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SHORT COMMUNICATION

# Analysis of Bacteriological Quality and Antibiogram of *Escherichia coli* and *Staphylococcus aureus* in Raw Milk Sold in Janakpurdham

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## ABSTRACT

**Objectives:** To assess the microbiological quality of raw milk sold in Janakpurdham, Nepal, by determining the Total Viable Count (TVC) and Total Coliform Count (TCC), isolating *Escherichia coli* and *Staphylococcus aureus*, and evaluating its antibiotic susceptibility pattern.

**Methods:** A total of 74 raw milk samples were aseptically collected from dairies and farmers in Janakpurdham from March to June 2025. TVC was determined using the pour plate method on Nutrient Agar, and TCC/*E. coli* isolation on Eosin Methylene Blue Agar. *S. aureus* isolation was done on mannitol salt agar after enrichment. Conventional biochemical tests were used to confirm the isolates. Antimicrobial susceptibility of the isolates was performed using the Kirby-Bauer disk diffusion method.

**Results:** Mean TVC was $1.13 \times 10^9$ CFU/ml (range: $2.075 \times 10^8$–$2.76 \times 10^9$), and mean TCC was $7.40 \times 10^7$ CFU/ml (range: $2.0 \times 10^5$–$5.28 \times 10^8$). *E. coli* was isolated from all seventy-four samples. Susceptibility was highest to gentamicin (100%) and chloramphenicol (85.13%), but 100% resistance was observed to ampicillin and amoxicillin-clavulanate. 60.81% isolates were multidrug-resistant. *S. aureus* was detected in 48 samples (65%). Antibiotic susceptibility testing revealed 100% resistance to ampicillin and 72.9% to cefoxitin, while varying resistance was observed for linezolid, erythromycin, clindamycin, and others.

**Conclusion:** Raw milk in Janakpurdham exhibits high microbial contamination and antibiotic-resistant bacteria, posing public health risks; therefore, improved hygiene and antibiotic stewardship are essential.

**Keywords:** Raw milk, *Escherichia coli*, *Staphylococcus aureus*, Total Viable Count, Coliform Count, Antimicrobial resistance, Janakpurdham.

## INTRODUCTION

Raw milk contamination occurs during milking, handling, storage, or transportation from sources such as udder infections (mastitis), dirty equipment, or fecal matter (Jay et al., 2005). Poor pre-milking hygiene and lack of pasteurization increases the health risk in developing countries (Dhungel et al., 2019; Rahmatalla et al., 2016). Raw milk, which is unpasteurized milk from animals such as cows or buffaloes, can contain pathogens like *Escherichia coli*, *Salmonella*, and *Listeria monocytogenes* if not handled properly (Pal et al., 2016). The presence of pathogenic microorganisms in raw milk poses significant public health risks, including foodborne disease.

*Escherichia coli* indicates fecal contamination and poor sanitation. While most strains are harmless, pathogenic and resistant variants pose a risk. *S. aureus* can cause a variety of illnesses. Presence of both these

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organisms represents a significant burden on public health globally, particularly in low- and middle-income countries. Antimicrobial resistance (AMR) exacerbates the issues caused by antibiotic misuse in veterinary practices (Oliver et al., 2005). This study evaluated raw milk quality sold in Janakpur and isolated two important milk-contaminating bacteria to assess antibiotic resistance levels for public health implications.

## METHODS

**Study Site and Duration:** Raw milk samples (50 mL each) were collected aseptically in sterile glass bottles from dairies, farmers, and shops across 25 wards of Janakpurdham Sub-Metropolitan City during the morning hours (8-10 AM) from March to June 2025. Samples were transported in an ice box and processed within 3-4 hours.

**Microbial Analysis:** Serial ten-fold dilutions ($10^{-1}$ to $10^{-7}$) were prepared. Total Viable Count (TVC) was determined by pour plate method on Nutrient Agar (NA), incubated at 37°C for 24 hours (NDDB, 2001). Total Coliform Count (TCC) and *E. coli* isolation were performed using spread plates on Eosin Methylene Blue Agar (EMBA), incubated at 37°C for 24-48 hours. Colonies with a greenish metallic sheen were subcultured for purity. For *S. aureus* isolation, 1 ml of milk sample was inoculated into 9 ml of sterile peptone water. After enrichment, a loopful was streaked onto Mannitol Salt Agar to get golden yellow colonies of *S. aureus*.

**Biochemical tests:** Confirmation of *E. coli* and *S. aureus* was done by biochemical tests using methods described elsewhere (Isenberg, 2007; Cheesbrough, 2006).

