Eurasian Journal of Agricultural and Environmental Medicine

2023 January-June; 2(1): pages: 1-8
DOI: 10.22587/ejaem.2023.2.1.1
Home page: http://www.aensiweb.com/EJAEM

Research Article **AENSI Publications**

Study the effect of Lactoferrin Isolated and Purificated from Bovine milk and Colostrum on *E. coli 0.157* isolated from diarrheic sheep

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Received date: 12 March 2023, Accepted date: 22 April 2023

Abstract
Human hemolytic uremic syndrome (HUS) and hemorraghic colitis are both caused by Escherichia coli H7serotype O157:H7 strains. A source of *E. coli* O157:H7 is cattle. We investigated whether bovine lactoferrin, a naturally occurring antimicrobial protein found in milk, could prevent the H7 *E. coli* O157 expansion:. The results revealed that bovine lactoferrin, at respective dosages varying from 3 to 10 mg/ml, had a higher influbce on *E. coli* O157:H7. LF of Bovine had no impact on the development of *this* strain at respective dosages varying from 0.1 to 2 mg/ml.

Keywords: Lactoferrin, Bovine milk, Colostrum, E. coli 0.157

INTRODUCTION

Colostrum is a fluid produced and accumulated by the mammary glands within the first several days following calving and throughout the last trimester of pregnancy. It containshigh-qualitycontains high quality nutrition and physiologically active compounds to the calf. Colostrum is not only nourishing, stimulating, and protective, but it also possesses purgative properties. By causing meconium to evacuate through peristalsis, it stops the body from over-densifying and having excretion problems. Among the bioactive components with immune-stimulating properties present in Coarecontains lactoperoxi dase.lactalbumin, -lactoglobulin fat that transports vital vitamins, astibodies, polyunsaturated fatty acids, lactoferrin, and lysozyme (Puppel et al., 2019).

Intriguingly, Lactoferrin has anti-oxidant, antiviral, anti-inflammatory, immunomodulatory, and anti-cancer characteristics in addition to its ability to promote the development of probiotic bacteria like Bifidobacterium.. Various antifungal, antibacterial, and immunoregulating substances are also present (Christiansen and Kjelden, 2010). One of the most crucial proteins in mammalian milk is lactoferrin, an iron-binding protein. The most likely physiologically relevant action of lactoferrin is defense against gastroenteritis. Among the many pathogens present are the diarrhea-causing *Escherichia coli,* Rotavirus, Giardia, *Shigella*, and *Salmonella* (Ochoa & Cleary,2009).

less recent Sheep and goats, two small domestic ruminants, have emerged as significant reservoirs of E. coli O157:H7 human illness. because of the increased consumption of sheep and goat foods, such as unpasteurized cheese, and the popularity of petting farms (La Ragione et al., 2009). Escherichia coli O157:H7 is a significant reservoir in ruminants, hence decreasing the amount of E. coli O157:H7 excreted by these animals may be crucial in lowering human infections (Yekta et al., 2011).

MATERIAL AN METHODS

A total of 10ples from diarrheal sheep were collected between January and December 2019 from veterinary offices and slaughterhouses in Baghdad City. Samples were taken on average eight to ten times per month from both genders, and each sample was placed in separate sterile cotton swabs with transport media (phosphate buffer saline) to prevent

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contamination. Samples were then immedi transported to the lab in a cool box with ice packs. 45 ml of buffered peptone water (BPW) and one gram of enrichment were added, and the mixture was incubated for 24 hours at 37 c. An inoculum (100 ul) was applied to sorbitol MacConkey agar plates from the enrichments, which were subsequently cultured for a further 48 hours at 37°C, and 2.5 mg/l of tellurite and 0.05 mg/l of cefixime are added . Brain heart infusion broths (BHIB) containing 20%(vol/vol) glycerol were infused with suspected *E. coli O157* colony cultures and stored at -80°C until DNA extraction was needed. The 16S rRNA gene unique to E. coli was amplified to validate the isolates based on growth, colony morphology, oxidase response, Gram stain, and TSI.

