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Isolation, Identification and Antibiotic Susceptibility of Microorganisms from Pasteurized Milk Samples

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Abstract: ***Even though pasteurization is a widely recognized technique for eradicating pathogens, microbial contamination in pasteurized milk continues to pose a major public health issue. The objective of this research was to isolate, identify, and evaluate the antibiotic resistance of microorganisms found in pasteurized milk samples. The present investigation showed a total of 60 bacterial isolates from the milk samples. Escherichia coli was identified as the most prevalent pathogen, accounting for 31.6% of the isolates, followed by Pseudomonas spp. at 21.6%, Klebsiella oxytoca at 15%, and Enterococci spp. at 11.6%. The antibiotic susceptibility tests revealed notable resistance patterns among these pathogens. Escherichia coli exhibited significant resistance to Ampicillin (60%) and Ciprofloxacin (55%), yet demonstrated susceptibility to Amikacin (85%) and Gentamicin (80%). Enterococci spp. showed resistance to Ampicillin (70%) but remained susceptible to Levofloxacin (85%). Klebsiella oxytoca displayed resistance to Ampicillin (65%) while being susceptible to Ceftazidime (85%). Pseudomonas spp. demonstrated resistance to Ciprofloxacin (60%) and Tetracycline (50%), but retained susceptibility to Amikacin (80%).***

Keywords: Pasteurization, Susceptibility, contamination, multi-drug-resistant, antibiotic

1.Introduction

Milk is an essential component of the human diet, providing vital nutrients for growth and health. Pasteurization, a heat treatment process developed by Louis Pasteur in the 19th century, was introduced to kill harmful microorganisms in milk without significantly altering its nutritional value. The process involves heating milk to a specific temperature (usually 63-72°C) for a set period (15-30 seconds) to eliminate pathogens such as Escherichia coli, Salmonella, Listeria, and Mycobacterium tuberculosis (FSSAI, 2020). Pasteurization is crucial for ensuring that milk is safe for consumption while retaining its nutritional benefits. Despite the benefits, pasteurized milk can still become contaminated with pathogens, primarily due to post-pasteurization contamination during handling, packaging, or storage.

Objectives:

A. **To isolate microorganisms,** present in pasteurized milk samples of different brands.
B. **To identify the bacterial and fungal species** isolated from the pasteurized milk samples.
C. **To determine the antibiotic susceptibility** of the isolated bacterial strains using standardized methods
D. **To evaluate the presence of antibiotic-resistant strains**, particularly multi-drug-resistant organisms among the isolated bacteria.

2.Materials and Methods

2.1 Collection of samples

A total of eight samples were collected for this study, with the goal of ensuring a broad and representative dataset. Each sample was carefully labelled for tracking and analysis, with designations ranging from M1 to M8.

2.2 Isolation of Pathogenic Microorganisms

The process starts with inoculating HiChrome UTI and MacConkey Agar plates with milk samples,these media provide a comprehensive platform for identifying a broad spectrum of pathogens in milk samples (Ghosh et al., 2018).After inoculation, the plates are incubated at 37°C for 24 hours, the optimal temperature for growing common dairy-associated pathogens(Patil et al., 2019).Upon completion of the incubation period, colonies are observed for specific color changes on media.Once specific colonies are observed, pure cultures are isolated by transferring individual colonies to fresh HiChrome UTI or MacConkey Agar plates using sterile techniques. This step ensures that the colonies are free from contamination and represent single microorganisms. Pure cultures are incubated again at 37°C to confirm consistency in color and growth, as seen in the initial results (Vikram et al., 2017). After obtaining pure cultures, these isolates undergo further confirmatory tests. Biochemical tests, such as catalase or oxidase tests, and molecular tests like PCR can be used to precisely identify the pathogens and assess their potential health risks.

2.3 Identification by staining and cultural characteristics

2.3.1 Microscopic study

Identification of milk pathogens through staining techniques, particularly Gram staining and fungal staining, plays a crucial role in microbiological diagnostics. This classification is vital in the dairy industry, as it aids in identifying common milk-borne pathogens such as Escherichia coli, Staphylococcus aureus, and Klebsiella pneumoniae. Fungal staining techniques, on the other hand, are essential for detecting mycological pathogens in milk, which can significantly impact dairy quality and safety. Fungal pathogens, such as Candida spp.,

Aspergillus spp., and Penicillium spp., can proliferate in dairy products, leading to spoilage and potential health risks. Staining methods like the Lactophenol Cotton Blue stain allow for clear visualization of fungal structures under a microscope, revealing hyphal characteristics and spore formation that aid in species identification (Sullivan et al., 2019). The use of fluorescent stains, such as Calcofluor White, can also enhance the detection of fungal pathogens by binding to chitin in the cell wall, providing additional sensitivity and specificity (Feng et al., 2020).

