

# Efficiency of lactoferrin to eradicate multidrug resistant *Staphylococcus aureus* isolated from some dairy products

Sayed H. Al Habty

Animal Health Research Institute (AHRI)

Dina N. Ali (✉ [dinanour2010@yahoo.com](mailto:dinanour2010@yahoo.com))

Animal Health Research Institute (AHRI)

---

## Research Article

**Keywords:** MRSA, VRSA, sensory evaluation, yoghurt, ice cream and Damietta cheese

**Posted Date:** February 17th, 2022

**DOI:** <https://doi.org/10.21203/rs.3.rs-1354085/v1>

**License:** © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

---

# Abstract

**Back ground:** This study aimed to investigate *Staphylococcus aureus* (*S. aureus*) in some dairy products, Genotypically and phenotypically antibiotic resistance pattern as well as to evaluate the effectiveness of lactoferrin (LF) as a bio preservative for yoghurt.

**Methods:** A total of 150 samples from yoghurt, ice cream and Damietta cheese (50 for each) in Assiut city, Egypt. Antimicrobial susceptibility against antibiotics commonly used in human and animals was tested using the disc diffusion method, *S. aureus* isolates, PCR was applied on multidrug resistant (MDR) *S. aureus* isolates for detection of *blaz*, *mecA* and *VanA* genes

**Results:** The highest prevalence *S. aureus* was detected in yoghurt (38%) followed by Damietta cheese (30%) and ice cream (14%). Antimicrobial susceptibility against antibiotics *S. aureus* isolates showed high resistance to tetracycline, penicillin, oxacillin, ampicillin, streptomycin, amoxicillin/clavulanate and neomycin, in different percentages. *blaz*, *mecA* and *VanA* genes were detected at 60% for *blaz* gene, 40% for *mecA* gene and 20% for *vanA* gene. Lactoferrin has a satisfactory antibacterial activity MIC at 20mg/ml and MLC at 40mg/ml. The results revealed that 40mg/ml LF in yoghurt could inhibit MDR *S. aureus* at 2nd day while, 20mg/ml at the fourth day.

**Conclusion:** The study concluded that LF can be used as a bio preservative in yoghurt due to its highest antimicrobial activity and acceptable sensorial properties.

## 1. Background

*Staphylococcus aureus* is one of the world's ubiquitous, yet sophisticated and captivating microorganisms due to its special world epidemiology [1]. *S.aureus* has robust pathogenicity due to its wide distribution, excessive pollution rate and fast transmission. It reasons a variety of medical manifestations, from mild-local, superficial pores and skin lesions to serious invasive diseases, and may additionally even threaten life, [2].

Milk and milk products are known to be a source of *S. aureus* contamination whether they are collected from cows suffering from mastitis or from food handlers carrying the microbe because of poor personal hygiene [3].

Over the past decade, the hassle of antimicrobial resistance in the African continent has gained one-of-a-kind interest. However, little is regarded about the actual extent of the problem due to the fact surveillance for antimicrobial resistance is carried out in solely a few countries [4].

Bacterial resistance to most conventional antibiotics has become a clinical and public health problem. Infections due to multidrug resistant microorganisms, such as methicillin resistant *S. aureus* (MRSA) considered a challenge, which ought to be controlled due to its high treatment costs, therapeutic failure and death. Vancomycin is the mainstay treatment of MRSA invasive infection [5]. However, emerging isolates with reduced vancomycin susceptibility have also been detected [6]. Furthermore, MRSA and vancomycin resistant *S. aureus* (VRSA) isolates have been identified as an emerging pathogen in livestock animals that is readily transferable to humans in contact with livestock [1, 7]. However, occurrence of vancomycin-resistant MRSA strains is scary recorded in animals because the drug not used in veterinary practice in many countries.

The emergence of such resistant strains plays a necessary position in therapeutic failure in each human and animal infections. The uncontrolled use of antibiotics in human and animals, together with bad diagnostic techniques and inappropriate prescribing by way of unqualified physicians, exacerbates the problem [8] and constitutes a top-notch mission for the prevention and control of this pathogen.

Because of the accelerated issues of antibiotics as antibiotic resistance, direct toxicity, hyper sensitivity and fantastic infection, the consumers wanted to take natural compounds so, lactoferrin has attracted more attention because Food and Drug Administration (FDA) licensed the Lactoferrin (LF) as " Generally Recognized as Safe (GRAS)" [9].

Lactoferrin has been the center of attention of extra excessive research. Due to its unique antimicrobial, immune modulatory, and even antineoplastic properties, it looks to have terrific practicable in practical medicine. Nevertheless, a whole lot research and many experiments nonetheless need to be carried out in order to acquire a better understanding of its pastime and interactions and to allow the full and secure utilization of this glycoprotein [10]. Also, lactoferrin proved its ability for immunity enhancement in Egypt, [11].

Fortification of yoghurt with lactoferrin can add more health benefits, yogurt is one of the most popular fermented dairy products that have been traditionally consumed for their numerous potential health benefits [12].

So, the purpose of this study was to detect MDR *S. aureus* in some milk products. As well as, to assess the antibacterial activity of lactoferrin and its sensorial properties as a bio preservative in yoghurt.

## 2. Materials And Methods

### 2.1 Collection of samples:

A total of 150 samples of dairy products include (yoghurt, ice cream and Damietta cheese (50 each) were collected from supermarkets and dairy shops in Assiut city, Egypt. The samples were collected in sterile separate tubes, labeled and carried on ice tank to be transferred with a minimum delay to the laboratory for bacteriological examination.

### 2.2 Enumeration and identification of *Staph. aureus*:

Enumeration of *S. aureus* using paired barker agar according to [13]. A loopful from each colony was streaked onto blood agar and mannitol salt agar (MSA) (HiMedia) and incubated aerobically overnight at 37 °C for growth. The typical *Staphylococcus spp.* of golden yellow with hemolysis and yellow colonies on blood agar and MSA, respectively were picked for further biochemical investigations. The biochemical investigations included microscopic appearance with Gram stain, catalase test, hemolysin test, and coagulase test [14].

