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Comparative Antibacterial Efficacy of Olea Africana and Acacia Etbaica Wood Smoke in Raw Milk from Southern Ethiopia

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HIGHLIGHTS

- Wood smoke of *Olea africana* reduces microbial loads in raw milk more effectively.
- Both *Olea africana* and *Acacia etbaica* smokes significantly inhibit bacterial growth.
- Container smoking improve raw milk safety by reducing pathogenic bacteria.
- *Olea africana* smoke decreases *Staphylococcus aureus* counts by over 2 log units.
- Pathogens like *Salmonella* and *Shigella* are more prevalent in non-smoked milk samples.

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Abbreviations
AMBC= Aerobic Mesophilic Bacterial Count
PCA= Plate Count Agar
PDA= Potato Dextrose Agar
SC= *Staphylococcus aureus* Count
TCC= Total coliform Count
WGCFNR= Wondo Genet College of Forestry and Natural Resources
YAMC= Yeast and Mold Count

ABSTRACT

Background: The rapid spoilage of milk can lead to foodborne illness, making effective preservation methods essential for ensuring food safety and extending shelf life. This study examined the antibacterial effects of wood smoke from *Olea africana* and *Acacia etbaica* on raw milk samples from Wondo Genet, Southern Ethiopia.

Methods: Raw milk samples were collected in February and April 2018, comprising 20 from a dairy farm and 20 from randomly selected smallholder farmers. The microbial loads of raw milk samples stored in smoked and non-smoked containers at 25 °C were compared. Microbial analysis included aerobic mesophilic bacterial count (AMBC), *Staphylococcus aureus* count (SC), total coliform count (TCC), and yeast and mold count (YAMC) over a 36-hour period. Pathogen screening targeted *Salmonella* spp. and *Shigella* spp. Statistical analysis was performed using one-way ANOVA followed by Tukey's post-hoc test to determine significant differences ($p < 0.05$). Analyses were conducted with SPSS Statistics software, version 26.0 (IBM Corp., Armonk, NY, USA).

Results: At 0 h, the dairy farm milk samples in non-smoked containers exhibited lower microbial loads than those from smallholder farmers, with significant differences ($p < 0.05$) in AMBC (5.74 vs. 6.04 $\log_{10}$ CFU/mL), SC (3.4 vs. 3.8 $\log_{10}$ CFU/mL), and TCC (2.71 vs. 3.51 $\log_{10}$ CFU/mL). Both wood smoke types effectively reduced microbial counts, with *Olea africana* showing greater efficacy. After 24 h, AMBC decreased by 0.9 log units for *Olea africana* compared with 0.47 for *Acacia*; SC decreased by 2.09 log units versus 1.14; and YAMC decreased by 1.51 log units compared with 0.76 log units. Significant reductions in TCC were observed only at 24 h. Pathogens such as *Salmonella* (30%) and *Shigella* (32.5%) were more prevalent in samples stored in non-smoked containers.

Conclusion: These results underscore the antimicrobial potential of traditional wood-smoking practices, especially those using *Olea africana*, in reducing microbial contamination in milk.

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Introduction

Milk and dairy products remain central to human nutrition, serving as key elements of a balanced diet. At the global level, cow's milk accounts for nearly four-fifths of total milk production, making it the dominant source. Buffalo milk represents the second largest share, while goat and sheep milk contribute only minor proportions to overall output (Mukherjee *et al.*, 2026). Camel milk, though culturally significant in certain regions, represents a negligible proportion of the global market (Ait El Alia *et al.*, 2025; Rakhmatulina *et al.*, 2024). Cow's milk is recognized as a nutrient-dense food, supplying high-quality proteins along with key vitamins such as riboflavin (B2) and cobalamin (B12). It also delivers essential minerals, including calcium, iodine, potassium, and phosphorus, which collectively support numerous physiological functions. These attributes provide the foundation for its health-promoting properties and its enduring role in diets across the globe (Scholz-Ahrens *et al.*, 2020; Voronina *et al.*, 2022).

Yet this same nutritional richness renders raw milk highly susceptible to pathogenic and spoilage microorganisms, which not only threaten consumer health but also limit its storage stability. Even aseptically drawn milk can contain a significant number of microorganisms (Ózsvári and Ivanyos, 2022). The teat skin and teat canal of cows naturally harbor various microbes that are difficult to eliminate through disinfection alone. In addition, exogenous contaminants can enter the milk at various points along the value chain (Yan *et al.*, 2022). Sources of contamination include handlers with poor hygiene, utensils, and the environment (Berhe *et al.*, 2020; Fusco *et al.*, 2020).

Harmful microorganisms that contaminate raw milk can lead to health problems and product defects (Gume *et al.*, 2023). Common milk-associated pathogens include Campylobacter jejuni, *Salmonella* enterica, Brucella abortus, Escherichia coli, Coxiella burnetii, Cryptosporidium parvum, Yersinia enterocolitica, *Staphylococcus aureus*, and Listeria monocytogenes (Keba *et al.*, 2020). Milk-borne diseases are a significant global public health concern, accounting for about 4% of all foodborne illnesses and particularly affecting low- and middle-income countries, which bear over 90% of the disease burden (Kapoor *et al.*, 2023). Raw milk also contains spoilage microorganisms that lead to significant product losses. Psychrotrophic bacteria such as Pseudomonas fluorescens and Pseudomonas fragi thrive at low temperatures and produce heat-stable enzymes that degrade milk proteins and fats, even after pasteurization (Saha *et al.*, 2024). Spore-forming bacteria such as Bacillus and Clostridium can survive pasteurization and proliferate during refrigeration, contributing to spoilage (Enayaty-Ahangar *et al.*, 2021). Yeasts and molds further exacerbate these issues by causing off-flavors and visible deterioration in dairy products (Shi and Maktabdar, 2021). These facts underline the need for high-quality and safe milk production to meet growing consumer demand.

Ethiopia is the second most populous nation in Africa and possesses vast livestock resources with the potential to transform its dairy sector. As of 2021, the country had a cattle population of approximately 70 million, ranking first in Africa and fifth globally (ESS, 2023a; Legese *et al.*, 2023). Despite this potential, Ethiopia faces a significant annual milk shortage. Total bovine milk production in 2021 was 3.957 billion tons (FAO, 2023), which was lower than that of Kenya (4.69 billion tons) and Egypt (5.277 billion tons). The country requires approximately 22.73 billion tons of milk per year for self-sufficiency, but production falls short at just 7.45 billion tons, resulting in a shortage of approximately 15.5 billion tons (ESS, 2023a).

