buffalo milk	10	9(90%)
Cow milk	10	9(90%)
Goat milk	5	4(80%)

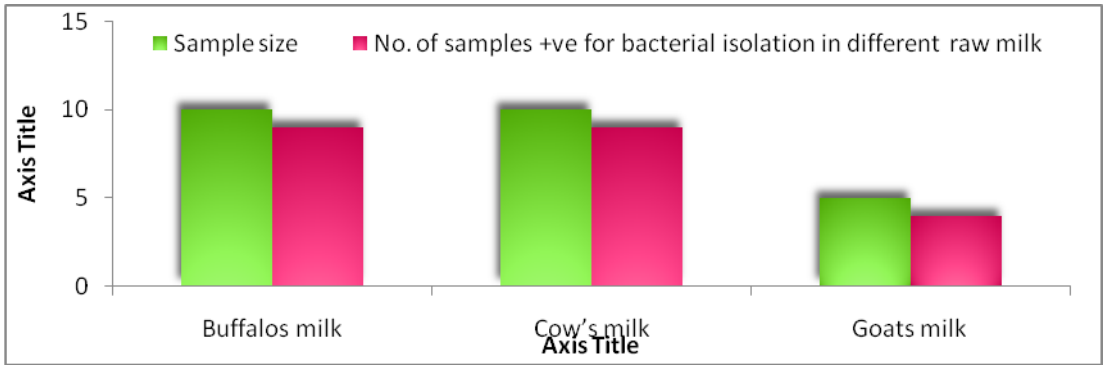

Figure 3; Incidence of bacterial isolates from raw milk with respect to types of milk sample

Incidence of bacterial isolates from pasteurized milk with respect to types of milk samples

Out of fifteen Branded pasteurized milk samples were studies, ten of them were contaminated by bacterial isolates co-responding to 66.66% of samples contaminated. Out of ten Local pasteurized milk samples, seven had labels of positive bacterial isolates. These values can be seen as 70%. Likewise the data found in the study **Oliveira *et al.* (2011)** found exactly similar result as table 4whereas**Ribeiro *et al.* (2009)** found that 25.7 of samples were contaminated.

Table 4 Incidence of bacterial isolates from pasteurized milk with respect to types of milk samples

Milk source	Sample size	No. of samples +ve for bacterial isolation in different pasteurized milk
Branded	15	10(66.66%)
Local	10	7(70%)

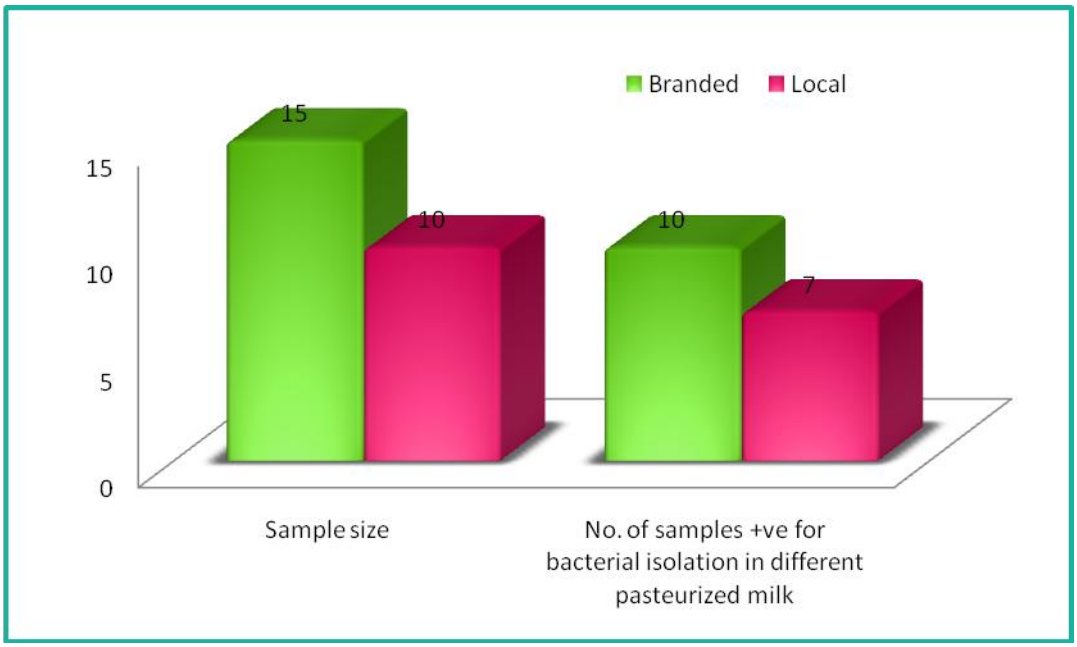

Figure 4 Incidence of bacterial isolates from pasteurized milk with respect to types of milk samples

