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Newly Method for Preparation of Live Attenuated Vaccine from Local Methicillin-resistant Staphylococcus aureus Isolate and Study its Effect on Immune Response in Rats

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THIS study was aimed to prepare a live attenuated vaccine by a new method from local methicillin-resistant *Staphylococcus aureus* (MRSA) isolate as a trial to control bovine mastitis. The isolated MRSA was confirmed by PCR, then live attenuated vaccine was prepared by a new method using 3% H_2O_2 followed by serial passage thirty times. Wistar albino rats were used as an experimental animal model to estimate the immune response against prepared vaccine. ELISA assay was used to detect CD4+ and CD8+ T-cells concentrations while RT-qPCR was used to detect IL-2 and IFN-γ gene expression to estimate immune response results from vaccination by the prepared vaccine. Results showed a significant increase both in CD4+ and CD8+ T-cells concentrations using ELISA. Also, RT-qPCR showed up-regulation of each of IL-2 and IFN-γ gene expression by increasing in change folds reached to 2612.11 and 704.28 times respectively for both of them. CD4+ T-cells can enhance the humoral immune response by differentiation to Th2 cells and cell-mediated immunity (CMI) by differentiation to Th1 cells, in addition, to enhancing effect of IFN-γ through a positive feedback mechanism that amplifies the Th1 effect. CD8+ T-cells differentiated to Cytotoxic T Lymphocytes (CTLs) that are responsible for cell-mediated immunity by the killing of infected cells by intracellular pathogens, in addition to IL-2 that enhance the effect of CTLs also. It concluded that prepared live attenuated vaccine stimulated both humoral and potent cell-mediated immunity promising it as a good new method for live attenuated vaccine production.

Keywords: *Staphylococcus aureus*, MRSA, Live attenuated vaccine, ELISA, RT-qPCR, Rats.

Introduction

Bovine mastitis is considered one of the serious and crucial diseases that affect milk production in the world due to decreased milk production amount with bad and unacceptable quality, severe economic loss due to discarded milk, increase cost of animal care and antibiotic treatment, premature culling, also it may lead to a serious problem for public health and increase risk of antibiotic resistance by microorganisms [1]. *S. aureus* can cause a wide range of infections both in humans and animals and it is one of the important causing of mammary gland infection in bovine which is called bovine mastitis. Mastitis can be caused by a whole association of pathogenic microorganisms including *S. aureus* which may be considered the number one pathogen causing mastitis in cattle and buffalo [2]. *S. aureus* frequently gives rise to persistent and chronic infection [3]. Mastitis associated with *S. aureus* leans towards the sub-clinical and chronic form that leads to low response for antibiotic therapy due to several causes [4]. Subclinical mastitis is characterized by the absence of true clinical signs of mastitis, also quality and yield of milk production are not affected but still the presence of a microorganism in the milk [5,6]. The chronic infection of *S. aureus*

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can be attributed to the ability of this bacteria to produce biofilm [7]. Staphylococci secrete many several mechanisms to avoid any toxic material in their environment such as antiseptic, disinfectants, and antibiotics [8]. Making control of *S. aureus* mastitis is very difficult [9]. vaccination was used as an effective method to control bovine mastitis caused by *S. aureus* [10-12]. So, the current study aimed to prepare a live attenuated vaccine from locally isolated MRSA.

Materials and Methods

Bacterial strain

Locally isolated biofilm-producing methicillin-resistant *S. aureus* (MRSA) from bovine mastitis was used for preparing live attenuated vaccine.

Confirmation of MRSA

MRSA was confirmed by polymerase chain reaction (PCR). Four primers were used for this purpose: *nuc* gene-specific for *S. aureus*, *mec* A gene specific for antibiotic resistance, *ica A* and *ica D* genes specific for biofilm production.