Several factors hinder the development of the dairy sector in Ethiopia, including low productivity of local breeds, inadequate feed, low adoption of improved technologies, and limited access to services (Legese *et al.*, 2023). In addition, significant losses occur along the milk production and supply chain, with estimates suggesting that 20–35% of the total milk produced is lost due to mishandling (Shiferaw, 2025). Main causes include improper milk handling, spoilage, lack of nearby markets, lack of refrigerators, transportation, and poor road infrastructure (Fekata *et al.*, 2023a). The dual nature of milk, being both a nutritious food and a suitable growth medium for microorganisms, emphasizes the need for effective preservation and handling practices.

To address the challenges of raw milk spoilage and safety, smallholder dairy farmers in Ethiopia—though not aware of the microbial causes—have long relied on traditional practices such as thorough cleaning and smoking of milk containers. A study revealed that 74% of respondents wash and flavor their milking vessels, while 79% smoke them (Zegeye and Teklehaymanot, 2016). These traditional practices aim to reduce milk spoilage and losses in the absence of modern technologies. However, a comprehensive approach combining traditional and modern methods may be necessary for a significant reduction in milk losses.

Although smoking of milk containers is a long-standing cultural practice in Ethiopia and other regions, its antibacterial efficacy has not been systematically validated under controlled laboratory conditions. Previous reports have largely emphasized sensory attributes or general preservation benefits, with limited focus on quantifying microbial load reduction using standardized microbiological methods (Hadero *et al.*, 2014; Welearegay *et al.*, 2012). Moreover, comparative data between smoked and non-smoked containers under identical experimental conditions remain scarce, and mechanistic insights into the antimicrobial action of smoke constituents have not been adequately linked to

microbial enumeration outcomes.

The present study addresses these gaps by applying routine, standardized microbiological techniques to evaluate the antibacterial effect of container smoking on raw milk. Unlike earlier investigations, this work simultaneously assessed aerobic mesophilic bacteria, *Staphylococcus aureus*, coliforms, yeasts, molds, and the pathogenic species *Salmonella* and *Shigella*. Importantly, the study is innovative in directly comparing the antimicrobial efficacy of smoke generated from two different woody plant species (*Olea africana* and *Acacia etbaica*), tested on raw milk samples sourced from both a modern dairy and smallholder farms. By demonstrating measurable reductions in microbial load and highlighting species-specific differences in smoke preservation, this study provides scientific validation of a culturally embedded, low-cost preservation method. The findings contribute to food safety by offering baseline data for integrating traditional smoking practices into modern milk hygiene protocols, thereby bridging indigenous knowledge with evidence-based microbiological standards.

Materials and method

Materials

All reagents and culture media (HiMedia, India) used in this study were of analytical grade and were procured through institutional (Hawassa University) procurement from licensed suppliers in Addis Ababa. Reagents and solutions (HiMedia, India): sodium hypochlorite (3%), ethanol (70%), sterile physiological saline (0.85% NaCl), nutrient broth, and glycerol (20%) were used. Potato dextrose agar (PDA) was supplemented with chloramphenicol and tetracycline (1% w/v each) for selective yeast and mold isolation.

All culture media (HiMedia, India): Plate Count Agar (PCA) for aerobic mesophilic bacteria; Mannitol Salt Agar (MSA) for *Staphylococcus aureus*; Violet Red Bile Agar (VRBA) for coliforms; PDA for yeasts and molds; Nutrient Agar for purification of isolates; *Salmonella–Shigella* Agar (SSA) for pathogen detection; Triple Sugar Iron (TSI) agar, Sulfide–Indole–Motility (SIM) agar, Urea Agar, and Lysine Iron Agar (LIA) for biochemical characterization. Biochemical and staining reagents (HiMedia, India): Gram staining reagents (crystal violet, iodine, alcohol, safranin), hydrogen peroxide for the catalase test, Kovac's reagent for the indole test, methyl red reagent, Voges–Proskauer reagents (α-naphthol and KOH), and citrate utilization medium were employed for phenotypic identification of isolates.

Description of the study area

The study was conducted in Wondo Genet District, Basha Town, the administrative center of the district. The town is located 13 km southeast of Hawassa City and 263 km south of Addis Ababa. Geographically, it lies between latitudes 7°01′ and 7°15′ N and longitudes 38°56′ and 39°13′ E. The average elevation of the district is 1,723 m above sea level, with an average annual rainfall of 1,160 mm. The mean monthly temperature of the area ranges from 19 °C to 25 °C. The main land-use types in the sub-catchment are natural forest, plantation forest, woodland, khat plantation, sugarcane plantation, agriculture, and settlements (Taddese *et al.*, 2022). According to the latest estimates from the Ethiopian Statistical Service (ESS, 2023b), the district has a total population of 240,182, comprising 122,399 males and 117,783 females.

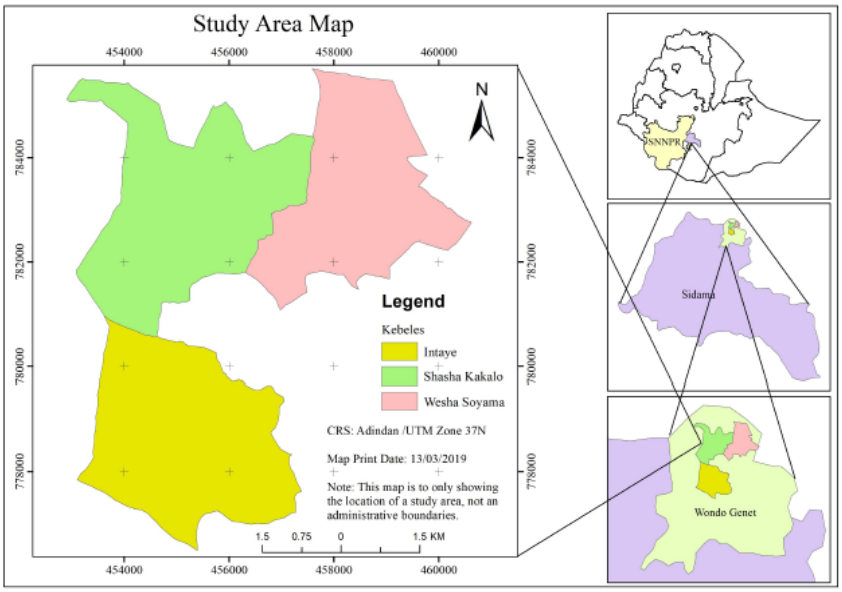


Figure 1: Map of study location, Wondo Genet town sub-urban kebeles, Sidama, Ethiopia

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The district is divided into 15 suburban rural kebeles, and three of them were purposely selected as sampling locations by considering the presence of residents who keep small-scale dairy farms. These were Intaye, Wosha-soyama, and Wosha-kakalo (Figure 1).

