Studies on prevalence of spore-forming and non-spore forming bacteria from milk samples

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Abstract

Milk serves as a complete nutritional source for human consumption, yet its high water content and neutral pH make it a prime environment for microbial proliferation. Contamination typically originates from the animal's udder, unhygienic handling, or substandard equipment. While pasteurization aims to ensure safety, the persistence of certain bacterial strains remains a significant public health concern. This study investigated the microbial quality of milk by analyzing 20 samples (10 pasteurized and 10 unpasteurized) collected from various locations in Akola city. A total of 51 bacterial isolates were recovered under aerobic and anaerobic conditions. Under aerobic conditions, 30 bacteria were isolated. Of these, 69% (20) were non-spore formers—predominantly found in unpasteurized milk (18) compared to pasteurized milk (2). The remaining 31% (10) were facultative anaerobic spore formers. Identification revealed that *Escherichia coli* had the highest prevalence (26.67%), followed by *Staphylococcus aureus* (20%), *Salmonella spp.* (10%), *Pseudomonas aeruginosa* (6.67%), and *Shigela spp.* (3.34%). Among aerobic spore formers, *Bacillus subtilis* (20%) and *Bacillus cereus* (13.34%) were the most common. Under anaerobic conditions, 21 bacteria were isolated. In contrast to aerobic results, spore-forming bacteria showed a significantly higher prevalence at 76% (16), while non-spore formers accounted for only 24% (5). The most prevalent anaerobic spore former was *Clostridium perfringens* (33.34%), followed by *Clostridium titani* (28.58%), *Bacillus licheniformis* (9.53%), and *Clostridium sporogenes* (4.77%). Anaerobic non-spore formers included *Lactobacillus spp.* (14.29%) and *Escherichia coli* (9.52%).The findings highlight a diverse bacterial load in both milk types, with a notably higher concentration of pathogens in unpasteurized samples and a dominance of *Clostridium* and *Bacillus* species among spore-forming isolates.

Key words -Milk, Spore former, Non- spore former

Introduction

Milk is one of the most essential and nutritionally complete foods consumed by humans. It contains high-quality proteins, fats, carbohydrates (mainly lactose), vitamins (A, D, B-complex), minerals (calcium, phosphorus, potassium), and water. The microbial load in milk is greatly increased by contaminated equipment, mastitis infection, poor storage conditions, and poor milking hygiene (Oliver et al., 2009). The demand for dairy products including curd, paneer, cheese, butter, and milk powder, as well as growing urbanization, have made preserving microbiological safety a top public health priority. The U.S. Food and Drug Administration emphasized that contaminated milk and milk products might spread diseases like *Salmonella species, Escherichia coli,* and *Listeria monocytogenes* (FDA, 2023). To ensure food safety, it is crucial to investigate the microbial predominance in dairy products.

The microbial flora of milk consists of beneficial, spoilage-causing, and pathogenic microorganisms. These microorganisms are broadly classified into spore-forming bacteria and non-spore-forming bacteria, based on their ability to produce endospores. Spore-forming bacteria were defined by Logan and De Vos in Bergey's Manual of Systematics of Archaea and Bacteria as Gram-positive species that can produce extremely resistant endospores in adverse environmental conditions (Logan & De Vos, 2015). These spores are resistant to chemical disinfectants, heat treatment, dehydration, and radiation. According to Postollec et al., spores are challenging to eradicate during dairy processing due to their resistance to pasteurization and potential for germination during storage (Postollec *et al*., 2015). Sporulation increases environmental persistence and contributes to food contaminations (Arnesen *et al*., 2018).

In contrast, non-spore-forming bacteria are more susceptible to heat treatment and do not create spores. Many of these are significant diseases or markers of hygiene, though. Because of fecal contamination and sick udders, raw milk often contains Salmonella, Escherichia coli, and Staphylococcus aureus (Oliver *et al.*, 2009). Dairy products are frequently linked to Listeria and Staphylococcal food poisoning outbreaks, according to EFSA (2022). The significance of both spore-forming and non-spore-forming bacteria in dairy microbiology is thus established.