2.3.2 Cultural characters

To study the cultural characteristics of microbial isolates, samples were inoculated onto various agar media and incubated at 37°C for 24 hours. This incubation period allows for the growth of colonies, which are subsequently examined for their morphological traits, including size, shape, color, and texture (Baron et al., 1994). Such observations are essential for the preliminary identification of microorganisms, as different species exhibit unique colony characteristics on specific media (Murray et al., 2003). The morphological assessment involves not only visual characteristics but also the consistency and elevation of colonies, which provide valuable information for classification (Berthelsen et al., 2007). Moreover, the choice of agar medium influences the growth and appearance of colonies, as some media are selective for certain types of bacteria, thereby aiding in differentiation (Levinson et al., 2004). Thus, a thorough examination of colony morphology contributes significantly to the accurate identification and classification of microbial isolates (Zhang et al., 2016).

2.4 Biochemical Tests

Biochemical tests are essential for the accurate identification of pathogens in milk, providing valuable insights into their metabolic and enzymatic activities. These tests help differentiate between microbial species based on their biochemical properties, which is crucial for ensuring food safety. IMVic test, Indole test, Methyl Res test, Voges-Proskauer test, Citrate test, Triple sugar iron test.

2.5 The VITEK 2 System for Microbial Identification in Milk

This system offers rapid and accurate identification of bacteria and fungi based on their biochemical properties, utilizing colorimetric methods to determine microbial presence. It enables the identification of pathogens commonly found in milk. Moreover, the VITEK 2 system can also provide antimicrobial susceptibility results, which are critical for guiding targeted therapy against identified pathogens (Hawser et al., 1998).

2.6 Antibiotic Susceptibility Testing in Milk

2.6.1 Disk Diffusion Method (Kirby-Bauer Test)

Its role in detecting antibiotic resistance in pathogens from milk products underscores its value in clinical and food microbiology settings, helping to ensure food safety and proper antibiotic therapy (Murray et al., 1996).

3.Results

3.1 Collection of samples

Pasteurized milk samples (M1-M8) from various brands were collected.

3.2 Isolation of pathogens from Pasteurized

Pathogens from pasteurized milk samples (M1-M8) were isolated using the serial dilution method. The diluted milk samples were plated on HiChrome UTI agar and MacConkey agar, which facilitated the growth of distinct colonies shown in Figure 1(a)(b).

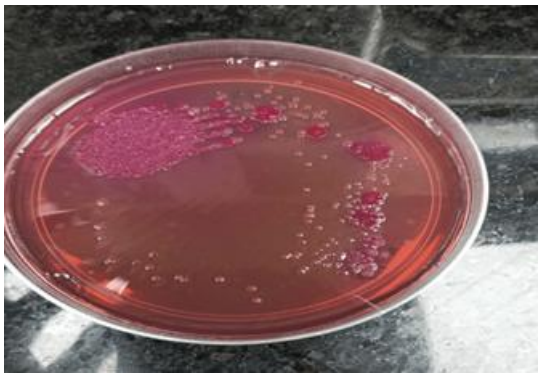

Figure 1 (a)

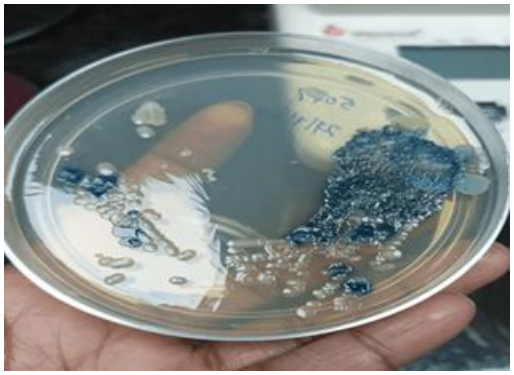

Figure 1 (b)

Table 1: Number of isolates from each milk sample

Milk samples	E. coli	Enterococci sps	Klebsiella oxytoca	Pseudomonas sps
M1	_	_	_	_
M2	_	_	9	_
M3	11	7	_	_
M4	15	_	_	10
M5	5	_	_	_
M6	_	_	_	_
M7	_	_	_	_
M8	_	_	_	3

3.3 Identification and Characterization of Isolates

3.3.1 Cultural characteristics

The cultural characteristics of the 60 isolates were first studied using HiChrome UTI and MacConkey agar media. All isolates exhibited luxuriant growth on these media, with distinct colony morphologies observed on HiChrome UTI agar, including variations in color and texture.

3.3.2 Microscopic Observation

Gram staining was performed on all 60 isolates to determine their cell wall structure. Out of these, 53 isolates were identified as Gram-negative bacteria, the remaining 7 isolates were Gram-positive. This differentiation was essential for guiding further biochemical and diagnostic analyses.