Study design

A cross-sectional and experimental study was conducted on the effect of container smoking on the microbial quality and/or safety of raw milk, based on microbiological laboratory analysis of milk samples collected from randomly selected smallholder farmers and the dairy farm of Wondo Genet College of Forestry and Natural Resources (WGCFNR).

Sample collection

During the period between February and April 2018, a total of 40 raw cow milk samples were collected, consisting of 20 from randomly selected smallholder farmers and 20 from the dairy farm of WGCFNR. All milk samples were collected aseptically (approximately 500 mL per sample) in the morning using sterile glass bottles at the time of milking. Each sample was labeled, placed in an icebox containing ice packs, and transported as soon as possible to the Microbiology Laboratory of WGCFNR, where all experimental analyses were conducted.

Preparation of smoked containers and experimental setup

Woody branches of *Olea africana* and *Acacia etbaica* (≈500 g each) were collected, sun-dried, and splintered. These species are traditionally used by local farmers to smoke milk containers. Sterile 250 mL glass bottles were designated as controls (non-smoked) or treatments ("AE" for *Acacia*, "OA" for *Olea*). Bottles were cleaned and sterilized, then exposed to smoke generated at 185 °C in a digital muffle furnace (Model SX3-10-12, Faithful Instrument Co., Ltd., Hebei, China) for 30 min (Hadero *et al.*, 2014). Raw milk (500 mL per sample) was aseptically divided into three portions (~166 mL each) and transferred into the labeled bottles. Samples were stored at ambient temperature (~25 °C), and microbial load was assessed from 10 mL aliquots taken at 0, 24, and 36 h.

Preparation of culture media and tenfold serial dilution of raw milk

All culture media were prepared according to the manufacturer's instructions (HiMedia, India). Changes in microbial load during the experiment were assessed using the standard plate count method. Milk samples from smoked and non-smoked bottles were manually agitated, and 10 mL aliquots were aseptically transferred into dilution blanks containing 90 mL sterile physiological saline (0.85% NaCl). From these, tenfold serial dilutions were prepared by transferring 1 mL aliquots into 9 mL saline tubes (10^{-2} to 10^{-7}). Vortex mixing was performed between transfers to ensure homogeneity.

Enumeration of microbial loads and detection of bacterial pathogens

The standard plate count method (Guodong *et al.*, 2026) was used to evaluate the milk samples for aerobic mesophilic bacteria (AMBC), *Staphylococcus aureus* count (SC), total coliform count (TCC), and yeast and mold count (YMC). Dominant AMBC and the occurrence of pathogens such as *Salmonella* and *Shigella* were also determined.

AMBC: 0.1 mL aliquots from appropriate dilutions were spread-plated on Plate Count Agar (PCA, HiMedia) in duplicate and incubated at 37 °C for 48 h. Colonies were counted using a Quebec dark-field colony counter, and averages were calculated from plates with 25–250 colonies (Guodong *et al.*, 2026).

The average microbial load across plates was calculated using the formula (Guodong *et al*., 2026):

$N = \sum C / (n_1 + 0.1n_2)\, d$

Where, $\sum C$ is the total colony count from the retained plates, n1 the number of plates from the first dilution, n2 the number from the second dilution, and d the dilution factor. Colony counts were transformed into $\log_{10}$ CFU/ml for ease of manipulation.

SC and TCC: 0.1 mL aliquots were spread-plated on Mannitol Salt Agar (MSA) and Violet Red Bile Agar (VRBA), respectively, in duplicate (Sandra *et al.*, 2016). Plates were incubated at 37 °C for 48 h, and typical golden colonies of *S. aureus* on MSA and pink coliform colonies on VRBA were counted from plates with 25–250 colonies. Average values were calculated as for AMBC.

YMC: 0.1 mL aliquots were spread-plated on PDA supplemented with 1% (w/v) chloramphenicol and tetracycline in duplicate (Valerie *et al.*, 2001). Plates were incubated at 25 °C for 5 days, and counts were taken from plates with 25–250 colonies. Average values were calculated as for AMBC.

Determination of the dominant flora and detection of Salmonella and Shigella species

To identify dominant aerobic mesophilic bacteria (AMB), 5–10 well-isolated colonies were picked from countable AMBC plates, purified by repeated subculturing on nutrient agar (Bekele et al., 2022), and preserved in 20% glycerol vials at −20 °C until phenotypic characterization. Isolates were putatively identified using Gram staining, catalase, and biochemical tests including triple sugar iron (TSI), indole, methyl red (MR), Voges–Proskauer (VP), and citrate utilization (Feng *et al.*, 2020).

For pathogen detection, milk samples (1:10 dilution)

were streaked onto *Salmonella–Shigella* Agar (SSA). To resuscitate injured cells, 0.1 mL aliquots from the same dilution were first cultured overnight in nutrient broth before re-streaking (Damena *et al.*, 2022). Plates were incubated at 37 °C for 48 h, and typical black colonies were purified by subculturing. Presumptive isolates were confirmed using SIM agar, TSI, urea agar, and lysine iron agar (LIA). *Salmonella* species were identified by the following traits: Gram-negative small rods, hydrogen sulfide positive, indole negative, motile, non-lactose fermenting, and lysine decarboxylase positive (Feng *et al.*, 2020).

