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In Vitro Antimicrobial Activity of Camel Urine Lactoferrin

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Abstract

Lactoferrin, an iron-binding glycoprotein found in various bodily fluids, is widely recognized for its broad-spectrum antimicrobial properties. This study evaluates the *in vitro* antibacterial and antifungal activity of lactoferrin isolated from camel urine against selected microbial strains. The purified lactoferrin demonstrated notable antibacterial efficacy, producing zones of inhibition ranging from 12–19 mm against *Staphylococcus aureus*, 10–16 mm against *Bacillus subtilis*, and 13–21 mm against *Escherichia coli*. Additionally, antifungal activity was observed against *Candida albicans*, with inhibition zones ranging from 11–18 mm. The antimicrobial mechanisms are attributed to iron sequestration and disruption of microbial cell membranes in bacteria, and inhibition of fungal growth via interference with cell wall synthesis and membrane integrity. These findings underscore the potential of camel urine-derived lactoferrin as a natural antimicrobial agent and contribute to the growing body of research exploring its biochemical and therapeutic properties.

Keywords: Lactoferrin; Antibacterial; Antifungal; *In Vitro* Activity; Microbial Inhibition

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INTRODUCTION

Lactoferrin, is a multifunctional protein which belongs to the transferrin family(Chasteen and Woodworth, 2024; Ianiro *et al.*, 2023). It is renowned for its antimicrobial properties, which includes antibacterial and antifungal activities (Sienkiewicz *et al.*, 2022). Lactoferrin have been isolated from many unconventional sources which includes Camel urine(Avalos-Gómez *et al.*, 2022; Bobreneva and Rokhlova, 2021; Kowalczyk *et al.*, 2022). Lactoferrin have garnered significant attention over the years due to its unique biochemical composition and therapeutic potential(Mayeur *et al.*, 2016; Pan *et al.*, 2021; Sienkiewicz *et al.*, 2022). Camel urine, which is mostly used traditionally in certain cultures for medicinal purposes, contains many bioactive compounds which includes lactoferrin that contributes to its antimicrobial efficacy(Abdel Gader and Alhaider, 2016). Lactoferrin consists of a single polypeptide chain folded in two symmetric, globular lobes (N- and C-lobes), each able to bind one ferric ion (Hong *et al.*, 2024; Kowalczyk *et al.*, 2022; Ongena *et al.*, 2024). This glycoprotein, found in physiological fluids of mammals, plays an important role in immune regulation and in defense mechanisms against bacteria, fungi, parasites, and viruses (Brouwer *et al.*, 2025).

The antibacterial activity of lactoferrin stems from its ability to sequester iron, which is an essential nutrient for growth of bacteria, thereby inhibiting the proliferation of a wide range of pathogenic bacteria(Hong *et al.*, 2024; Kowalczyk *et al.*, 2022; Ongena *et al.*, 2024). Lactoferrin also disrupts bacterial cell membranes and modulates immune response, which enhances its bactericidal effects(Abd El-Hack *et al.*, 2023; Gruden and Poklar Ulrih, 2021). Lactoferrin antifungal activity is attributed to direct interactions with fungal cell walls, interference with fungal metabolic pathways, and also the generation of reactive oxygen species that damage the fungal cells(Lai et al., 2016; Wang et al., 2019).

These properties make lactoferrin derived from Camel urine a promising candidate for the development of novel antimicrobial agent, particularly in the face of rising antibiotic resistances. Several studies have led to the development of vaccines, antimicrobial drugs, and oral healthcare products using lactoferrin as the main agent. Lactoferrin is used in oral care products (e.g., toothpaste, mouth rinses, and chewing gums) to inhibit the growth of oral pathogens (e.g., Streptococcus mutans) and suppress oral malodor (Wang *et al.*, 2019). Lactoferrin is also used in vaccines and antimicrobial drugs (Sherman et al., 2015). The administration of bovine and human lactoferrin have proven to help in generation of a

stronger T-cell helper 1 response and boosted the effectiveness of vaccines against certain bacteria and fungi (Hwang *et al.,* 2017). No detrimental effects have been reported when lactoferrin is in above mentioned and other vaccines. Regarding its application as a drug, the therapeutic potential of lactoferrin has been demonstrated against several diseases caused by bacteria and fungi (Wang et al., 2019).

In this present study lactoferrin isolated from camel urine would be used for invitro activity, the zone of inhibition would be measured against certain bacteria and fungi isolates.

MATERIAL AND METHODS

Lactoferrin

Lactoferrin was purified from Camel urine using acetone precipitation, dialysis, ion exchange chromatography, and gel filtration.