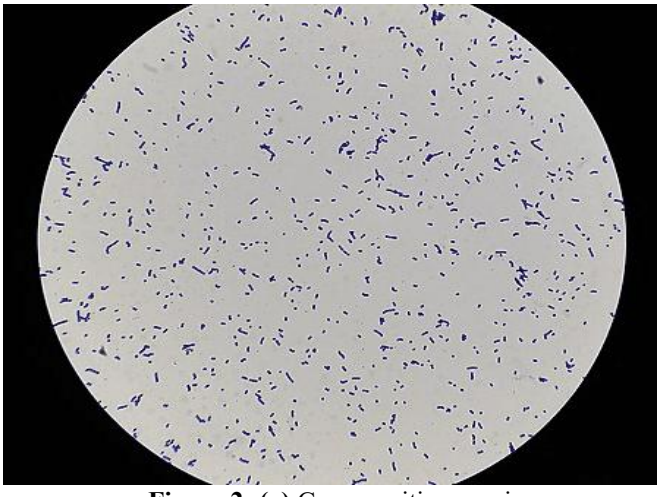

Figure 2: (a) Gram positive cocci

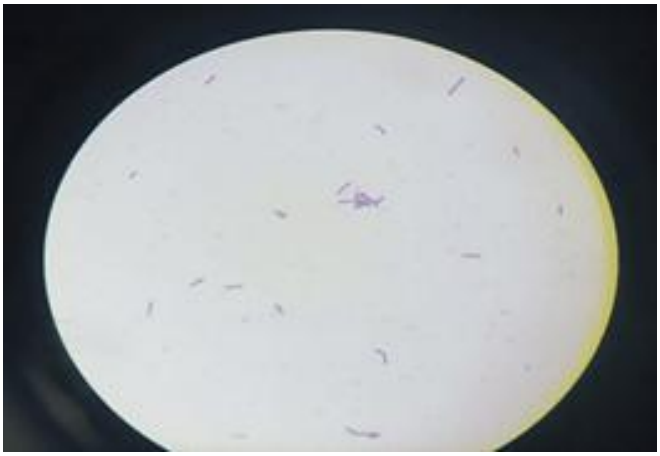

Figure 2: (b) Gram positive bacilli

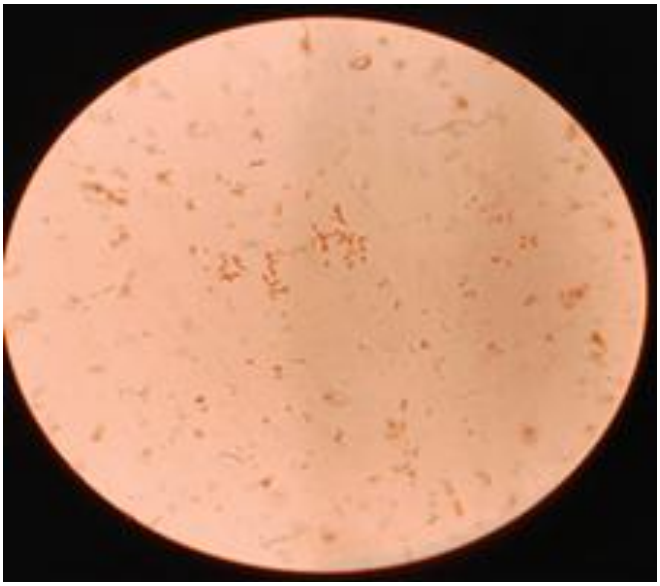

Figure 2: (c) Gram negative cocci

3.4 Biochemical Tests

Biochemical test results of isolates are shown below in table 2.

Table 2: Biochemical test of different microorganisms

Test	E. coli	Enterococci Sp	K. oxytoca	Pseudomonas sp
Indole	Positive	Negative	Positive	Negative
Methyl Red	Positive	Negative	Negative	Negative
Voges-Proskauer	Negative	Negative	Positive	Negative
Citrate	Negative	Negative	Positive	Positive
Catalase	Positive	Positive	Positive	Positive
Urease	Negative	Negative	Positive	Negative
Triple Sugar Iron Test	Acid Slant, Acid Butt with Gas, No H2S	Alkaline Slant, Acid Butt, No Gas	Acid Slant, Acid Butt with Gas	Alkaline Slants, Alkaline butt with no Gas

3.5 Vitek Test

The system also tests antimicrobial susceptibility by observing bacterial growth in the presence of antibiotics, helping to determine effective treatment options. Widely used in clinical microbiology, the VITEK system offers efficient and accurate bacterial identification and antibiotic resistance profiling.

