Prevalence and verification of antibiotic resistant bacteria isolated from cow and buffalo milk samples in Limda, Gujarat, India

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Abstract— Milk is a highly nutritious commodity. The presence of all types of nutrients at a neutral pH brings about a favourable environment for the growth of various microorganisms, including bacteria, fungi, and molds. In this study, raw milk from 10 buffalos and 10 cows was collected from a dairy station in Limda, Gujarat, India. The milk was obtained from containers. These samples were then used to isolate various bacteria present. Using culture-dependent techniques, bacteria were isolated and were checked for their resistance to common antibiotics in the market. Amoxycillin-clavulanic acid, azithromycin and clindamycin were selected to verify the antibiotic resistance of these bacteria. Amoxycillin-clavulanic acid and azithromycin were in the form of tablets. Clindamycin was in the form of 1% gel. Antibiotic solutions were prepared and susceptibility testing was done using disk diffusion method. The results showed that over 85% of the 20 samples were resistant to clindamycin, 10% were resistant to azithromycin, and all samples were sensitive to amoxycillin-clavulanic acid. Further identification of these bacterial species is required to assess the major bacterial species in each sample.

Keywords: Antibiotics, antibiotic susceptibility, culture-dependent methods, pathogenic bacteria, spoilage bacteria

I. INTRODUCTION

Milk has a high beneficial and wholesome food that is suitable for human eating and can be sourced from a range of animals, including cows, goats, and buffalos. The high level of nutriens, comprising of proteins, fats, carbohydrates, vitamins, minerals, and essential amino acids, all at a near neutral pH and at a high-water activity provides an ideal environment for the growth of many important, pathogenic and spoilage microorganisms [1, 2]. These microorganisms can cause milk spoilage and affect the shelf life by producing extracellular proteases and lipases [3].

Before pasteurization of milk, lactic acid bacteria (LAB), a group of bacteria that ferment lactose to lactate, are a dominant population in bovine, goat, sheep, and buffalo milk. The most common LAB include *Lactococcus*, *Leuconostoc*, *Lactobacillus*, *Streptococccus*, and *Enterococcus*.[1] The major bacterial species in raw milk include *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, and *Escherichia coli* [4].

Despite the proposed health benefits of raw milk for humans, the disease-causing bacteria commonly found in raw milk include *Campylobacter* spp., Shiga toxin-producing *Escherichia coli*, *Salmonella enterica*, *Mycobacterium bovis*, *Brucella* species, *Corynebacterium diphtheria*, *Pseudomonas* spp., and *Coxiella burnetti* [5, 6]. Along with the presence of potential pathogenic bacteria, raw milk also contains antibiotic-resistant microbes. Thus, the incorporation of raw milk into daily diet may facilitate the dissemination of antimicrobial resistance genes (ARGs) to the human gastrointestinal tract [5].

The native microflora in raw milk has a direct impact on the subsequent development of dairy products. It has mostly focused on the milk at farms or during transportation [5, 7]. Dairy locations and milking practices, including housing, feed, faeces, bedding type and milk containers, bacterial populations present on cow teats, on dust, and in air in the milking area ultimately contribute to the raw milk microbiome [6, 7].

II. MATERIALS AND METHOD

II.I. Sample Collection

20 samples of raw milk were collected from a dairy station in Limda Village, Waghodia, Gujarat. The samples were collected during a period of 1 month, from December, 2022 to January, 2023. 10 raw milk samples were collected from buffalos (B) and 10 raw milk samples were collected from cows (C). The samples were taken from individual containers that were brought by the farmers to this dairy station. The samples were collected in the morning between 7AM-8AM.

Figure 1. Sample collection centre and collection of sample

II.II. INOCULATION

Serial dilutions were prepared for each milk sample. Samples were inoculated in Brain Heart Infusion Broth (BHI, M210-500G, HIMEDIA®). After an incubation period of 24 hours, each sample was subcultured on Typtic Soy Agar (TSA, M1968-500G, HIMEDIA®).

II.III. ANTIBIOTIC SUSCEPTIBILITY TEST

0.1 mL of bacterial culture inoculated in BHI broth was spread onto Mueller-Hinton Agar (MHA, M173-500G, HIMEDIA®). Amoxycillin-clavulanic acid (650 mg), Azithromycin (500 mg), Clindamycin Phosphate Gel (1% clindamycin) were selected as the antibiotics for this study. Solutions were prepared for these antibiotics. 6 mm paper disks were prepared from Whatman filter paper. The paper disks were made to absorb the antibiotic solutions and were placed on the media. Paper disks absorbed in distilled water were used as control.

III. Result and Discussion

III.I. INOCULATION: Results of bacterial growth on TSA for buffalo samples and cow samples showed that all colonies were creamy white in colour. B9, B10, C1, and C2 showed the presence of pink colonies. However, identification of individual bacteria has not been conducted in this study.

Figure 2. Growth of microorganism

II.II. ANTIBIOTIC-RESISTANCE

8 out of 10 buffalo samples (80%) were resistant to Clindamycin. 1 out of 10 samples (10%) was resistant to Azithromycin. All samples were sensitive to Amoxycillin-clavulanic acid. All bacteria were more sensitive to Amoxycillin-clavulanic acid than Azithromycin as shown in fig 3 and table 1.