Milking procedures, processing conditions, storage temperature, animal health, and ambient sanitation all have an impact on the predominance of spore-forming and non-spore-forming bacteria in milk and dairy products. Raw milk can be directly contaminated with pathogenic and spoiling microorganisms due to poor udder health, such as mastitis. Furthermore, land, feed, water, milking equipment, and handlers can all get contaminated if appropriate hygiene practices are not followed. The impact of poor hygiene and handling procedures during production and distribution, showed higher microbial counts in raw milk compared to pasteurized milk samples taken from urban markets. Singh *et al*. (2024) Microbial load is also greatly influenced by processing conditions. Clarified that while bacterial spores from genera like *Bacillus and Clostridium* can withstand heat treatment and may germinate in the right circumstances, pasteurization is successful in eliminating the vegetative cells of non-spore-forming bacteria. The temperature at which food is stored also affects the growth of germs since inadequate refrigeration causes surviving bacteria to proliferate quickly, which can cause spoiling and health hazards (Walstra *et al*. 2016). In order to reduce microbial contamination, rigorous hygienic procedures, efficient sanitation programs, and appropriate cold chain management throughout production, transportation, and storage are crucial. EFSA (2022). Thus present study was undertaken to check the microbial quality and presence of spore formers and non-spore formers that could be potential health risk from milk samples collected from Akola city.

Material and Methods

- **Collection of milk samples**. :

  Each sample was collected from different area in Akola city. A total of 20 samples were collected in sterilized bottles 10 pasteurized & 10 unpasteurized samples carried to the laboratory immediately in an ice-box and stored into the refrigerator at 4°C for further use.

- **Isolation of the spore-forming and non-spore-forming bacteria from pasteurized and unpasteurized milk samples.**
- **Isolation of non-spore-forming bacteria from pasteurized & unpasteurized milk samples**-

In the study for isolation of non-spore forming all the pasteurized and unpasteurized milk samples were firstly diluted by serial dilution technique. The sample for last dilution was then inoculated onto nutrient agar plates and all plates were incubated at 37°C for 24 hrs. under aerobic condition and into desiccator by candle jar method for anaerobic condition.

- **Isolation of spore-forming bacteria from pasteurized & unpasteurized milk samples**-

For isolation of spore forming bacteria from pasteurized and unpasteurized milk samples were first heated at 80°C for 10 min to destroy all vegetative cells. After heating samples were diluted and then inoculated onto nutrient agar plates. All the plates were then incubated at 37°C for 24 hrs under aerobic condition and into desiccator by candle jar method for anaerobic condition.

- **Identification of isolates:**

All the isolates for aerobic and anaerobic conditions isolates under were separately maintained on nutrient agar slants at 4°C these isolates identified by their morphological- gram staining, endospore staining and motility by cultural characteristics- size, shape, color, margin, opacity, texture, elevation & biochemical characteristics which includes sugar fermentation test, IMViC test and various enzymes tests.

Results

In the present study 10 pasteurized and 10 unpasteurized samples were collected from the various locations of Akola city. A total of 51 bacteria were isolated from 20 milk samples under aerobic and anaerobic conditions. A total of 30 bacteria were isolated under aerobic condition from pasteurized and unpasteurized milk samples. It was found that 69% (20) of aerobic non-spore formers were isolated from milk samples which include 2 from pasteurized milk and 18 from unpasteurized milk. While 31% (10) aerobic, facultative anaerobic spore former were isolated from milk samples which include 3 from pasteurized and 7 from unpasteurized milk (Table 1 & fig. 1).

All the 30 isolates were then identified by various morphological, cultural and biochemical characteristics (Table 2 & 3). From the results it was found that amongst the non-spore-forming bacteria prevalence *Escherichia coli* was high (26.67%) followed by *Staphylococcus aureus* (20%), *Salmonella spp.* (10%), *Pseudomonas aeruginosa* (6.67%) and *Shigela spp.* (3.34%) while amongst the spore forming bacteria *Bacillus subtilis* was predominantly isolated (20%) followed by *Bacillus cereus* (13.34%) (figure 2).

In a similar way anaerobic spore forming and non-spore forming bacteria from milk samples were also isolated from milk samples. It was found that a total 21 bacteria were isolated from pasteurized and unpasteurized milk samples under anaerobic condition 76% (16) of spore-forming bacteria were isolated which is high as compared to 24%(5) non-spore-forming bacteria from milk samples (Table 4 & fig. 3).