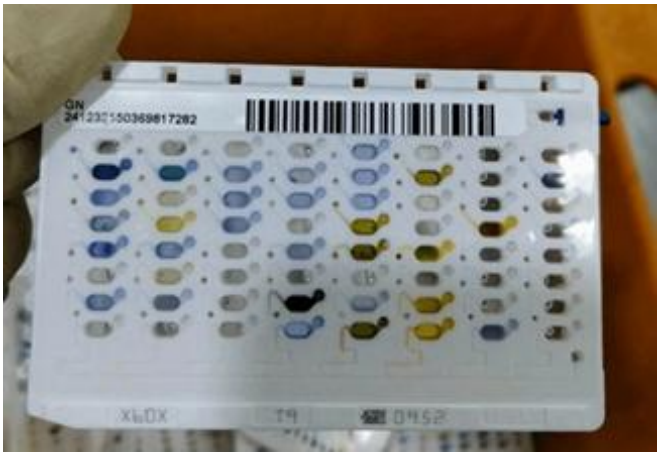

Figure 3: Vitek card showing biochemical reaction results

Table 3: Incidence of microorganism through Vitek test

Organism	Colony Aspects	Gram +/-	Shape	Motility	Isolate Number	% of incidence
E. coli	Round, smooth, moist	Gram-negative	Rod	Motile	31	51
Enterococci sp.	Small, smooth	Gram-positive	Cocci	Non-motile	7	11
K. oxytoca	Mucoid, large	Gram-negative	Rod	Non-motile	9	15
Pseudomonas sp.	Round, flat, and colorless colonies	Gram-negative	Rod	Motile	13	23

3.6 Antibiotic test

An antibiotic susceptibility test was performed on 60 bacterial isolates from milk samples, using commonly prescribed antibiotics. The results provided insight into the resistance and susceptibility patterns of the bacteria, aiding in determining appropriate treatment options and ensuring the safety of dairy products.

Table 4: Antibiogram results of isolated organisms

Isolate No.	AMK (mm)	AMP (mm)	FOX (mm)	CAZ (mm)	CTX (mm)	CHL (mm)	CIP (mm)	TMP-SMX (mm)	GEN (mm)	LEV (mm)	STR (mm)	TET (mm)
1	R (12)	S (22)	R (14)	S (20)	I (16)	S (22)	R (13)	S (21)	I (15)	S (23)	R (12)	S (22)
2	S (18)	I (17)	S (20)	I (16)	R(11)	R (12)	S (21)	I (15)	R(10)	I (16)	S (21)	R (10)
3	I (15)	R (10)	I (15)	R (12)	S (21)	I (15)	I (17)	R(12)	S (21)	R (11)	I (15)	I (15)
4	S (17)	S (21)	S (21)	S (22)	R(10)	S (21)	R (12)	S (23)	R(11)	S (21)	R (13)	S (23)
5	R (11)	R (12)	R (10)	R (11)	S (22)	R (10)	S (22)	R(11)	S (23)	R (10)	S (23)	R (11)
6	S (20)	S (23)	S (23)	S (23)	I (15)	S (23)	I (16)	S (22)	I (14)	S (22)	I (16)	S (21)
7	I (14)	I (16)	I (13)	I (14)	R(13)	I (14)	R (11)	I (14)	R(12)	I (15)	R (10)	I (16)
8	S (19)	R (11)	S (18)	R (13)	S (23)	R (11)	S (23)	R(10)	S (22)	R (12)	S (22)	R (12)
9	R (13)	S (22)	R (12)	S (19)	R(12)	S (22)	I (15)	S (21)	I (16)	S (23)	I (14)	S (22)
10	R (12)	S (22)	R (14)	S (20)	I (16)	S (22)	R (13)	S (21)	I (15)	S (23)	R (12)	S (22)
11	S (18)	I (17)	S (20)	I (16)	R(11)	R (12)	S (21)	I (15)	R(10)	I (16)	S (21)	R (10)
12	I (15)	R (10)	I (15)	R (12)	S (21)	I (15)	I (17)	R(12)	S (21)	R (11)	I (15)	I (15)
13	S (17)	S (21)	S (21)	S (22)	R(10)	S (21)	R (12)	S (23)	R(11)	S (21)	R (13)	S (23)
14	R (11)	R (12)	R (10)	R (11)	S (22)	R (10)	S (22)	R(11)	S (23)	R (10)	S (23)	R (11)
15	S (20)	S (23)	S (23)	S (23)	I (15)	S (23)	I (16)	S (22)	I (14)	S (22)	I (16)	S (21)
16	I (14)	I (16)	I (13)	I (14)	R(13)	I (14)	R (11)	I (14)	R(12)	I (15)	R (10)	I (16)
17	S (19)	R (11)	S (18)	R (13)	S (23)	R (11)	S (23)	R(10)	S (22)	R (12)	S (22)	R (12)
18	R (13)	S (22)	R (12)	S (19)	R(12)	S (22)	I (15)	S (21)	I (16)	S (23)	I (14)	S (22)
19	R (12)	S (22)	R (14)	S (20)	I (16)	S (22)	R (13)	S (21)	I (15)	S (23)	R (12)	S (22)
20	S (18)	I (17)	S (20)	I (16)	R(11)	R (12)	S (21)	I (15)	R(10)	I (16)	S (21)	R (10)
21	I (15)	R (10)	I (15)	R (12)	S (21)	I (15)	I (17)	R(12)	S (21)	R (11)	I (15)	I (15)
22	S (17)	S (21)	S (21)	S (22)	R(10)	S (21)	R (12)	S (23)	R(11)	S (21)	R (13)	S (23)
23	R (11)	R (12)	R (10)	R (11)	S (22)	R (10)	S (22)	R(11)	S (23)	R (10)	S (23)	R (11)
24	S (20)	S (23)	S (23)	S (23)	I (15)	S (23)	I (16)	S (22)	I (14)	S (22)	I (16)	S (21)
25	I (14)	I (16)	I (13)	I (14)	R(13)	I (14)	R (11)	I (14)	R(12)	I (15)	R (10)	I (16)
26	S (19)	R (11)	S (18)	R (13)	S (23)	R (11)	S (23)	R(10)	S (22)	R (12)	S (22)	R (12)
27	R (13)	S (22)	R (12)	S (19)	R(12)	S (22)	I (15)	S (21)	I (16)	S (23)	I (14)	S (22)
28	R (12)	S (22)	R (14)	S (20)	I (16)	S (22)	R (13)	S (21)	I (15)	S (23)	R (12)	S (22)
29	S (18)	I (17)	S (20)	I (16)	R(11)	R (12)	S (21)	I (15)	R(10)	I (16)	S (21)	R (10)
30	I (15)	R (10)	I (15)	R (12)	S (21)	I (15)	I (17)	R(12)	S (21)	R (11)	I (15)	I (15)
31	S (17)	S (21)	S (21)	S (22)	R(10)	S (21)	R (12)	S (23)	R(11)	S (21)	R (13)	S (23)
32	R (11)	R (12)	R (10)	R (11)	S (22)	R (10)	S (22)	R(11)	S (23)	R (10)	S (23)	R (11)