Bacterial strains and fungal strains culture conditions

Three bacterial species including one Gram-negative (*Escherichia coli*) and Gram-positive strains (*Staphylococcus Aureus* and *Bacillus Subtilis*) was used. One fungal specie (*Candida albicans)* was used. The molten agar was poured into sterile petri dishes and allowed to solidify. A small amount of the microorganism was spread on the surface of the agar using a sterile swab. A sterile cook borer was used to create 4 wells on the agar plate. The purified lactoferrin concentration of 200 µL, 400 µL, 600 µL, and 800 µL was poured into separate wells. The agar plates was incubated in an incubator at an appropriate temperature (37∘C for bacteria and 30∘C for fungi) for 24 hours. The zone of inhibition was measured using a ruler, and the diameter was measured in mm. (Usman and Osuji, 2007; Volleková *et al.*, 2001)

RESULTS

Antibacterial activity of Camel urine lactoferrin against bacterial isolates

This is presented in table 1. The antibacterial activity of camel urine lactoferrin result shows a dose-dependent inhibition with an increase in the concentration of the purified lactoferrin. As concentration increases from 200 to 800 µL, inhibition zones grow

larger: *Staphylococcus Aureus* from 12 to 19 mm, *B. Subtilis* from 10 to 16 mm , and *E.coli* 13 to 21 mm. *Staphylococcus Aureus* zone of inhibition values are higher than that of *B. Subtilis*, but lower than those of *E.coli* (except at 200 µL). There is also an increase in the zone of inhibition (3-4mm) that is consistent across concentrations, indicating a steady, dose-dependent response.

Antifungal activity of Camel urine lactoferrin against fungal isolates

The result is presented in table 2. The antifungal activity of Camel urine lactoferrin result reveals that as concentration rises from 200 to 800 µL, inhibition zones increases: *C. Albicans* from 11 to 18 mm. Moreover, it shows an increasing inhibition with lactoferrin concentration.

Table 1- Antibacterial activity of Camel urine lactoferrin against bacteria isolates at four concentrations

Concentration (µL)	Zone of inhibition diameter (mm)		
	Staphylococcus Aureus	Bacillus Subtilis	Escherichia coli
200	12	10	13
400	15	12	16
600	17	14	18
800	19	16	21
Standard Antibiotic control (Ampiclox) 50%	25	20	30

Table 2. - Antifungal activity of Camel urine lactoferrin against fungal isolates at four concentrations

Concentration (µL)	Zone of inhibition of diameter (mm)
	Candida Albicans
200	11
400	14
600	16
800	18
Standard Antifungal control (Ketoconazole) 50%	24

DISCUSSION

The antibacterial activity of camel urine lactoferrin is noteworthy. It has been suggested that the antibacterial activity of lactoferrin occurs at C-lobe region, and this is related to its iron-binding activity or the presence of antibacterial peptides (Gruden and Poklar Ulrih, 2021; Piacentini *et al.*, 2024). Furthermore, compared with the N-lobe, the C-lobe of lactoferrin has shown stronger growth inhibitory activity against *Escherichia coli*, indicating its potential antibacterial properties (Jin *et al.*, 2022). Additionally, synthetic peptides containing the C-lobe sequence of lactoferrin have exhibited inhibitory effects on bacterial growth, highlighting the antibacterial potential of the C-lobe (Gruden and Poklar Ulrih, 2021; Jin *et al.*, 2022; Piacentini *et al.*, 2024). In this study also, Camel urine lactoferrin appears to be more effective against *Escherichia coli*, moderately effective against *Staphylococcus aureus*, and less effective against *Bacillus Subtilis*. The effectiveness against the bacteria strains could be due to iron sequestration by lactoferrin, disruption of bacterial membranes, inhibition of bacterial adhesion to host cells and antioxidant properties of lactoferrin. These phenomenon's usually occurs at the C region binding site of lactoferrin (Ashraf *et al.*, 2024; Kim *et al.*, 2025; Piacentini *et al.*, 2024). This could also align with the study by Jin *et al.*, (2022) which provides valuable insights into the antibacterial activity of the C-lobe of lactoferrin. The study by Jin *et al.*, (2022) highlights that lactoferrin, as an iron-binding protein, restricts the availability of free iron, which is essential for the growth of microorganisms. This limitation of free iron may contribute to the antibacterial activity of the purified lactoferrin from Camel urine C-lobe. This result is consistent with research by Gruden and Poklar Ulrih (2021), Hong *et al.* (2024), and J. W. Kim *et al.* (2025), which highlights the antibacterial potential of lactoferrin's C-lobe. The C-lobe's ability to bind Fe3+ is a key mechanism driving its antibacterial activity. By sequestering iron, the C-lobe restricts bacterial growth and proliferation, thereby mediating its antibacterial effects

Another perspective on the antibacterial activity of lactoferrin could be due to lactoferrin-binding protein B which is transported to the outer leaflet of the outer membrane in Gram-negative bacteria via numerous translocation and processing phases. Upon reaching its mature state, its integration in the membrane is facilitated by the addition of a palmitoyl group to the far N-terminal cysteine (Ostan *et al.*, 2021). A key characteristic of lactoferrin-binding protein B across various organisms is a region in its C-terminal lobe that can counteract lactoferrin's antimicrobial activity. The mechanisms underlying this effect differ, as evidenced by the structural variability in their C-terminal regions. Initial

studies proposed that negatively charged amino acid sequences (Asp, Glu) in lactoferrin-binding protein B enable the sequestration of cationic antimicrobial peptides derived from lactoferrin (Morgenthau *et al.*, 2014). These regions are highly dynamic, making them difficult to crystallize and study structurally.

