# Immunostimulatory, Antibacterial and antibiofilm activity of purified Donkey colostrums lactoferrin on multidrug resistance *Serratia liquefaciens* producing *Intl* gene

**Likaa, H. Mahdi** [*] **BSc, MSc, PhD (Pathogenic bacteria)**
**Neihaya, H. Zaki** [*] **BSc, MSc, PhD (bacterial physiology)**
**Istabreq, M. A. Salman**[*] **BSc, MSc (microbiology)**
**Luma, A. H. Zwain**** **BSc, MSc, PhD (microbiology)**

*Fac Med Baghdad*
*2017; Vol.59, No.3*
*Received: May 2017*
*Accepted: Oct.2017*

**Abstract:**

**Background:** Modern methods of biofilm prevention depended on the utilize of normal foodstuffs can solve antibacterial and antibiofilm problems.

**Objective:** To purify donkey lactoferrin, and evaluate antibiotic resisted *Serratia liquefaciens* which producing *Intl* gene and investigate the inhibitory action of lactoferrin on biofilm and stimulate immune response.

**Methods:** lactoferrin extracted from donkey milk, and purified by ammonium sulfate and Sephadex chromatography. Antibacterial and antibiofilm activities of lactoferrin on *Serratia liquefaciens* were assayed, and effect of lactoferrin on the innate immune response of mice was determined.

**Results:** Lactoferrin contains 9.88% carbohydrates, 128 ppm iron, and molecular weight was 85kDa. It reduced *S.liquifaciens* in lung tissues of mice, and TNF-α and IL-6 cytokine levels decreasing by oral uptake of donkey milk.

**Conclusion:** All isolates resistance to Amoxicillin-clavulanic acid, and *Intl* gene was 160 bp. Lactoferrin concentration was about 2.77mg/ml. lactoferrin increase innate immune response from *S. liquefaciens* infection. These outcomes can be evidence for the way to original comprehensions regarding the use of Donkey colostrums lactoferrin as an oral adjuvant for a wide range of diseases.

**Keywords:** *S.liquefacien*s; Donkey milk, Lactoferrin; Purification; *Intl* gene; antibiofilm; immunostimulator**.**

## Introduction:

*Serratia* spp. is opportunistic bacteria, and it is commonly caused bacteriemia. They are responsible for a diversity of infections, like bacteremia, pneumonia, intravenous catheter-associated infections, osteomyelitis, endocarditis, and, infrequently, endogenous and exogenous endophthalmitis (1). Two mechanisms concerned with the progress of antibiotic resistance, mutation and achievement of resistance genes by gene transfer horizontally (2). Genetic rudiments are (integrons) or DNA accomplished of capturing genes by site-specific recombination revenue that frequently hold gene cassettes, have antibiotic resistance genes (3). Numerous types of integrons identified, clinical isolates of gram-negative bacteria have type 1 integrons generally scattered in it, and the incidence of integron class 2 has increased (4). Type II of integrons established in transposon and occurrence of pathogens having resistance to antibiotics constitute a main difficulty of health (5).

[*]*Dept.of Biology, college of Science, Al-Mustansiriyah University.*
*Email: neihaya_2008@yahoo.com*
*** Dept. of Biology /College of Education Ibn Al Haitham/Baghdad University*

Bacterial biofilm creation and expansion function has been recommended to be a significant step in the pathogenesis of many types of bacterial (6).For the past century the beneficial role of normal yields were described and many clinical benefits to these materials were studied like diarrhea treatment, antimicrobial action, immune inflection (7and 8). Also it has been exposed to be flourishing for the prevention of biofilm creation by normal yields (9 and10). Donkey milk is composite of high lactose content, casein, whey protein, non-protein nitrogen (NPN), and amino acids. Among the functional proteins detected in donkey milk, there are molecules active in antimicrobial protection such as lysozyme and lactoferrin. In donkey mammary secretion, defatted or not, growth factors and hormones have also been determined. In detail, donkey mammary secretions contain human-like leptin. The bioactive peptides insulin like growth factor 1, ghrelin and tri-iodothyronine were also found in frozen donkey milk (11). Lactoferrin is glycoprotein binds to iron that has serum transferrin and ovotransferrin (12). It is found on mucosal surfaces, within strict granules of polymorphonuclear leukocytes, and in biological fluids, demonstrating that it may take part in a protective part as response of innate immune (13). Lactoferrin has an activity against

a variety of bacteria and it was established, it shows that lactoferrin kill bacteria only when its without iron, and iron flooded lactoferrin has a compact function against microbes (14). Multiple tissue types including the gastrointestinal (GI) tract, liver, and lung of mice contain receptors for lactoferrin. Lactoferrin has targeted control of some cellular processes and can act as a transcription factor, regulating granulopoiesis and DNA synthesis in certain cells types. Administration of lactoferrin is suppressed tumor formation in a transgenic mouse lung tumor model. Lactoferrin can also alter circulating levels of white blood cells and direct differentiation of monocytes (15). Lactoferrin is also considered a cell-secreted intermediary between both kinds of immune responses. Recent healing searches on infectious diseases prominence on the intonation to response of immunity, make apply of lactoferrin to beneficially modulate the immune system has really improved, therefore this study aims to produce purified and characterized Donkey lactoferrin, and evaluated its function on antibiotic resisted bacteria of *Serratia liquefaciens* that producing *Intl* gene and investigate alternative therapeutic protocols by using inhibitory action of donkey lactoferrin on development of biofilm and stimulate immune response.