Replication note

Microbial loads were determined for a total of 40 independent raw milk samples. These comprised 20 samples collected on different days from the institutional dairy herd at WGCFNR and 20 samples obtained from 20 individual smallholder farmers. Each sample represented a biological replicate, and plating was performed in duplicate to provide technical replication. Mean colony counts from duplicate plates were used for statistical analysis.

Data analysis

Differences in microbial loads between smoked and non-smoked containers were assessed using one-way ANOVA in SPSS Statistics, version 26.0 (IBM Corp., Armonk, NY, USA). The microbial loads of milk samples stored in containers treated with *Olea africana* and *Acacia etbaica* smoke were analyzed at 0, 24, and 36 h. Analyses were based on 40 independent milk samples, and mean differences were considered significant at $p \leq 0.05$.

Result and discussion

Antibacterial comparison of Olea africana and Acacia etbaica smoke at zero hour

The results showed that at zero hour (immediately after milk transfer to non-smoked and smoked containers), all average microbial load parameters were lower in milk samples from smoked containers than in those from non-smoked containers (control). This trend was observed for milk samples collected from both the WGCFNR dairy farm (Table 1) and smallholder farmers (Table 2). Interestingly, the microbial load–lowering effect was more pronounced in the milk samples exposed to smoke from *Olea africana* wood than in those exposed to *Acacia etbaica* wood smoke. This suggests that smoke from *Olea africana* wood was more effective in reducing microbial contamination in the milk samples.

The AMBC of the raw milk samples at zero hour

At zero hour, the AMBC of raw milk samples collected from the WGCFNR dairy farm exceeded 5 $\log_{10}$ CFU/mL across all treatments (Table 1). Control samples consistently showed the highest values, while smoke-treated containers exhibited modest reductions, with *Olea africana* yielding slightly lower counts than *Acacia etbaica*. These differences, though small, suggest a mild inhibitory effect of smoke exposure at the point of collection.

Consistent with these findings, milk samples from both the WGCFNR farm (Table 1) and smallholder farmers (Table 2) demonstrated AMBC levels above 5 log units/mL at zero hour. This observation aligns with previous reports that raw milk, even when aseptically drawn and handled under hygienic conditions, inherently contains substantial microbial populations (Chiarlone *et al.*, 2025). The presence of microorganisms is attributed to the natural microbiota of the teat and skin surface, as well as unavoidable contamination during milking and handling (Quigley *et al.*, 2013; Yan *et al.*, 2022).

From a regulatory perspective, the European Commission (2010) stipulates that raw cow's milk intended for processing should not exceed 300,000 CFU/mL (≈5.48 log units). The mean AMBC values observed in this study, particularly in the non-smoked controls, were above this threshold, indicating compromised hygienic quality. Elevated AMBC levels are widely recognized as indicators of poor hygiene during production, storage, and processing, and they increase the risk of pathogen presence and spoilage organisms. As highlighted by Welearegay *et al.* (2012), factors such as milking practices, animal health, and storage conditions critically influence microbial counts. The slight reductions observed with smoke exposure, though insufficient to meet regulatory standards, point to a potential role of traditional smoking practices in moderating microbial contamination.

Importantly, the average AMBC in smallholder milk was 6.04 ± 0.39 log units (Table 2), significantly higher than the 5.74 ± 0.17 log units observed in WGCFNR dairy milk (Table 1). This difference was statistically significant ($p < 0.05$; Table 3), underscoring the impact of management practices on microbial quality at the point of collection. Overall, the data suggest that while smoke treatment exerts a mild inhibitory effect, baseline microbial loads remain high across sources, reflecting the inherent microbial ecology of raw milk.

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Table 1: The mean microbial load of milk samples from WGCFNR dairy farm at 0 h in $\log_{10}$ CFU/mL stored in non-smoked and smoked bottles

Treatment	AMBC (mean ± SD)	SC (mean ± SD)	TCC (mean ± SD)	YAMC (mean ± SD)
Control	5.74 ± 0.17^{a}	3.40 ± 0.31^{a}	2.71 ± 0.13^{a}	3.54 ± 0.05^{a}
Acacia etbaica	5.66 ± 0.07^{ab}	3.30 ± 0.28^{ab}	2.61 ± 0.06^{ab}	3.34 ± 0.02^{b}
Olea africana	5.54 ± 0.11^{b}	3.20 ± 0.32	2.49 ± 0.07^{b}	3.16 ± 0.21^{b}

Values are mean ± SD (n = 20). Means within a column followed by different superscript letters differ significantly. AMBC – Aerobic mesophilic bacterial count, SC – *Staphylococcus aureus* count, TCC – Total coliform count, YAMC – Yeast and mold count.

This disparity reflects the institutional setting of the WGCFNR farm, where trained handlers and regulated hygienic protocols are employed (Feyisa *et al.*, 2024). In contrast, milking and handling practices in the informal sector in rural Ethiopia often lack hygienic measures (Assen and Abegaz, 2024). Institutional oversight generally ensures better hygiene, while informal rural handling practices remain vulnerable to contamination. Nonetheless, the mean AMBCs from both sources exceeded the 5.48 log units limit set by the European Commission (2010), indicating compromised hygienic quality.

Comparatively, the values reported here were lower than those documented in Hawassa, where Welearegay *et al.* (2012) found mean AMBCs of 6.87 and 7.36 log units in milk from large- and small-scale farms, and Mikru *et al.* (2021) reported 8.21 log units in small-scale dairies. Similarly, Tassew and Seifu (2011) observed higher counts (7.61 and 8.12 log units) in Bahir Dar Zuria. In contrast, Tegegne and Tesfaye (2017) reported lower values (4.59–4.77 log units) in Gondar, reflecting differences in hygienic handling and storage practices. These comparisons highlight that while the AMBCs in the present study were relatively moderate, they remain above acceptable limits, reinforcing the need for improved hygiene interventions across both institutional and smallholder contexts.

The SC of the raw milk samples

At zero hour, the average SC in raw milk samples from the WGCFNR dairy farm stored in non-smoked containers ranged from 2.90 to 3.70 $\log_{10}$ CFU/mL, with a mean of 3.40 ± 0.31 log units (Table 1). Only one of the 20 samples exceeded 4 log units. In contrast, milk stored in *Acacia etbaica* wood–smoked containers showed slightly lower values (2.90–3.65 log units; mean 3.30 ± 0.28), while *Olea africana* smoke exposure yielded the lowest counts (2.60–3.55 log units; mean 3.20 ± 0.32).

