

# Review of: "Clinical and Subclinical Bovine Mastitis: Staphylococcus aureus Isolation and Identification from Dairy Farms Located in and Around Hawassa Town, Southern Ethiopia"

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Potential competing interests: No potential competing interests to declare.

The study includes an epidemiological panel of the prevalence of mastitis - both clinical and subclinical - on farms in Ethiopia, and interestingly provides information on the odds ratio of variables such as the animals' age, frequency of hygienic methods on the farm, and others.

The abstract article is concise and provides sufficient information about the study.

Bacterial identification **targeting** *S. aureus* was done, and this agent was identified in 60 (47.2%) milk samples. - I suggest changing the bold word, because it was already used in the former sentence.

Infected cows and quarters are normally identified through the bacteriological examination of milk samples (Artursson et al., 2010). ***S. aureus* is transmitted from an infected to an uninfected mammary gland during the milking process. Shared equipment, udder cloths, and even milkers' hands can transmit *S. aureus* between cows if good hygienic practices are not followed.** Environmental factors, such as bedding, housing, and feedstuffs, can also be contaminated and play a role in spreading *S. aureus* infection. **Transmission occurs mainly at milking time through contaminated milking machines, clothes, and hands of milkers or machine operators (Abera et al., 2010).** - The highlighted sequences are repetitive. I suggest choosing one of them and discarding the other.

**When the milk and CMT reagent are mixed in equal amounts, the CMT reagent dissolves or disrupts the outer cell wall and the nuclear cell wall of any leukocyte, which are primarily fat (detergent dissolves fat). DNA is now released from the nuclei. DNA will string or gel together to form a stringy mass. As the number of leukocytes increases in a quarter, the amount of gel formation will increase in a linear fashion (Melleneger, 2001).** - I believe the explanation about the method is unnecessary and could be checked by the reader in the references.

The number of animals analyzed was good and above what was expected by the sample size calculation.

**Transportation was done to the HU-FVM lab by keeping an icebox containing ice packs.** - This sentence could be simplified, e.g., "transported in low temperature."

The following sentences are repeated in the **Isolation and Identification** section, as they were previously described in the **Udder Cleaning** section. Both sections could be merged into one single paragraph. Repeated sequences to discard:

**Udder cleaning was performed with clean water, soap, and dried with a clean towel, followed by teat disinfection with 70% alcohol before milk sampling. Ten milliliters of milk was taken after discarding the forestrip milk from each dairy cow. Transportation was done to the HU-FVM lab by keeping an icebox containing ice packs.**

The following sequences are not necessary since they refer to basic microbiological tests, such as the catalase test, coagulase test, and Mannitol salted media. They should be discarded: **A catalase test using 3% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) was performed to identify catalase-positive and catalase-negative bacteria. The colonies that were identified by Gram staining and catalase tests were subcultured on Mannitol Salt Agar (MSA) plates and incubated at 37°C, examined after 24–48 hrs for growth and a change in the color of the medium. The presence of growth and a change of pH in the media (red to yellow color) were regarded as a confirmative identification of the salt-tolerant staphylococci. The fermentation of mannitol by *S. aureus* causes a yellow discoloration of the medium (Quinn et al., 2002).**

**The tube coagulase test was conducted according to the method of Robertson et al. (1999). Accordingly, 0.1 ml of fresh cultures of suspected staphylococci grown on Nutrient Broth for 18-24 hours was added to 0.5 ml of 1/10 diluted sterile rabbit plasma (Sigma) in the test tube. The tube was incubated at 37°C and examined every 4-24 hours to see the presence of clotting. The reaction was considered coagulase-positive if any degree of clotting was visible (Tallent et al., 2001). The suspected culture was also inoculated on a purple agar base with 1% maltose media and incubated at 37°C for 24 hrs. Samples were considered positive for *S. aureus* when the suspected isolates were catalase-positive, coagulase-positive, and showed rapid fermentation of maltose on the PAB (Quinn et al., 2002).**

**The prevalence of mastitis was calculated by dividing the number of mastitis-positive cows (clinical and subclinical) by the total number of animals examined.** = "The prevalence of mastitis-positive cows was calculated," should be enough, as a prevalence calculation is a known equation.

Results are well described and analyzed.

The discussion is fairly written and contains sufficient references.

**The higher prevalence of mastitis (61.9%) was reported in cows kept in houses cleaned twice per day (less frequently cleaned) than in cows kept in houses cleaned four times or more per day (more frequently cleaned).** - For better reading, I suggest using the “less/more frequently cleaned” in the sentence rather than between parentheses, instead of the words “twice” and “four times.”

Recommendations are precise and adjusted to the results shown in the study.