



# Bovine Milk as a Source of Antibiotics?

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## Introduction

Antimicrobial resistance continues to drive the search for effective treatments against common bacterial pathogens (Joseph et al. 2013, Godinez et al. 2019). Previous studies have shown that select natural substances have antibiotic properties comparable to traditional antibiotic medications. Lactoferrin, an iron-binding glycoprotein from bovine milk (Gruden and Ulrich 2021), as well as honey, tea tree oil, and garlic, can inhibit bacterial growth (Jazani et al. 2007, Al-Naama 2009, Thomsen et al. 2011). This study tested the hypothesis that lactoferrin will yield significantly larger zones of inhibition of bacterial growth of *Staphylococcus aureus* and *Escherichia coli* compared to honey, tea tree oil, and garlic and not significantly different than antibiotics ampicillin and tetracycline.

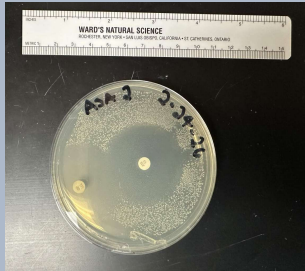


Figure 1. Pictured is *S. aureus* + Antibiotics (ampicillin & tetracycline) Trial #2

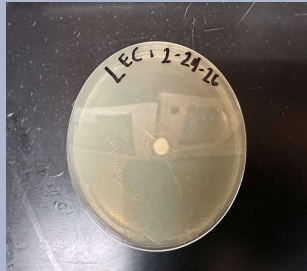


Figure 2. Pictured is *E. coli* + Lactoferrin Trial #1

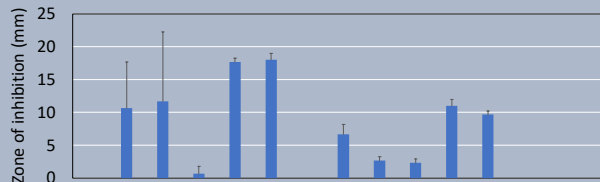
## Materials & Methods

The researchers grew *Staphylococcus aureus* (Gram+ coccus) and *Escherichia coli* (Gram- bacillus) bacteria in Petri dishes with nutrient agar incubated at 37°C. Petri dishes encompassed 3 replicates of each treatment:

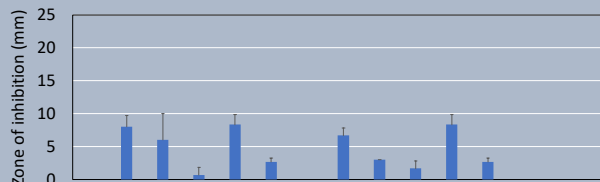
- Bacterial species + Lactoferrin
  - + Tea tree oil
  - + Honey
  - + Garlic
  - + Ampicillin & Tetracycline
- Bacteria alone (positive control)
- No bacteria (negative control)

Using the Kirby–Bauer Method, the zones of inhibition were measured every 24 hours for 3 days.

### Zone of Inhibition at 24 hrs



### Zone of Inhibition at 48 hrs



### Zone of Inhibition at 72 hrs

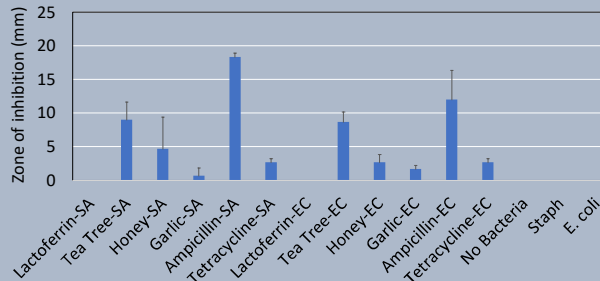


Figure 3. Zones of inhibition of *S. aureus* and *E. coli* grown on nutrient agar with lactoferrin, tea tree oil, honey, garlic, ampicillin, tetracycline, and alone (n=3, mean ± SD).

## Results

At 24 hours, the zones of inhibition of *S. aureus* with tea tree oil, honey, ampicillin, and tetracycline were significantly larger than other treatments (1-way ANOVA,  $F_{5,12}=6.85$ ,  $p<0.05$ , Figure 3), and *E. coli* with tea tree oil, ampicillin, and tetracycline were significantly larger (1-way ANOVA,  $F_{5,12}=80.75$ ,  $p<0.05$ , Figure 3). At 48 hours, *S. aureus* with tea tree oil, honey, and ampicillin were significantly larger (1-way ANOVA,  $F_{5,12}=10.52$ ,  $p<0.05$ , Figure 3), and *E. coli* with tea tree oil and ampicillin were significantly larger (1-way ANOVA,  $F_{5,12}=33.51$ ,  $p<0.05$ , Figure 3). At 72 hours, *S. aureus* with tea tree oil, honey, and ampicillin were significantly larger (1-way ANOVA,  $F_{5,12}=27.39$ ,  $p<0.05$ , Figure 3), and *E. coli* with tea tree oil and ampicillin were significantly larger (1-way ANOVA,  $F_{5,12}=16.75$ ,  $p<0.05$ , Figure 3).

## Discussion

Contrary to original expectations, the research showed that bovine lactoferrin was a weak bacterial inhibitor. This result is consistent with findings showing that commercial bovine lactoferrin will exhibit no antibacterial activity unless under the right conditions such as when the strain itself is sensitive to lactoferrin, or when there are incredibly high levels of lactoferrin (Shlykova et al., 2025). For further research, the researchers advise to use a larger concentration of lactoferrin per mL of water, and to ensure usage of a highly purified sample. Garlic was ineffective at inhibiting growth of *S. aureus* and *E. coli*, but tea tree oil and honey were effective bacterial inhibitors. This result is consistent with previous findings showing that tea tree oil and honey can produce significant levels of bacterial inhibition (Godinez et al. 2019, Joseph et al. 2013). While not as effective as traditional antibiotic medications of ampicillin and tetracycline, tea tree oil and honey are natural substances with antibiotic properties that are easily accessible and low cost.



Figure 4. Pictured is the Petri dishes in the incubator

## Literature Cited

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