Milk samples from smallholder farmers exhibited higher SC values overall. In non-smoked containers, counts ranged from 2.81 to 4.62 log units, with a mean of 3.80 ± 0.72 log units (Table 2). Eight of the 20 samples (40%) exceeded the 4 log unit threshold. Exposure to *Acacia etbaica* smoke reduced the mean to 3.50 ± 0.78 log units, with six samples (30%) ≥ 4 log units, while *Olea africana* smoke lowered the mean further to 3.30 ± 0.71 log units, with only two samples (10%) ≥ 4 log units. Overall, SC values were consistently lower in WGCFNR farm milk than in smallholder milk, and both smoke treatments exerted a modest suppressive effect.

These findings confirm that wood smoke exposure can reduce SC levels, though not uniformly below the recommended maximum permissible limit of ≤ 4 log CFU/mL for raw milk intended for human consumption (European Commission, 2005; Oliveira *et al.*, 2022). Elevated SC levels are often associated with mastitis in lactating cows (Kadariya *et al.*, 2014) and pose risks of intoxication through enterotoxigenic strains. In this study, 40% of smallholder samples in non-smoked containers exceeded the regulatory limit, but smoke exposure reduced this proportion to 30% (*Acacia etbaica*) and 10% (*Olea africana*). Comparable reductions have been reported by Wanjala *et al.* (2016), who observed a ~2 log unit decrease in SC following *Olea africana* smoke treatment.

Relative to other Ethiopian studies, the mean SCs observed here (< 4 log units) were lower than the 4.35 log units reported in Tigray (Tarekgne *et al.*, 2015), suggesting comparatively better hygienic quality. Nonetheless, the persistence of samples above the permissible limit underscores the need for improved mastitis control and hygienic handling, particularly among smallholder farmers. The modest reductions achieved by smoke exposure highlight its potential as a traditional intervention, though it is insufficient as a standalone measure for ensuring compliance with international safety standards.

Table 2: The mean microbial load of milk samples from smallholder farmers at 0 h in $\log_{10}$ CFU/mL stored in non-smoked control and smoked bottles

Treatment	AMBC (mean ± SD)	SC (mean ± SD)	TCC (mean ± SD)	YAMC (mean ± SD)
Control	6.04 ± 0.39^{a}	3.80 ± 0.72^{a}	3.51 ± 0.17^{a}	3.59 ± 0.47^{a}
Acacia etbaica	5.93 ± 0.25^{ab}	3.50 ± 0.78^{ab}	3.44 ± 0.19^{ab}	3.37 ± 0.30^{ab}
Olea africana	5.79 ± 0.29^{b}	3.30 ± 0.71^{b}	3.40 ± 0.17^{b}	3.23 ± 0.30^{b}

Values are mean ± SD (n = 20). Means within a column followed by different superscript letters differ significantly.

Legend: AMBC – Aerobic mesophilic bacterial count, SC – *Staphylococcus aureus* count, TCC – Total coliform count, YAMC – Yeast and mold count, StDev – standard deviation, Mini – minimum, Maxi – maximum.

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-The total coliform bacterial count (TCC) of the raw milk samples

At zero hour, raw milk samples from the WGCFNR dairy farm stored in non-smoked containers exhibited total coliform counts (TCC) ranging from 2.30 to 2.84 $\log_{10}$ CFU/mL, with a mean of 2.71 ± 0.13 log units (Table 1). In contrast, smallholder farmer samples in non-smoked containers showed markedly higher values (2.50–4.26 log units; mean 3.51 ± 0.17), a statistically significant ($p < 0.05$) difference between the two sources (Table 3).

Exposure to wood smoke reduced TCC in both settings. At the WGCFNR farm (Table 1), Acacia etbaica smoke lowered counts to 2.61 ± 0.06 log units, while *Olea africana* smoke produced the lowest mean (2.49 ± 0.07). Similarly, smallholder milk (Table 2) exposed to Acacia etbaica and *Olea africana* smoke showed reduced averages of 4.25 ± 0.19 and 4.20 ± 0.17 log units, respectively (Table 2). Although reductions were modest, the inhibitory effect of smoke was more pronounced with *Olea africana*.

The mean TCCs observed here were lower than those reported in Hawassa, where Welearegay *et al.* (2012) documented values of 5.26 and 4.24 log units in large- and small-scale farms, and lower than the 4.41–4.94 log units reported in Bahir Dar Zuria and Mecha district (Tassew and Seifu, 2011). However, the values in this study remained above the maximum permissible level of 2 log units set by the International Farm Comparison Network (IFCN, 2006), indicating suboptimal hygienic quality.

During storage, TCC increased steadily over 36 h, with the slowest rise observed in milk stored in *Olea africana*–smoked containers, consistent with Wanjala *et al.* (2016). Coliforms are fast-growing organisms with short generation times (Ramanujam *et al.*, 2021), and their proliferation can quickly compromise milk quality. While high coliform counts do not necessarily confirm fecal contamination, they are strong indicators of unhygienic handling and are associated with off-odors and flavor defects that reduce consumer acceptability.

Overall, the findings highlight both the persistent hygiene challenges in smallholder systems and the modest protective role of traditional smoke treatments, particularly with *Olea africana*. Yet, the counts exceeding international standards underscore the need for improved hygienic practices and stricter quality control measures.

