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Bacteriological and molecular studies on *Staphylococcus aureus* isolated from mastitic cows using Vitek2 compact system

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Abstract

Staphylococcus aureus is the most frequently bacterial pathogen isolated from cattle and buffaloes mastitic milk. In this study, 100 cow's milk samples suffered from clinical mastitis were collected and subjected to bacteriological and molecular characterization of *Staphylococcus aureus.* The results revealed that *50 Staphylococcus aureus* was isolated in an incidence of 50% of which 36 (72%) were coagulase positive and 14 (28%) were coagulase negative.

The virulence genes of *Staphylococcus aureus* have been effectively detected using multiplex PCR. The detected virulence genes were *coa, spa, hlg*, and *clfA* as 5, 6, 3 and 3 positive strains out of 8 tested isolates by the amplification at 630bp, 226bp, 937pb, and 638bp, respectively.

Also, the antibiotic resistant genes *mecA*, and *blaZ* of *Staphylococcus aureus* have been effectively detected as 6 and 8 positive strains out of 8 tested isolates by the amplification at 310bp and173bp, respectively. The results were fully discussed and field recommendations were pointed out.

Keywords: *Staphylococcus aureus, Mastitis, Virulence genes and Cows.*

Introduction

Generally, milk is one of few foodstuffs consumed as it is in its natural state. There are many microorganisms may contaminate milk via different sources including hands of milkers, udder, air, dust, flies, rodents and polluted water supply. Therefore the presence of organisms in milk constitutes a public health hazards and may produce dreadful diarrhea, gastritis, epidemic diarrhea in infants and food poisoning.

Staphylococcus aureus is one of the most common etiological pathogens, causing intra mammary infections in dairy herds leading to severe economic losses in worldwide industry **(Rubin *et al.,* 1999; Shorr, 2007 and Saleh *et al.,* 2016).** Accurate identification of the *Staphylococcus aureus* is therefore of great importance in bacteriological laboratory, The vitek2 system used with Gram positive (GP) identification card **(Biomereux, 2000)** is an automated machine designed to provide rapid and accurate phenotypic identification for most clinical staphylococci **(Funke and Funke, 2005).** *Staphylococcus aureus* has been exhibit several and different phenotypic properties that causing a variable response to antibiotics treatment. This organism produces a large number of potential virulence factors which have important roles in the pathogenesis **(Hassan *et al.*, 2010 and Mohammad *et al.*, 2015).** These include, coagulase (coa) (bound and free coagulases) which clots plasma and coats the bacterial cell so prevent phagocytosis, it considered as one of the most important virulence factors, this enzyme enables staphylococci to captured within a fibrin meshwork, dissemi-

nate and resist opsonophagocytic clearance mechanism of the host immune cells **(Duran *et al.*, 2012 and Enany *et al.*, 2013).** Staphylococcal protein A (spa) is a membrane-bound exoprotein of bacterial cell wall that bind to the immunoglobulins through impaired opsonisation by serum complement and phagocytosis of poly morophnuclear leukocyte (PML). *Staphylococcus aureus* developed a high resistance against a wide variety of antibiotics which increase their virulence. The most important antibiotic resistance genes of *S. aureus* were B-lactamase that may be mediated by the *blaZ* and *mecA* genes which designated for methicillin resistance (MRSA) that coded for penicillin binding protein 2a **(Katayama *et al.*, 2005).** Most bovine staphylococcal strains produce a and b-hemolysin, encoded by *Hla* has been suggested to be involved in gangarenous bovine mastitis from epidemiological point the presence of hyper virulent strains of *Staphylococcus aureus* showed the need to detect and characterize these strains for better monitoring the dissemination and suitable therapeutic for reduction of disease severity **(Cremieux *et al.*, 2009).**

Milk is consider as the most perfect single food that contains all digestible nutrients needed by the body in a proper and well balanced proportions.

Therefore, the main aim from this work is to characterize *S. aureus* isolated from cow's milk samples and detect their virulence and antibiotic resistance genes.

Materials and Methods

Milk samples

A total 100 milk samples were collected from mastitic lactating cows at Giza governorate from small-holders farmers. The samples were collected according the procedure described by **Brown *et al.* (1981).**

Staphylococcus aureus isolation and identification

Milk samples were incubated aerobically for 24 hours (h) at 37°C to achieved initial bacterial growth and a loopfull was taken from each sample and streaked onto blood agar and Baired parker agar medium. All plates were incubated aerobically at 37°C for 24-48 h. Bacterial colonies were identified by cultural, morphological and biochemical characters as described by **Quinn *et al.* (2002).**

Tube coagulase test

The tube coagulase test was done according to the American Public Health Association **(A.P.H.A.,1992)**.