All the 21 isolates were then identified by standard conventional methods which includes morphological, cultural and biochemical characteristics (Table 5 & 6). Results showed that most of the spore-formers under anaerobic condition were belongs to *Bacillus spp*. Amongst the anaerobic spore formers highest prevalence of *Clostridium perfringens* 33.34% (7) was observed followed by *Clostridium titani* 28.58 % (6), *Lactobacillus spp.* 14.29 % (3), *Bacillus licheniformis* 9.53 % (2) and *Clostridium sporogenes* 4.77 % (1). While amongst the non-spore-forming bacteria a facultative anaerobic *Escherichia coli* was only isolated from milk sample in a less percentage of 9.52 as compare to others non-spore formers (figure 4).

Table 1: isolation of spore forming and non-spore forming bacteria from pasteurized and unpasteurized milk sample under aerobic condition.

Milk samples	Spore formers	Non-spore formers
Pasteurized	3	2
unpasteurized	7	18
Total	10	20

Figure 1: Occurrence of spore formers & non-spore formers.

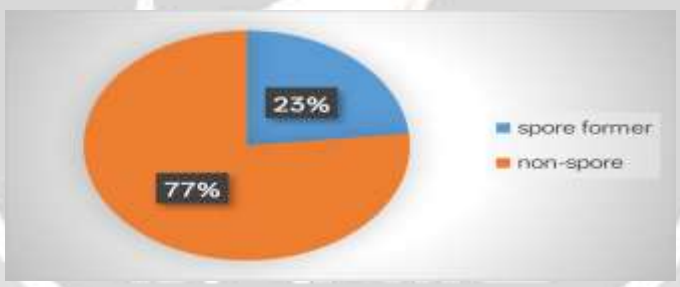


Table 2: Morphological charecteristics & cultural charecteristics of isolates from milk samples, under aerobic condition.

S.N.	isolats	Morphological characters			Cultural characters							
		Gram reaction	Spore formation	Motility	Size	Shape	Color	Opacity	Texture	Margine	Oxygen requirement	Elevation
1	A1	+ve	+ve	motile	2-4	irregular	Cream white	opaque	Dry rough	lobate	aerobic	Flat
2	A2	+ve	+ve	motile	3-5	Large rod	White gray	opaque	Dry rough	irregular	aerobic	flat
3	A3	-ve	-ve	motile	1-3	rod	Grayish white	opaque	smooth	entire	Facultative Anaerobic	Convex
4	A4	+ve	-ve	Non-motail	2-4	circular	Golden yellow	opaque	Smooth buttery	entire	Facultative Anaerobic	Convex
5	A5	-ve	-ve	Non-motile	1-3	Short rod	white	opaque	smooth	entire	Facultative Anaerobic	Convex
6	A6	+ve	+ve	motile	3-5	Large rod	White gray	opaque	Dry rough	irregular	aerobic	flat
7	A7	-ve	-ve	motile	1-3	rod	Greyish white	opaque	smooth	entire	FacultativeAnaerobic	Convex
8	A8	+ve	-ve	Non-motile	2-4	circular	Golden yellow	opaque	Smooth buttery	entire	FacultativeAnaerobic	Convex
9	A9	-ve	-ve	motile	2-5	irregular	Bluish green	opaque	smooth	entire	aerobic	flat
10	A10	+ve	+ve	motile	3-5	Large rod	White gray	opaque	Dry rough	irregular	FacultativeAnaerobic	flat
11	A11	+ve	-ve	Non-motile	2-4	circular	Golden yellow	opaque	Smooth buttry	entire	FacultativeAnaerobic	Convex
12	A12	-ve	-ve	motile	1-3	rod	Greyish white	opaque	smooth	entire	FacultativeAnaerobic	Convex
13	A13	-ve	-ve	motile	2-4	circular	Grayish white	opaque	smooth	entire	Facultative Anaerobic	Convex
14	A14	+ve	+ve	motile	2-4	irregular	Cream white	opaque	Dry rough	lobate	aerobic	flat
15	A15	+ve	-ve	Non-motile	2-4	circular	Golden yellow	opaque	Smoothbuttry	entire	FacultativeAnaerobic	Convex
16	A16	+ve	+ve	motile	3-5	Large rod	White gray	opaque	Dry rough	irregular	FacultativeAnaerobic	flat
17	A17	-ve	-ve	motile	1-3	rod	Greyish	opaque	smooth	entire	FacultativeAnaerobi	Convex

