

Article

# Prevalence and Identification of Major Mastitis Pathogens in Dairy Cattle: Focus on *Escherichia coli* and *Staphylococcus aureus*

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**Abstract:** The inflammatory disease of the mammary gland known as mastitis has many different possible sources of infection such as bacteria, fungi or other causes. Dairy cattle that are affected by mastitis can suffer financially from decreased milk production and poor milk quality, thus it is necessary to provide effective prevention and treatment methods for mastitis within the dairy industry. The objective of this research was to isolate and identify those bacterial pathogens responsible for causing bovine mastitis in dairy cattle suspected of being infected. Twenty milk samples were obtained from dairy cattle that were believed to be suffering from bovine mastitis. All twenty samples were then tested using bacterial cultures with selective and differential media. Following testing with conventional microbiological techniques, the VITEK 2 Compact system was used to verify the bacterial identification. Out of the twenty milk samples tested, eighteen samples (90%) demonstrated some form of bacterial growth, whereas only two samples (10%) showed no evidence of any type of bacterial growth. The most common bacteria isolated out of 18 different bacterial strains was *Escherichia coli*, which accounted for 38.88% of all isolates, followed closely by *Staphylococcus aureus*, accounting for 33.33% of the total, *Klebsiella pneumoniae*, accounting for 11.11% of the total, *Aeromonas veronii*, accounting for 11.11% of the total, and *Rahnella aquatilis*, accounting for 5.55% of the total. Bacterial pathogens were identified in most of the cow's milk samples tested that were suspected of developing mastitis. *Escherichia coli* and *Staphylococcus aureus* were identified as the two primary bacterial strains present in the milk samples. Isolation of bacteria and identification of the bacteria can assist with diagnosing, preventing, and managing bovine mastitis.

**Keywords:** Mastitis, Bacterial Isolation, Bacteriological Identification, Milk Microbiology, Veterinary Microbiology, Infectious Diseases

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## 1. Introduction

The prevalence and economic impact of bovine mastitis make it one of the largest problems facing dairy producers globally. Mastitis results in decreased milk yield, decreased milk quality, increased veterinary costs and treatments, and early removal of affected cows from the herd, also the mastitis creates significant issues regarding animal welfare and the safety of dairy products. Thus, preventing and controlling mastitis must be given top priority by the global dairy industry [1], [2].

Bacterial pathogens are among the most important infectious causes of bovine mastitis; fungi and viruses can also induce the disease. Commonly detected pathogenic bacteria include *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae*. The distribution and prevalence of this disease are constrained by four categories of factors, including geographical location and cattle herd management [3], [4].

Precise isolation and identification of pathogens related to mastitis is a core prerequisite to grasp the epidemiological characteristics of this disease and formulate appropriate prevention and control strategies. Currently, traditional bacterial culture remains the gold standard for isolating such pathogens, and automated identification systems such as the VITEK® 2 Compact can also provide reliable results for strain confirmation [5].

This study aimed to isolate and identify bacterial species recovered from milk samples collected from dairy cows clinically suspected of mastitis.

## **2. Materials and Methods**

### **2.1 Sample Collection**

A total of 20 milk samples were collected from dairy cows clinically suspected of mastitis across several smallholder dairy farms (approximately 3–4 farms) in Tikrit, Saladin Governorate, Iraq, during January and February 2025. Milk samples were collected from a single quarter per cow, involving 7 cows. Before taking each sample, the teat end was thoroughly sanitized using 70% alcohol. After discarding the initial stream(s) of milk, approximately 10 milliliters (mL) of milk was collected from each teat in a sterile container, under strictly controlled aseptic conditions. Each sample was immediately placed in a refrigerated insulated container with ice, and was transported to the laboratory within a short period of time, where it would be processed for bacteriological testing.

### **2.2 Bacteriological Isolation**

For every milk sample, a 10 µL aliquot was quantitatively streaked onto Blood Agar, Nutrient Agar, MacConkey Agar, Mannitol Salt Agar and Eosin Methylene Blue (EMB) Agar using standard bacteriological loop procedures. An additional enrichment medium, Brain Heart Infusion (BHI) Agar, was employed to enhance recovery of fastidious organisms. All inoculated plates were incubated aerobically at 37°C for 24-48 hours prior to examination of bacterial growth, colony morphology, and pure isolation subculture.

### **2.3 Identification of Bacterial Isolates**

Presumptive identification of the bacterial isolates was performed based on colony morphology, Gram staining characteristics, and growth patterns on selective differential media. Confirmed species-level identification of the isolated bacteria was subsequently performed using the VITEK® 2 Compact system in accordance with the manufacturer's instructions, following the protocols described by [5].

### **2.4 Data Analysis**

The frequency and percentage of each bacterial isolate were calculated using descriptive statistics. The distribution and prevalence of the identified bacterial species associated with bovine mastitis were reported accordingly.