Preparation of live attenuated vaccine

A new method for attenuation of MRSA by using 3% H_2O_2 with serial passage was used as follows: a single colony from growing MRSA on mannitol salt agar was elected to produce a live attenuated vaccine. It has been cultured on ten plates of nutrient agar and incubated for 24 hours at 37 ^oC, then they harvested on 100 ml of autoclaved PBS, the concentration of bacteria adjusted to be 1.2 X 10^9 CFU \ ml by a spectrophotometer which was equal to (0.976) at OD 600, then an equal volume of 3% H_2O_2 was added to the bacterial suspension and incubated for 2 hours at 37 ^oC after that the bacterial suspension centrifuged by cold centrifuge at 4100 xg for one hour, the supernatant was removed and the pellet was washed three times by sterile PBS then centrifuged by cold centrifuge at 4100 xg for ten minutes then the pellet was dissolved in 100 ml of sterile PBS and vortexed well to form a homogenous bacterial suspension. One drop of it was cultured on 5% of sheep blood agar and incubated for 24 hours at 37 ^oC. A single colony of growing bacteria on 5% sheep blood agar was elected to further attenuation by serial passage on nutrient agar every 24 hours for thirty times. After that single colony of attenuated bacteria returned to grow at 5% of sheep blood agar for a second time for 24 hours at 37 ^oC then it was harvested and preserved as a bacterial vaccine seed of live attenuated MRSA at deep freeze (-80 ^oC) to be used later. Bacterial vaccine seed was prepared by culturing it at 5% of sheep blood agar and incubated for 24 hours at 37 ^oC to reactivate it, then it was cultured in ten plates of nutrient agar for another 24 hours at 37 ^oC, then growing bacterial masses were harvested on 50 ml of sterile PBS and it is concentration was adjusted to be 1.2 X 10^9 CFU/ml by a spectrophotometer which was equal to (0.976) at OD 600 which was used in vaccination of experimental animals as a live attenuated vaccine.

Animal's model

It has been used fifty-five male Wistar rats their weights ranged 200-225 grams /animal and ages at eight weeks. Forty-five of them have been divided into three equal groups by fifteen animals for every group used in the vaccination program whereas reminding ten animals were used in the determination of safety tests for the prepared live attenuated vaccine.

Study design

Groups division

Forty-five animals were divided into three main groups and each main group was divided into three sub-groups: the first group (with their three sub-groups) were used experimentally to study the effect of the prepared live attenuated vaccine on the immune response while the second group (with their three subgroup animals) were used as positive control which they were vaccinated with inactivated commercial vaccine and third group (with their three subgroup animals) were used as negative control groups which they treated with PBS only.

Vaccination schedule

Prepared live attenuated vaccine: Prime vaccination at zero-day (0.3 ml) by S/C injection route without using of adjuvant nor booster dose. Inactivated MASTIVAC® OVEJERO commercial polyvalent vaccine as (control positive group) prime vaccination at zero-day (0.3ml) by S/C injection route, first booster dose at 14 days (0.25ml), second booster dose at 28 days(0.2ml). PBS as control negative group prime treatment with PBS only at zero-day, first treatment as an indicator for the first booster at 14 days, second treatment as an indicator for the second booster at 28 days.

Blood and tissue collection

At 5 days after administration of prime dose vaccine for each group, first sub-group animals were killed according to animal ethics

committees. Blood was collected from them and serum was separated to detect each of CD4+ and CD8+ T-cells concentrations by ELISA and liver tissue from each animal was collected to detect gene expression and concentrations each of IL-2 and IFN-γ by RT-qPCR. At 19 days the second subgroup animals were killed after blood was collected from them and serum separated to follow up of CD4+ and CD8+ T-cells concentrations and liver tissue from each animal was collected to follow each of IL-2 and IFN-γ concentrations. At 33 days the last remaining subgroup animals were killed after blood was collected from them and serum separated to follow up of CD4+ and CD8+ T-cells concentrations and liver tissue from each animal was collected to follow each of IL-2 and IFN-γ concentrations.

DNA extraction

DNA was extracted using a commercial DNA extraction kit from Geneaid company/ United Kingdom type Presto^{Tm} Mini g DNA Bacterial kit according to manufacturer's instruction.

Primers sequences used in PCR: nuc gene:

F 5′-GCGATTGATGGTGATACGGTT-3′, R 5′-AGCCAAGCCTTGACGAACTAAAGC-3′ with product size 279 bp [13], *ica A* gene: F5′-GAGGTAAAGCCAACGCACTC-3′, R5′-CCTGTAACCGCACCAAGTTT-3′ with product size 151 bp [14], *ica D* gene: F 5′-ACCCAACGCTAAAATCATCG-3′, R 5′-GCGAAAATGCCCATAGTTTC-3′with product size 211 bp [14], *mec A* gene: F 5′-AAAATC GATGGTAAAGGTTGGC-3′, R 5′- AGTTCTGCAGTACCGGATTTGC-3′ with product size 533 bp [15].