Additional research has demonstrated that both bovine lactoferrin (Avalos-Gómez *et al.*, 2022; Blais *et al.*, 2024) and human lactoferrin (Avalos-Gómez *et al.*, 2022; Ostan *et al.*, 2021; Sienkiewicz *et al.*, 2022) can bind and release lipopolysaccharides (LPS) from gastric Gram-negative bacilli, indicating direct interactions with bacterial cell membranes. Consequently, lactoferrin's interaction with bacterial outer membrane components, such as lipopolysaccharides and porins, likely plays a critical role in its antimicrobial activity. Specifically, lactoferrin's binding to the lipid A component of LPS inhibits neutrophil priming for enhanced formyl-Met-Leu-Phe-triggered superoxide release and may also reduce cytokine production following LPS exposure (Gruden and Poklar Ulrih, 2021; J. W. Kim *et al.*, 2025; Sienkiewicz *et al.*, 2022).

Lactoferrin exhibits varying antifungal activity across species, primarily due to differences in the C-lobe region of the protein (Kim *et al.*, 2025; Zhang *et al.*, 2025). The antifungal efficacy of lactoferrin, even with similar purity levels, can differ significantly depending on its production source, ranging from moderately active to inactive against the same test organism (Kaczmarek *et al.*, 2025; Ostrowska *et al.*, 2025). In this study, camel urine-derived lactoferrin showed moderate effectiveness against *Candida albicans*. While the precise mechanism of lactoferrin's antifungal action remains unclear, bovine lactoferrin has been extensively documented for its activity against various human and plant pathogenic fungi. Among the *Candida* species, *Candida albicans*, *Candida tropicalis*, and *Candida krusei* show the highest susceptibility to lactoferrin, while *Candida glabrata* is the least susceptible (Sachivkina *et al.*, 2021). Early research attributed lactoferrin's antifungal effects to its iron-sequestering ability, which induces a fungistatic effect, with growth inhibition in Candida reversible by iron supplementation (Długosz *et al.*, 2025).

More recent studies suggest that lactoferrin's primary antifungal mechanism is iron-independent, involving direct interaction with the fungal cell surface, leading to membrane damage and leakage

(Akdaşçi *et al.*, 2024; Casu *et al.*, 2025; Gruden and Poklar Ulrih, 2021). Supernatant protein assays and propidium iodide staining have revealed that lactoferrin increases cell

surface permeability in *Candida albicans*, *Candida krusei*, and *Cryptococcus neoformans*, resulting in cell death (Chang *et al*., 2021; Długosz *et al*., 2025). Scanning electron microscopy has shown physical alterations in *Candida* isolates, including protein leakage, surface bleb formation, swelling, and cell collapse (Yalçın and Bonabian, 2025). Additional effects observed in *Candida albicans* after lactoferrin treatment include potassium ion release, cytosolic acidification, altered membrane potential, accumulation of intracellular reactive oxygen species, and chromatin condensation, all indicative of an apoptosis-like process (Fernandes and Carter, 2017). However, the specific mechanisms by which lactoferrin triggers these apoptosis-like processes in fungi remain poorly understood.

The experimental findings provide a comprehensive view of lactoferrin's activity against bacterial and fungal strains, highlighting its cellular targets and inhibitory mechanisms. Lactoferrin appears to exert its effects by binding to bacterial and fungal proteins or by occupying cellular membrane receptors, thereby disrupting microbial activity. These results, supported by zone inhibition studies, underscore lactoferrin's broad therapeutic potential and pave the way for future research into lactoferrin-based therapeutics.

CONCLUSION

The results suggest that isolated lactoferrin from camel urine exhibits a significant antimicrobial activity against a wide range of bacterial and fungal pathogens. Lactoferrin exhibited a strong inhibitory effect against *E. coli*, moderate effectiveness against *S. aureus* and *C. albicans*, and lower efficacy against *B. subtilis*.

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Author Contributions

K.A. Ahmad, and A.U. Wurochekke: Conceptualization; K.A. Ahmad: Formal analysis; K.A. Ahmad: Investigation; K.A. Ahmad: Funding; K.A. Ahmad and A.U. Wurochekke: Methodology; A.U. Wurochekke: Project administration; A.U. Wurochekke and M.S. Jada: Supervision; A.U. Wurochekke and M.S. Jada: Validation; K.A. Ahmad: Visualization; K.A. Ahmad: Writing - original draft; A.U. Wurochekke and M.S. Jada: Writing - review & editing. All authors have read and agreed to the final version of this manuscript

Conflict of Interest

The authors have no competing interest to declare.

Data availability

K.A. Ahmad, will provide the data that support the study's findings upon request.

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