Table 3: Comparison of the microbial load parameters of the milk samples at zero h in $\log_{10}$ CFU/mL from WGCFNR dairy farm and smallholder farmers in Wondo Genet District, Ethiopia

Microbial parameter	Milk sample source	N	Mean ±SD	Mini	Maxi	Sig.
AMBC	WGCF dairy farm	20	5.74 ±0.22^{a}	5.3	6.05	0.007
	Smallholder farmers	20	6.06 ±0.46^{b}	5.2	6.77	
	Total	40	5.90 ±0.39	5.2	6.77	
SC	WGCF dairy	20	3.42 ±0.32^{a}	2.9	3.95	0.018
	Smallholders	20	3.77 ±0.54^{b}	2.8	4.62	
	Total	40	3.60 ±0.47	2.8	4.62	
TCC	WGCF dairy	20	2.68 ±0.24^{a}	2.3	3.05	0.00
	Smallholder farmers	20	3.51 ±0.43^{b}	2.5	4.26	
	Total	40	3.10 ±0.54	2.3	4.26	
YAMC	WGCF dairy farm	20	3.54 ±0.24^{a}	3.1	3.95	0.656
	Smallholder farmers	20	3.59 ±0.41^{a}	2.8	4.12	
	Total	40	3.56 ±0.34	2.8	4.12	

Different superscript letters of each mean (a, b) in the column indicate statistically significant differences ($P \leq 0.05$), while similar superscript letters of each mean (a) in the column indicate no statistically significant difference ($P \leq 0.05$). SD: standard deviation, Mini: minimum, Maxi: maximum. AMBC: Aerobic mesophilic bacterial count, SC: *Staphylococcus aureus* count, TCC: Total coliform count, YAMC: Yeast and mold count, WGCFNR: Wondo Genet College of Forestry and Natural Resources

-The YAMC of the raw milk samples

At zero hour, raw milk samples from the WGCFNR dairy farm stored in non-smoked containers exhibited YAMC ranging from 3.13 to 3.65 $\log_{10}$ CFU/mL, with a mean of 3.65 ± 0.05 log units (Table 1). Exposure to Acacia etbaica wood smoke slightly reduced values (2.95–3.45 log units; mean 3.34), while *Olea africana* smoke produced the lowest counts (2.69–3.30 log units; mean 3.16 ± 0.21).

For smallholder farmer milk samples, exposure to Acacia etbaica smoke yielded a mean YAMC of 3.37 ± 0.30 log units (range 2.81–4.12), while *Olea africana* smoke reduced counts further to 3.23 ± 0.30 log units (range 2.30–3.80) (Table 2). Overall, smoke treatments exerted a modest inhibitory effect, with *Olea africana* consistently showing greater reductions.

The mean YAMC values observed in non-smoked containers were lower than those reported in Hawassa, where Welearegay *et al.* (2012) documented 4.82 log units in large farms and 4.27 log units in small farms. In Nigeria, Nwankwo *et al.* (2015) reported a mean of 3.09 log units in raw milk from milking buckets, which was lower than the values observed here (> 3.5 log units). Conversely, Ethiopian studies have reported comparable or higher

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YAMC values. For instance, Mamo *et al.* (2016) observed similar dynamics during the fermentation of raw milk into homemade ergo in North Shewa, highlighting the variability of yeast and mold proliferation under different handling and storage conditions.

Yeasts and molds are opportunistic contaminants that thrive in nutrient-rich substrates such as milk, contributing to spoilage and off-flavors. Their presence at levels exceeding 3 log units indicates suboptimal hygienic handling and storage practices. The modest reductions achieved by smoke exposure suggest that traditional smoking may slow fungal growth, but not sufficiently to ensure compliance with quality standards. These findings reinforce the importance of improved hygienic interventions to limit fungal contamination in both institutional and smallholder milk systems.

- Antibacterial comparison of Olea africana and Acacia etbaica smoke during storage

The effect of container smoking on microbial dynamics was monitored over a 36 h storage period for all milk samples (n = 40). Across treatments, microbial proliferation followed the order *Olea africana* < *Acacia etbaica* < control. At zero hour, exposure to *Acacia etbaica* and *Olea africana* smoke reduced the mean AMBC by 0.13 and 0.28 log units, respectively, compared with non-smoked controls (Table 4). These reductions became more pronounced at 24 h, reaching 0.47 and 0.90 log units, respectively. By 36 h, however, differences between smoked and non-smoked samples were no longer statistically significant, reflecting convergence in microbial loads over extended storage.

Distinct growth trends were observed. In control bottles, AMBC rose sharply during the first 24 h, consistent with rapid bacterial proliferation in the absence of inhibitory factors, but declined by 36 h, likely due to nutrient depletion, acidification, and accumulation of toxic metabolites. In contrast, smoked containers exhibited slower but steady increases in AMBC, suggesting that phenolic compounds, aldehydes, and organic acids in smoke residues moderated bacterial multiplication (Bintsis *et al.*, 2026; Wanjala *et al.*, 2016). This restrained growth delayed the onset of self-limiting conditions, thereby extending microbial stability.

For SC, smoke exposure reduced initial loads by 0.18 (*Acacia etbaica*) and 0.36 (*Olea africana*) log units. By 24 h, reductions reached 1.14 and 2.09 log units, respectively, compared with controls, and at 36 h *Olea africana* smoke maintained a > 2 log unit reduction. These differences were statistically significant ($p < 0.05$) throughout storage, underscoring the strong inhibitory effect of *Olea africana* on staphylococcal growth.

Table 4: The effect of container smoking with two plant species on the microbial load of milk samples collected from WGCFNR dairy and smallholders (n=40) during 36 h storage time

Exposure time in h	Treatments and mean microbial counts in log_{10} CFU/mL		
	Control	***Acacia etbaica***	***Olea africana***
	Mean AMBC in log_{10} CFU/mL		
0	5.90 ±0.39[a]	5.77 ±0.36[a]	5.62 ±0.35[b]
24	9.31 ±0.52[a]	8.84 ±0.48[b]	8.41 ±0.52[c]
36	9.26 ±0.51[a]	9.24 ±0.50[a]	9.20 ±0.50[a]
	Mean SC in log_{10} CFU/mL		
0	3.60 ±0.47[a]	3.42 ±0.46[a]	3.24 ±0.44[b]
24	4.51 ± 0.45[a]	3.37 ±0.51[b]	2.42 ±0.65[c]
36	3.40 ±0.39[a]	2.59 ±0.82[b]	1.26±1.04[c]
	Mean TCC in log_{10} CFU/mL		
0	3.10 ±0.54[a]	3.04 ±0.54[a]	2.98 ±0.52[a]
24	6.25 ±1.29[a]	5.73 ±1.29[a]	5.27 ±1.25[b]
36	6.19 ±1.27[a]	6.12 ±1.28[a]	6.08 ±1.27[a]
	Mean YMC in log_{10} CFU/mL		
0	3.56±0.34[a]	3.35±0.36[b]	3.19±0.37[c]
24	4.28±0.39[a]	3.52±0.42[b]	2.77±0.63[c]
36	3.36±0.40[a]	2.82±0.63[b]	1.89±0.96[c]

Values are mean ± SD. Different superscript letters of each mean ± standard deviation (a, b, c) in each row indicate statistically significant differences ($P \leq 0.05$). AMBC: Aerobic Mesophilic Bacteria Count, SC: *Staphylococcus aureus* count, TCC: Total coliform count, YMC: Yeast and mold count.