9 out of 10 cow samples (90%) were resistant to Clindamycin. 1 out of 10 samples (10%) was resistant to Azithromycin. All samples were sensitive to Amoxycillin-clavulanic acid. All bacteria were equally sensitive to Amoxycillin-clavulanic acid and Azithromycin as shown in fig 4 and table 2.

Overall, out of 20 samples, all samples showed sensitivity to Amoxycillin-clavulanic acid. 2 samples were resistant to Azithromycin (10%). 17 samples were resistant to Clindamycin (85%).

Table 1. Antibiotic susceptibility test results for buffalo samples; (+) denotes sensitivity to the drug, (-) denotes resistance to the drug.

Sample No.	Amoxycillin-clavulanic acid	Azithromycin	Clindamycin
B1	+	+	-
B2	+	+	-
B3	+	+	-
B4	+	+	-
B5	+	+	-
B6	+	-	-
B7	+	+	+
B8	+	+	-
B9	+	+	-
B10	+	+	+

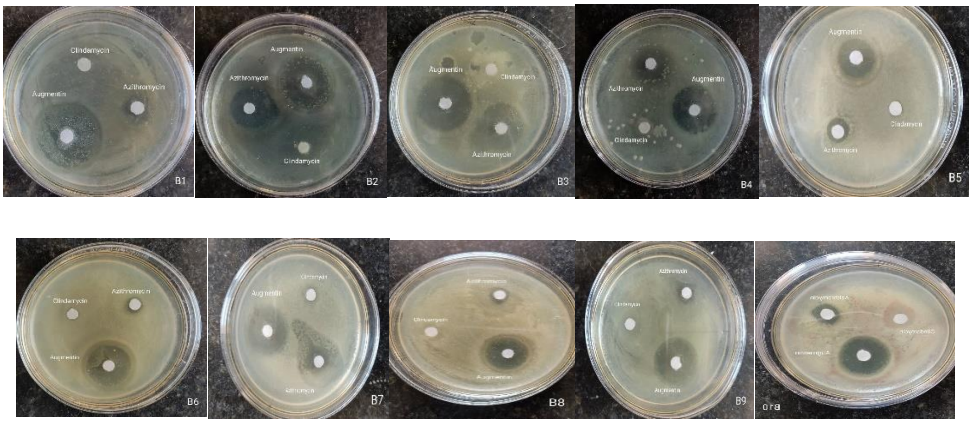

Figure 3. Antibiotic Susceptibility test on MHA. Buffalo samples B1-B10

Table 2. Antibiotic susceptibility test results for cow samples; (+) denotes sensitivity to the drug, (-) denotes resistance to the drug.

Sample No.	Amoxycillin-clavulanic acid	Azithromycin	Clindamycin
C1	+	+	-
C2	+	+	-
C3	+	+	-
C4	+	+	-
C5	+	-	-
C6	+	+	-

C7	+	+	-
C8	+	+	-
C9	+	+	-
C10	+	+	+

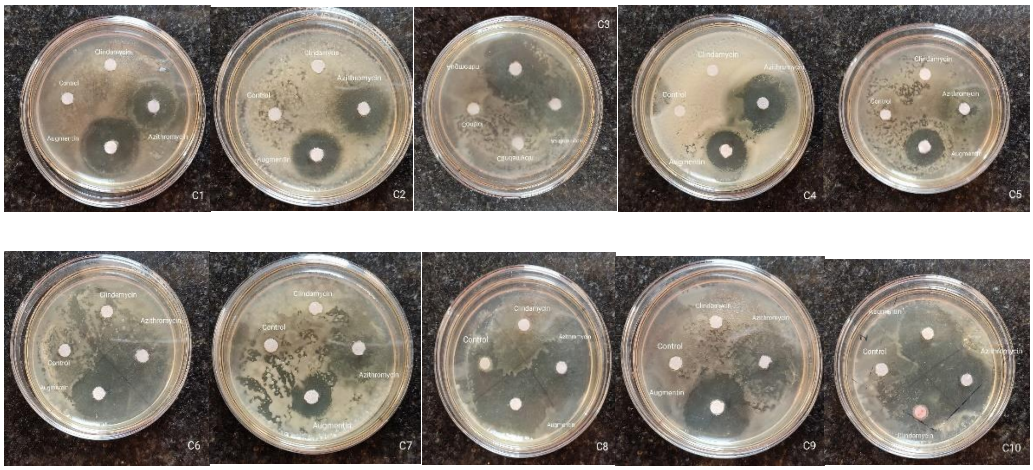

Figure 4. Antibiotic Susceptibility test on MHA. Cow samples C1-C10

IV. CONCLUSION

This research was conducted to identify and isolate the bacteria in raw milk from cows and buffalos. Although there are health benefits to drinking raw milk, the microbiota in milk is heavily affected by the environment around the milk, transportation and preservation processes. Moreover, the microbiota can also contain antimicrobial resistance genes. Our study found that the bacteria isolated were resistant to clindamycin. Changing the concentration of clindamycin to a higher dose may or may not show resistance. Amoxycillin-clavulanic acid was found to be more effective against bacteria than azithromycin. The identification of bacterial species using culture-independent techniques such as gene sequencing is highly recommended to identify the major bacterial populations in each sample.

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