Samples of bovine colostrum interacting

samples obovine colostrum sampleslected from various locations across the Baghdad Governorate and promptly frozen before being kept at 18°C. Using centrifugation for 15-20 minutes at 4°C with an acceleration of 8,000 revolutions per minute, the frozen materials were defrosted.. After the casein from the skimmed colostrum was precipitated with 1 mol/L HCl at pH 4.2, the samples of skimmed bovine colostrum were frozen and kept at 18°C. and colostral whey was produced when microfiltration was used to remove the precipitated casein.. Then, cloistral whey was packaged in plastic containers and kept overnight at 4°C before usage. After being diluted ten times with distilled water, the cloistral whey was ultrafiltered (at 20 °C) in a bench-top setup using a 50 kD membrane as the cut-offostrum whey.

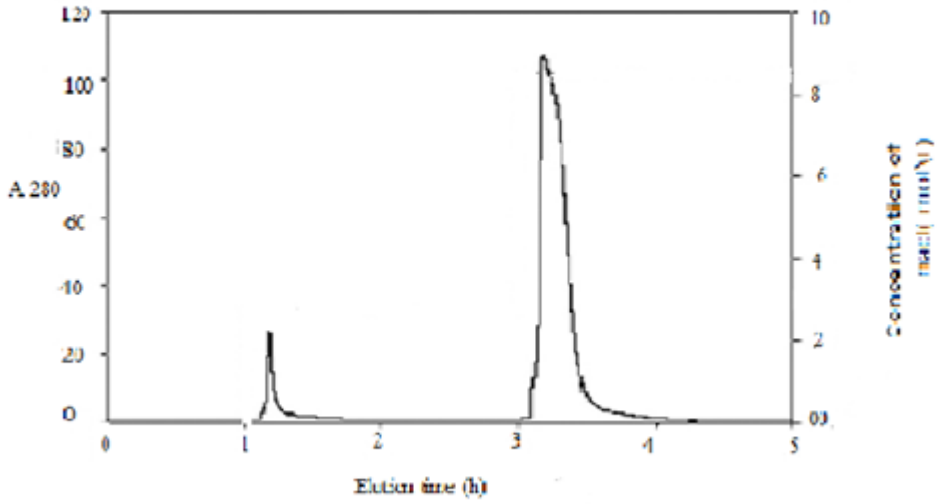

Figure 1. LF was isolated from the ultrafiltrated colostrum whey using a CM-sepharose FF column (1.6 25 cm) and cation-exchange chromatography

Lactoferrin separations using ion exchange chromatography

CM Separose FF and DEAE Sepharose FF were used to absorb LF. The ultrafiltrated whey underwent cation exchange chromatographic separation on a column made of CMsepharose FF measuring 1.6 25 cm, followed by anion exchange chromatographic separation using a DEAE-sepharose column with a diameter of 1.67 cm.. After the anion exchange columns had been cleansed with deionized water, stepwise sodium chloride solutions of increasing molarities were employed to elute them. The LF fractions were then dialyzed in sequence for an entseparates at the same speed. The cation was freeze-dried at 63°C and tested against pure water Figure 2.