Polymerase chain reaction (PCR)

The contents of PCR reaction were put in micro-centrifuge tube 200 µl (Eppendorf tubes) included 4 µl of the extracted genomic DNA of MRSA + 1 µL from each of forward and reverse primer + 12.5 µL of master mix type (Green mix) PROMEGA USA + 6.5 µL of nuclease-free water PROMEGA USA. The tube has been mixed well by a mini centrifuge. Then it entered to thermocycler according to soft program for each gene as follow: *nuc* gen: 1 cycle of primary denaturation at 94 ˚ for 5 min, 34 cycles of three stages (denaturation at 94˚for 1 min, annealing at 55 ˚ for 1 min, extension at 72 ˚ for 1 min), then 1 cycle of final extension at 72 ˚ for 10 min. *ica A* gene and *ica D* gene: 1 cycle of primary denaturation at 95 ˚ for 5 min, 40 cycles of three stages (denaturation at 95˚for 20 seconds, annealing at 60 ˚ for 20 seconds, extension at 72 ˚ for 20 seconds), then 1 cycle of final extension at 72 ˚ for 3 min. *mecA* gene: 1 cycle of primary denaturation at 95 ˚ for 5 min, 34 cycles of three stages (denaturation at 94˚for 30 seconds, annealing at 55 ˚ for 30 seconds, extension at 72 ˚ for 1 min), then 1 cycle of final extension at 72 ˚ for 5 min. Then PCR product was electrophoresed in 2% of an agarose gel that stained with ethidium bromide and DNA ladder with 11 fragments 100-1000 bp plus a band at 1500 bp in size (PROMEGA/USA).

RNA extraction

Gene expression levels of mRNA for each IL-2, IFN-γ genes have been extracted by using commercial RNA extraction kit (SV Total RNA Isolation System) from Promega company/ USA. RNA has been extracted according to the manufacturer's instructions.

Converting of extracted RNA to cDNA

The extracted RNA has been converted into cDNA by using the commercial kit of reverse transcriptase enzyme system from Promega (GoScript^{Tm} Reverse Transcriptase System/ Promega) according to the manufacturer's instruction using conventional PCR.

RT-qPCR test:

Eco 48 Real-Time PCR system apparatus (PCR max limited)/United Kingdom has been used in RT-qPCR. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) has been used as a housekeeping gene, amplification was performed by adding 20µl for each well as 10 µl of master mix type (Go Script™ RT-qPCR master mix) from Promega / USA + 2.5 µl from each of forward and reverse primers + 5 µl of cDNA, the same amounts used with housekeeping gene GAPDH. All components were mixed well and the plate was sealed and placed directly in the RT-qPCR thermocycler which was set previously by it is software, finally, it runs with a specific program used for this amplification. The running program consist of initial denaturation 95 ^{o}C for 2 minutes/1 cycle, 40 cycles of three stages (denaturation 95 ^{o}C for15 second/, annealing 60 ^{o}C for1 minute, extension 60 ^{o}C for 1 minute). IL-2 and IFN-γ primers used in RT-qPCR were designed and blotted according to NCBI reference design code, using Rattus norvegicus IL-2 and IFN-γ mRNA GenBank sequence with accession numbers of NM_053836.1 and NM_138880.3 for each of them respectively.

Primers sequences used in RT-qPCR

IL-2 and IFN-γ primers used in the RT-qPCR test were designed and blotted according to NCBI reference design code, using Rattus norvegicus IL-2 and IFN-γ mRNA GenBank sequence with accession numbers of NM_053836.1 and NM_138880.3 for each of them respectively, housekeeping gene used according to Laukova *et al.* [16]. Their sequences as, IL- 2 gene: F 5′-TGCAGCGTGTGTTGGATTTG-3′, R 5′-TGGCTCATCATCGAATTGGC-3′; IFN-γ gene: 5′- ATCGAATCGCACCTGATCAC -3′, R 5′- TTGGCGATGCTCATGAATGC -3′; GAPDH: 5›-AGATCCACAACGGATACATT- 3′, R 5›-TCCCTCAAGATTGTCAGCAA- 3′.