### 2.3 Antimicrobial susceptibility test :

The Clinical Laboratory Standards Institute's Kirby-Bauer disc diffusion method was used to determine antimicrobial susceptibility in vitro [15]. Each *S. aureus* isolate was cultured for 4-5 hours at 37 degrees Celsius in Mueller Hinton broth, and the turbidity was compared to a 0.5 McFarland reference solution. Each isolate's pure broth culture was placed on Mueller Hinton agar with sterile cotton swabs and allowed to dry. The antimicrobial discs were put and incubated for 24 hours at 37 degrees Celsius. The diameter of the inhibitory zone was measured with a caliper, and the results were documented and interpreted using CLSI criteria [15]. The isolates were tested against 16 antibiotic discs that corresponded to eight different classes of Oxoid-supplied chemotherapeutic drugs. The used antibiotic discs were Penicillin (PEN) Ampicillin (AMP), Oxacillin (OXA), Amoxicillin /clavulanate (AMC), Sulphamethoxazole-trimethoprim (SXT), Tetracycline (TET), Neomycin (N), Streptomycin (S), Gentamicin (GEN), Erythromycin (ERY), Norfloxacin (NOR), Ciprofloxacin (CIP), Marbofloxacin (MAR), Cefotaxim (CTX), Ceftiofur, (XNL) and Vancomycin (VA).

### 2.4 Multiple Antibiotic resistant Index (MARI)

Resistance was judged by the inhibition zone diameter to determine the MAR index that was defined as a/b, where (a) represents the number of antibiotics to which the isolated strain was resistant and (b) represents the number of all tested antibiotics. [16]. Isolate showing resistance to one agent in at three or more different classes of antimicrobials were considered multi drug resistant (MDR) [17]. Isolates with MARI values of more than 0.2 or 20% were considered highly resistant.

### 2.5 Molecular confirmation of penicillin resistant *S.aureus*, MRSA and VRSA isolates:

The isolates were sent to the Reference Laboratory for Veterinary Quality Control on Poultry Production in Animal Health Research Institute, Dokki, Giza, Egypt for detection of *23S rRNA gene*, *blaz gene*, *mecA gene* and *vanA gene*. Characteristic to *S. aureus*, penicillin resistance, methicillin resistance and vancomycin resistance, respectively. DNA extraction from samples was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Primers used were supplied from Metabion (Germany) are listed in table (A)

**Table (A): Primers sequences, target genes, amplicon sizes and cycling conditions.**

Target gene	Primers sequences	Amplified segment (bp)	Primary denaturation	Amplification (35 cycles)			Final extension	Reference
				Secondary denaturation	Annealing	Extension		
<i>S. aureus</i> 23S rRNA	ACGGAGTTACAAAGGACGAC	1250	94°C	94°C	55°C	72°C	72°C	[18]
	AGCTCAGCCTTAACGAGTAC		5 min.	30 sec.	1 min	1.2 min.	12 min.	
<i>blaZ</i>	TACAACTGTAATATCGGAGGG	833	94°C	94°C	50°C	72°C	72°C	[19]
	CATTACACTCTTGGCGGTTTC		5 min.	30 sec.	40 sec.	50 sec.	10 min.	
<i>mecA</i>	GTA GAA ATG ACT GAA CGT CCG ATA A	310	94°C	94°C	50°C	72°C	72°C	[20]
	CCA ATT CCA CAT TGT TTC GGT CTA A		5 min.	30 sec.	30 sec.	30 sec.	7 min.	
vanA	CATGACGTATCGGTAAAATC	885	94°C	94°C	50°C	72°C	72°C	[21]
	ACCGGGCAGRGTTATTGAC		5 min.	30 sec.	40 sec.	50 sec.	10 min.	

## 2.6 Determination of minimum inhibitory concentration (MIC) of lactoferrin:

The MIC and minimum lethal concentration (MLC) of lactoferrin were carried out using broth dilution method, as described by [22]. Lactoferrin was purchased from Canada, lactovegetarian, EN: 131947. Item number AOR 04110. The following concentrations of lactoferrin solution were prepared, 0.65, 1.25, 2.5, 5, 10, 20 and 40mg/ml in distilled water and sterilized by 0.45 mm filter and freshly used. Genotyped strains of MDR *S. aureus* were sub-cultured onto 5% sheep blood agar plates and incubated aerobically at 37 °C for 24h. Selected 3-4 colonies and inoculated in tryptic soy broth then incubated at 37 °C for 2-6 h. Suspensions turbidity was adjusted to match of 0.5 McFarland standard then diluted to obtain a final concentration of 10<sup>5</sup> CFU /ml approximately. Two-fold serial of lactoferrin (0.65, 1.25, 2.5, 5, 10, 20 and 40 mg/ ml (w/v) were prepared separately using sterile Muller Hinton broth. Each tube was inoculated with a suspension of the 100 µL from CFU/ml. The inoculated tubes together with the control tube (tubes contained broth only) were incubated aerobically at 37 °C for 24h. The MIC of lactoferrin was detected by lowest concentration of lactoferrin that inhibits growth of the organism with lack of visible turbidity. To determine the MLC, 100 µL from each clear tube (no visible growth) was spread onto sterile Muller Hinton agar (Oxoid, UK) for 24 hours incubation. MLC was detected as the lowest concentration of LF that killed the tested MDR Staphylococcus organisms (No growth on the plate). The mean MIC and MLC was recorded from triple readings in each test.

## 2.7 Agar well diffusion test:

The antibacterial test was conducted using the agar diffusion method summarized as the following. A concentration of 10<sup>5</sup> CFU /ml bacterial strain culture of MDR *S. aureus* was prepared and spread uniformly on the dried surface of a MHA plate by using sterile cotton swab. Multiple wells of 6 mm were made in the agar plate by using sterile cork pourer 50 µL of LF were added to each of the wells containing concentrations of 0.65, 1.25, 2.5, 5, 10, 20 and 40mg/ml The plates were incubated for 24 h at 37°C ± 1°C, under aerobic conditions. After incubation Inhibition of the bacterial growth was measured in mm. The tests were made in triplicate [23].

## 2.8 Anti-bacterial properties of lactoferrin on MDR *S. aureus* in yoghurt:

### 2.8.1 Bacterial suspension inoculation:

A fresh culture of MDR *S. aureus* isolate was adjusted to give an initial count of approximately a MacFarland 0.5 standard. The growth density was adjusted to match (4.5 log). [24].