Deoxyribonuclease (DNase) activity

Deoxyribonuclease (DNase) activity was done according to **Murray *et al.* (2003).**

Identification method with Vitek2 system

The test panels (ID-GPC, biomerieux) were automatically filled by a culture suspensions in 0.45 saline, sealed and inserted into the Vitek2 reader-incubator module (biomerieux) and subjected to a kinetic fluorescence measurement every 15 min. The results were interpreted by the ID-GPC database and final results were obtained automatically according to **Chatzigeoriou *et al.* (2011)**.

Antibiotic sensitivity test of the isolates was carried out by Vitek2 compact system using AST-GN card **Chatzigeoriou *et al.* (2011).**

Multiplex PCR detection of the virulence genes of *S. aureus* isolates (*coa, spa, hlg* and *clfA* genes) as well as (*blaZ* and *mecA*) resistance genes.

DNA extraction

DNA extraction from random eight *S. aureus* isolates was done using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations and according to **Sambrook *et al.* (1989).** Briefly, 200 µl of the sample suspension was added to 10 µl of the proteinase K and 200 µl of the lysis buffer and incubated at 56˚C for 10 min. Then, 200 µl of 100% ethyl alcohol was added to the lysate. After washing and centrifuging the sample, 100 µl of elution buffer that provided by the kit was used to elute the nucleic acid.

PCR amplification

PCR amplification was applied on 8 random isolates of *S. aureus* using primers that revealed to (*coa, spa, hlg*, and *clfA*) genes as virulent genes and two resistance genes of *S. aureus* as (*blaZ* and *mecA*). This PCR amplification of these genes was carried out using the following primers (Table1). These primers were utilized in a 25 µl reaction containing 12.5 µl of PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentration, 5.5 µl of nuclease-free water, and 5 µl of DNA template. The reaction was performed in an (Applied Biosystem Thermal Cycler). Cycling conditions of the different primers during the PCR amplification following the manufacturer's recommendations: primary denaturation: 94°C-5 min., secondary denaturation: 94°C-30 sec., annealing: 55°C-45 sec., extension: 72°C-45 sec., no. of cycles: 35 and final extension: 72°C-10 min.

Analysis of the PCR Products

The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 20 µl of the PCR products was loaded in each gel well. The fragments sizes were determined using a gelpilot 100bp and 100bp plus DNA Ladders (Qiagen, Germany, GmbH). The gel was photographed by a gel documentation system (Alpha Innotech, Biometra).

Table (1). Primers used for the detection of virulent genes and resistance genes of *S. aureus,* F: Forward and R: Reverse.

Target genes	Primers sequences	Amplified Segment (bp)	Reference
coa	F:ATAGAGATGCTGGTACAGG	*350, 430, 570 or 630	**Lyer and Kumosani (2011)**
	R:GCTTCCGATTGTTCGATGC		
spa	F:TCAACAAAGAACAACAAAATGC	226	**Wada *et al.* (2010)**
	R:GCTTTCGGTGCTTGAGATTC		
hlg	F-GCCAATCCGTTATTAGAAAATGC	937	**Kumar *et al.* (2009)**
	R-CCATAGACGTAGCAACGGAT		
clfA	F-GCAAAATCCAGCACAACAGGAAACGA	638	**Scherrer *et al.* (2004)**
	R-CTTGATCTCCAGCCATAATTGGTGG		
blaZ	F:ACTTCAACACCTGCTGCTTTC	173	**Duran *et al.* (2012)**
	R:TGACCACTTTTATCAGCAACC		
mecA	F:GTAGAAATGACTGAACGTCCGATAA	310	**McClure *et al.* (2006)**
	R:CCAATTCCACATTGTTTCGGTCTAA		

* Four different bands may be detected 350bp, 430bp, 570bp or 630bp**.**

Results

Staphylococcus aureus was isolated from 50 out of 100 mastitic cow's milk samples with an incidence of 50%. *S. aureus* isolates were identified by cultural, morphological and biochemical characters, they shown to be positive by hemolysis on blood agar plates, and black on Baird-parker medium. The incidence of coagulase positive *Staphylococcus aureus* isolated was 36 isolates out of the 50 positive *S. aureus* as shown in Table (2). Also, the isolates showed to be positive for the DNase activity on DNase agar medium. Where, the appearance of clear zone around the colonies indicates the production of DNase.