Data analysis

After amplification was completed, all results of target and housekeeping genes were analyzed by using fold change 2^ΔΔCT [17].

ELISA assay

ELABSCIENCE®/USA ELISA kits were used for determining the concentrations of CD4+ and CD8+T-cells according to the manufacturer's instructions.

Statistical analysis

SPSS software was used (One-way analysis ANOVA) with LSD and Duncan applied for data analysis. Significance estimated at P-value ≤ 0.05 with mean ±Standard Deviation.

Results

MRSA confirmation by PCR

The PCR result showed that the local isolated was methicillin-resistant *Staphylococcus aureus* (MRSA). It was successfully amplified each of *nuc gene* by using of specific set primers appears as a clear band with 279 bp in size and *mec A gene* by using of a specific set of primer appears as a clear band with 533 bp in size also both of *ica A* and *ica D gene*s were confirmed by using of a specific set of primers and appeared as a clear band with 151 bp and 211 bp in size respectively for each of them, after agarose gel electrophoresis has been done for PCR product indicating that *S. aureus* was positive for all genes used in the study Fig. (1).

Safety test

The result of the safety test showed no any undesirable side effects nor systemic reaction and also no granuloma formation or any other post-vaccination reaction or effects, indicating the safety of the vaccine.

CD4+ T-cells concentration

The results showed by using ELISA a significant increase in the concentration of CD4+ T-cells in vaccinated groups with prepared live attenuated vaccine at each of 5 and 33 days only whereas groups vaccinated with inactivated commercial vaccine showed increasing in CD4+ T- cells at all times 5,19,33 days respectively when they compared with the non-vaccinated

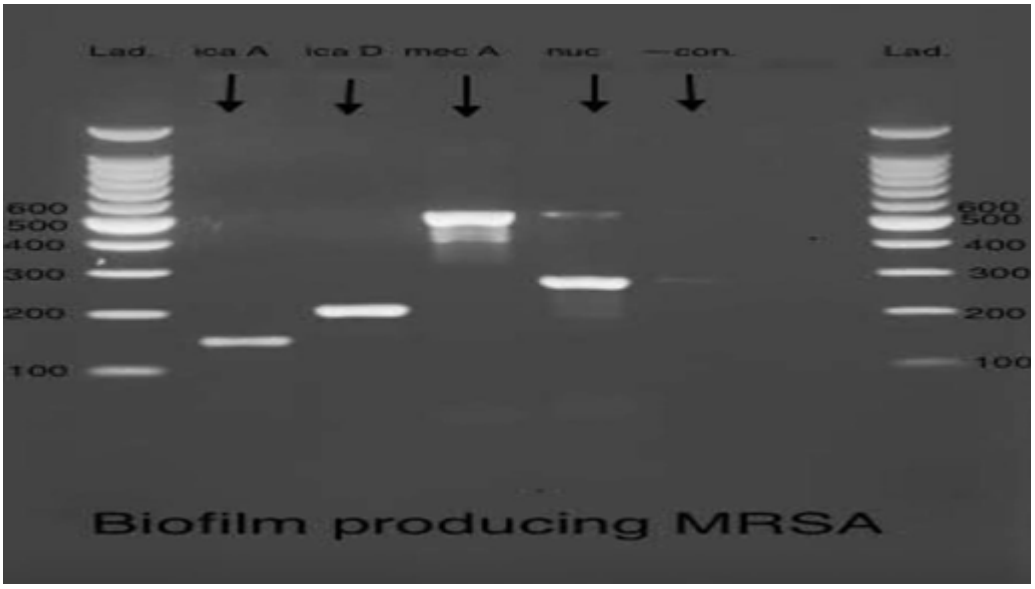

Fig. 1. Amplification result as a clear band with 151 bp, 211 bp, 533, and 279 bp in size for each of *ica A* gene, *ica D* gene, *mec A gene* and *nuc* gene by using a specific set of primers for each gene. Lane 1 and Lane 8: Ladder, Lane 2: *ica A* gene, Lane 3: *ica D* gene, Lane 4: *mec A* gen, Lane 5: *nuc gene*, Lane 6: control negative.

control group but with the progression of time, prepared live attenuated vaccine showed a significant increase at 33 days in compared to 19 days (Table 1)

CD8+ T-cells concentrations

The results of ELISA showed a significant increase in the concentration of CD8+ T-cells in vaccinated groups with prepared live attenuated vaccine at each of 5 and 33 days respectively whereas groups vaccinated with inactivated commercial vaccine showed increasing at 5 days only when they compared with a non-vaccinated control group (Table 2).