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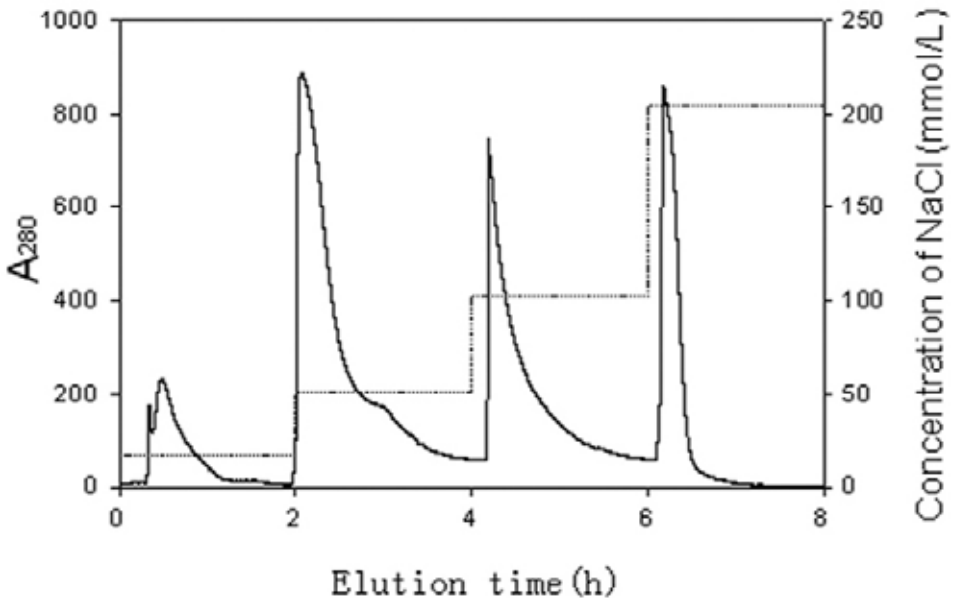

Figure 2: Anion-exchange chromatography with a DEAE-sepharose FF column (1.6 75 cm) separates LF from ultrafiltrated colostrum whey.

MicrobiologiTo order to produce overnight cultures, a colony from a single well was inoculated into a 10-ml tube with Luria bro. Thehe tube was incubated at 37°C for 24 hours while being shaken at 200 rpm. Each of the four strains of E. coli O157:H7 was tested for the antibacterial activity of LF. Centrifugation (11 337 g, 5 min) was used to pellet *E. coli* O157:H7 overnight cultures into 1 was subsequently reconstituted in 1 ml of LB medium. 100 CFU/ml of bacteri*a* and then were cultured in LB broth with various amounts of bovine LF (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 3 and 10 mg/ml) for 16 hours at 37°C.

RESULTS AND DISCUSSION

Results of Percentage of Isolation of *E.coli o157*

The results of bacterial isolation showed five bacterial isolates, from one hundred diarrheic fecal samples which were identified as *E.coli* and represent 5%, while 95 samples were gave negative results for bacterial culture from various urban and rural farm animals from different area in Baghdad governorate.

Genomic DNA extraction

The results demonstrate that the DNA of 5 isolaes was extracted favorably and appears as a compact band using a simple protocol genomic DNA Extraction kit (G-SPIN kit) **Figure 3.**

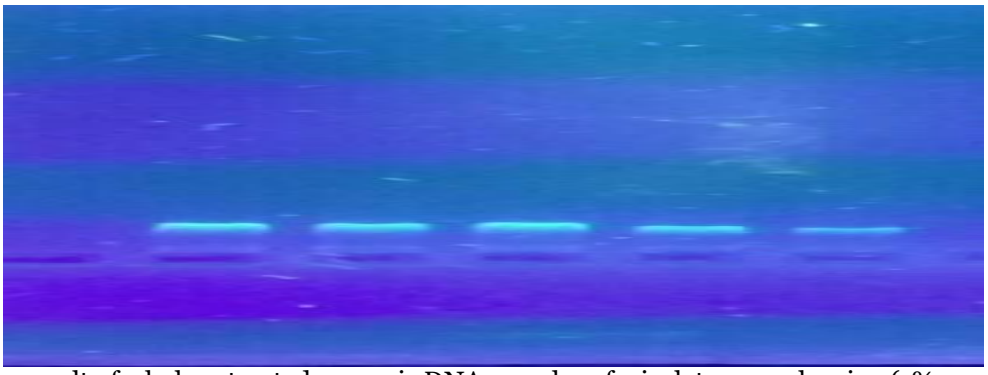

Figure 3: Show, result of whole extracted genomic DNA samples of 5 isolates on gel, using (1% agarose, TBE buffer (1X), 5 V/cm. for one hour, electrophoresis stained with red safe stain.