### 2.8.2 Yoghurt preparation:

Raw milk was once boiled for 10 min. then unexpectedly cold and inoculated with 2% yoghurt way of life at 45°C. One ml of MDR *S.aureus* pressure suspension (which organized as before) blended with 100 ml of organized milk and divided into suitable sterile jars, lactoferrin was once added at concentrations 10, 20 and 40 mg/ ml for every respectively, and every other tremendous control jars except lactoferrin incubated at 40 °C until curdling. Control jar (free from pressure suspension, lactoferrin as a terrible control) used to be additionally stored. The jars have been saved at refrigerator temperature (5±2 °C). The inoculated jars have been examined bacteriologically for the remember of *S.aureus* using Baird-Parker media (37°C for 24-48h) at time zero, after curdling and, every 2 days until the cease of the experiment.

### 2.8.3 Sensory evaluation:

Control yoghurt jars (free from the preceding microorganism however inoculated with lactoferrin concentrations of 10, 20 and 40mg/ml respectively) had been prepared as beforehand mentioned and every one was once subjected to the preceding treatments. Thirty panelist had been selected in groups of one-of-a-kind ages, intercourse (18 females and 12 males), and education to style the trials.

The grasp of shoppers toward samples with a range of conc. of lactoferrin was once studied with recognize to three one-of-a-kind attributes (odor, taste and over all acceptability (OAA) [25]. The stage of settlement used to be scored as percentages.

### 2.8.4 Statistical Analysis

The statistical analysis was performed using programs GraphPadPrism 5.04 (GraphPad, Inc., San Diego, USA) and Statistical 12.0 (Dell, Inc., Tulsa, USA). The bacterial count represented by mean  $\pm$ SE. The data represented by using the Microsoft Excel Spreadsheet.

## 3. Results

**Table 1.** Statistical analytical results of *S. aureus* examination of examined dairy products.

Samples types	Results of <i>S. aureus</i> counts (log <sub>10</sub> cfu/ g)				
	Positive Samples		Min.	Max.	Mean $\pm$ SE
	No./50	%			
yoghurt	19	38	2.6	4.3	3.5 $\pm$ 2
Ice Cream	7	14	1.6	3.3	2.9 $\pm$ 1.5
Damietta Cheese	15	30	1	4.3	3.2 $\pm$ 2.1
Total	41	27.3	5.2	11.9	9.6 $\pm$ 5.6

**Table 2.** The inhibitory effect of different concentrations of lactoferrin on MDR *S. aureus* isolates:

Concentrations	Zone of inhibition (mm)
40mg/ml	29 $\pm$ 1.7
20mg/ml	22 $\pm$ 2.1
10mg/ml	13.4 $\pm$ 2.7
5mg/ml	0
2.5mg/ml	0
1.25mg/ml	0
0.65mg/ml	0

## 4. Discussion

*Staphylococcus aureus* is a versatile bacterium that causes a wide range of diseases in humans and animals. It causes mild skin infections to life-threatening disorders including pneumonia and meningitis [26]. Milk and milk products are known to be a source of *S. aureus* contamination, whether gathered from mastitis-affected cows or food workers who have the organism due to poor personal hygiene. Therefore, this study investigated the presence of *S. aureus* in some dairy products in Assiut city, Egypt. *S. aureus* could be detected in Yoghurt, Ice cream and Damietta Cheese samples at percentages of 38, 14 and 30% respectively. The staphylococcus count ranged from 2.6 to 4.3 with a mean value of 3.5 $\pm$ 2 log<sub>10</sub>cfu/ g in yoghurt samples while in ice cream samples ranged from 1.6 to 3.3 with a mean value 2.9 $\pm$ 1.5 log<sub>10</sub>cfu/ g and in Damietta Cheese samples it ranged from 1 to 4.3 with a mean value 3.2 $\pm$ 2.1 log<sub>10</sub>cfu/ g as shown in Table (1). These results were agreed with those reported by [27, 28, 29]. But lower than [30] and higher than [31, 32]. The high incidence in raw milk could be attributed to environmental pollution, cross contamination between the milk and each other and poor handling during transportation, besides, shedding of *S. aureus* from infected animals is another cause of contamination of milk and dairy food [33, 34].

The massive consumption of antibiotics by human and animals resulted in increased number of bacterial populations' resistance thus creating critical public health problems. For the present study *S. aureus* isolates showed resistance to most antibiotics tested as shown in Figure ( 1 ).The resistance rate was 100%, 95.1%, 92.7%, 87.8%, 85.4%, 80.5% and 73.2% for tetracycline, penicillin, oxacillin, ampicillin,

streptomycin, amoxicillin/clavulanate and neomycin, respectively. For tetracycline our results were agreed with those of [35, 36] who their results were 100% and 80%, respectively. For penicillin the present results agreed with results of [37, 38] who their results were 94.4%, and 96%, respectively. Moreover, our results for Oxacillin and Ampicillin were nearly similar to with that recorded by [36, 38] (86%) and (93%), respectively. The resistance against large number of antibiotics may be related to long term of sub dose applications and even misuse usage. The differences in the results of antibiotic resistances among studies may be attributed to antibiotic resistances not only from country to another but also from area to other according to the lows of drug handling and abuse. It is interesting to note that resistance rates were observed against marbofloxacin, ceftiofur and vancomycin was (0.00%, 2.4% and 7.3%, respectively. These results were in agreement with that obtained by [39, 40, 41, 42]. The highly efficacy of these antibiotics may be attributed to their recent use and expensive costs in veterinary medicine.

Determination of the multiple antibiotic resistance index (MARI) of the isolates shows that most of isolates were resistant to three or more antibiotics with over all mean value about 0.54. This is nearly agreed with previous work of [43, 44] who cited that MARI was 0.61 and 0.48, respectively. On the other hand lesser index was reported by [45, 42] as 0.23 and 0.3, respectively. The high MARI confirming that there were high antibiotic misuses in such area or country.

The use of PCR considers an excellent method for diagnosing of suspected pathogen due to its rapid and sensitive results [46]. Suspected isolates which show multi drug resistance phynotypically were examined by PCR for genotypically assessments of *23S rRNA gene*, *blaz gene*, *mecA gene* and *vanA gene*. Our results clarified that all tested isolates were harbored *23S rRNA* as in Photo (1).