RT-qPCR results

IL-2 gene expression change fold

The results showed that the vaccinated group with the live attenuated vaccine showed up-regulation in IL-2 gene expression and very sharp increase in change folds reached to 2612.11 and 82.48 at 5 and 19 days respectively returned to decrease in their values to reach for 1.46 times change folds at 33 days while the vaccinated group with inactivated commercial vaccine also showed up-regulation of IL-2 gene expression and increase in change folds reached to only 5.98, 2.58, 2.43 times at 5,19,33 days respectively (Table 3) and (Fig. s 2) respectively.

IFN-γ gene expression change fold

The RT-qPCR results showed that that group vaccinated with the live attenuated vaccine was showed up-regulation of IFN-γ gene expression with a sharp increase in change folds reached to 704.28 and 23.87 times at 5 and 19 days respectively returned to down-regulation with no any change at 33 days while group vaccinated with the inactivated commercial vaccine showed down-regulation in the IFN-γ gene expression with no any changes at any time (Table 4) and (Fig. 3), respectively.

TABLE 1. The CD4+ T-cells concentration (pg/ml) at 5, 19 and 33 days in vaccinated and control groups.

Time / Group	5 days	19 days	33 days
Control (non-vaccinated)	742.89± 86.83	815.31± 112.35	767.84± 63.25
Inactivated commercial vaccine	1941.43 ± 130.28^{A}	1540.39 ± 374.72^{A}	1825.09 ± 390.27^{A}
Live attenuated vaccine	1499.08 ± 306.47^{Aa}	1077.28 ± 259.88$^{a\,*}$	1590.78 ± 253.67 $^{A\,**}$

Data were presented as mean ± SD
Significance value under P ≤ 0.05
Capital letters mean the presence of significant differences between vaccinated and non-vaccinated control groups in the same column
Small litters mean presence significance between groups each to other at the same column
* and ** mean presence significance inside the same group with time progression at the same row

TABLE 2. The CD8+ T-cells concentration (pg/ml) at 5, 19 and 33 days in vaccinated and control groups

Time / Group	5 days	19 days	33 days
Control (non-vaccinated)	44.17 ± 11.33	43.23 ± 9.29	42.12 ± 9.24
Commercial vaccine	66.44 ± 14.33^{A}	53.42 ± 20.99	59.85 ± 23.26
Live attenuated vaccine	61.23 ± 14.87^{A}	56.79 ± 14.20	72.60 ± 11.82^{A}

Data were presented as mean ± SD
Significance value under P ≤ 0.05
Capital letters mean presence significance between vaccinated and non-vaccinated control groups at the same column

TABLE 3. IL-2 gene expression change fold in vaccinated, control groups and housekeeping gene .

Group	Day's post vaccination	Mean CT	GAPDH mean CT	ΔCT Sample	ΔCT control	ΔΔCT	2^ΔΔCT
Live att. V.	days 5	27.69	17.38	10.30	21.65	-11.35	2612.11
	days 19	32.67	17.38	15.29	21.65	-6.37	82.48
	days 33	38.49	17.38	21.11	21.65	-0.54	1.46
Comm. V.	5 days	36.456	17.384	19.07	21.65	-2.58	5.98
	days 19	37.67	17.384	20.29	21.65	-1.37	2.58
	days 33	37.755	17.384	20.37	21.65	-1.28	2.43
Control		39.04	17.38	21.65	21.65	0.00	1.00