							white				c	
18	A18	-ve	-ve	motile	2-5	irregular	Bluish green	opaque	smooth	entire	Aerobic	flat
19	A19	+ve	+ve	motile	2-4	irregular	Cream white	opaque	Dry rough	lobate	aerobic	flat
20	A20	-ve	-ve	motile	1-3	rod	Greyish white	opaque	smooth	entire	FacultativeAnaerobic	Convex
21	A20	+ve	-ve	Non motile	2-4	circular	Golden yellow	opaque	Smoothbuttry	entire	FacultativeAnaerobic	Convex
22	A22	-ve	-ve	motile	2-4	circular	Greyish white	opaque	smooth	entire	FacultativeAnaerobic	Convex
23	A23	+ve	+ve	motile	2-4	irregular	Cream white	opaque	Dry rough	lobate	aerobic	Flat
24	A24	-ve	-ve	motile	1-3	rod	Greyish white	opaque	smooth	entire	FacultativeAnaerobic	Convex
25	A25	-ve	-ve	motile	2-4	circular	Greyish white	opaque	smooth	entire	FacultativeAnaerobic	Convex
26	Ap26	-ve	-ve	motile	1-3	rod	Greyish white	opaque	smooth	Entire	FacultativeAnaerobic	Convex
27	Ap27	+ve	-ve	Non-motile	2-4	circular	Golden yellow	opaque	Smoothbuttry	entire	FacultativeAnaerobic	Convex
28	Ap28	+ve	+ve	motile	2-4	irregular	Cream white	opaque	Dry rough	lobate	Aerobic	flat
29	Ap29	+ve	+ve	motile	2-4	irregular	Cream white	opaque	Dry rough	lobate	Aerobic	flat
30	Ap30	-ve	-ve	motile	1-3	rod	Greyish white	opaque	smooth	entire	FacultativeAnaerobic	Convex

Table 3: Biochemical characteristics of isolates from milk samples under aerobic condition.

S. N.		IMViC characters				Enzyme tests						Sugar fermentation A/G				Identified Isolates
	Isolate	I	M	V	C	Urease	Catalase	Oxidase	Protase	Amylase	Gelatinase	Maltose	Lactose	Sucrose	Dextrose	
1	A1	-	-	+	+	-	+	+	+	+	+	+/-	-/-	+/-	+/-	*B. subtilis*
2	A2	-	+	+	+	-	+	+	+	+	+	-/-	-/-	+/-	+/-	*B. cereus*
3	A3	+	+	-	-	-	+	-	-	-	-	+/+	+/+	+/-	+/+	*E. coli*
4	A4	-	-	+	-	+	+	-	+	-	+	+/-	+/-	+/-	+/-	*S. aureus*
5	A5	+	+	-	-	-	+	-	-	-	-	+/-	-/-	-/-	+/-	*Shigella spp.*
6	A6	-	-	+	-	+	+	-	+	-	+	+/-	+/-	+/-	+/-	*B. cereus*
7	A7	+	+	-	-	-	+	-	-	-	-	+/+	+/+	+/-	+/+	*E. coli*