Table (2). The prevalence of coagulase positive *Staphylococcus aureus* isolated from the mastitic cow's milk.

No. examined samples	No. of *S. aureus* isolates	Coagulase positive		Coagulase negative	
		N	%	N	%
100	50	36	72%	14	28%

% was calculated according to total number of isolates and N is the number of the isolates.

Also, its biochemical identification was done using VITEK2 compact system and the results are shown in Table (3). Also, the antibiotic sensitivity test was done using VITEK2 compact system and the results showed that the isolates were resistant to oxacillin, rifampicin, and tetracycline. While these isolates were sensitive to ciprofloxacin, clindamycin, doxycycline, erythromycin, gentamicin, levofloxacin, moxifloxacin, nitrofurantion, tigecycline, trimethoprim/sulfamethoxazle and vancomycin as shown in table (4).

Table (3). Biochemical identification of *Staphylococcus aureus* using VITEK2 compact system

Biochemical details of *Staphylococcus aureus*																
2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	-	9	BGAL	-	11	AGLU	-
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	-
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	-	27	BGUR	-
28	AlaA	-	29	TyrA	-	30	dSOR	-	31	URE	-	32	POLYB	-	37	dGAL	-
38	dRIB	-	39	ILATk	-	42	LAC	-	44	NAG	-	45	Dmal	-	46	BACI	+
47	NOVO	-	50	NC6.5	-	52	dMNE	+	53	dMNE	+	54	MBdG	+	56	PUL	-
57	dRAF	-	58	O129R	-	59	SAL	+	60	SAC	+	62	dTRE	+	63	ADH2s	-

Table (4). Result analysis showed antibiotic sensitivity for *Staphylococcus aureus*

Antibiotic	Strength potency	Sensitive		Resistant	
		N	%*	N	%*
Ciprofloxacin	5µg	40	80	10	20
Clindamycin	25µg	38	76	12	24
Doxycycline	30µg	40	80	10	20
Erythromycin	10µg	40	80	10	20
Gentamicin	30µg	40	80	10	20
Levofloxacin	25µg	39	78	11	22
Moxifloxacin	30µg	41	82	9	18
Nitrofurantion	20µg	38	76	12	24
Oxacilin	1µg	10	20	40	80
Rifampicin	30µg	12	24	38	76
Tetracycline	30µg	12	24	38	76
Tigecycline	32µg	38	76	12	24
Trimethoprim /sulfamethoxazle	32µg	38	76	12	24
Vancomycin	32µg	38	76	12	24

*Percentages were calculated according to the No. of tested isolates (50).

The results of both virulence and antibiotic resistance genes in (8) *S. aureus* isolates from clinical mastitic milk are illustrated in Table (5). These results revealed that the antibiotic resistant gene *blaZ* is the most prevalent among the eight *S. aureus* isolates with a rate of 100%, followed by the antibiotic resistance gene *mecA* and the virulence gene *spa* with 75% each. Then, followed by the virulence gene *coa* with 62.5%. However, the virulence genes *hlg* and *clfA* were found in 37.5% each of the 8 isolates.

The *S. aureus* virulence genes based on *coa, spa, clfA* and *hlg* primers which revealed to *coa, spa, clfA* and *hlg* virulence genes of *S. aureus*, give amplicon at 630bp for *coa* gene Fig (1), 226bp for *spa* gene as in Fig (2), 638bp for *clfA* gene and 937bp for *hlg* gene as in Fig (3). While, the *S. aureus* antibiotic resistance genes based on *blaZ* and *mecA* primers which revealed to *blaZ* and *mecA* antibiotic resistance genes of *S. aureus* which give amplicon at 310bp for *mecA* and 173bp for *blaZ* gene as in Fig (4).

Table (5). Occurrence of some virulence and resistance genes *coa, spa, hlg, clfA, blaZ* and *mecA* in the eight *S. aureus* isolates from clinical mastitis.