**Methods:**

Preparation of Bacteria: Pathogenic isolates of *Serratia liquefaciens* (which isolated from sputum specimens of patients suffered from respiratory tract infection and resisted to broad range of antibiotics) were obtained from postgraduate laboratory in Biology department–college of Science/AL-Mustansiriyah University. Bacterial isolates inoculated on the surface of prepared Nutrient agar plates, and incubation at 35°C for 18-24 hours. Antibiotic Susceptibility: Kirby-Bauer (disk diffusion) technique used to check the antibiotic propensity of *Serratia liquefaciens* strains under study. Muller-Hinton agar (Hi media company/ India) and 13 diverse discs of antibiotics (Bioanalyse/Turkey) used in this test. Inhibition zones of antibiotics calculated, and then compared with the standard zones (16). Estimation of MIC: MICs of Ceftazidime and Lactoferrin toward *Serratia liquefaciens* isolates were resolute by broth micro-dilution method (16). Serial dilutions for Ceftazidime used at concentration from 0.125 to 1024 µg/ml in Muller-Hinton broth. Genotypic detection of *Intl* gene: Extraction of genomic DNA: DNA of *Serratia liquefaciens* isolates obtained by suspending 2-3 colonies of each strain in 500 µl of lysis buffer that prepared according to (17) and heating by using water bath for 10 min at 90 °C, then the samples were spun for 10 min at 10000 rpm. Detection *Intl* gene by PCR: The sequences of *Intl* (Alpha DNA, Canada) primer used were:(5'-CAG TGG ACA TAA GCC TGT TC-3') used as forward primer, and (5'-CCC GAG GCA TAG ACT GTA-3') used as a Reverse primer (17). The conditions of PCR reaction including: an initial denaturation for 10 min at 94°C, followed by 30 cycles of denaturation at 94°C for 30 seconds, while annealing at 50°C for 30 seconds, and extension at 72°C for 30 seconds. Final extension was at 72°C for 5 min. Electrophoresis with 1.5% agarose for 2hr. at 7v/cm used to detect PCR products, and use of UV-transilluminator documentation system by the aid of Ethidium bromide stains (18). Donkey colostrums collection: Donkey colostrums obtained from AL-Orfelly in Baghdad city, samples collected after donkey parturition (within earliest 4 days), and were at once chilly at -18°C until occupy. Extraction of Donkey colostrums: Donkey colostrums milk skimmed by centrifugation in a (Sigma MA3-18) centrifuge at 4000 g/min for half hour at 4°C. Colostrums whey equipped by precipitation of casein from skimmed colostrums in low pH with gradual adding of 1N HCl to pH 4.6, the precipitated casein detached by centrifugation at 10000g/min for quarter of hour in 4°C. The supernatant (whey) used to be pH 6.8 with 1N NaOH and dialyzed against distill water for 18 hr, and then stored at -18°C until utilize as a crude lactoferrin (19). Purification of Donkey colostrums: Additional proteins precipitated by using Ammonium sulfate in two steps, and after centrifugation in 10000×g, 30 min at 4°C, the precipitate dissolved in phosphate buffer (20 mM), then filtrated, and finally lactoferrin dried by lyophilization. It purified by carboxymethyl Sephadex-C50 chromatography (FPLC/ USA) using phosphate buffer (0.2 M, pH 7.7), and NaCl from 0.0 to 0.5 M. During purification, eluents have protein recorded by U.V (280 nm) (20), while protein estimated by Lowry method (21). Determination of Carbohydrate content: Phenol-Sulfuric acid procedure used in determined carbohydrate in Donkey colostrums lactoferrin (22). Determination of Iron content: It determined as method of (23). Affinity membrane chromatography used to establish total iron concentration in purified Donkey colostrums Lactoferrin calculated as fallow: Purified Total iron concentration (ppm) = (Atomic absorption sample - Atomic absorption for blank) x final volume / weight of sample (gm) x dilution factor. Molecular weight of purified donkey lactoferrin was predictable comparing with standard labeled proteins (Bovine serum albumin, Ova-transferrin, Aldolase, catalase) in the standard curve which represented the correlation of log of M.W of standard proteins versus (Ve/ Vo). Antibacterial effect of Lactoferrin on *Serratia liquifaciens:* The agar well diffusion method used to detect antibacterial effect of Lactoferrin on *Serratia liquifaciens* strains at the concentrations (100, 50, 25, and 12.5) µg/ml, and the plates cultured without lactoferrin under the same condition were used as controls (24). Biofilm assay

**1-** Biofilm formation by *Serratia liquefaciens*

It achieved as described by (25), and *Psudomonas aeruginosa* was used as positive control.