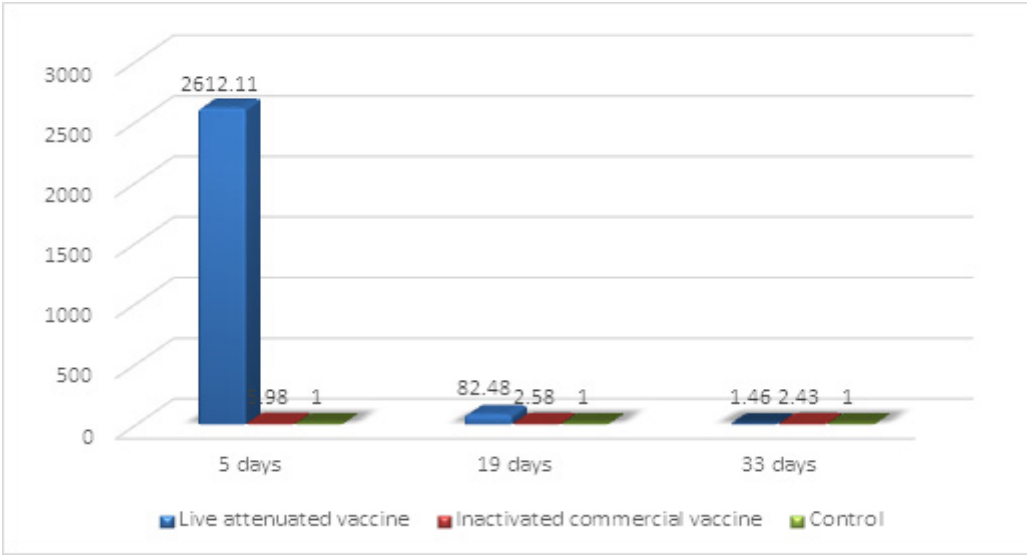

Fig. 2. IL-2 gene expression change fold in a vaccinated groups: live attenuated vaccine, inactivated commercial vaccine and control group

TABLE 4. IFN-γ gene expression change fold in vaccinated, control groups and housekeeping genes

Groups	Day's post vaccination	Mean CT	GAPDH mean CT	ΔCT Sample	ΔCT control	ΔΔCT	2^ΔΔCT
Live-attenuated .v	days 5	25.192	17.571	7.62	17.08	-9.46	**704.28**
	days 19	30.075	17.571	12.50	17.08	-4.58	**23.87**
	days 33	36.876	17.571	19.31	17.08	2.22	**0.21**
Commercial v.	5 days	37.518	17.571	19.95	17.08	2.87	**0.14**
	days 19	36.567	17.571	19.00	17.08	1.92	**0.27**
	days 33	35.524	17.571	17.95	17.08	0.87	**0.55**
Control		34.65	17.571	17.08	17.08	0.00	**1.00**

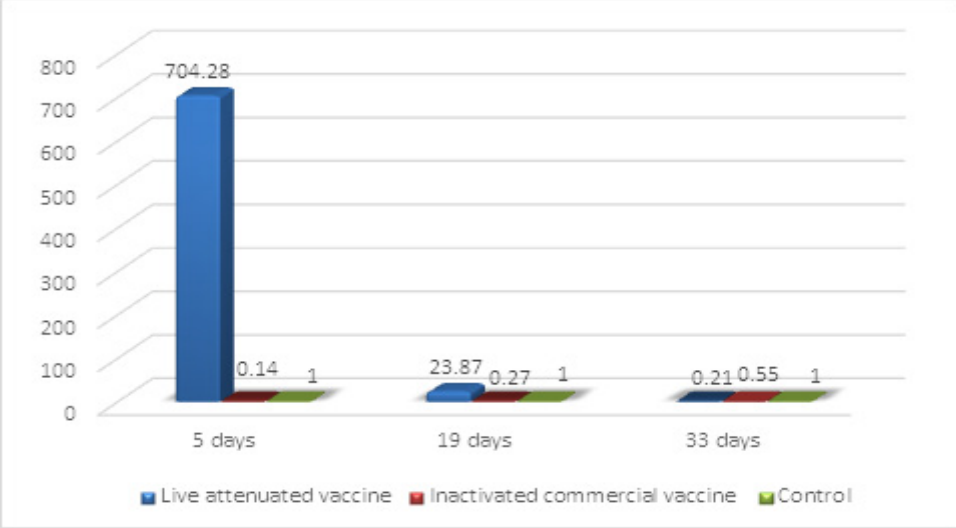

Fig. 3. IFN-γ gene expression change fold in a vaccinated groups: live attenuated vaccine, inactivated commercial vaccine and control group.