8	A8	-	-	+	-	+	+	-	+	-	+	+/-	+/-	+/-	+/-	*B. subtilis*
9	A9	-	-	-	+	-	+	+	+	-	+	-/-	-/-	-/-	-/-	*P. aeruginosa*
10	A10	-	+	+	+	-	+	+	+	+	+	-/-	-/-	+/-	+/-	*B. cereus*
11	A11	-	-	+	-	+	-	-	+	-	+	+/-	+/-	+/-	+/-	*S. aureus*
12	A12	+	+	-	-	-	+	-	-	-	-	+/+	+/+	+/-	+/+	*E. coli*
13	A13	-	+	-	+	-	+	-	-	-	-	+/+	-/-	-/-	+/+	*Salmonella spp.*
14	A14	-	-	+	+	-	+	+	+	+	+	+/-	-/-	+/-	+/-	*B. subtilis*
15	A15	-	-	+	-	+	+	-	+	-	+	+/-	+/-	+/-	+/-	*S. aureus*
16	A16	-	+	+	+	-	+	+	+	+	+	-/-	-/-	+/-	+/-	*B. cereus*
17	A17	+	+	-	-	-	+	-	-	-	-	+/+	+/+	+/-	+/+	*E. coli*
18	A18	-	-	-	+	-	+	+	+	-	+	-/-	-/-	-/-	-/-	*P. aeruginosa*
19	A19	+	+	-	-	-	+	-	-	-	-	+/+	+/+	+/-	+/+	*B. subtilis*
20	A20	+	+	-	-	-	+	-	-	-	-	+/+	+/+	+/-	+/+	*E. coli*
21	A21	-	-	+	-	+	+	-	+	-	+	+/-	+/-	+/-	+/-	*S. aureus*
22	A22	-	+	-	+	-	+	-	-	-	-	+/+	-/-	-/-	+/+	*Salmonella spp.*
23	A23	-	-	+	-	+	+	-	+	-	+	+/-	+/-	+/-	+/-	*B. subtilis*
24	A24	+	+	-	-	-	+	-	-	-	-	+/+	+/+	+/-	+/+	*E. coli*
25	A25	-	+	-	+	-	+	-	-	-	-	+/-	-/-	-/-	+/-	*Salmonella spp.*
26	Ap26	+	+	-	-	-	+	-	-	-	-	+/+	+/+	+/-	+/+	*E. coli*
27	Ap27	-	-	+	-	+	+	-	+	-	+	+/-	+/-	+/-	+/-	*S. aureus*
28	Ap28	-	-	+	+	-	+	+	+	+	+	+/-	-/-	+/-	+/-	*B. subtilis*
29	Ap29	-	-	+	-	+	+	-	+	-	+	+/-	+/-	+/-	+/-	*S. aureus*
30	Ap30	+	+	-	-	-	+	-	-	-	-	+/+	+/+	+/-	+/+	*E. coli*

(+: present, -:Absenent)

Figure 2: Identified spore formers & non spore formers from milk sample.

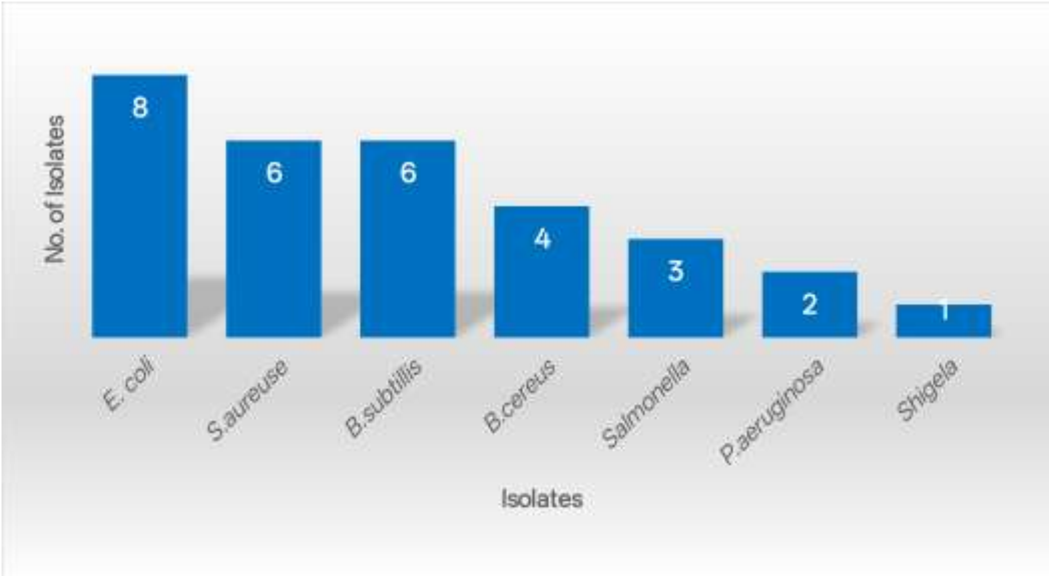


Table 4:

isolation of anaerobic spore forming & non-spore forming bacteria from milk sample.

Sr. no.	Milk sample	Spore formers	Non-spore formers
1	Pasteurize	06	02
2	unpasteurized	10	03
Total		16	05

Figure 3: Occurrence of spore formers & non spore formers.