2- Antibiofilm of donkey lactoferrin and ceftazidime on *S. liquefaciens*

For the inhibition of biofilm assay, the highest biofilm producing isolates of *S. liquefaciens* (S2, S5, S7, and S8) were selected to be assayed. Before the staining step, the previously prepared ceftazidime and purified donkey lactoferrin added to biofilm containing wells. After incubation (24 hr, 37ºC) wells washed and stained, and then viable count was approved out according to (25). The plate cultured without lactoferrin under the same condition was used as control. Immunomodulatory, antibacterial activity of Donkey lactoferrin on *Serratia liquefaciens* in vivo.

Culture conditions of bacteria: *S.liquefaciens* was grown on Luria-Bertani (LB) agar plates (LB-Lennox formulation, consisting of 10g tryptone, 5g yeast extract, and 5 g NaCl / liter) incubated at 30°C for 18 hr. bacterial isolates inoculated in LB broth at 30°C incubation with gently sloping overnight. After 24 hr, culture was diluted (1:50) in LB broth for 3h at 30°C. (75-l volume) of bacterial inoculums diluted with sterile phosphate-buffered saline (PBS) to the final preferred concentration. CFU quantity was diluted and culturing on LB plates for all experiments. Bacteria accustomed to about 1x $10^6$ CFU/ml. Infection of mice with *Serratia liquefaciens and* (experimental design): BALB/c mice aged 4-5 weeks old (18-20 gm) obtained from Animal House of Medicine College/ Baghdad University and housed under typical environment (animal experiments performed in agreement with the NIH Guide). Oropharyngeal aspiration done according to (26), mice separated to 2 groups (10 mice in each one): untreated control (C) orally administered normal saline. Treatment group: mice orally administered Donkey colostrums lactoferrin 1mg/ day for 7days before infection and 6 days post infection. Animal were observed and evaluated for up to 6 days, mice by exposure to CO2 on day 6 post infection. CFU determined in lungs by sacrified mice, and lungs were aseptically removed, rinsed, and PBS homogenizing in (TH homogenizer). Tissue homogenates diluted and applied to N.A plates then incubated in 30°C for 18 hr. CFU values from tissue were extra polated from colony counts. Broncho-alveolar lavage (BAL) According to procedure of (27) samples of BAL were obtained. Cytokine assay. TNF-α and interleukin-6 (IL-6) concentrations detected by ELISA in BAL fluid via mouse Quantikine kits (R&D Systems, Inc., Minneapolis, MN, USA). Statistical analysis. All the assays were compared using ANOVA and the Tukey LSD test. Differences well thought-out were considerable when $P< 0.05$.

**Results:**

Bacterial Isolates: Nine pathogenic isolates of *Serratia liquefaciens* (isolated from respiratory tract infection) were obtained from post-graduate laboratories in Department of Biology-College of Science in AL-Mustansiriyah University. Pattern of Antibiotic Susceptibility: *Serratia liquifaciens* isolates showed pattern of antibiotic susceptibility toward 13 different antibiotics. Results showed that all isolates resisted to Amoxicillin- clavulanic acid except no.2, while all isolates resisted to Ampicillin-Sulbactam and Ceftriaxone except isolate no.1 and 7.

Estimation of MIC: The MIC of ceftazidime for all nine *Serratia liquifaciens* isolates tested, and the break point for ceftazidime is = 4 (g/ml according to [CLSI]. The result showed that 2 isolates of *Serratia liquifaciens* (number 4 and 9) have 2 µg/ml MIC for ceftazidime, while other seven isolates of *Serratia liquifaciens* were gave MIC values more than break point for ceftazidime. The MICs of ceftazidime in *Serratia liquifaciens* isolates ranged from 32 µg/ml to 256 µg/ml (Table-1). Genotypic detection of *Intl* gene: *Intl* gene obtained by PCR amplification for isolates (1, 6, and 8) of *Serratia liquifaciens* which appeared in gel wells number (6, 4 and 3) respectively. PCR fragments size was 160 bp comparing to the ladder (100-1000 bp) as in (Fig.1). Purification of Donkey colostrums lactoferrin: There were three peaks appearances after purification of Donkey colostrums Lactoferrin by from Cation exchange chromatography. The second peak is Donkey colostrums Lactoferrin which eluted in 0.4-0.5 of NaCl (1M) (Fig. 2). Concentration of lactoferrin indomitable by Lowry method and it's about 2.77mg/ml. Molecular weight of purified donkey lactoferrin: (Fig.3) showed that the molecular weight of purified donkey lactoferrin was 85 KDa which estimated by gel filtration chromatography. The carbohydrate and iron contents in lactoferrin: The results exhibited 9.88% of carbohydrates while iron content was 128 ppm from purified Donkey colostrums lactoferrin. Antibacterial effect of Donkey Lactoferrin on *Serratia liquifaciens:* Antibacterial effect of lactoferrin against *Serratia liquifaciens* isolates (inhibition zones determined around colonies), and this activity decreased with the decrease of concentration of lactoferrin as shown in (Table-2). Biofilm formation of *Serratia liquefaciens:* The results of biofilm production from *S. liquefaciens* isolates existed in (Table-3). It's showed that each isolate exhibited a different potential to form biofilm under the same situation of testing. Four (%) isolates were high producers, while 5 (%) isolates were good in biofilm production and none of the testes isolates were poor in production. What's more, *S. liquefaciens* isolates; S2, S5, S7 and S8 produced the thickest biofilm; 1.281, 1.227, 1.104 and 1.698, respectively. Obviously, *S. liquefaciens* S8 achieved the highest biofilm thickness. In vitro inhibitory activity of purified lactoferrin and ceftazidime on *S. liquefaciens* biofilm Treating biofilms of *S. liquefaciens* S2, S5, S7, S8 with purified donkey lactoferrin and ceftazidime,