Penicillin used to be notably very positive in opposition to most staphylococcal infections, but *S. aureus* started out producing  $\beta$ -lactamase enzyme in the mid1940s, which destroys the penicillin  $\beta$ -lactam ring. Later, more than 90% of *S. aureus* strains had been penicillin resistant [47]. So, to detect the resistance to penicillin through the production of  $\beta$ -lactamase due to the presence of *blaz* gene that coded for an alteration of penicillin-binding protein 2a which reduced the affinity for  $\beta$ -lactam antibiotics [48].

Results obtained in this study represent resistance to penicillin by using *blaz* gene in (60%) of examined MDR isolates, Bands with approximate size of 833 bp were detected for *blaz* gene. Photo 2. In agreement with our study the *blaz* gene was detected in 59.2%, 60, 65 and 97% [49, 50, 51, 52] Christine et al.,2021 . High *blaz* genes might indicate an increased use, and possibly misuse of  $\beta$  lactams in the study farms [53, 5, 54] encoding for the beta-lactamases *blaz* gene in staphylococci at 42.9% and 28.8% of *S.aureus* isolates, respectively.

Antimicrobial resistance in methicillin-resistant strains of *S.aureus* (MRSA) is related with the acquisition of a cell genetic element referred to as the staphylococcal cassette chromosome *mec*, which carries the *mecA* gene, encoding the low-affinity penicillin-binding protein 2a and confers resistance to the  $\beta$ -lactam antibiotics [55]. So, our outcomes revealed the presence of *mecA* gene in (40%) of the examined samples. Bands with approximate size of 310 bp had been detected for *mecA* gene, Photo (3). These were lower than [29, 51] who revealed that the presence of *mecA* gene was 66.7 and 75% of MDR *S. aureus*, respectively. Also [49, 50] who found a very high ratio of MRSA reach at 77% to 100%.

Glycopeptides such as vancomycin has become the cornerstone for treating MRSA infections over the ultimate twenty years [56]. Conceivably, the excessive utilization of vancomycin outcomes in the alarming significant of its resistance and the existence of two kinds of glycopeptide resistant *S. aureus*. The first type, vancomycin intermediate resistant *S. aureus* (VISA) [57]. The 2nd one, vancomycin-resistant *S. aureus* (VRSA) is related with the acquirement of *vanA* operon from *Enterococcus faecalis* by a horizontal gene transfer [58].

Vancomycin has been an effective agent in opposition to the MRSA infections for decades [7]. But in July 2002, the situation changed when the Centers for Disease Control and Prevention (CDC) in the USA documented the first sample of *S.aureus* that used to be resistant to both vancomycin and methicillin [59]. In the present work the examined isolates showed 20% vancomycin resistant by using *vanA* gene detection. Bands with approximate dimension of 885 bp had been detected for *vanA* gene as in Photo (4). This results was resemble that reported by [60] who revealed that ratio of *vanA* gene was 20.3%. On the other hand high results were recorded by [61] as 40% in milk and [62] as 27.6% and 54.5% for camel meat and abattoir workers respectively.

The presence of resistance to many antimicrobials, emphasizing the need for new herbal anti-microbial retailers to deal with *S. aureus* infection, Lactoferrin is a protein that takes place naturally in milk and nowadays is increasingly supplemented in foods for its a couple of features and its application into meals upkeep is gaining superb interest due to consumers' vogue [63].

The MIC and MLC of lactoferrin were carried out using broth dilution method. Lactoferrin proved to have antibacterial activity against MDR *S. aureus* at 20 mg/ml for MIC and 40 mg for MLC. Our result of MIC was agreed that reported by [64]. Generally, results of MIC of LF were extremely differed from study to another and from pathogen to another. Lower results of MIC were recorded (2.67 mg/ ml, 4 mg /ml and 4 mg /ml) by [65, 66, 67] respectively. On the other hand, higher result was recorded by [68] who reported that MIC was 112.5 mg/ ml for *Salmonella enterica*.

The antibacterial activity of Lactoferrin was performed using agar well diffusion method as in Table (2). The results revealed that the concentration of 40 mg / ml gave the highest observed zone of inhibition ( $29 \pm 1.7$  mm diameter) followed by 20 mg and 10 mg as  $22 \pm 2.1$  mm and  $13.4 \pm 2.7$  mm, respectively. Our finding ( $22 \pm 2.1$  mm) was similar to that recorded by [66, 67]. The latter authors cited that inhibitory effect of camel LF against *E. coli* and *S. aureus* was observed to be the very potent. According to zone diameter interpretation charts of [15] Marbofloxacin ( $>24$  mm), Ceftiofur, ( $>26$  mm) and Vancomycin ( $>18$  mm) which consider the highly susceptible antibiotics against MDR Staphylococci as in Figure (1). In Table (2) 40 mg/ml LF come superior than the highly susceptible previously mentioned antibiotics against MDR Staphylococci in regarding to zone of inhibition diameter. The good results of LF may be due to it exhibits a broad spectrum antibacterial activity through main mechanisms, its iron chelating capacity that reduce the iron by bacteria, and binding to the bacterial membrane, changing its stability and consequently, the viability of bacteria [69].

As shown in Figure 2 we used three concentrations of lactoferrin 40, 20 and 10mg/ml. 40 mg/ml reduced the count of *S.aureus* at 1<sup>st</sup> day and completely inhibit its growth at 2<sup>nd</sup> day but 20 and 10mg/ml inhibit *S.aureus* growth at 4<sup>th</sup> and 6<sup>th</sup> day, respectively. While in positive control *S.aureus* could survive in yoghurt till the 6<sup>th</sup> day with a mean count  $2.4 \log_{10}$  CFU/gm, and that may be due to increase the acidity of yoghurt, *S.aureus* organisms are the most sensitive bacterial species to acidity [70]. Moreover, [71] cited that increased degree of LF, the bacterial load reduced dramatically in contrast to the control, thereby growing the shelf life of some dairy product. The results of sensory evaluation of the present study revealed that the samples treated with different concentrations of lactoferrin were generally acceptable; moreover the samples treated with 10, 20 and 40mg/ml concentrations of lactoferrin were given the highest score during the whole period of experiment as shown in Figure (3). Addition of bovine LF to yoghurt did not substantially affect the physicochemical properties of the product and increased the growth of lactic acid bacteria [72]. Also, [11] found that fortification of milk with lactoferrin did not interfere with yoghurt manufacture and the sensory properties of produced yoghurt was acceptable.