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PCR results of *16srRNA* of *E.coli 0157*

The 5 suspected isolates of *E.coli 0157* were confirmed by convential PCR technique, amplification of bacterial genomic DNA was conducted by using two primers Under these optimal conditions, the expected fragment approximately 1250 bp of *16srRNA* gene were successfully amplified for as *E.coli 0157* which were confirmed by the electrophoresis analysis The results showed that the investigated *E.coli 0157* isolates had been correctly identified by genus When amplified PCR products, that created using the universal bacterial *16srRNA* primers, Figure 4.

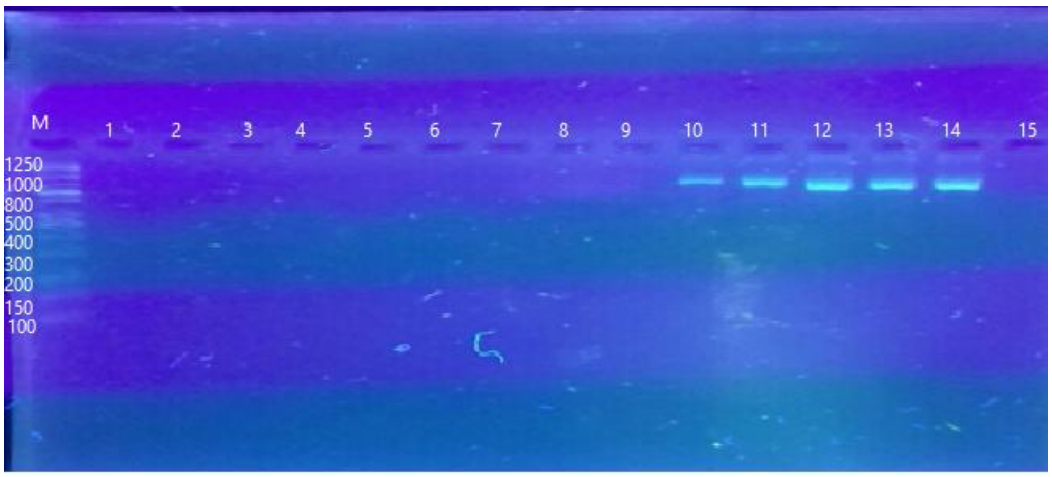

Figure 4: PCR product the band size 1250 bp 16SrRNA. The product was electrophoresed on 1.5% agarose avoltsvolt/cm2. 1x TBE buffer for 1:30 hours. M: DNA ladder (100 -2000).: lane (1-30) represent (E.coli 0157humans in human) iUVlized under U.V light **.**

Analysis of The Nucleotide Sequence of Partial *16srRNA* Gene of *E.coli 0157*

Result analyses of Nucleotide Sequence of Partial *16srRNA* Gene of 78 isolates of *E.coli 0157* that submitted in GenBank database showed 99% similarity or compatibility with the reference strains in GenBank, by using Blast and Bio edit Sequence alignment and can be found under the accession number at NCBI-GEN Bank as follow five accession numbers were obtained from *16SrRNA* and registration of 15 sequences of *16SrRNA* gene for human isolates include submission ID: CP044148.1 , C,3539.1 ,: MK791711.1, C022050.2 : CP015832.1 belong to E.coli 0157 Figure(5).

Table 1: Analysis of The Nucleotide Sequence of Partial 16srRNA Gene of E.coli 0157.

	Accession	Country	Host	Source	Compatibility
1.	ID: CP044148.1	USA	----------	Escherichia coli O157	99%
2.	ID: CP043539.1	United Arab Emirates	camel	Escherichia coli O157	99%
3.	ID: MK791711.1	Pakistan	chicken	Escherichia coli O157	99%
4.	ID: CP022050.2	USA: DC	Homo sapiens	Escherichia coli O157	99%
5.	ID: CP015832.1	United Kingdom	Homo sapiens	Escherichia coli O157	99%