O.D. and viable count exhibited significant differences (P<0.05) between before and post treatment (table-4). Purified donkey lactoferrin left no live cells except for one case; S5, but ceftazidime with three cases S5, S2 and S7. Furthermore, the cognate O.D. reading perhaps referred to the remaining exopolysaccharids.

Inhibition activity of Donkey colostrums lactoferrin on *S. liquefaciens* in vivo

In lung tissues numbers of *S. liquefaciens* exhibited an important decrease (100- to 1000) fold in Donkey lactoferrin-treated mice after 6 day of infection, compared with control (Fig. 4). However, bacterial numbers in lungs exhibited significant differences (P < 0.05) between the two groups after 6 days. In tested mice, Donkey colostrums lactoferrin caused pathogen clearance.

Immunomodulatory effect of Donkey colostrums lactoferrin in vivo.TNF-α and IL-6 cytokines Levels in lungs were high significantly by *S. liquefaciens* infection, demonstrating the forced pulmonary inflammation induced by the infection (Fig.5). The TNF-α and IL-6 cytokines level showed significantly decreasing by the oral uptake of Donkey colostrums lactoferrin compared with control group at day 6.

**Table-1: MIC of Ceftazidime and Donkey Lactoferrin against *S. liquefaciens* Biofilm**

| S. liquefaciens isolates | ceftazidime MIC (µg/ml) | lactoferrin MIC (µg/ml) |
|---|---|---|
| S2 | 64 | 32 |
| S5 | 32 | 256 |
| S7 | 128 | 64 |
| S8 | 32 | 128 |

***MIC= Minimum Inhibitory Concentration**

**Table-2: Antibacterial Activity of Purified Donkey Lactoferrin against *Serratia liquefaciens***

| Treatment | concentration µg/ml | Inhibition zone (mean ± SD) | | |
|---|---|---|---|---|
| Control (D.W.) | 0 | 0 ± 0 | | |
| Lactoferrin | 100 | 27.19 ±1.77 | p1 | a |
| | 50 | 21.55 ± 2.01 | p1 | b |
| | 25 | 13.68 ± 1.09 | p1 | c |
| | 12.5 | 10.41 ± 2.64 | p1 | d |

**p1: probability compared to control, p< 0.05**
**a-d: different letters in the same column refers to significant differences p< 0.05.**

**Table-3: Absorbance for *S. liquefaciens* Biofilm at 540 nm And Statistical Analysis.**

| Isolate number | Absorbance ± SD |
|---|---|
| S1 | 0.601 ±0.079 |
| S2 | 1.281±0.355 |
| S3 | 0.742 ± 0.124 |
| S4 | 0.686 ±0.051 |
| S5 | 1.227±0.335 |
| S6 | 0.507 ±0.398 |
| S7 | 1.104 ±0.115 |
| S8 | 1.698±0.322 |
| S9 | 0.821±0.278 |

***Each datum is a mean of triplicate. SD= standard deviation. LSD=0.267. P= 5.07E-07**

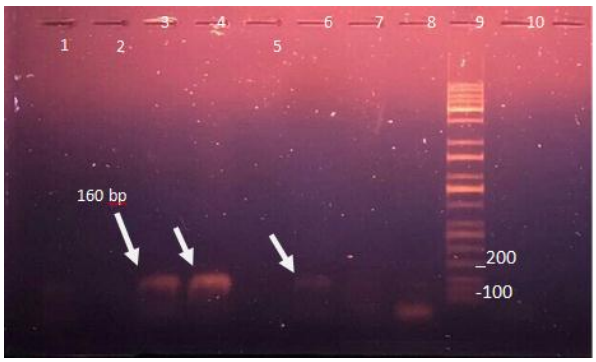



**Figure (1): Recognition of Integron by Extension of *Intl* Gene. Lane 9, DNA Ladder (100-1000 bp); lanes 3, 4, and 6 (160 bp); Lane 1, 2, 5, 7, 8 and 10 were Integron1 Negative. (Agarose 1.5% for 2hr. at 7 v/cm)**