Discussion

Methicillin-resistant *Staphylococcus aureus* (MRSA) is considered a major challenge in the livestock sector; it was an important causative agent of bovine mastitis which causes large economic loss. MRSA has a wide range of virulence factors leading to increased severity of disease and ease evasion of host immune mechanisms leading to difficulty of control such as antibiotic resistance and biofilm formation and excretion of exotoxins making it very worse to treat it and control its effect changing the disease to chronic form [18]. So, vaccination is considered an easy and simple suitable method for controlling bovine mastitis caused by MRSA. The existing study aimed to produce a local vaccine from an MRSA isolate as an important method to control bovine mastitis caused by MRSA. In newly method live attenuated vaccine production, MRSA was attenuated first by adding 3%H_2O_2 for two hours which lead to exhaustion of it then serial passage for thirty times lead to complete attenuation of this bacteria. Our results revealed that attenuated vaccine stimulated both humoral and cell-mediated immunity by increasing CD4+ and CD8+ T-cells concentrations. Naïve CD4+ cells can be differentiated into functionally different types of sub-population helper T-cells [19], the important types are Th1, Th2. Th2 cells are responsible for the stimulation of humoral immunity by producing several types of cytokines such as IL-4 that affect B-cells directly leading to promote it is differentiation to more specific cells called plasma cells responsible for the production of specific antibodies, also produces IL-5, IL-13 which they play an important role in promoting humoral immunity and tissue repair in addition to their role in defense against extracellular pathogens and clearance of them [20].While Th1 cells are responsible for cell-mediated immunity by producing important cytokines such as IL-2, IFN-γ, GM-CSF, and TNF-alfa [21,22]. IFN-γ plays an important role in the activation of phagocytic cells that promote the killing activity of these cells for intracellular pathogens, also it can promote humoral immune response by helping B-cells to induce specific antibodies through antibody-dependent system phagocytosis, also it helps the activation of CD8+ T-cells indirectly [23] the results of existing study agreed with Lin et al., [23] which they revealed that vaccination of mice with adjuvanted Als3p-N vaccine enhanced CD4+ T-cells production that promoted Th1 cells production. Unsophisticated CD8+ T-cells can be differentiated to an activated type of T-cells called cytotoxic T cells (CTLs) which are responsible for the killing of target infected cells by releasing cytolytic enzymes. Kotfis et al., [24] referred that CD8+ T-cells were very important in the elimination of intracellular pathogens such as viruses and intracellular bacteria, and these cells are called cytotoxic T-cells. *S. aureus* bacteria may consider now a day non-classical facultative intracellular bacterium giving them the chance to behave as intracellular and extracellular behavior in the host at the same time [25]. So, the ability

of *S. aureus* to live intracellular pathogen gives the chance for CD8+ T-cells to differentiate to CTLs that kill these cells and eliminate these intracellular pathogens. Results of this current study revealed that the prepared live attenuated vaccine showed up-regulation of IL-2 gene expression by a sharp increase in their change folds in vaccinated groups by it, reached 2612.11 times. Also, IFN-γ showed up-regulation in IFN-γ gene expression by increasing their change folds in the vaccinated group with prepared live attenuated vaccine reached 704.28. Whereas groups vaccinated with inactivated commercial vaccine which was used as control positive vaccine showed upregulation but with a very slight increase in IL-2 gene expression reached only to 5.98 times in change folds with down-regulation and switch off IFN-γ gene expression when they compared with groups vaccinated with a prepared live attenuated vaccine which they showed a very sharp increase in both IL-2 and IFN-γ up-regulation. So, these results indicate and lead to a fact that prepared live attenuated vaccine was able to potent stimulation of cell-mediated immunity. The main function of IL-2 was activation of CMI through enhancing the effect of Cytotoxic T-lymphocytes CTLs and Natural killer cells NKs to the killing of infected cells by promoting secretion each of cytolytic enzymes such as perforins and granzymes, in addition to their enhancing ability to the secretion of other cytokines [26,27], furthermore, it plays an important role in the proliferation of CD8+ cells finally generation of a lineage of CD8+ memory cells which they are very important in the future [28]. Another fact promotes and corresponds with these results above is that differentiated CD4+ T-cells into Th1 linage cells, that plays a crucial role in CMI by producing each of IL-2 and IFN-γ with high levels that finally promote an immune response against intracellular pathogens, virally infected cells, other inflammatory diseases that initiated by pathogens [29]. In addition to Th1 cells as a main source of IFN-γ also CD8+ T-cells contribute with it in this function, also NKs and NK T-cells secrete it at the innate immune response [30]. Our results agreed with Yu *et al.,* [31] that revealed stimulation of each Th1 and Th2 by significant increases in their cytokine profiles among them IFN-γ level in mice. Also, Kolla *et al.,* [32] designated multi-epitope vaccine was stimulated both humoral and cell-mediated immunity with an increase in antibody levels and increasing levels of IL-2 and IFN-γ with other cytokines through simulation study which was agreed with our result by increasing secretion each of IL-2 and IFN-γ.