## **3. Results**

### **3.1 Bacterial Growth in Milk Samples**

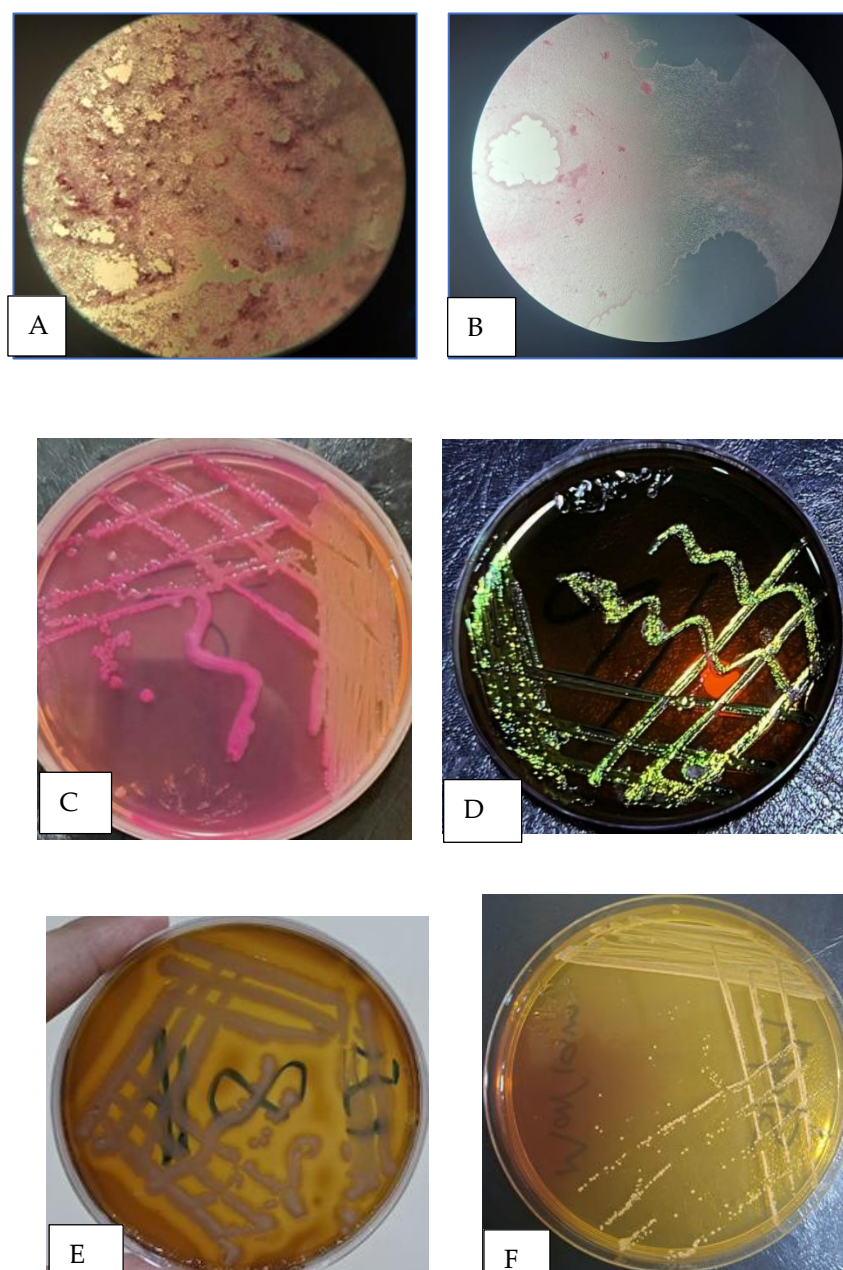
A total of 20 milk samples collected from cows with suspected mastitis were subjected to bacteriological examination. Bacterial growth was detected in 18 (90%) samples, whereas no bacterial growth was observed in 2 (10%) samples.

The distribution of bacterial species identified among the positive isolates is summarized in Table 1.

**Distribution of Bacterial Isolates** > The distribution of bacterial species identified among the positive isolates is summarized in Table 2. *Escherichia coli* was the most predominant pathogen, accounting for 38.89% (7/18) of the isolates, followed by *Staphylococcus aureus* at 33.33% (6/18). *Klebsiella pneumoniae* and *Aeromonas veronii* each represented 11.11% (2/18), whereas *Rahnella aquatilis* was the least isolated species at 5.56% (1/18).

**Table 1.** Frequency and percentage of bacterial isolates recovered from mastitic milk samples.

| Bacterial species            | Number of isolates | Percentage (%) |
|------------------------------|--------------------|----------------|
| <i>Escherichia coli</i>      | 7                  | 38.88          |
| <i>Staphylococcus aureus</i> | 6                  | 33.33          |
| <i>Klebsiella pneumoniae</i> | 2                  | 11.11          |
| <i>Aeromonas veronii</i>     | 2                  | 11.11          |
| <i>Rahnella aquatilis</i>    | 1                  | 5.55           |
| Total                        | 18                 | 100            |

**Figure 1.** Representative Gram-stained bacterial isolates and cultural characteristics of pathogens recovered from mastitic milk samples:

A. Gram-stained smear showing Gram-positive bacterial morphology (magnification: 1000×, oil immersion).

- B. Gram-stained smear showing Gram-negative bacterial morphology (magnification: 1000×, oil immersion).
- C. *Escherichia coli* colonies displaying a characteristic metallic green sheen on Eosin Methylene Blue (EMB) agar.
- D. *Escherichia coli* pink colonies on MacConkey agar.
- E. *Aeromonas* spp. colonies showing hemolysis on Blood agar
- F. *Staphylococcus* spp. colonies growing on Mannitol Salt agar (MSA).