Isolates	Virulent gene				Antibiotic resistant gene	
	coa	***spa***	***hlg***	***clfA***	***blaZ***	***mecA***
1	+	+	-	-	+	+
2	+	+	+	+	+	+
3	+	+	+	+	+	+
4	-	+	+	+	+	-
5	+	+	-	-	+	+
6	-	-	-	-	+	+
7	-	-	-	-	+	-
8	+	+	-	-	+	+
No. of positive	5	6	3	3	8	6
%*	62.5	75	37.5	37.5	100	75

* %: Calculated according to the No. of tested isolated isolates (8)

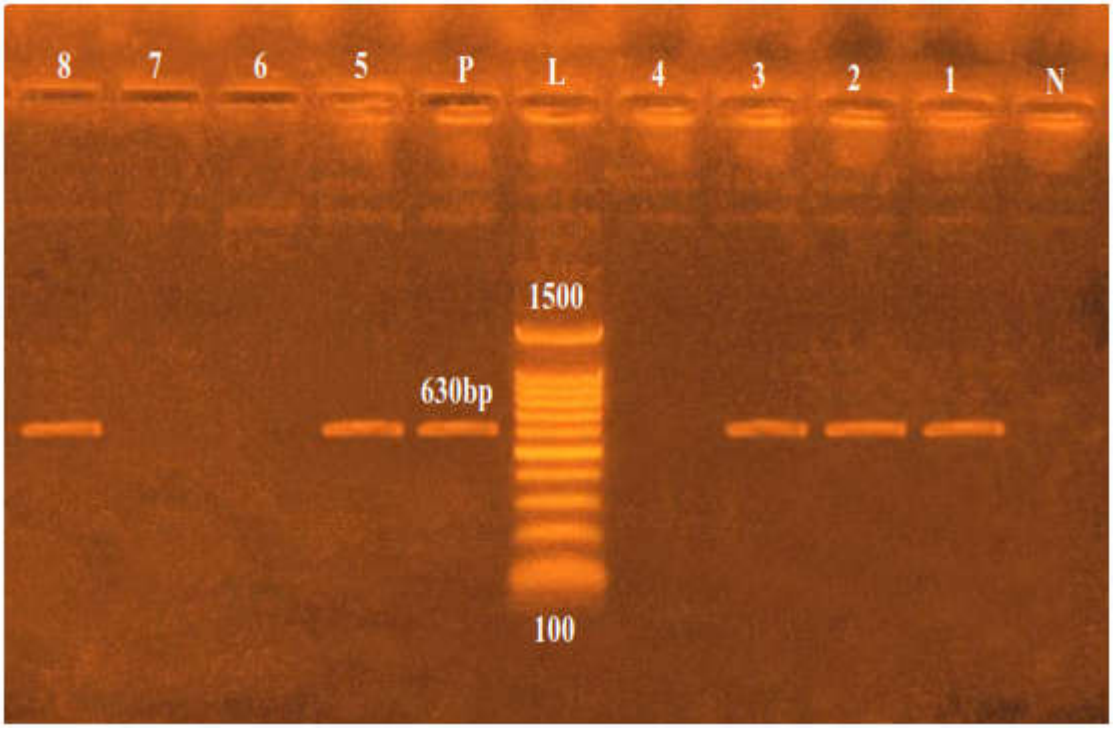

Fig. (1): Amplification of 630bp fragment of *coa* gene of *S. aureus isolates* (samples1, 2, 3, 5 and 8 are positive), N: negative control, P: positive control and L: DNA ladder (100-1500bp).