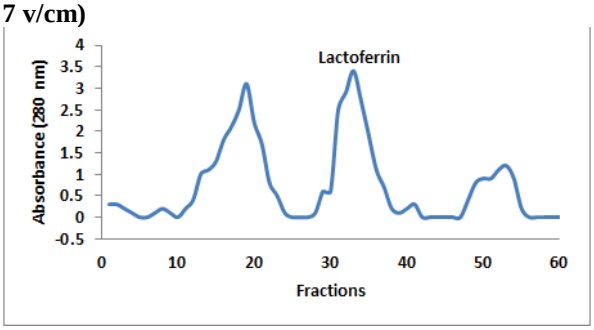



**Figure (2): Purification Graph; Second Peak is Lactoferrin (0.4-0.5 of 1M Nacl elution).**

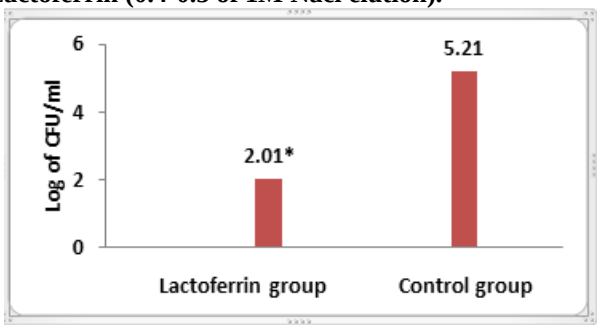



**Figure (3): Molecular Weight of Purified Donkey Lactoferrin**

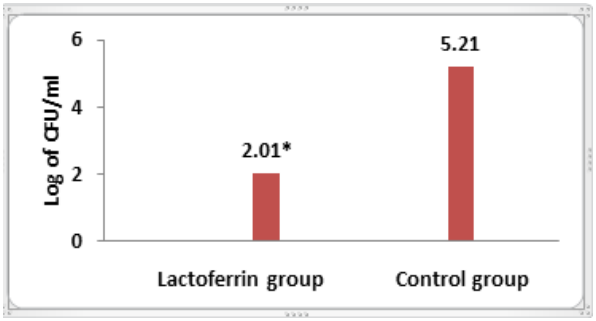
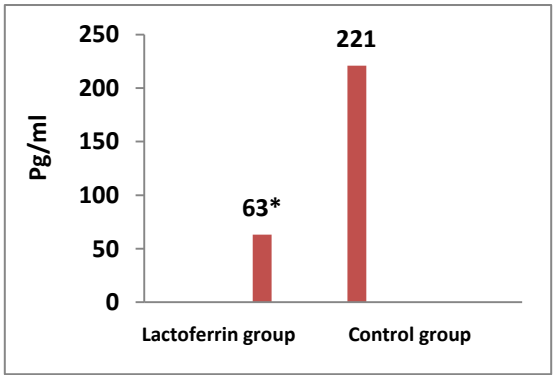



**Figure (4): *S. liquefaciens* Numbers in Mice Lungs; *Statistically Significant Difference in (Infected Mice Treated with Lactoferrin) Compared With Control Group (Infected Mice Treated With Saline).**

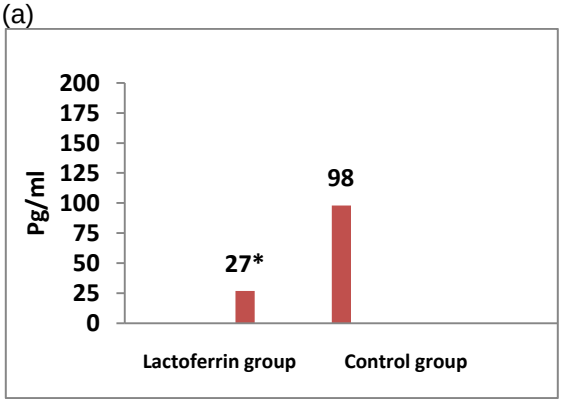
**Table-4: Optical Density, Viable Count (CFU/ml) And Statistical Analysis of *S.liquifacience* Biofilm After Treatment With Ceftazidime And Purified Lactoferrin[1, 2]**

| S.liquifacience isolate | Parameter | Before treatment | After treatment with | | | |
|---|---|---|---|---|---|---|
| | | | Donkey lactoferrin | ceftazidime | P value | LSD |
| S.2 | OD | 0.903 ± 0.238 a | 0.485 ± 0.042 b | 0.65 ± 0.016c | 0.006497 | 0.125 |
| | VC | 658668 ± 7765.45 a | ND a | 5433±1997.5 b | 2.00E-10 | 5643 |
| S.5 | OD | 2.224± 0.89 a | 0.591 ± 0.074 b | 1.054 ± 0.075 c | 0.019822 | 0.461 |
| | VC | 8466662 ± 208169 a | 447.665± 30.913b | 945410 ± 14640.13 c | 4.00E-10 | 108909.22 |
| S.7 | OD | 0.531 ± 0.192a | 0.164 ± 0.028 b | 0.466± 0.107 a | 0.025 | 0.28 |
| | VC | 348332± 3055.04 a | ND b | 5565 ± 208.17c | 4.00E-12 | 2113 |
| S.8 | OD | 0.615 ± 0.127 a | 0.434 ± 0.078b | 0.72 ± 0.181 c | 0.113 | 0.097 |
| | VC | 446668 ± 4142.45 a | ND b | ND b | 9.00E-10 | 3874 |