## 5. Conclusion

The present study concluded that most of the isolated *S. aureus* in dairy products are MDR against several antibiotic groups. A high prevalence of *S. aureus* in milk and some milk products is considered a public health hazard and might be involved as a cause of high prevalence of human food poisoning. Moreover, LF considers a suitable food preservative in yoghurt due to powerful antibacterial activity and its good sensorial properties.

## Abbreviations

LF: lactoferrin, MRSA: methicillin resistant Staphylococcus aureus, VARSA: vancomycin resistant Staphylococcus aureus, MDR: multi drug resistant.

## Declarations

### Acknowledgment:

All authors would like to thank [me.elsherif@yahoo.com](mailto:me.elsherif@yahoo.com) for helping us in collection of samples.

### Competing interests:

The authors declare that they have no competing interests.

**Funding:** This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors

**Conflict of interest:** none.

**Ethics approval:** This research did not involve experiments on humans or animals and received the ethical approval of the Animal Health Research Institute, Agriculture Research Center, Egypt.

**Consent to participate:** Not applicable

**Consent for publication:** Not applicable

**Availability of data and material:** Main data available on request. Figures from lab. work are included in main manuscript.

**Code availability:** Not applicable

**Authors' contributions:** All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Dina Nour- Eldin Ali and Sayed Al Habyt. The first draft of the manuscript was written by Dina Nour- Eldin Ali and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

## References

1. Kest H, Kaushik A . Vancomycin-Resistant *Staphylococcus aureus*: Formidable Threat or Silence before the Storm? J Infect Dis Epidemiol 2019, 5:093 Volume 5 | Issue 5 DOI: 10.23937/2474-3658/1510093.
2. Turner N A, Sharma-Kuinkel B K, Maskarinec S A, Eichenberger E M, Shah P P, Carugati M, et al. Methicillin-resistant *Staphylococcus aureus*: an overview of basic and clinical research. 2019. Nat. Rev. Microbiol. 17, 203–218. doi: 10.1038/s41579-018-0147-499(13)70231-7.
3. Bingol EB, Cetin O, Colak H, Hampikyan H. Presence of enterotoxin and verotoxin in Turkish cheeses sold in Istanbul. Turkish J. Vet. Anim. Sci.,2012, 36: 424–432.
4. WHO (World Health Organization) . Antimicrobial Resistance: Global Report on surveillance. Geneva; 2014. <https://www.who.int/drugresistance/documents/surveillance-report/en>.
5. Wang S, Yu Z , Wang J, Ho H, Yang Y, Fan R, Du Q, Jiang H, Han R. Prevalence, Drug Resistance, and Virulence Genes of Potential Pathogenic Bacteria in Pasteurized Milk of Chinese Fresh Milk Bar .J Food Prot(2021) 84 (11): 1863–1867.
6. Elsayy S, Elsherif W M , Hamed R. Effect of silver nanoparticles on vancomycin resistant *Staphylococcus aureus* infection in critically ill patients. Pathogens and Global Health. <https://doi.org/10.1080/20477724.2021.1914412>
7. Cong Y, Yang S, Rao X. Vancomycin resistant *Staphylococcus aureus* infections: A review of case updating and clinical features. Journal of Advanced Research 21 (2020) 169–176.
8. Kimang'a A N. A situational analysis of antimicrobial drug resistance in Africa: are we losing the battle? Ethiop J Health Sci. 2012;22:135–43.
9. El-Hafez S M A, Ismael A B, Mahmoud M B , Elaraby A K A. Development of new strategy for non-antibiotic therapy: bovine lactoferrin has a potent antimicrobial and immunomodulator effects. Adv. Infect. Dis.2013, 3: 185-192. <https://doi.org/10.4236/aid.2013.33027>.
10. Al-Mogbel M S , Godfred A M , Mohamed T Elabbasy , Manal M Alkhulaifi , Ashfaque Hossain , Mushtaq A Khan . Effect of Synergistic Action of Bovine Lactoferrin with Antibiotics on Drug Resistant Bacterial Pathogens. Medicina 2021, 57, 343. <https://doi.org/10.3390/medicina57040343>
11. Zakaria A M , Zakaria H M , Abdelhiee E Y , Fadl S E, Ombarak R A. The Impact of Lactoferrin Fortification on the Health Benefits and Sensory Properties of Yogurt. Journal of Current Veterinary Research. 2020. ISSN: 2636-4026 Journal homepage: <http://www.jcвр.journals.ekb.eg>.
12. Tsukahara, T, Fujimori A, Misawa Y, Oda H, Yamauchi K, Abe F, Nomiyama T. The preventive effect of lactoferrin-containing yogurt on gastroenteritis in nursery school children— intervention study for 15 weeks. International Journal of Environmental Research and Public Health, 2020. 17(7). <https://doi.org/10.3390/ijerph17072534>.
13. AOAC "Association of Official Analytical Chemists" . International: Official Methods of Analysis method 2001. *aureus* count plate method for rapid detection of Staph. aureus in selected foods. J. AOAC Int. 84, 1431.
14. Goldman E, Green L H. Practical handbook of microbiology,2008. 2nd ed. CRC Press of Taylor & Francis Group, New York, USA.
15. CLSI, M100 2018. Performance standards for antimicrobial susceptibility testing: twenty-third informational supplement, Clinical Laboratory Standard Institute, Wayne, PA, USA, 2018.
16. Kumar S, Manoharan M, Ilanchezian S, et al. Plasmid analysis and prevalence of Multidrug resistant *Staphylococcus aureus* reservoirs in Chennai city, India. The internet journal of microbiology. 2012;7–1. 40.
17. Magiorakos A P, Srinivasan A, Carey R B, Carmeli Y, Falagas M E, Giske C G. Multidrug-resistant, extensively drug-resistant and pandrug-resistant Bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin. Microbiol Infect. Mar;2012, 18(3): 268–81. <https://doi.org/10.1111/j.1469-0691.2011.03570.x>.
18. Bhati T, Nathawat P, Sharma SK, Yadav R, Bishnoi J, Kataria A K. Polymorphism in *spa* gene of *Staphylococcus aureus* from bovine subclinical mastitis. Veterinary World, 2016;9:421-424.
19. Bagcigil A B, Taponen S, Koort J, Bengtsson B, Myllyniemi A, Pyörälä S. Genetic basis of penicillin resistance of *aureus* isolated in bovine mastitis. Acta Veterinaria Scandinavica 2012, 54:69.
20. McClure JA, Conly JM, Lau V, Elsayed S, Louie T, Hutchins W, Zhang K. Novel multiplex PCR assay for detection of the staphylococcal virulence marker Panton-Valentine leukocidin genes and simultaneous discrimination of methicillin-susceptible from -resistant staphylococci. 2006. J ClinMicrobiol 44: 1141-114.