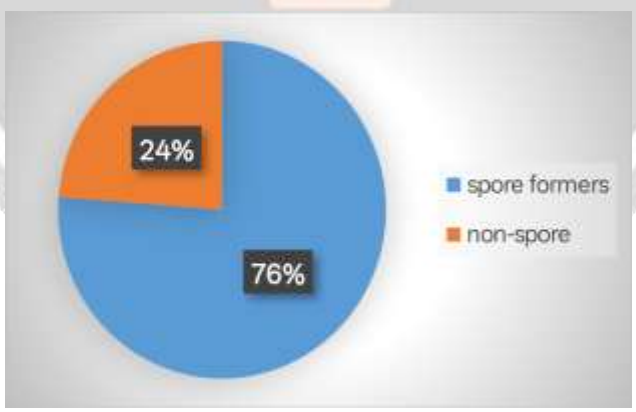


Table 5: Morphological and cultural characters of isolates from milk samples under anaerobic condition.

S. N.	Isolate	Morphological characters			Cultural characters							
		Gram reaction	Spore formation	Motility	Size	Shape	Color	Opacity	Texture	Margine	Oxygen requirement	Elevation
1	Aa1	+ve	+ve	motile	1-3 mm	irregular	grayish	translucent	soft	irregular	Obligate anearobic	flat
2	Aa2	+ve	+ve	Non-motile	2-4 mm	circular	grayish	opaque	smooth	smooth	anearobic	raised
3	Aa3	+ve	+ve	motile	2-5 mm	irregular	Grayish white	opaque	Soft rough	irregular	anearobic	raised
4	Aa4	+ve	+ve	motile	1-3 mm	irregular	grayish	translucent	soft	irregular	Obligate anearobic	Flat
5	Aa5	+ve	+ve	motile	1-3 mm	irregular	grayish	translucent	soft	irregular	Obligate anearobic	Flat
6	Aa6	+ve	-ve	Non-motile	1-3 mm	circular	Creamy white	opaque	smooth	entire	Facultative Anaerobic	convex
7	Aa7	+ve	+ve	Non-motile	2-4 mm	circular	grayish	opaque	smooth	smooth	anearobic	raised
8	Aa8	+ve	+ve	Non-motile	2-4 mm	circular	grayish	opaque	smooth	smooth	anearobic	raised
9	Aa9	-ve	-ve	motile	1-3	rod	Greyish white	opaque	smooth	entire	Facultative Anaerobic	convex
10	Aa10	+ve	+ve	motile	2-4	irregular	Light brown	opaque	Dry rough	lobate	Facultative Anaerobic	flat
11	Aa11	+ve	+ve	motile	1-3 mm	irregular	grayish	translucent	soft	irregular	Obligate Aanearobic	flat
12	Aa12	+ve	+ve	Non-motile	2-4 mm	circular	grayish	opaque	smooth	smooth	Anearobic	raised
13	Aa13	+ve	-ve	Non-motile	1-3 mm	circular	Creamy white	opaque	smooth	entire	Facultative Anaerobic	convex
14	Aap14	+ve	+ve	motile	1-3 mm	irregular	grayish	translucent	soft	irregular	Obligate anearobic	flat
15	Aap15	-ve	-ve	motile	1-3	rod	Greyish white	opaque	smooth	entire	Facultative Anaerobic	convex
16	Aap16	+ve	+ve	Non-motile	2-4 mm	circular	grayish	opaque	smooth	smooth	anearobic	raised
17	Aap17	+ve	+ve	motile	2-4	irregular	Light brown	opaque	Dry rough	lobate	Facultative Anaerobic	flat
18	Aap18	+ve	+ve	Non-motile	2-4	circular	grayish	opaque	smooth	smooth	anearobic	raised