[1]**O.D. = optical density, VC= viable count, LSD= least significant difference. Each datum is the mean of triplicate.**
[2]**Similar letters in the same raw refer to insignificant differences.**




(a)




(b)

**Figure (5): Concentrations of Inflammatory Cytokines on Day 6 in BAL Fluid of Mice Infected with *S. liquefaciens* And Treated with Saline or Donkey Lactoferrin. (a) Tumor Necrosis Factor-α (b) Interleukin-6. *Statistically Significant Difference (P < 0·05) Compared With Control.**

**Discussion**:

Results of search (28) agreed with this study and showed that antibiotic susceptibility of *Serratia liquefaciens* isolates as following**:** Piperacillin/ Tazobactam 12%, Imipenem/ Meropenem 1%, Cefotaxime 18%, Ceftazidime 16%, Ciprofloxacin 8%, and Gentamicin 2%. Also results of MIC helps to establish which kind of antibiotic is mainly effective, and can guide to an suitable option of an antibiotic that will boost chances of treatment achievement, and help in the struggle to slow resistance of antibiotic (29). Integrons determined in some types, but integrons type 1 was the majority common and disseminated among gram-negative bacteria (3) and this agreed with our results. (7) Exhibited that integron type 1 (intI1) determined in *P. aeruginosa* and *A.baumannii*, and intI1 detection rates as high as 71.4z and 79.5z among *Acinetobacter* spp. Lactoferrin is an iron-binding protein that has great types of biological functions as, regulation of iron homeostasis, cellular growth, antimicrobial and antiviral effects, and anticancer (30). (31) Described that Donkey lactoferrin purified by a cationic exchange chromatography, and analysis with 12% SDS–PAGE. Carbohydrates content in Lactoferrin ranged between 7-11.5% (this result agreed with ours), and differs from other types of mammals, and this can lead to difference in lactoferrin molecule (32). Absence of iron in the environment forces bacteria to move; as a result, they cannot adhere to surfaces (33). lactoferrin may exist useful to prevent intestine infections in infants because its ability of antimicrobial and bacteriostatic, and their act may expand the maintenance of fresh milk from donkey and the relative potential marketable distribution (34). An assured bactericidal effect on *S. liquefaciens*, about ($1\times\log^{10}$ CFU/ ml) of bacteria turn down (8), while (7) exhibited that lactoferrin has tough activity against both kinds of bacteria, however, it was more effective on Gram-positive rather than gram-negative bacteria (7)**.** Although modern methods to biofilm management based on utilize of biological-based liquids with activity against microbes, and specificity to lead in prevent the biofilm resistance issue (35). There is a lack of information regarding the function of purified donkey lactoferrin in inhibition of biofilms of *S. liquefaciens*. lactoferrin used as therapeutic agent against different kinds of bacteria, this effect resulted by inducing excess of pro-inflammatory intermediaries through diseases (36). There is a significant role of lactoferrin in host immunity, and have the capacity to alter cytokine production. Capacity to product and activity of reactive oxygen, allows lactoferrin to provide as an exclusive regulator to a broad range of responses, including those occupied in septic shock, inflammation, and subsequent progress of disease

(37).TNF-α and IL-6 Intrapulmonary levels were high in the BAL fluid by *S. liquefaciens*, demonstrating occurrence of aggravated pulmonary inflammation. The decreased induction of these cytokines by Donkey colostrums lactoferrin -taken orally- administration clearly indicates the useful role of Donkey colostrums lactoferrin to recover the tissue damage caused by limited inflammation (27).

**Conclusion:**

Nine pathogenic isolates of *Serratia liquefaciens* isolated from respiratory tract infections, and most of isolates exhibited resistance to Amoxicillin- clavulanic acid and Ampicillin-Sulbactam and Ceftriaxone. Most of *Serratia liquifaciens* isolates gave MIC values more than break point for ceftazidime. Fragment size was 160 bp to *Intl* gene for *Serratia liquifaciens* (1, 6, and 8) isolates by PCR. Donkey Lactoferrin concentration was about 2.77mg/ml, and contains 9.88% carbohydrates, and 128 ppm iron. Donkey lactoferrin concentration (100, 50, 25, and12.5) µg/ml, have effect vis. *Serratia liquifaciens* isolates. From these outcomes, oral management of Donkey colostrums lactoferrin might defend host animals from *S. liquefaciens* infection by increase innate immune response. Exhaustive searches are necessary to explain mechanisms essential in role of Donkey colostrums lactoferrin in pneumonia and other nosocomial infection in humans.