21. Patel R, UHL JR, Kohner P, Hopkins MK , Cockerill FR. Multiplex PCR Detection of *vanA*, *vanB*, *vanC-1*, and *vanC-2/3* Genes in Enterococci. 1997. Journal of clinical microbiology, P. 703–707.
22. Stephen J C. Manual of antimicrobial susceptibility testing, American Society for Microbiology, 2005. Library of Congress Cataloging-in-Publication Data, USA
23. Valgas C, De.Souza SM, Smânia E A, Smânia A J. Screening methods to determine antibacterial activity of natural products. 2007. Braz. J. Microbiol. 38 (2), 369-380, <https://doi.org/10.1590/S1517-83822007000200034>
24. Ruparelia J P, Chatterjee A K, Duttagupta SP, Mukherji S. Strain specificity in antimicrobial activity of silver and copper nanoparticles. 2008. Acta Biomater. 4, 707–716.
25. Fernandes J C, Tavaría F K, Soares J C, Ramos O S, Joao Monteiro M, Pintado M E, Xavier Malcata F. Antimicrobial effects of chitosans and chito oligosaccharides, upon *Staphylococcus aureus* and *Escherichia coli*, in food model systems. 2008. Food Microbiol. 25(7): 922-8.
26. Jangra P , Singh A. *Staphylococcus aureus* hemolysin neutralizing single-domain antibody isolated from phage display library of Indian desert camel. Asian Pac J Trop Med 2010; 3(1): 1-7.
27. Ibrahim G A, Sharaf OM, Abd El-Khalek A B. Microbiological Quality of Commercial Raw Milk, Domiati Cheese and Kareish Cheese. 2015. Middle East J. Appl. Sci., 5(1): 171–176.
28. Ahmed AA, Maharik NMS, Valero A, Kamal SM. Incidence of enterotoxigenic *Staphylococcus aureus* in milk and Egyptian artisanal dairy products. 2019 Food Control, 104: 20–27.
29. Zeinhom M M , Ahmed A Abed H . Prevalence, Characterization and control of *Staphylococcus aureus* isolated from raw milk and Egyptian soft cheese. Journal of Veterinary Medical Research, 2021; 27 (2): 152–160 <https://doi.org/10.21608/JVMR.2021.146885>.
30. Hanaa M Fadel , Jehan Ismail. Occurrence and Zoonotic Importance of Methicillin-Resistant *Staphylococcus aureus* in raw Milk and Some Dairy Products at Ismailia City, Egypt. Zagazig Veterinary Journal Volume 43, Number 3, p. 95-104, 2015.
31. Sasidharan S, Prema B, Yoga Latha L. Antimicrobial drug resistance of *Staphylococcus aureus* in dairy products. Asian Pac J Trop Biomed 2011; 1(2): 130-132.
32. Alghizzi M, Shami A. The prevalence of *Staphylococcus aureus* and methicillin resistant *Staphylococcus aureus* in milk and dairy products in Riyadh, Saudi Arabia. Saudi Journal of Biological Sciences 28 (2021) 7098–7104.
33. Addis M, Pal M, Kyule M. Isolation and identification of *Staphylococcus* species from raw bovine milk in Debre zeit. 2011. Ethiopia Vet. Res., 4: 45–49.
34. Rahimi E. Enterotoxigenicity of *Staphylococcus aureus* isolated from traditional and commercial dairy products marketed in Iran. 2013. Braz. J. Microbiol., 44(2): 393–399
35. Islam MR, Ahamed S, Alam S, Rahman M, Sultana T, Seok RohY, Kim B. Identification and Antibiotic Sensitivity of the Causative Organisms of Sub-clinical Mastitis in Sheep and Goats, 2012. Pak.Vet.J.32 (2) ,179-182.
36. Eid S, Nasef S, Erfan A. Multi drug resistant bacterial pathogens in eggs collected from backyard chickens. 2015. Assiut Vet. Med. J. Vol. 61 No 144.
37. Abera M, Demie B, Aragaw K, Regassa F, Regassa A. Isolation and identification of *Staphylococcus aureus* from bovine mastitic milk and their drug resistance patterns in Adama town, 2010. Ethiopia, Journal of Veterinary Medicine and Animal Health Vol. 2(3), pp. 29-34.
38. Pati B K, Mukherjee R. Characterization of *Staphylococcus aureus* Isolates of Bovine Mastitis Origin and Antibiotic. 2016. 10.1089/mdr.2019.020. Sensitivity Pattern from Northern Plains of India. J Vet Res Ani Husb. Vol. 1. Issue. 1. 34000105.
39. Firouzi R, Rajaian H, Tabaee M I , Saeedzadeh A. In vitro antibacterial effects of marbofloxacin on microorganisms causing mastitis in cows J. 2010.Vet. Res. 65, 1:51-55.
40. Kroemer S, Galland D, Guérin-Faublée V, Giboin H, Woehrlé-Fontaine F. Survey of marbofloxacin susceptibility of bacteria isolated from cattle with respiratory disease and mastitis in Europe. 2012. Veterinary Record, 170, 53 doi: 10.1136/vr.100246.
41. Idriss SH E, Foltys V, Tancin V, Kirchnerova K, Tancinova D , K. Zjecaú . Mastitis pathogens and their resistance against antimicrobial agents in dairy cows in Nitra , Slovakia, Slovak J. Anim. Sci., 47, 2014 (1): 33-38.
42. Aliyu Y, Abdullah I O, Whong CZ, Olalekan BO, Reuben. Occurrence and antibiotic susceptibility of methicillinresistant *Staphylococcus aureus* in fresh milk and milk products in Nasarawa State, North-Central Nigeri, Journal of Microbiology and Antimicrobials, 2020. Vol. 12(1), pp. 32-41.
43. Bakheet AA, Ali MN, Al- Habaty S H , Nasef S. Detection of Disinfectant resistant aerobic bacteria in unhatched chicken eggs. 2017. BVMJ -32 (2): 284-259.
44. Sineke N, Asante J, Amoako D, King Abia A L, Perrett K, Linda A, Bester LA, Essack *Staphylococcus aureus* in Intensive Pig Production in South Africa: Antibiotic Resistance, Virulence Determinants, and Clonality. Pathogens 2021, 10, 317, <https://doi.org/10.3390>.