Conclusion

It was concluded from this study that MRSA was exhausted by using 3% H_2O_2 for two hours then it completely attenuated by serial passage for thirty times. Prepared live attenuated vaccine by this newly method proved their ability to stimulate immune response both humoral and potent cell-mediated immunity in rats and this promising result may candidate it to be used in clinical stages of vaccine production in the future.

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Conflict of Interest

The authors revealed that there is no conflict of interest.

Funding statement

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Ethical consideration

The study was done according to animal ethics committees AECs which included adequate care, matters relevant to animal welfare, support on the welfare at the time of the killing and euthanized killing of animals were taken into consideration at the time of research**.**

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طريقة جديدة لتحضير اللقاح الحي المضعف من العزلة المحلية لجرثومة المكورات العنقودية الذهبية المقاومة للميثيسيلين ودراسة تأثيرها على الاستجابة المناعية في الجرذان

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استهدفت هذه الدراسة تحضير لقاح حي مضعف من العزلة المحلية للمكورات العنقودية الذهبية المقاومة للميثيسيلين (MRSA) بطريقة جديدة كمحاولة للسيطرة على التهاب الضرع البقري. اكدت عزلة MRSA بواسطة اختبار تفاعل البلمرة المتسلسل PCR، وبعدها حضر اللقاح الحي المضعف منها بطريقة جديدة اعتمدت على استخدام 3% H_2O_2 متبوعًا بالتمريرات المتسلسلة لثلاثين مرة. استخدم ذكور الجرذان البيضاء كحيوانات تجربة. واستخدم اختبار مقايسة الممتز المناعي المرتبط بالإنزيم ELISA للكشف عن تراكيز الخلايا التائية +CD4 و+CD8 في حين استخدم اختبار تفاعل البلمرة المتسلسل بالزمن الحقيقي RT-qPCR للكشف الكمي عن التعبير الجيني لكل من جينات IL-2 وIFN-γ لتقدير الاستجابة المناعية المتولدة نتيجة التلقيح. أظهرت النتائج زيادة معنوية في تراكيز الخلايا التائية +CD4 و+CD8 باستخدام اختبارELISA. وأظهر اختبار RT-qPCR تنظيمًا ايجابيا في التعبير الجيني لكل من جينات IL-2 وIFN-γ وزيادة حادة في تركيزهما وصلت إلى ٢٦١٢,١١ و٧٠٤,٢٨ ضعفا لكل منهما على التوالي. زيادة الخلايا التائية +CD4عززت الاستجابة المناعية الخلطية بتمايزها إلى الخلايا التائية المساعدة من النوع الثاني Th2 وعززت المناعة الخلوية ايضا بتمايزها إلى الخلايا التائية المساعدة من النوع الاول Th1، إضافة الى تعزيزها لعمل IFN-γ من خلال آلية ردود الفعل الإيجابية والتي بدورها ضخمت عمل Th1. زيادة الخلايا التائية +CD8 عززت الاستجابة المناعية الخلوية عن طريق تمايزها الى الخلايا الليمفاوية التائية السامة للخلايا (CTLs) والتي ساهمت في قتل الخلايا المصابة بالمسببات المرضية الداخل الخلوية، بالإضافة إلى تأثير IL-2 التي عززت تمايز هذه الخلايا CTLs أيضًا. استنتجت من هذه الدراسة أن اللقاح التجريبي المحضر محليا بهذه الطريقة حققت نتائج واضحة وملموسة في تحفيزها للمناعة الخلطية والخلوية بشكل واضح، وتعتبر هذه الطريقة من الطرق الجديدة في إنتاج اللقاح الحي المضعف.