Prevalence of different bacterial pathogens from raw milk samples with respect to types of milk samples

This study investigated the prevalence of different types of bacterial pathogen was recorded. Laboratory results indicated in Cow milk, *E.coli* (33.33%) as the most prevalence bacteria being applicator this is similar to the finding of **Kewler *et al.* (1992).** The isolation of *S.aureus* (33.33%) in this work is in agreement with that of **Anklo and Sterneojo (2006).** Who isolated 20.4% and 15.4% respectively from healthy Cow in Kaniya.The implication of the presence of *S.typhi* is that it causes tiered which cause a series health problem to the consumers. *S.typhi* (22.22%) were isolated from ten Cow, higher percentage of *P.aeruginosa* (34.6%) were obtains by **Akoglu *et al.* (2012)** and 20% of *S.typhi* were studies in **Ekici *et al.* (2004).**A total of ten Buffalo milk samples were examine for the isolation of bacteria, nine isolates were isolated among which 44.44% were *E.coli* , 33.33% were positive for *S.aureus*, 11.11% for *S.typhi* and 11.11% for *P.aeruginosa* were isolated. On analyzing the data, the isolates were found to be statistically non significant P>0.05 (Table 4.5).The incidence of different types of bacteria isolated from Buffalo milk samples co-related with the finding of **Maniruzzaman *et al.* (2010), Kosietal (2000)** and **Beizhong et al. (2007)** with slight variation.Our results indicate that four samples were positive out of five Goat milk samples, *E.coli* (50%), *S.aureus* (25%), *S.typhi* (25%) were isolated, while *P.aeruginosa* was not detected in Goat milk.Data analyzed by **Ekici *et al.* (2004)** showed as *E.coli* 40%, *S.aureus* 24% were observed. While in case of **Sharma *et al.* (2011)** studied 17.39% incidence of *S.typhi*.

Table 5 Prevalence of different bacterial pathogens from raw milk samples with respect to types of milk samples

Types of milk	No .of isolates	Bacterial Pathogens			
		E.coli	*S.aureus*	*S.typhi*	*P. aeruginosa*
Buffalo	9	4(44.44%)	3(33.33%)	1(11.11%)	1(11.11%)
Cow	9	3(33.33%)	3(33.33%)	2(22.22%)	1(11.11%)
Goat	4	2(50%)	1(25%)	1(25%)	0

Due to sample F_{cal} =4.102<F_{tab} =19.30, NS= Non significant.
Due to Pathogen F_{cal} =6.81 < F_{tab} =19.30, NS= Non significant.

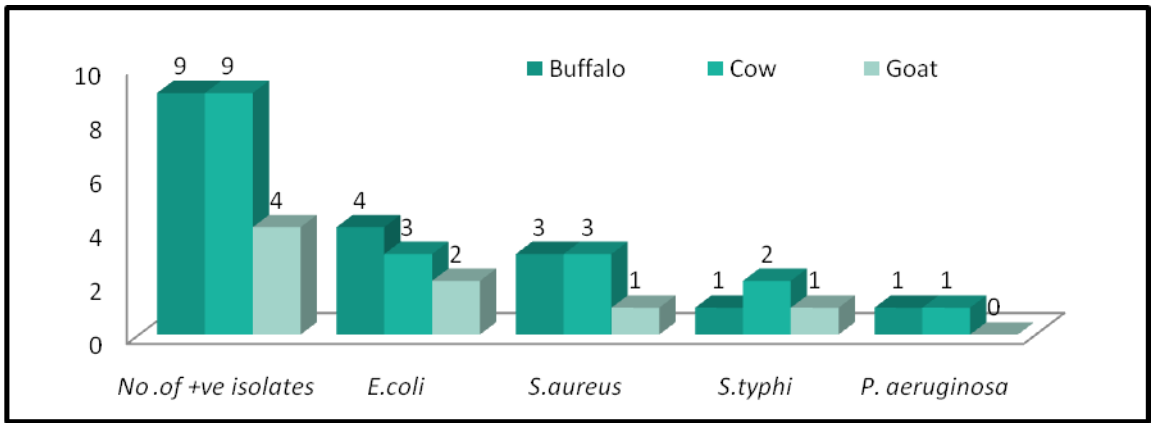

Figure 5 Prevalence of different bacterial pathogens from raw milk samples with respect to types of milk samples

Prevalence of different bacterial pathogens from pasteurized milk with respect to types of milk samples