Organism Quantity:

Selected Organism : Staphylococcus aureus

Source:

Collected:

Comments:

|                            |                 |                       |         |       |
|----------------------------|-----------------|-----------------------|---------|-------|
| Identification Information | Analysis Time:  | 4.88 hours            | Status: | Final |
| Selected Organism          | 92% Probability | Staphylococcus aureus |         |       |
| ID Analysis Messages       | Bionumber:      | 050422033763031       |         |       |

|                     |      |   |    |       |   |    |       |   |    |      |   |    |       |   |    |       |   |
|---------------------|------|---|----|-------|---|----|-------|---|----|------|---|----|-------|---|----|-------|---|
| Biochemical Details |      |   |    |       |   |    |       |   |    |      |   |    |       |   |    |       |   |
| 2                   | AMY  | - | 4  | PIPLC | - | 5  | dXYL  | - | 8  | ADH1 | + | 9  | BGAL  | - | 11 | AGLU  | + |
| 13                  | APPA | - | 14 | CDEX  | - | 15 | AspA  | - | 16 | BGAR | - | 17 | AMAN  | - | 19 | PHOS  | + |
| 20                  | LeuA | - | 23 | ProA  | + | 24 | BGURr | - | 25 | AGAL | - | 26 | PyrA  | + | 27 | BGUR  | - |
| 28                  | AlaA | - | 29 | TyrA  | - | 30 | dSOR  | - | 31 | URE  | + | 32 | POLYB | + | 37 | dGAL  | - |
| 38                  | dRIB | + | 39 | ILATk | + | 42 | LAC   | - | 44 | NAG  | + | 45 | dMAL  | + | 46 | BACI  | + |
| 47                  | NOVO | - | 50 | NC6.5 | + | 52 | dMAN  | + | 53 | dMNE | + | 54 | MBdG  | + | 56 | PUL   | - |
| 57                  | dRAF | - | 58 | O129R | - | 59 | SAL   | - | 60 | SAC  | + | 62 | dTRE  | + | 63 | ADH2s | - |
| 64                  | OPTO | + |    |       |   |    |       |   |    |      |   |    |       |   |    |       |   |

**Figure 2.** VITEK 2 identification report for *Staphylococcus aureus* with a 92% probability.

#### 4. Discussion

The high bacterial recovery rate (90%) observed in this study underscores the prominent role of bacterial pathogens in the etiology of bovine mastitis in the targeted region [13]. The small proportion of culture-negative samples (10%) may be attributed to prior antimicrobial therapy, intermittent bacterial shedding, low pathogen loads below detectable limits, or the presence of fastidious/non-bacterial microorganisms [3], [6]. The high isolation frequency found here agrees with earlier studies conducted on a regional scale that have reported higher culture-positive rates for clinical mastitis cases [4], [7]. Differences between studies were primarily due to variations in herd management practices, milking hygiene standards, geographic location, and sensitivity of diagnostic methods used [1], [8].

In this study, *Escherichia coli* were found to be the most common bacteria (38.89%). Since it is an environmental pathogen [14], its presence indicates fecal contamination and poor hygiene on the farm such as dirty bedding, manure, water sources, and/or lack of proper pre-milking teat preparation [7]. The difference in *E. coli* isolation rates between various studies is generally due to environmental factors and how animals are cared for [1].

*Staphylococcus aureus* is the second highest isolated organism, accounting for 33.33% of all isolates. It has been recognized as a primary contagious pathogenic organism [15], and can easily colonize the mammary gland, resulting in long-term sub-clinical or chronic infections that are generally spread from cow to cow during milking. The findings from this study are in line with the current body of knowledge regarding *S. aureus* as a significant cause of bovine mastitis [9], [12].

Also, there were some smaller amounts of *Klebsiella pneumoniae*, *Aeromonas veronii*, and *Rahnella aquatilis* present. The presence of these opportunistic bacteria demonstrates how complex the polymicrobial dynamic is that is associated with bovine mastitis. There are many variables that can influence whether these types of bacteria will be found in a cow's udder, including the amount of contact the cow has had with the

environment, the level of cleanliness at the dairy facility, and the overall strength of the cow's immune system [10], [11].

## 5. Conclusion

Dairy cow mastitis is primarily caused by bacterial pathogens. Two of the most common strains that we isolated from the milk samples of cows suffering from mastitis were *Escherichia coli* and *Staphylococcus aureus*. There were also three other strains (*Klebsiella pneumoniae*) that had a lower frequency than the previous two. Traditional bacteriological testing has been verified to be able to accurately identify each of these strains. These results can aid in providing routine diagnostic and epidemiological surveillance of mastitis and can assist dairy farms in developing preventative and controlling strategies.

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