**Authors' Contributions:**

Dr.Likaa, H. Mahdi: Study conception, Data collection and study design.

likaahamied@uomustansiriyah.edu.iq

Dr.Neihaya, H. Zaki: drafting manuscript and critical revision.

dr.neihayahz@ uomustansiriyah.edu.iq

Istabreq, M. A. Salman: Acquisition of data analysis and interpretation of data

stsy@uomustansiriyah.edu.iq

Dr.Luma, A. H. Zwain: Acquisition of data analysis and interpretation of data

**References:**

*1-Van-Houdt, R.; Givskov, M.; and Michiels, C. W. Quorum sensing in Serratia. FEMS Microbiology Reviews, 2007; 31(4): 407-424.*

*2-Martinez, J.L. The role of natural environments in the evolution of resistance traits in pathogenic bacteria. Proc. Biol. Sci. 2009; 276: 2521–2530.*

*3-Eraç, F.; goär-Limoncu, M.; Ermertcan, S.; Tas, F. and Aydemir, S. Prevalence of blaPER-1 and Integrons in Ceftazidime-Resistant Gram-Negative Bacteria at a University Hospital in Turkey. Jpn. J. Infect. Dis. 2013; 6: 146-148.*

*4-Kamalbeik, S.; Kouchek, M.; Salehi,M.; Fallah,F.; Malekan,M.; Talaie, H. Prevalence of Class 2 Integrons in Multidrug-Resistant Acinetobacter baumannii in Toxicological ICU Patients in Tehran. Iranian Journal of Toxicology, 2013; 7 (22).*

*5-JAPONI, S.; JAPONI, A.; FARSHAD, J.; ALI, A. and JAMALIDOUST, M. Association between Existence of Integrons and Multi-Drug Resistance in Acinetobacter Isolated from Patients in Southern Iran. Polish Journal of Microbiology, 2011; 60(2): 163–168.*

*6-Lavender, H.; Jagnow, R. and Clegg, S. Biofilm Formation In Vitro and Virulence In Vivo of Mutants of Klebsiella pneumoniae. Infection and Immunity, 2004; 72(19): 4888–4890.*

*7-Mahdi, L. Shafiq, S; and Ajaa, H. Effects of Crude and Purified Bacteriocin of Pediococcus pentosaceus on the Growth and Zearalenone Production by Fusarium graminearum. Int. J. of Curr. Engin. and Technol.2013; 4(1): 2277 – 4106.*

*8-Mahdi, L; AL-Kakei, S, and Zwain, L. The Effect of Pediococcus pentosaceus Bacteriocin on Listeria monocytogenes in Soft Cheese. Int'l Conf. on Med. Genetics, Cellular & Molecular Biology, Pharmaceutical & Food Sciences (GCMBPF) Istanbul. 2015, 105-108.*

*9-Mahdi, L. and Husain, N, S. Antagonstic Effect of Lactobacillus fermentum Supernatant Against Enterococcus faecium and Enterococcus faecalis In Vitro. Fac. Med. Baghdad, 2012; 54 (2).*

*10-Mahdi. L; Abd Alkareem. S and Musafer, H. Immunomodulatory and antagonistic effect of Lactobacillus reuteri and its purified characterized bacteriocin against Salmonella enterica and Shigella flexnerii. J. Advance Nature Applie. Scien. 2016; 10(17): 155-167.*

*11-Guo, H.Y.; et al. (April 2007). "Composition, physiochemical properties, nitrogen fraction distribution, and amino acid profile of donkey milk.". Journal of Dairy Science. Journal of Dairy Science. 90 (4): 1635–43*

*12-Al-Musawii, M, H; Hussein, H, N; and Taha, M, B. Synergestic effect of colostrums lactoferrin and purified characterized Brevicin on ESBL producing Pantoea agglomerance isolated from blood cultures of infants in Baghdad. Euro. J. of Biomed. and Pharma. Scien. 2016; 3(10): 2349-8870.*

*13-Conesa, C.; Rota, C.; Castillo, E.; Pérez, M. D.; Calvo, M. and Sánchez, L. Effect of heat treatment on the antibacterial activity of bovine lactoferrin against three foodborne pathogens. International Journal of Dairy Technology, 2010; 63(2): 209-215.*

*14-Ahmed, M.; Khaled, A.; Magzoub, M.; and Hassan, M. Effect of heat treatment on the chromatographic detection, electrophoretic pattern and antibacterial activity of lactoferrin purified from camel milk. Journal of Food, Agriculture & Environment.2014; 12(2): 20-23.*

*15-Tung,Y.T, Chen,H.L, Yen,C.C, Lee, P.Y, Tsai,H.C, Lin,M.F, and Chen,C.M. Bovine lactoferrin inhibits lung cancer growth through suppression of both*