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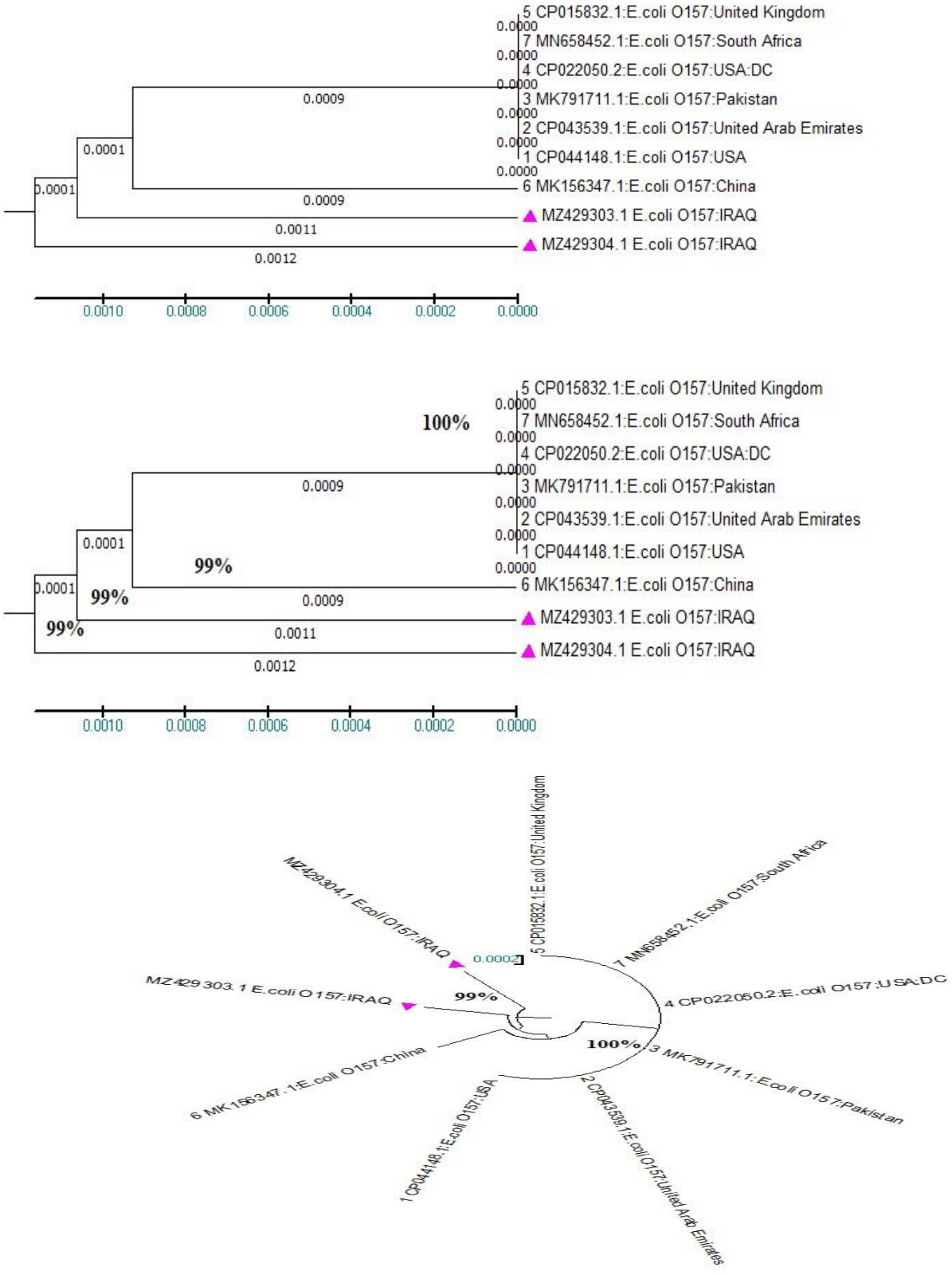

Figure 5: Evolutionary relationships of taxa of E.coli 0157

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Results of cultural characteristics

E.coli. Produce distinct colonies on selective media emerged, whereas on chrome agar, the colonies were ro,und blthe ue while Colony morphology of isolates of *E.coli* appear as smooth , small, pale and rounded colonies on sorbitol MacConkey agar (**Figure 6)**