Coliform dynamics were less responsive at zero and 36 h, but significant reductions were observed at 24 h. Compared with controls (6.25 ± 1.29 log units), exposure to *Acacia etbaica* and *Olea africana* smoke lowered mean TCC to 5.73 ± 1.29 and 5.27 ± 1.25 log units, respectively, corresponding to reductions of 0.52 and 0.98 log units. These findings align with Kenyan studies in which smoked containers suppressed coliform proliferation in raw camel milk (Gitau *et al.*, 2022; Wanjala *et al.*, 2016).

For example, Wanjala *et al.* (2016) investigated the antimicrobial effect of smoking milk-handling containers in pastoral systems in Kenya. The study demonstrated that smoking the inner surfaces of plastic milk containers significantly reduced microbial proliferation in raw camel

milk stored under ambient conditions. Specifically, milk held in smoked containers exhibited markedly lower total viable counts (4.64 $\log_{10}$ CFU/cm²) compared with non-smoked controls (5.99 $\log_{10}$ CFU/cm²). In addition, coliform growth was suppressed in smoked containers, indicating that smoke residues deposited on container surfaces exerted a protective effect against contamination. The authors attributed this antimicrobial activity to phenolic compounds and organic acids present in wood smoke, which inhibit bacterial multiplication.

YAMC declined progressively with storage, with the most pronounced reductions in *Olea africana*–smoked containers. At 24 h, reductions reached 0.76 log units (*Acacia etbaica*) and 1.51 log units (*Olea africana*), indicating significant antifungal activity. This trend corroborates earlier Ethiopian reports in which smoking vessels improved physicochemical stability and extended shelf life. Hadero *et al.* (2014) noted that fumigating pots with *Olea africana* smoke improved physicochemical parameters such as pH stability and decreased microbial loads, indicating enhanced shelf life and safety of stored raw milk. The use of wood smoke from these plant species for fumigating milk containers has proven to be an effective and culturally integrated method for enhancing raw milk safety in Ethiopian pastoral communities (Lemma *et al.*, 2024). These findings support traditional fumigation as a viable intervention for improving milk hygiene without reliance on modern refrigeration or chemical sanitizers.

The antimicrobial effects of container smoking are attributed to smoke's complex chemical composition, which includes over 400 compounds such as acids, alcohols, carbonyls, esters, furans, lactones, and phenols (Rigling *et al.*, 2023). Phenolic compounds, in particular, exert bacteriostatic effects by disrupting cellular functions. This study demonstrated that fumigating milk containers with wood smoke from *Olea africana* and *Acacia etbaica* significantly reduces microbial counts and the occurrence of common pathogens in raw milk. Wood smoke has been used for centuries as a traditional food preservation method, leveraging its antimicrobial and antioxidant properties (Amenu *et al.*, 2019). Specifically, *Olea africana* possesses antibacterial, antifungal, and antioxidant activities due to its phytoconstituents, while various *Acacia* species show antimicrobial effects against a range of bacteria (Adhikari and Rangra, 2023; Sadiq *et al.*, 2017). Traditional use of *Olea africana* and *Acacia etbaica* for fumigating milk vessels thus represents a culturally integrated preservation method that enhances microbial safety without reliance on refrigeration or chemical sanitizers.

Overall, the results demonstrate that wood smoke fumigation significantly reduces microbial loads and delays spoilage, with *Olea africana* showing superior efficacy. While not sufficient to fully meet international microbial safety standards, this practice provides a valuable traditional intervention for improving raw milk hygiene in Ethiopian pastoral communities.

-The dominant bacterial flora and occurrence of Shigella and Salmonella species

From the 40 raw milk samples analyzed, a total of 138 aerobic mesophilic bacterial isolates were recovered, comprising 55 from smoked containers and 83 from non-smoked controls. Gram staining, microscopy, and biochemical profiling identified 134 isolates belonging to 11 bacterial genera, while four were provisionally categorized as other Gram-negative rods (Figure 2). Gram-negative bacteria predominated, accounting for 50.9% of isolates in smoked containers and 63.9% in non-smoked containers. The most frequently encountered Gram-negative species were *Escherichia coli* (18 isolates), *Shigella* (13), and *Salmonella* (12). Among Gram-positive isolates, *Lactobacillus* (18), *Staphylococcus* (15), and *Bacillus* (14) species were most common.

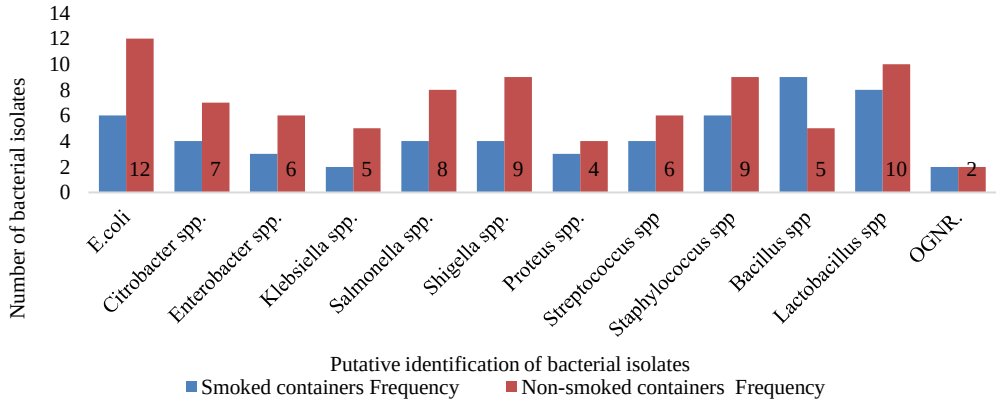


Figure 2: Frequency distribution of the dominant aerobic mesophilic bacterial flora of the raw milk samples kept in smoked and non-smoked containers. Values represent the total number of bacterial isolates identified in each group. Error bares are not shown because the data represent observed counts rather than means of replicate measurements.