45. Amoako D G, Somboro AM, Akebe LK, Molechan C, Perrett K , Bester L, Sabiha Y E. Antibiotic Resistance in *Staphylococcus aureus* from Poultry and Poultry Products in uMgungundlovu District South Africa, Using the "Farm to Fork" Approach, Article in Microbial Drug Resistance• August 2019, DOI:
46. Váradi L, Luo JL, Hibbs DE, Perry JD, Anderson RJ, Orenge S, Groundwater W. Methods for the detection and identification of pathogenic bacteria: past, present, and future, Chem. Soc. Rev., 2017, 46, 4818-4832.
47. Khan A, Hussain R, Javed MT, Mahmood F. Molecular analysis of virulent genes (coa and spa) of *Staphylococcus aureus* involved in natural cases of bovine mastitis.2013. J. agric. Sci., 50: 739-743.
48. Pereira V, Lopes C, Castro A, Silva J, Gibbs P, Teixeira P. Characterization for enterotoxin production, virulence factors, and antibiotic susceptibility of *Staphylococcus aureus* isolates from various foods in Portugal. Food Microbiology, 2009, 26: 278-282.
49. Shukla SK, Stemper ME, Ramaswamy SV, Conradt JM, Reich R, Graviss EA, Reed Molecular characteristics of nosocomial and Native American community-associated methicillin-resistant *Staphylococcus aureus* clones from rural Wisconsin. J. Clin. Microbiol. 2004. 42:3752-3757.
50. Naas T, Fortineau N, Spicq C, Robert J, Jarlier V, Nordmann Three-year survey of community-acquired methicillin-resistant *Staphylococcus aureus* producing Pantone-Valentine leukocidin in a French university hospital. J. Hosp. Infect. 2005. 61, 321–329.
51. Christine M bindyo , George C Gitao , Paul Joseph Plummer, Benard W Kulohoma , Charles M Mulei , Rawlynce Bett. Antimicrobial Resistance Profiles and Genes of *Staphylococci* Isolated from Mastitic Cow's Milk in Kenya. 2021. Antibiotics,10,772.<https://doi.org/10.3390/antibiotics10070772> <https://www.mdpi.com/journal/antibiotics>
52. Ahmed H, Abeda , Al Sayed R, Attiab , Ahmed A. Genotyping of  $\beta$ -lactams resistant staphylococci isolated from bovine subclinical mastitis. Beni-Suef University Journal of Basic and Applied Sciences 7 (2018) 499–504.
53. Yang F, Wang Q, Wang X R, Wang L, Li XP, Luo JY, Zhang SD, Li HS. Genetic characterization of antimicrobial resistance in *Staphylococcus aureus* isolated from bovine mastitis cases in Northwest China. J. Integr. Agric. 2016, 15, 2842–2847.
54. Schmidt T, Marleen M, Kock, Marthie M Ehlers. Molecular Characterization of *Staphylococcus aureus* Isolated from Bovine Mastitis and Close Human Contacts in South African Dairy Herds: Genetic Diversity and Inter-Species Host Transmission ,Front Microbiol. 2017; 8: 511.
55. Katayama Y, Ito T, Hiramatsu K. A new class of genetic element, staphylococcus cassette chromosome mec, encodes methicillin resistance in *Staphylococcus aureus*. Antimicrob Agents Chemother. 2000 Jun;44(6):1549–55.
56. Tarai B, Das P, Kumar D. Recurrent challenges for clinicians: Emergence of methicillin resistant *Staphylococcus aureus*, vancomycin resistance, and current treatment options. J Lab Physicians 2013; 5: 71–8.
57. Berger-Bachi B, McCallum N. State of the knowledge of bacterial resistance. Injury 2006; 37: S20–S25.
58. Zhu W, Murray PR, Huskins WC, Jernigan JA, McDonald LC, Clark NC, Anderson KF, McDougal LK, Hageman JC, Olsen-Rasmussen M, Frace M, Alangaden GJ, Chenoweth C, Zervos MJ, Robinson-Dunn B, Schreckenberger PC, Reller LB, Rudrik JT, Patel JB. Dissemination of an Enterococcus Inc18-Like vanA plasmid associated with vancomycin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 2010; 54: 4314–20.
59. CDC Centers for Disease Control and Prevention. *Staphylococcus aureus* resistant to vancomycin–United States. MMWR Morb Mortal Wkly Rep. 2002;51:565.
60. El-Zamkan MA, Mubarak AG, Omran A. Prevalence and phylogenetic relationship among methicillin- and vancomycin-resistant *Staphylococci* isolated from hospital's dairy food, food handlers, and patients. J. Adv. Vet. and Anim. Res. 2019. 6,4, 463–473.
61. Radwan IA, Mahdy W, Hegazy E, Salam HS. Vancomycin resistance among methicillin-resistant *Staphylococcus aureus* isolates from animal milk, J. Vet. Med. Res., 2017. 24 (2): 303-310.
62. Al-Amery k, Elhariri M, Elsayed A, El-Moghazy G, Elhelw R, Heba El-Mahallawy H, Mohamed El Hariri M, Dalia Hamza D. Vancomycin-resistant *Staphylococcus aureus* isolated from camel meat and slaughter house workers in Egypt, Antimicrobial Resistance and Infection Control, 2019. 8:129, 2
63. Niaz B, Saeed F, Ahmed A, Imran M, Maan A A, Khan M K I, Tufail T, Anjum F M, Hussain S, Suleria H A R. Lactoferrin (LF): a natural antimicrobial protein. Int. J. Food Prop. 2019 22(1): 1626–1641. <https://doi.org/10.1080/10942912.2019.1666137>.
64. Aguila A, Herrera AG, Morrison D, Cosgrove B, Perojo A, Montesinos I, n Pe´rez J, Sierra G, Gemmell CG, Brock JH. Bacteriostatic activity of human lactoferrin against *Staphylococcus aureus* is a function of its iron-binding properties and is not influenced by antibiotic resistance. FEMS Immunology and Medical Microbiology, 2001, 31, 145-152.
65. Kutila T, Pyörälä S, Saloniemi H, Kaartinen L. Antibacterial effect of bovine lactoferrin against udder pathogens. Acta vet. scand. 2003, 44, 35-42.
66. Niaz B, Tahir Zahoor T, Randhawa MA, Jamil J. Isolation of Lactoferrin from Camel Milk through Fast Protein Liquid Chromatography and its Antagonistic Activity against *Escherichia coli* and *Staphylococcus aureus*. Pakistan J. Zool., 2017,49(4),1307-1313,