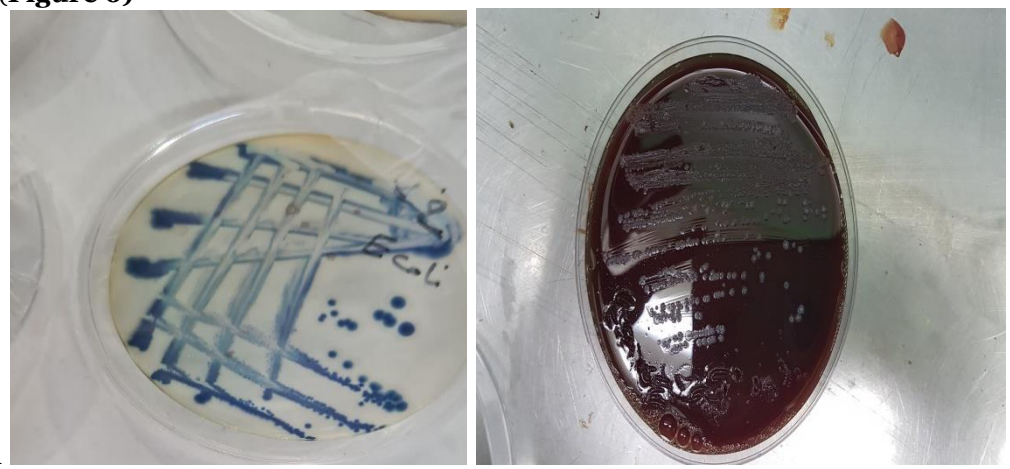
A 

Figure 6: Colony characteristics of *E.coli 0157* on selective media in (A) on chrome agar and (B) sorbitol MacConkey agar.

Lactoferrins' impact on the development of E. coli O157:H7

Both in vitro and in vivo investigations have demonstrated that lactoferrin inhibits the development of various harmful bacteria, including E. coli (Shashikumar and Puranik 2011).The development of E. *coli O157:H7* bacteria was observed in various concentrations of bovine LF. Using (3 to 10 mg/ml, respectively), (figure 7) Growth of *Escherichia coli O157*:H7 was significantly inhibited between four and seven hours after incubation. Thus, *E. coli O157*:H7 was more effectively inhibited by bLF. Bovine LF, at doses ranging from 0.1 to 2 mg/ml, did not affect the growth of *E. coli O157*:H7. After removing the LF after 8 hours, bacteria were let to grow again in fresh medium.

The only levels of LF that stopped bacterial growth for an hour were 8 to 10 mg/ml. As a result, it is possible that bovine lactoferrins had bacteriostatic effects on E. coli O157:H7. From four to seven hours after incubation, lactoferrins significantly (at 3 to 10 mg/ml) inhibited growth. As a result, a considerable anti-microbial effect takes time to become apparent, as was also noted by Kawasaki et al. (2000). Also our study in computable with study by Rybarczyk *et al.,* (2017) They concluded that lactoferrin had an antibacterial effect against E. coli O157:H7 in both in vitro and in vivo experiments. This could be because of how slowly LF interacts with bacterial LPS, which is known to kill bacteria. Bacterial outer membranes frequently have phospholipids in the inner leaflet and lipopolysaccharide (LPS), a polyanionic glycolipid, in the outer leaflet.

Lactoferrin's ability to bind iron and decrease its availability in the growing environment is the reason for this., it displayed bacteriostatic effects at high doses (8 to 10/ml), which made its direct antibacterial action on E. coli O157:H7 greater our study matching with the study staed by(HASSAN *et al.,* 2022;Orsi, 2004). Who demonstrated that *Ecoli 0157* can be treated with high consternation of lactoferrin's In a different research, In broth from three to six hours after incubation, Atef Yekta et al. (2010) demonstrated that the growth of E. coli O157:H7 was considerably reduced when using 0.5 to 10 mg/ml and 0.1 to 10 mg/ml of human or bovine lactoferrin, respectively.