*inflammation and expression of vascular endothelial growth factor.2013, J. Dairy Sc. 96: 2095–2106.*

*16-CLSI, (Clinical and Laboratory Standards Institute). Performance standard for antimicrobial susceptibility testing, Twenty-First informational supplement; M100-S2, 2011; 31(1).*

*17-Reischl, U; Linde, H.; Metz, M.; Leppmeier, B.; and Lehn, N. Rapid identification of methicillin-resistant Staphylococcus aureus and simultaneous species confirmation using real-time fluorescence PCR. Clin. Microbiol. 2000; 38(6): 2429-35.*

*18-Koeleman J.; Stoof, J.; Van Der bijl, M.; Vandenbrouck, G. and Savelkoul, P. Identification of epidemic strains of Acinetobacter baumannii by integrase gene PCR. J.Clin. Microbiol. 2001; 39: 8-13.*

*19-Sambrook, J. and Rusell,D.W. Molecular cloning:a laboratory manual. Cold spring Harbor, NY. Cold spring Harbor laboratory Press.2001.*

*20-Al-Hatim, R.R. Separation and purification Lactoferrin from bovine and ovine colostrums and studying of some its properties and applications. Master's Thesis, College of Agric. university of Basra-Iraq, 2012.*

*21-Lowry, O. H.; Reschrough, N. J.; Earr, A. and Randull, R. J. Protein measurement with Folin reagent. J. Biol. Chem. 1951; 193: 265-275.*

*22-Dubois, M.; Gilles, K.; Hamiton, J.; Robers, P. and Smith, F. Colorimetric method for determination of sugars and substances. Analytical and Chem. 1956; 28: 350-356.*

*23-Lee, K. and Clydesdale, F. Quantitative determination of the Elemental, ferrous, ferric, soluble a complexes iron in food. J. Food Sci. 1979; 44: 549-554.*

*24-Natascia, M.; Capucchio, B. M.; Biasibetti, E.; Pessione, E.; Cirrincione, S.; Giraudo, L.; Corona, A. and Dosio, F. Antimicrobial Activity of Lactoferrin-Related Peptides and Applications in Human and Veterinary Medicine. Molecules. 2016; 21(752): 1-25.*

*25-Harley, J. and Prescott, H. Laboratory Exercises in Microbiology, 5th Edition .The McGraw−Hill Companies, 2002.*

*26-Könninger, U.; Hobbie, S.; Benz, R.; Braun, V. The haemolysin secreting ShlB protein of the outer membrane of Serratia marcescens: determination of surface-exposed residues and formation of ion permeable pores by ShlB mutants in artificial lipid bilayer membranes. Mol. Microbiol. 1999; 32: 1212-1225.*

*27-Villena, S.; Racedo, G.; Agüero, E.; Bru, M.; Medina, A. and Alvarez, S. Lactobacillus casei improves resistance to pneumococcal respiratory infection in malnourished mice. J. Nutr. 2005; 135(6): 1462-9.*

*28-Public health England. Voluntary surveillance of bacteraemia caused by Enterobacter spp, Serratia spp. and Citrobacter spp. in England, Wales and Northern Ireland: 2009-2013. Health protection report. 2014; 8(40): 17-40.*

*29-IDEXX- Reference Laboratories. Microbiology Guide to Interpreting Minimum Inhibitory Concentration. Laboratories, Inc. 2013; 1-14.*

*30-Nazzaro,F.; Orlando, P.; Fratianni, F. and Coppola, R. Isolation of Components with Antimicrobial Property from the Donkey Milk: A Preliminary Study. The Open Food Science Journal, 2010; 4: 43-47.*

*31-Vincenzetti, S.; Amici, A.; Pucciarelli, S.; Vita, A. and Polidori, P. A Proteomic Study on Donkey Milk. Biochem Anal Biochem.2012; 1(2).*

*32-Van-Veen, H.A.; Geerts, M.; Van-Berkel, P. and Nuijens, J. The role of N-linked glycosylation in the protection of human and bovine lactoferrin against tryptic proteolysis. Eur. J. Biochem. 2004; 27: 678-684.*

*33-Jahani, S.; Shakiba , A.; and Jahani, L. The Antimicrobial Effect of Lactoferrin on Gram-Negative and Gram-Positive Bacteria. Int J Infect. 2015; 2(3).*

*34-Mariani, P.A. Donkey milk nutraceutical characteristics: a biochemical evaluation of nutritious and clinical properties. University of Camerino School of Advanced Studies, 2008.*

*35-Simões, M.; Simões, L. and Vieira, M. A review of current and emergent biofilm control strategies. LWT - Food Sci. Technol. 2010; 43: 573–583.*

*36-Foster T.J. Immune evasion by Staphylococci. Nat Rev Microbiol. 2005; 3: 948–58.*

*37-Actor, J.; Hwang, N. and Kruzel, L. Lactoferrin as a Natural Immune Modulator. Curr Pharm Des. 2009; 15(17): 1956–1973.*