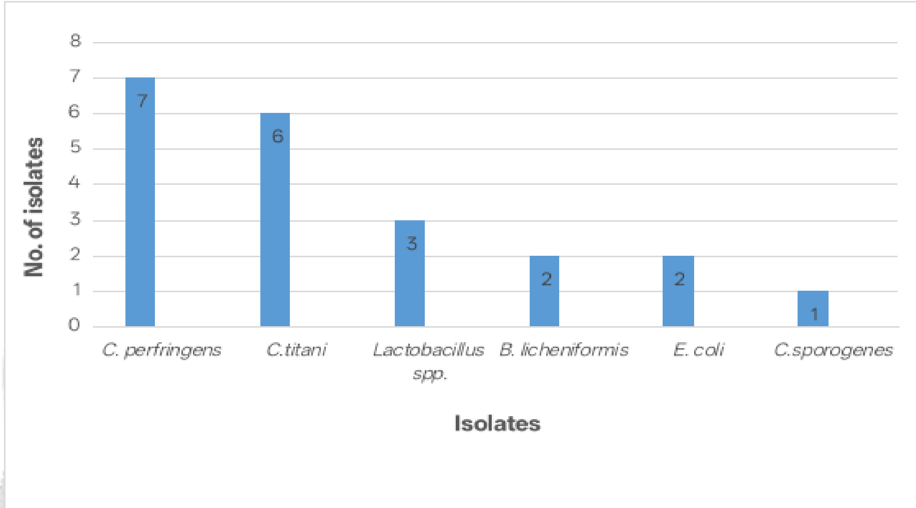
					mm							
19	Aap19	+ve	+ve	motile	1-3 mm	irregular	grayish	translucent	soft	irregular	Obligate anearobic	flat
20	Aap20	+ve	-ve	Non-motile	1-3 mm	circular	Creamy white	opaque	smooth	entire	Facultative Anaerobic	convex
21	Aap21	+ve	+ve	Non-motile	2-4 mm	circular	grayish	opaque	smooth	smooth	anearobic	raised

Table 6: biochemical characteristics of isolates from milk samples under anaerobic condition.

S. N.		IMViC characters				Enzyme tests						Sugar fermentation A/G				Identified Isolates
	Isolate	I	M	V	C	Urease	Catalase	Oxidase	Protease	Amylase	Gelatinase	Maltose	Lactose	Sucrose	Dextrose	
1	Aa1	-	-	-	-	-	-	-	+	-	+	-/-	-/-	-/-	-/-	*C. titani*
2	Aa2	-	-	-	-	-	-	-	+	+	+	+/+	+/+	+/+	+/+	*C. perfringens*
3	Aa3	-	-	-	-	-	-	-	+	-	+	-/-	-/-	-/-	+/-	*C. sporogenes*
4	Aa4	-	-	-	-	-	-	-	+	-	+	-/-	-/-	-/-	-/-	*C. titani*
5	Aa5	-	-	-	-	-	-	-	+	-	+	-/-	-/-	-/-	-/-	*C. titani*
6	Aa6	-	-	-	-	-	-	-	-	-	-	+/-	+/-	+/-	+/-	*Lactobacillus spp.*
7	Aa7	-	-	-	-	-	-	-	+	+	+	+/+	+/+	+/+	+/+	*C. perfringens*
8	Aa8	-	-	-	-	-	-	-	+	+	+	+/+	+/+	+/+	+/+	*C. perfringens*
9	Aa9	+	+	-	-	-	+	-	-	-	-	+/+	+/+	+/-	+/+	*E. coli*
10	Aa10	-	-	+	+	+	+	+	+	+	+	+/-	-/-	+/-	+/-	*B. licheniformis*
11	Aa11	-	-	-	-	-	-	-	+	-	+	-/-	-/-	-/-	-/-	*C. titani*
12	Aa12	-	-	-	-	-	-	-	+	+	+	+/+	+/+	+/+	+/+	*C. perfringens*
13	Aa13	-	-	-	-	-	-	-	-	-	-	+/-	+/-	+/-	+/-	*Lactobacillus spp.*
14	Aap14	-	-	-	-	-	-	-	+	-	+	-/-	-/-	-/-	-/-	*C. titani*
15	Aap15	+	+	-	-	-	+	-	-	-	-	+/+	+/+	+/-	+/+	*E. coli*
16	Aap16	-	-	-	-	-	-	-	+	+	+	+/+	+/+	+/+	+/+	*C. perfringens*
17	Aap17	-	-	+	+	+	+	+	+	+	+	+/-	-/-	+/-	+/-	*B. licheniformis*
18	Aap18	-	-	-	-	-	-	-	+	+	+	+/+	+/+	+/+	+/+	*C. perfringens*
19	Aap19	-	-	-	-	-	-	-	+	-	+	-/-	-/-	-/-	-/-	*C. titani*
20	Aap20	-	-	-	-	-	-	-	-	-	-	+/-	+/-	+/-	+/-	*Lactobacillus spp.*
21	Aap21	-	-	-	-	-	-	-	+	+	+	+/+	+/+	+/+	+/+	*C. perfringens*

(+:Present,-:Absent)

Figure 4: Identified spore formers & non spore formers from milk sample.