Additionally, Lactoferrin at concentrations of 10 and 20 mg/ml significantly decreased the viability of E. coli isolated after 72 hours in broth at all doses, according to (Taha *et al.* 2019).Also The bactericidal effects of LF binding to the surface of Gram-negative bacteria are initiated by the release of lipopolysaccharide (LPS) from the membrane (Ellison *et al.,* 1988; Orsi, 2004). The selective penetration of ions and disruption of the bacterial TTSS caused by the serine protease activity of LF are additional antibacterial actions attributed to it (Ochoa et al., 2003). On the other side, E. coli O157:H7 may have also devolved a bacterial defense mechanism that prevented lactoferrins from binding. This may help to explain why low doses of LF (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2 ml/ml) either had no impact or only temporarily inhibited development. According to Senkovich *et al.* (2007) and Kieckens *et al.*,2018 LF blockage may result from a contact with a bacterial surface protein or from porins being protected from LF interaction by LPS. (figure8).

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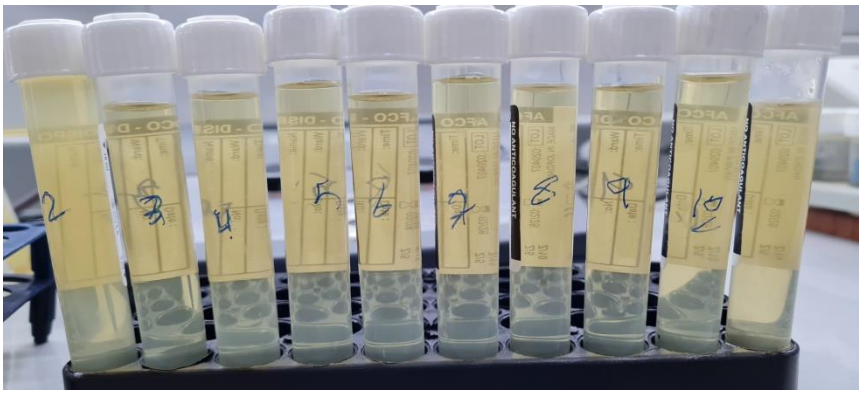

Figure 7: concentrations of bovine LF. Using (3 to 10 mg/ml

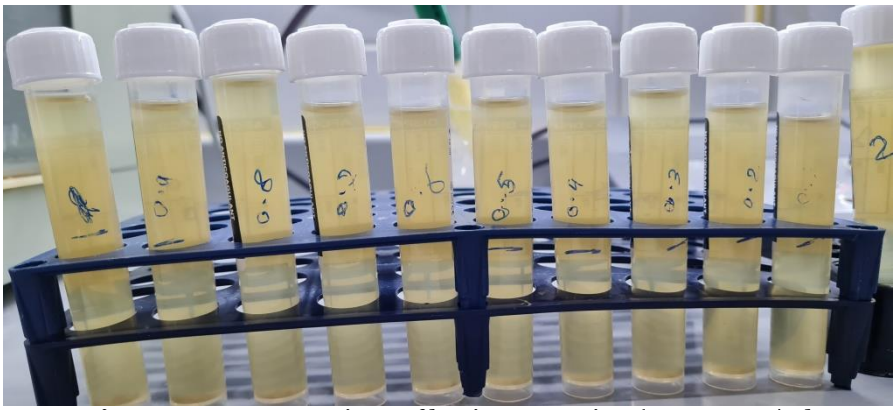

Figure 8: Concentrations of bovine LF. Using (0.1to 2 mg/ml

CONCLSUION

In summary, this study contributes to the growing body of evidence supporting lactoferrin's potential as an effective antimicrobial agent against harmful bacteria like E. coli O157:H7. The results underscore the importance of dosage and duration in achieving significant inhibitory effects. Further research could delve into the specific interactions between lactoferrin and bacterial components, shedding light on the precise mechanisms underlying its antibacterial properties and potentially leading to the development of novel antimicrobial strategies**.**

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