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Pathogen incidence was notable. *Salmonella* spp. were detected in 30% (12/40) of samples, while *Shigella* spp. were present in 32.5% (13/40). Co-occurrence of both pathogens was observed in 10% of WGCFNR farm samples and 15% of smallholder samples (Figure 3). The frequency of *E. coli*, *Shigella*, and *Salmonella* isolates was significantly lower in smoked containers (6, 4, and 4 isolates, respectively) than in non-smoked controls (18, 13, and 12 isolates), highlighting the inhibitory effect of smoke residues.

The predominance of *Enterobacteriaceae* contrasts with reports elsewhere, where lactic acid bacteria (*Lactococcus*, *Lactobacillus*, *Leuconostoc*) and spoilage-associated genera (*Pseudomonas*, *Acinetobacter*) dominate raw milk flora (Quigley *et al.*, 2013; Yan *et al.*, 2022). The selective enrichment of *Lactobacillus* spp. in smoked containers suggests that smoke residues may favor acid-tolerant commensals (Wanjala *et al.*, 2016). Persistence of *Bacillus* spp. reflects their spore resistance to environmental stressors (Cho and Chung, 2020).

Raw milk is inherently vulnerable to microbial hazards due to contamination from handlers, diseased animals, unsanitized equipment, soil, and fecal matter (Martin *et al.*, 2023; Ntuli *et al.*, 2023). Mastitic udders can shed *Staphylococcus aureus* and *Streptococcus agalactiae*, while biofilms of *Enterobacteriaceae* and *Bacillus* spp. on equipment surfaces contribute to persistent contamination (Quigley *et al.*, 2013). Airborne spores and dust further influence microbial composition.

The detection of *E. coli*, *Shigella*, and *Salmonella* in this study aligns with findings from Addis Ababa, where *E. coli* (26.3%) and *Salmonella* spp. (18.4%) were dominant in raw milk (Garedew *et al.*, 2012). In Bahir Dar, *Klebsiella* spp. were reported as prevalent contaminants (Tassew and Seifu, 2011), underscoring regional variability linked to hygienic practices and environmental conditions. Importantly, the International Farm Comparison Network (IFCN, 2006) stipulates that *Salmonella* and *Shigella* must not be detected in 25 mL of raw milk intended for human consumption. Their presence here raises significant public health concerns. *Shigella*, a strict human pathogen, points to contamination from handlers (Oliver *et al.*, 2005). Pathogenic *E. coli*, particularly Shiga toxin–producing strains (STEC), can cause severe gastrointestinal illness and hemolytic uremic syndrome (Teshale *et al.*, 2025).

Salmonella enterica remains a leading cause of foodborne illness globally, with contamination often occurring at production and collection stages (Bedassa *et al.*, 2023). *Shigella* species, though less common in milk, are highly infectious and associated with dysentery in low-resource settings (Gu *et al.*, 2022). Overall, the microbial profiling underscores both the diversity of aerobic mesophilic flora and the persistence of pathogenic *Enterobacteriaceae* in raw milk. While container smoking reduced pathogen frequency, the detection of *Salmonella* and *Shigella* above permissible limits highlights the urgent need for improved hygienic practices and pathogen control measures in Ethiopian dairy systems.

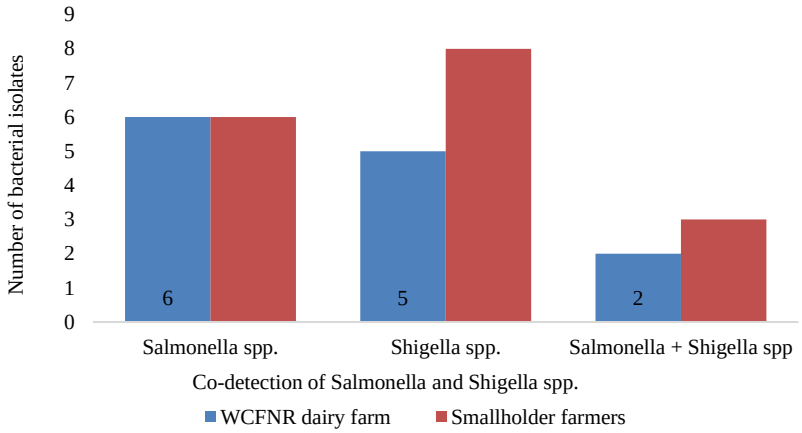


Figure 3: Incidence of *Salmonella* and *Shigella* species in raw milk samples collected from the Wondo Genet College of Forestry and Natural Resources dairy farm and smallholder farmers. Values represent the total number of bacterial isolates identified in each group. Error bars are not shown because the data represent observed counts rather than means of replicate measurements. Values represent the number of isolates identified per group. Because these are categorical frequency counts, error bars are not applicable.

Conclusion

This study demonstrated that fumigating milk containers with smoke from *Olea africana* and *Acacia etbaica* significantly improved the microbial quality and safety of raw cow milk. Both plant species reduced the levels of aerobic mesophilic bacteria, coliforms, yeasts, molds, and key pathogens such as *Staphylococcus aureus*, *Salmonella*, and *Shigella*. *Olea africana* showed slightly stronger antimicrobial effects, highlighting its value as a potential preservative. These findings validate the traditional practice of container smoking as a low-cost, culturally accepted method for reducing microbial contamination and extending the shelf life of raw milk. This is especially

relevant in rural communities, where refrigeration and modern preservation technologies are limited. However, the persistence of high microbial loads and pathogens in some samples, even after smoking, indicates that fumigation should be considered a complementary practice rather than a substitute for proper hygiene and safe handling. Strengthening awareness among smallholder farmers, alongside integrating traditional methods with modern safety standards, could play a vital role in reducing milk-borne diseases and postharvest losses. Future studies should focus on identifying the specific antimicrobial compounds in the smoke, optimizing fumigation protocols, and evaluating consumer acceptance of smoked milk.

Author contributions

A.A. participated in drafting the work plan and executed the laboratory work; A.M. conceived and planned the work, supervised the laboratory work, and wrote the draft manuscript; M.A. participated in the data analysis and edited the final draft of the manuscript. All authors read and approved the final manuscript.

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Conflicts of interest

The authors declare that there is no conflict of interest.

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Ethical consideration

Not applicable

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