**Antibiotic Susceptibility Test:** Antimicrobial susceptibility testing (AST) was performed using the Kirby-Bauer disk diffusion method on Mueller-Hinton Agar (MHA) following the CLSI guidelines (2023). Antibiotics tested were Ampicillin (10 µg), Amoxycillin-Clavulanate (30 µg), Gentamicin (10 µg), Ceftazidime (30 µg), Tetracycline (30 µg), Chloramphenicol (30 µg) and Ciprofloxacin (5 µg) for *E. coli*. For *S. aureus*, ampicillin, cefoxitin, linezolid, erythromycin, clindamycin, chloramphenicol, tetracycline and gentamycin were used. Multidrug resistance (MDR) was defined as resistance to ≥3 classes of antibiotics (Magiorakos et al., 2012).

**Data Analysis:** Data were entered into MS Excel 2016 and analyzed descriptively (mean, standard deviation, range, quartiles).

## RESULTS

All 74 samples exhibited microbial growth. The mean TVC was $1.13 \times 10^9$ CFU/ml (SD: $5.62 \times 10^8$), ranging from $2.075 \times 10^8$ to $2.76 \times 10^9$ CFU/ml. While the Mean TCC was $7.40 \times 10^7$ CFU/ml (SD: $7.72 \times 10^7$), ranging from $2.0 \times 10^5$ to $5.28 \times 10^8$ CFU/ml.

*E. coli* was isolated from all samples, confirmed biochemically. AST showed 100% sensitivity to gentamicin (74/74) and 85.13% to chloramphenicol (63/74); 79.23% to ceftazidime (59/74); 78.38% to tetracycline (58/74); 71.62% to ciprofloxacin (53/74). There was 100% resistance to ampicillin and amoxicillin-clavulanate while 28.38% to Ciprofloxacin, 21.62% to tetracycline, 20.77% to ceftazidime, 14.87% to chloramphenicol (Table 1). 60.81% (45/74) isolates are multi drug resistant showing resistance to ≥3 classes of antibiotics.

**Table 1. Antimicrobial Susceptibility pattern of *Escherichia coli* Isolates**

| Antimicrobial Agents | Concentration (µg) | Zone Diameter (mm) | | |
|---|---|---|---|---|
| | | Sensitive (n, %) | Intermediate (n, %) | Resistant (n, %) |
| Ampicillin (AMP/AP) | 10 | ≥ 17 (0, 0%) | 14–16 (0, 0%) | ≤ 13 (74, 100%) |
| Amoxycillin clavulanic (AMC/AUG) | 30 | ≥ 18 (0, 0%) | 14–17 (0, 0%) | ≤ 13 (74, 100%) |
| Tetracycline (T/TE) | 30 | ≥ 15 (58, 78.38%) | 12–14 (0, 0%) | ≤ 11 (16, 21.62%) |
| Ceftazidime (CAZ/C) | 30 | ≥ 21 (59, 79.23%) | 18–20 (0, 0%) | ≤ 17 (15, 20.77%) |
| Chloramphenicol (CMP/CAC) | 30 | ≥ 18 (63, 85.13%) | 13–17 (0, 0%) | ≤ 12 (11, 14.87%) |
| Gentamicin (GEN) | 10 | ≥ 15 (74, 100%) | 13–14 (0, 0%) | ≤ 12 (0, 0%) |
| Ciprofloxacin (CIP) | 5 | ≥ 26 (53, 71.62%) | 22–25 (0, 0%) | ≤ 21 (21, 28.38%) |

A total of 48 *S. aureus*-positive strains (65%, 48/74) were isolated and identified. The highest resistance was found to Ampicillin (100%) and cefoxitin (72.9%), followed by linezolid (31.25%), erythromycin (25%), clindamycin (25%), chloramphenicol (18.75%), tetracycline (14.5%) and gentamycin (12.5%) (Fig. 1). Out of the 48 verified S. aureus isolates, 18 (37.5%) were determined to be MDR.