Out of fifty samples Branded pasteurized milk studies, ten of them were contaminated by bacterial pathogen co-responding to 50% of *E.coli*, 30% of *S.aureus*, 20% of *S.typhi* were observed. Out of ten samples of Local pasteurized milk studied, seven of them were contaminated by bacterial pathogen among the total bacterial population the presage of organism in Local pasteurized milk were *E.coli* (40.85%), *S.aureus* (28.57%), *S.typhi* (14.28%) and *P.aeruginosa* (14.28). On analyzing the data, the isolates were found to be statistically non significant P>0.05 (Table: 6).**Dasilva *et al.* (2011)** found that 22.1% of *E.coli*. In case of **Oliveir *et al.* (2011)** shows 68% of *S.aureus* were contaminated while 12.12% of *P.aeruginosa*was analyzed positive in **Kumaresan and Villi (2008).**

Table 6 Prevalence of different bacterial pathogens from pasteurized milk with respect to types of milk samples

Types of milk	No. of isolates	Bacterial Pathogens			
		E.coli	*S.aureus*	*S.typhi*	*P. aeruginosa*
Branded	10	5(50%)	3(30%)	2(20%)	0(0%)
Local	7	3(40.85%)	2(28.57%)	1(14.28%)	1(14.28%)

Due to sample F_{cal} =3.27 < F_{tab} =230.16, NS= Non significant.
Due to Pathogen F_{cal} =1.42 < F_{tab} =230.16, NS= Non significant.

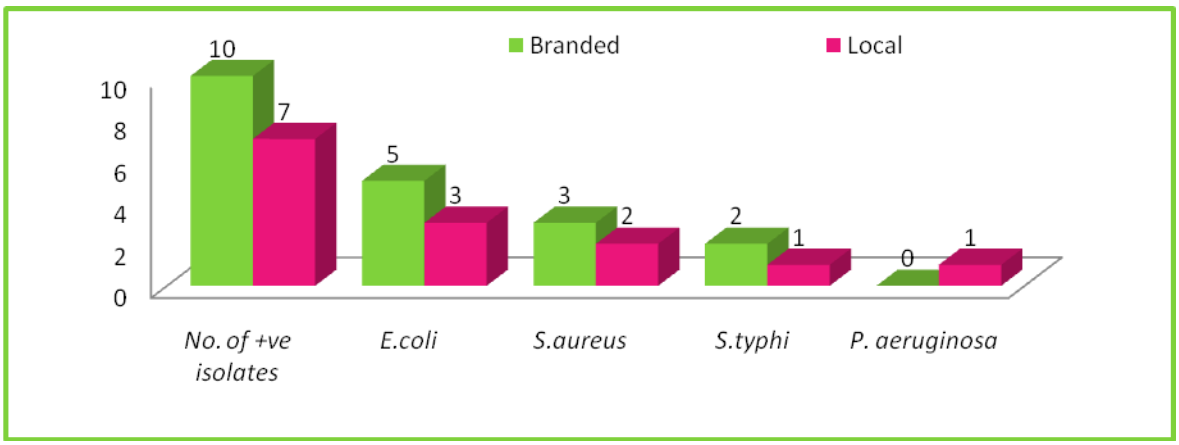

Figure 4.6; Prevalence of different bacterial pathogens from pasteurized milk with respect to types of milk samples

IV. SUMMARY AND CONCLUSION

25 raw milk samples (Buffalo-10 samples, Cow- 10 samples and Goat- 5 samples) and twenty five of pasteurized milk samples (fifteen local milk samples and ten branded milk samples) were collected from different dairy and shops of Allahabad city.Milk samples were collected in sterile sample bottles and samples were spread on surface of the Nutrient Agar medium, MacConkey's Agar medium, Mannitol salt Agar medium and CLED Agar medium and incubated at 37ºC for 24 hrs. The isolates were identified on the basis of cultural, morphological and biochemical characteristics. The isolates were subjected to colony forming unit and their associated factors were assessed using**chi square (χ^2) test, two way ANOVA, C.R.D and C.D**. Following results were obtained and the summary and conclusion was drawn.

- The bacterial count was highest in Buffalo milk i.e. 71 $\times 10^3$cfu/ml and lowest in Goat milk i.e. 50.2 $\times 10^3$cfu/ml.
- The bacterial count was highest in branded milk i.e. 20.53×10^3cfu/ml and lowest in local milk i.e.17.6×10^3 cfu/ml.
- 88% of pathogenic bacteria isolates were found to be positive, out of 25 samples from raw milk.
- 68% of pathogenic bacteria isolates were found to be positive, out of 25 samples from Pasteurized milk.
- Out of different sources maximum incident of bacterial isolates were shown in raw milk.

Higher incidences of different pathogenic microorganisms were observed in raw as well as pasteurized milk. Proper processing regarding pasteurization recommended to milk samples. Attention must be paid to save the raw milk as well as the pasteurized milk from the thread of milk quality related problems and public health concern. Further research is needed to make the strategies of eliminating the problems related to raw milk as well as pasteurized milk.

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