33	S (20)	S (23)	S (23)	S (23)	I (15)	S (23)	I (16)	S (22)	I (14)	S (22)	I (16)	S (21)
34	I (14)	I (16)	I (13)	I (14)	R(13)	I (14)	R (11)	I (14)	R(12)	I (15)	R (10)	I (16)
35	S (19)	R (11)	S (18)	R (13)	S (23)	R (11)	S (23)	R(10)	S (22)	R (12)	S (22)	R (12)
36	R (13)	S (22)	R (12)	S (19)	R(12)	S (22)	I (15)	S (21)	I (16)	S (23)	I (14)	S (22)
37	R (12)	S (22)	R (14)	S (20)	I (16)	S (22)	R (13)	S (21)	I (15)	S (23)	R (12)	S (22)
38	S (18)	I (17)	S (20)	I (16)	R(11)	R (12)	S (21)	I (15)	R(10)	I (16)	S (21)	R (10)
39	I (15)	R (10)	I (15)	R (12)	S (21)	I (15)	I (17)	R(12)	S (21)	R (11)	I (15)	I (15)
40	S (17)	S (21)	S (21)	S (22)	R(10)	S (21)	R (12)	S (23)	R(11)	S (21)	R (13)	S (23)
41	R (11)	R (12)	R (10)	R (11)	S (22)	R (10)	S (22)	R(11)	S (23)	R (10)	S (23)	R (11)
42	S (20)	S (23)	S (23)	S (23)	I (15)	S (23)	I (16)	S (22)	I (14)	S (22)	I (16)	S (21)
43	I (14)	I (16)	I (13)	I (14)	R(13)	I (14)	R (11)	I (14)	R(12)	I (15)	R (10)	I (16)
44	S (19)	R (11)	S (18)	R (13)	S (23)	R (11)	S (23)	R(10)	S (22)	R (12)	S (22)	R (12)
45	R (13)	S (22)	R (12)	S (19)	R(12)	S (22)	I (15)	S (21)	I (16)	S (23)	I (14)	S (22)
46	R (12)	S (22)	R (14)	S (20)	I (16)	S (22)	R (13)	S (21)	I (15)	S (23)	R (12)	S (22)
47	S (18)	I (17)	S (20)	I (16)	R(11)	R (12)	S (21)	I (15)	R(10)	I (16)	S (21)	R (10)
48	I (15)	R (10)	I (15)	R (12)	S (21)	I (15)	I (17)	R(12)	S (21)	R (11)	I (15)	I (15)
49	S (17)	S (21)	S (21)	S (22)	R(10)	S (21)	R (12)	S (23)	R(11)	S (21)	R (13)	S (23)
50	R (11)	R (12)	R (10)	R (11)	S (22)	R (10)	S (22)	R(11)	S (23)	R (10)	S (23)	R (11)
51	S (20)	S (23)	S (23)	S (23)	I (15)	S (23)	I (16)	S (22)	I (14)	S (22)	I (16)	S (21)
52	I (14)	I (16)	I (13)	I (14)	R(13)	I (14)	R (11)	I (14)	R(12)	I (15)	R (10)	I (16)
53	S (19)	R (11)	S (18)	R (13)	S (23)	R (11)	S (23)	R(10)	S (22)	R (12)	S (22)	R (12)
54	R (13)	S (22)	R (12)	S (19)	R(12)	S (22)	I (15)	S (21)	I (16)	S (23)	I (14)	S (22)
55	R (12)	S (22)	R (14)	S (20)	I (16)	S (22)	R (13)	S (21)	I (15)	S (23)	R (12)	S (22)
56	S (18)	I (17)	S (20)	I (16)	R(11)	R (12)	S (21)	I (15)	R(10)	I (16)	S (21)	R (10)
57	I (15)	R (10)	I (15)	R (12)	S (21)	I (15)	I (17)	R(12)	S (21)	R (11)	I (15)	I (15)
58	S (17)	S (21)	S (21)	S (22)	R(10)	S (21)	R (12)	S (23)	R(11)	S (21)	R (13)	S (23)
59	R (11)	R (12)	R (10)	R (11)	S (22)	R (10)	S (22)	R(11)	S (23)	R (10)	S (23)	R (11)
60	S (20)	S (23)	S (23)	S (23)	I (15)	S (23)	I (16)	S (22)	I (14)	S (22)	I (16)	S (21)