Discussion

In the study pasteurized and unpasteurized milk samples were col lected. It was found that under aerobic condition most of the non-spore-forming bacteria observe in both pasteurized and unpasteurized milk. In which the higher percent of non-spore formers was observed amongst unpasteurized milk samples. Which include mostly Gram-positive bacteria such as Staphylococcus aureus, & other Gram-negative Escherichia coli, Pseudomonas aeruginosa and Shigella spp. The reason may be the contamination of milk samples during milking procedures from milks vendors or use of contaminated utensils or water. Contamination with Escherichia coli, Salmonella and shigela spp. represents potential health risk of consumption of such milk samples which can lead to severe infections. Presence of Escherichia coli, Salmonella & Shigella spp. may also represent possible contamination with fecal matter handaling or use of contaminate water other studies have also reported presence of such harmful bacteria into milk samples. (Ahmed, Shimamoto, 2015), Similar findings were reported by Lingathurai and Vellathurai (2010) and Chye et al. (2004), who detected Staphylococcus aureus and E. coli in raw milk due to poor sanitation during milking and handling.

Amongst pasteurized milk, number of bacteria isolated were less as only 23% occurrence was observed in which 3 spore formers and 2 non-spore formers were isolated which includes Bacillus subtilis, & Bacillus cereus spore-forming bacteria, while non-spore-forming bacteria include Pseudomonas aeruginosa, & Staphylococcus aureus Chye et al.,2004). Occurrence of less number of bacteria indicates pasteurization was proper and effective in reduction of harmful bacteria from milk samples. (Lingathurai, S., & Vellathurai, P. (2010).Similar observations were reported by Meer et al. (1991), who stated that Bacillus cereus and other spore-forming bacteria are frequently detected in pasteurized milk because their spores can survive thermal processing. Likewise, Giffel et al. (2002) reported that Bacillus cereus is one of the most common spore-forming bacteria found in dairy products and processing environments due to its resistance to heat treatm subtilis in pasteurized milk samples, emphasizing that these organisms can survive pasteurization and grow during refrigerated storage.

In a similar way the milk samples were also utilized for isolation of spore formers and non-spore formers from milk samples under anaerobic condition to check strict anaerobes found in to the milk samples. It was found that 24% spore formers were isolated it was lasses compare to non-spore-formers (76%). Amongst the pasteurized & unpasteurized milk Gram-positive bacteria were predominant as compare to gram-negative bacteria. Which include mostly spore formers Clostridium perfringens, Clostridium titani and Lactobacillus spp. amongst the anaerobic bacteria isolated. The genus Clostridium spp. and Bacillus spp. Were found as potential health risk as Clostridium perfringens, Clostridium titani, and Clostridium sporogenes producing a resistant spores which helps to survive bacteria under pasteurized condition also, others studies have also reported presence of Clostridium spp into the milk samples. Similar findings were reported by Bassi et al. (2013) and Giffel et al. (2002), who observed that Clostridium species are commonly present in raw milk and dairy products due to environmental contamination and their ability to form highly resistant spores. The predominance of Gram-positive bacteria observed in the present study is consistent with the findings of Quigley et al. (2013), who reported that Gram-positive bacteria, particularly spore-forming genera such as Bacillus and Clostridium, are commonly associated with milk microbiota.

Conclusion

The present study was conducted to isolate and identify spore-forming and non-spore-forming bacteria from pasteurized and unpasteurized milk samples collected from different areas of Akola city. In results unpasteurized milk contained a higher number of bacterial isolates compared to pasteurized milk. Under aerobic conditions, non-spore-forming bacteria were more common, and Escherichia coli was the most predominant bacterium, followed by Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Salmonella spp, Pseudomonas aeruginosa, and Shigella spp. Similarly under anaerobic conditions, spore-forming bacteria were more prevalent, with Clostridium perfringens being the most dominant, followed by Clostridium titani, Lactobacillus spp., Bacillus licheniformis, Escherichia coli, and Clostridium sporogenes. Overall, it indicates that milk can be contaminated with different types of bacteria, especially in unpasteurized milk. Therefore, highlights the importance of proper pasteurization, hygienic handling, and suitable storage conditions to maintain the microbiological quality and safety of milk and to protect consumers from milk-borne infections.

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