67. Ombarak RA, Marwa A, Saad Abdel-Rahman M, Elbagory . Bio preservative Effect of Lactoferrin against Foodborne Pathogens Inoculated in Egyptian Soft Cheese “Karish Cheese”Alexandria. Journal of Veterinary Sciences www.alexjvs.com. Vol. 63 (2): 97-103 Oct. 2019 DOI: 10.5455/ajvs.76015
68. Biernbaum EN, Gnezda A, Akbar S, Franklin R, Venturelli P, McKillip J. Lactoferrin as an antimicrobial against Salmonella enterica and Escherichia coli O157:H7 in raw milk, JDS Communications , 2021, 2:92–97.
69. Farnaud S, Evans R. Lactoferrin: The conductor of the Immunological system? Pages 47–76 in New Research on Immunology. 2005.B. A. Veskler, ed. Nova Science Publishers, New York, NY.
70. Bergdoll MS, Lee Wong A.C. Staphylococcal intoxication. In: Foodborne Infections and intoxication, (Eds) H.P. Reimann and D.O.Clover, 2006. 523-562. Elsevier.
71. Shashikumar CSS , Puranik D. Study on Use of Lactoferrin for the Biopreservation of Paneer. Tropical Agricultural Research. 2011. Vol.23 (1):70-76.
72. Franco I, E Castillo , M D Pérez , M Calvo , L. Sánchez . Effect of bovine lactoferrin addition to milk in yogurt manufacturing. J. Dairy Sci.2010. 93:4480–4489 doi: 10.3168/jds.2009-3006.

## Photo

Photo 1 to 4 is available in supplementary section.

## Figures

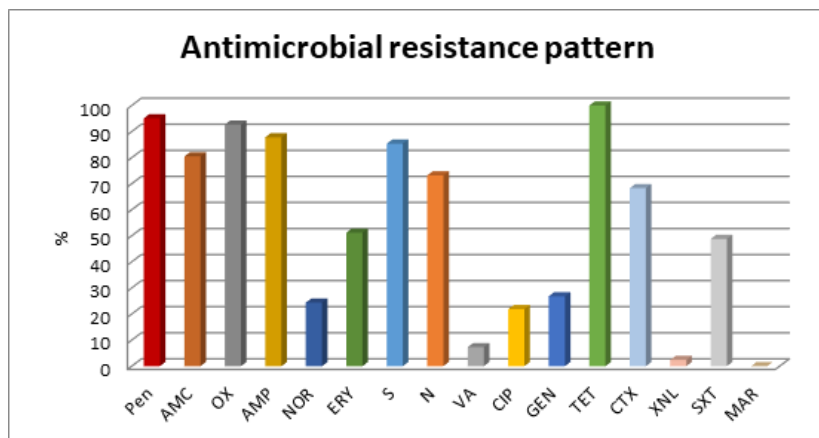


Figure 1

Antimicrobial resistance pattern for the isolated *S. aureus* isolates (n = 41).

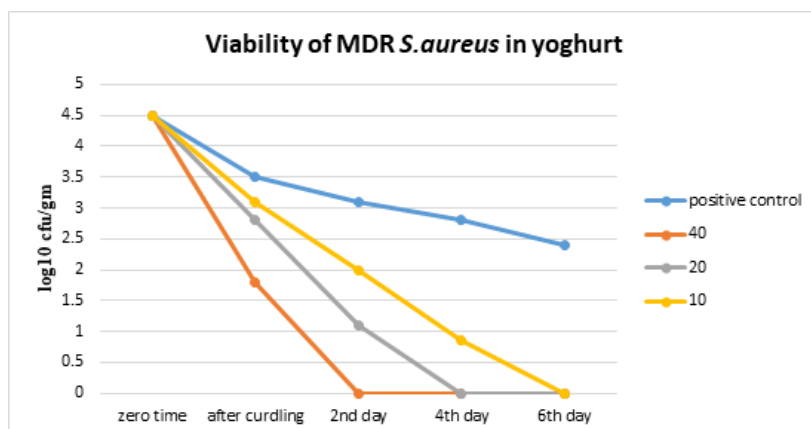
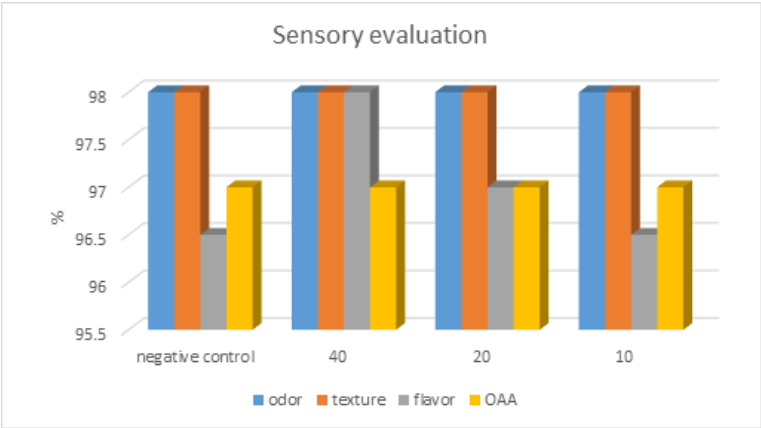


Figure 2



**Figure 3**  
Sensory evaluation of yoghurt after addition of different concentrations of lactoferrin.

**Supplementary Files**

This is a list of supplementary files associated with this preprint. Click to download.

- [Photo01.png](#)
- [Photo02.png](#)
- [Photo03.png](#)
- [Photo04.png](#)