Amikacin (AMK), Ampicillin (AMP), Cefoxitin (FOX), Ceftazidime (CAZ), Cefotaxime (CTX), Chloramphenicol (C), Ciprofloxacin (CIP), Cotrimoxazole (SXT), Gentamicin (GEN), Levofloxacin (LEV), Streptomycin (STR), and Tetracycline (TE).

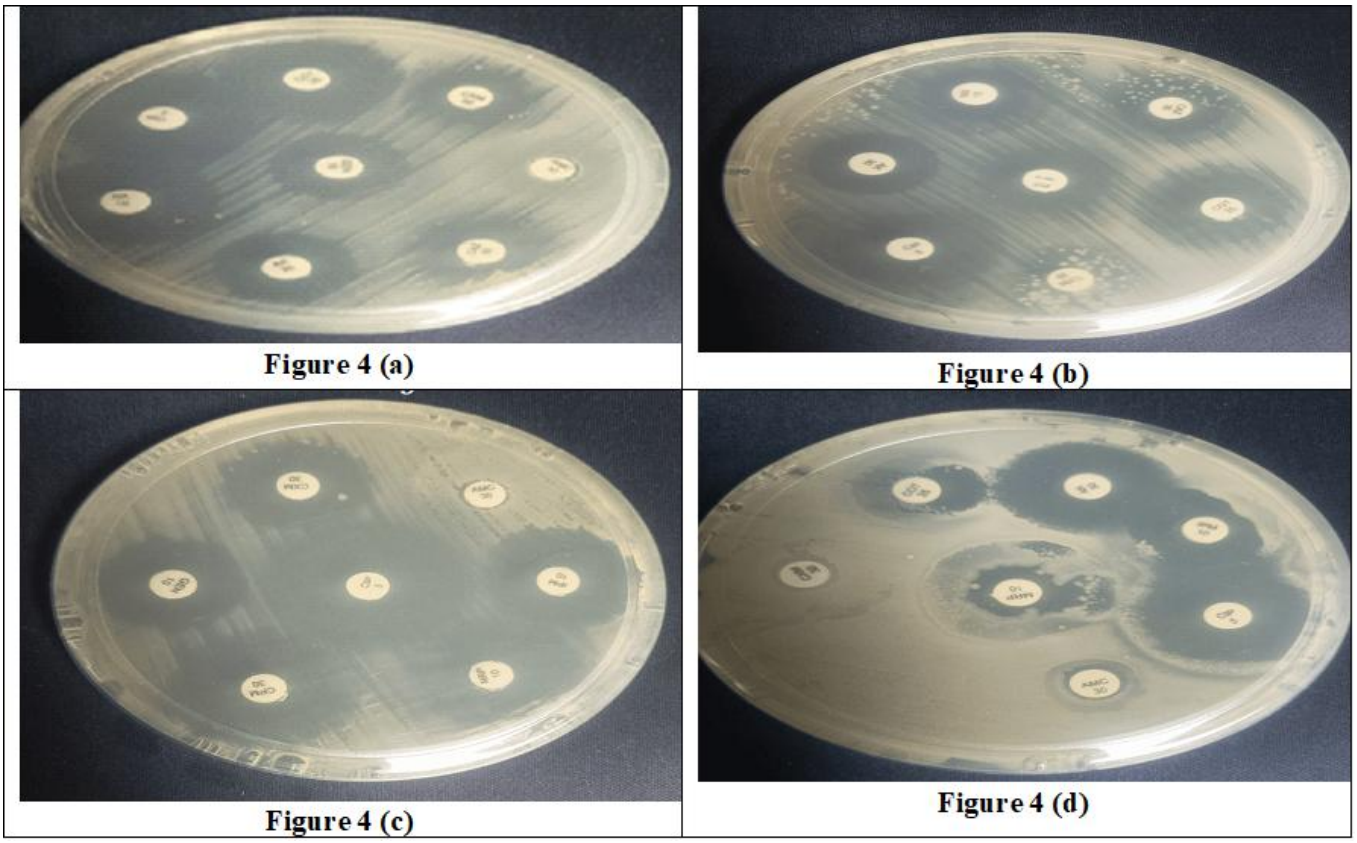

Figure 4: Antibiotic Susceptibility Test

3.7 Antibiotic prevalence microorganisms pattern

In the study of antimicrobial susceptibility patterns for microorganisms, the resistance, intermediate, and susceptible percentages for 12 antibiotics were meticulously analyzed. These antibiotics including Amikacin (AMK), Ampicillin (AMP), Cefoxitin (FOX), Ceftazidime (CAZ), Cefotaxime (CTX), Chloramphenicol

(CHL), Ciprofloxacin (CIP), Trimethoprim-Sulfamethoxazole (TMP-SMX), Gentamicin (GEN), Levofloxacin (LEV), Streptomycin (STR), and Tetracycline (TET), were tested to determine how effectively they combat microbial infection.

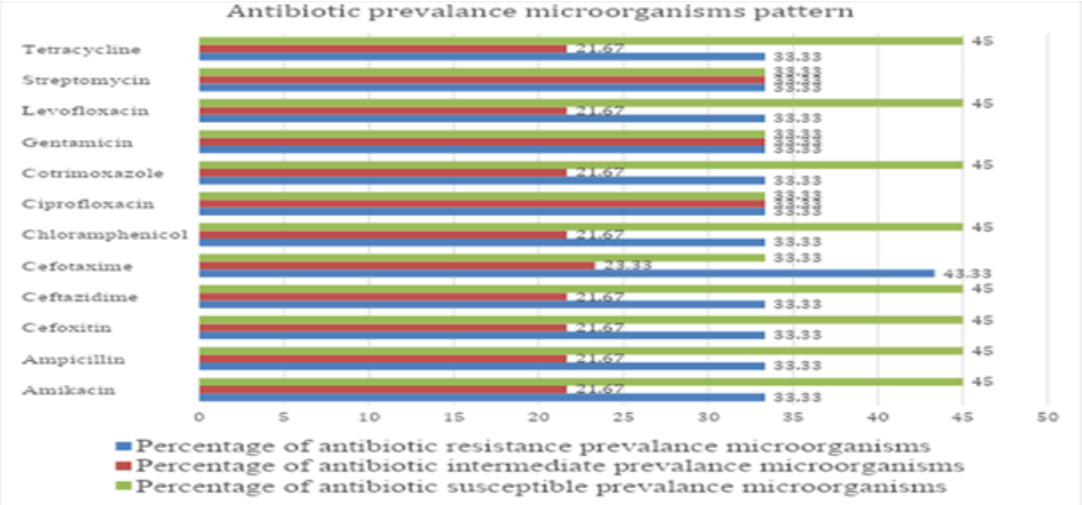

Figure 5: Antibiotic prevalence microorganisms pattern

3.7.1 Resistance Patterns:

Resistance, represented as a percentage of microorganisms that are fully resistant to a given antibiotic, serves as a critical indicator of antimicrobial ineffectiveness. In this dataset, **Amikacin**, **Ampicillin**, **Cefoxitin**, **Ceftazidime**, **Chloramphenicol**, **Cotrimoxazole**, **Levofloxacin**, and **Tetracycline** all shared the same resistance rate of **33.33%**, showing that a third of the microbial population is resistant to these antibiotics. This uniformity in resistance suggests that these drugs might not perform optimally when faced with resistant bacterial strains. Interestingly, **Cefotaxime** exhibited a slightly higher resistance rate at **43.33%**, indicating that nearly half of the microbial population might render this drug ineffective in treating infections. This elevated resistance rate makes Cefotaxime less favorable, especially in cases of severe or persistent infections where susceptibility is vital for treatment success. **Ciprofloxacin**, **Gentamicin**, and **Streptomycin** each showed **33.33%** resistance, suggesting moderate resistance levels. These findings underscore the challenge of using these drugs, as the likelihood of encountering resistant strains is significant.