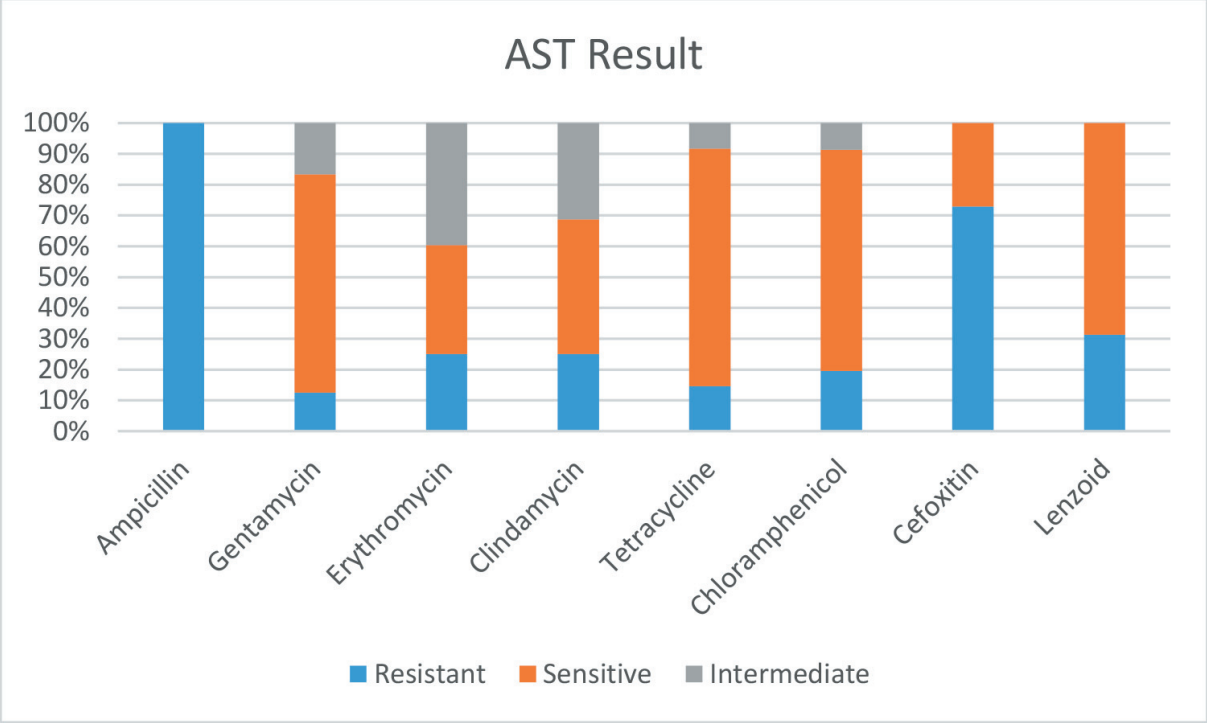


**Figure 1. Antimicrobial Susceptibility of *S. aureus* isolates**

## DISCUSSION

The nutrient-rich composition of raw milk makes it susceptible to contamination. The high TVC ($1.13 \times 10^9$ CFU/mL) and TCC ($7.40 \times 10^7$ CFU/mL) in this study exceeded the WHO/FAO limits ($<10^5$ CFU/mL TVC, $<10^2$ CFU/mL coliforms), indicating poor hygiene. Similar findings in Kathmandu (Bhattarai et al., 2017:$10^6$–$10^8$ CFU/mL) and Chitwan (Koirala & Joshi, 2019:$8.5 \times 10^8$ CFU/mL) suggest widespread issues in Nepal due to inadequate refrigeration and poor sanitation. In India, Sarkar (2015) reported $10^7$–$10^9$ CFU/mL, emphasizing the need for pasteurization.

*E. coli* detection signals fecal contamination. The antibiogram showed 100% resistance to beta-lactams, aligning with Sharma et al. (2021) in Nepal's Terai (100% ampicillin resistance). Hasan et al. (2015) in Bangladesh reported high resistance to Ampicillin (95%) and Tetracycline (60%). Lower resistance to gentamicin (0%) offers treatment options, but the 28.40% MDR rate is concerning and linked to veterinary antibiotic misuse.

Out of 74 milk samples, *Staphylococcus aureus* was detected in 48 samples, representing an overall prevalence of 65% (48/74). The isolation rate of *S. aureus* varies widely across different studies and regions.

Comparable results were reported by Alnakip (2009), El-Jakee et al. (2008), Jakeen et al. (2010), and Nassar (2013), who documented prevalence rates of 16% to 22.7% in cow milk. In contrast, significantly higher prevalence rates were reported by El-Gendy (2015), Ralls et al. (2008), and Wafy (2006), ranging from 60% to 90.4%. On the other hand, relatively lower isolation rates were found in studies by Amer et al. (2007) and Kivaria et al. (2006), which reported prevalence rates between 6.3% and 14.5%.

Liu et al. (2017) reported a 27.7% isolation rate of *Staphylococcus aureus* in 195 raw milk samples collected from northern China. Similarly, Zhao et al. (2020) found a 28.9% contamination rate in bulk tank milk samples from dairy farms in Shandong Province—both lower than the prevalence found in the current study. Contamination of raw milk with *S. aureus* commonly originates from mastitis-infected animals or human carriers. Poor hygiene practices during milking and processing significantly increase the risk of contamination (Schmidt et al., 2017).

## CONCLUSION

The present study has shown that *Staphylococcus aureus* and *E. coli* is widely prevalent in milk in Janakpur city. The high rate of isolation indicates the higher

public health risk among this region. The results also emphasize the importance of regular microbiological examination of milk and milk products for the production of quality and safe products.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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