3.7.2 Intermediate Patterns:

Intermediate susceptibility, where microorganisms are neither fully resistant nor fully susceptible, suggests that increased doses of the antibiotic might be necessary to achieve therapeutic success. For most of the antibiotics in this study, the intermediate prevalence was **21.67%**. This includes **Amikacin**, **Ampicillin**, **Cefoxitin**, **Ceftazidime**, **Chloramphenicol**, **Cotrimoxazole**, **Levofloxacin**, and **Tetracycline**. The moderate intermediate rates suggest that, while these drugs might still be effective, adjustments in dosage or combination therapies may be required for optimal outcomes. However, **Cefotaxime** displayed a slightly higher intermediate prevalence at **23.33%**, implying a greater proportion of cases where careful dose management would be required. **Ciprofloxacin**, **Gentamicin**, and **Streptomycin** had even higher intermediate susceptibility at **33.33%**, indicating significant variability in effectiveness. This increased intermediate prevalence suggests that a large portion of microbial populations could exhibit dose-dependent responses, thus requiring clinicians to monitor patient outcomes closely when these antibiotics are used.

3.7.3 Susceptibility Patterns:

Susceptibility refers to the percentage of microorganisms that are fully responsive to a given antibiotic, indicating effective treatment. Among the antibiotics analyzed, **Amikacin**, **Ampicillin**, **Cefoxitin**, **Ceftazidime**, **Chloramphenicol**, **Cotrimoxazole**, **Levofloxacin**, and **Tetracycline** showed the same susceptibility rate of **45%**. This relatively high susceptibility suggests that nearly half of the microbial population would respond well to these antibiotics, making them useful options for managing infections caused by susceptible strains. **Cefotaxime**, however, exhibited a lower susceptibility rate at **33.33%**, indicating reduced effectiveness against a third of the microbial population. **Ciprofloxacin**, **Gentamicin**, and **Streptomycin** also displayed a **33.33%** susceptibility rate, showing that while they are effective against a third of the cases, there remains a substantial proportion of microbes that may not respond fully to these treatments.

The analysis of antibiotic resistance, intermediate prevalence, and susceptibility across a range of antibiotics reveals notable patterns in microbial behavior. The uniform **33.33%** resistance rate observed for most antibiotics indicates that resistant strains are consistently present, posing a challenge to treatment. The intermediate prevalence, particularly for **Cefotaxime**, **Ciprofloxacin**, **Gentamicin**, and **Streptomycin**, highlights the need for careful dose adjustments in clinical settings. Additionally, while the **45%** susceptibility rate for several antibiotics is encouraging, the lower rates for others, such as **Cefotaxime**, suggest that alternative treatments or combination therapies may be necessary to improve patient outcomes.

4.Discussion

In the current study,the isolation rate of E. coli was 31.6%, while K. oxytoca accounted for 15%, Enterococci spp. 11.6%, and Pseudomonas spp. 21.6%.Antibiotic susceptibility testing was conducted on 60 isolates using common antibiotics, including Amikacin (AMK), Ampicillin (AMP), Cefoxitin (FOX), Ceftazidime (CAZ), Cefotaxime (CTX), Chloramphenicol (C), Ciprofloxacin (CIP), Cotrimoxazole (SXT), Gentamicin (GEN), Levofloxacin (LEV), Streptomycin (STR), and Tetracycline (TE).

The results revealed that E. coli exhibited high resistance to Ampicillin (60%), Ciprofloxacin (55%), and Cotrimoxazole (50%), while showing high susceptibility to Amikacin (85%) and Gentamicin (80%). Enterococci spp. showed notable resistance to Ampicillin (70%) and Ciprofloxacin (65%), but were generally susceptible to Cefoxitin (80%) and Levofloxacin (85%). Klebsiella oxytoca exhibited resistance to Ampicillin (65%) and Streptomycin (55%), while demonstrating susceptibility to Ceftazidime (85%) and Ciprofloxacin (75%). Pseudomonas spp. were highly resistant to Ciprofloxacin (60%) and Tetracycline (50%), but were susceptible to Amikacin (80%) and Levofloxacin (70%). The increase in Ciprofloxacin resistance, however, suggests a worrying trend in antimicrobial resistance among Pseudomonas spp.

The increasing resistance to commonly used antibiotics, particularly among E. coli and Pseudomonas spp., calls for renewed efforts in food safety practices and antibiotic stewardship in the dairy industry.

5.Conclusion

The study successfully isolated and identified significant bacterial pathogens from pasteurized milk samples collected. The findings confirm the continued presence of E. coli, Pseudomonas spp., K. oxytoca, and Enterococci spp. in pasteurized milk. Antibiotic susceptibility testing revealed concerning levels of resistance, particularly to Ampicillin and Ciprofloxacin, emphasizing the need for strict antimicrobial stewardship. Improved hygiene practices during milk processing and storage are essential to prevent contamination. Further research is needed to monitor the evolving patterns of antimicrobial resistance in milk-borne pathogens.

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Author Profile

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