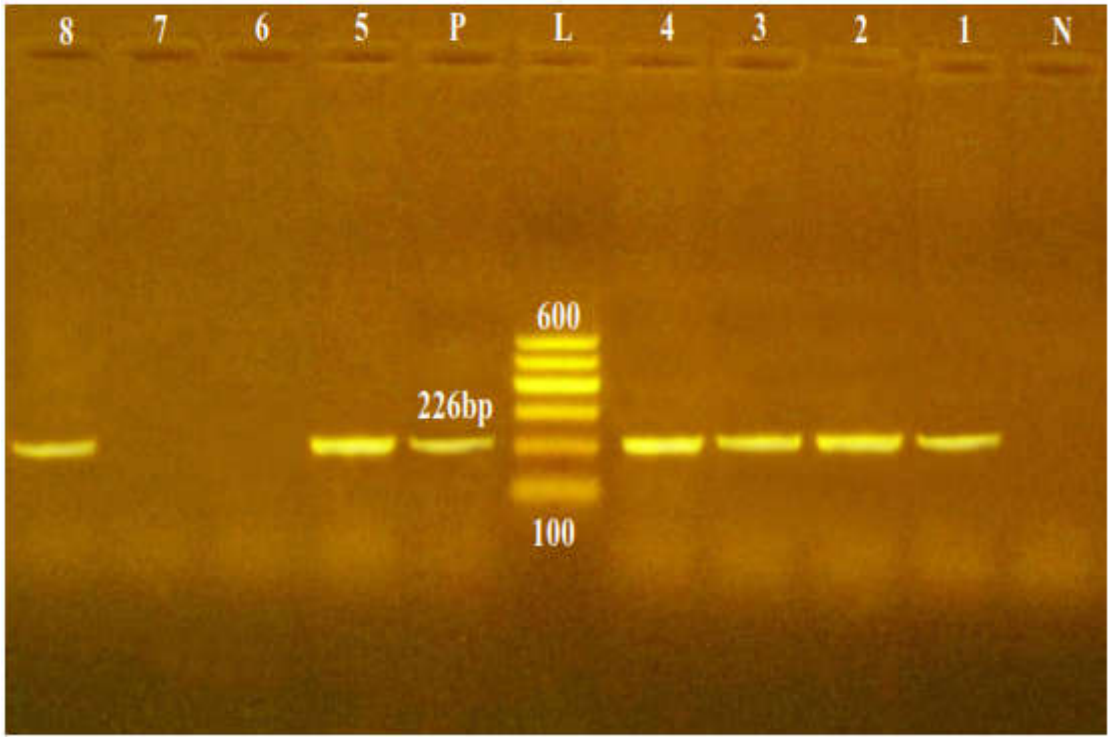

Fig. (2): Amplification of 226bp fragment of *spa* gene of *S. aureus* isolates (samples 1, 2, 3, 4, 5 and 8 are positive), N: negative control, P: positive control and L: DNA ladder (100-600bp).

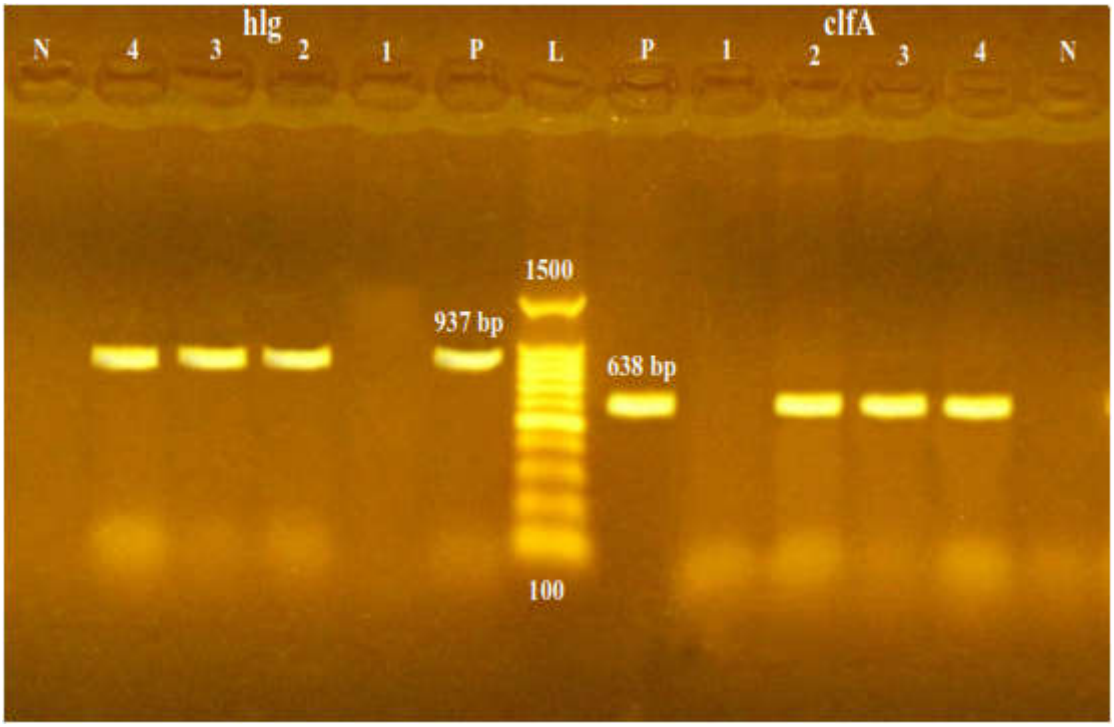

Fig. (3): Multiplex PCR amplification of *S. aureus* isolates extracted DNA for *hlg* and *clfA* genes, L: represents the molecular size marker (100- 1500bp DNA ladder), N: negative control, P: positive control of *hlg* (937bp) and 638bp for *clfA*, and Lane 2, 3 and 4are positive for both genes.

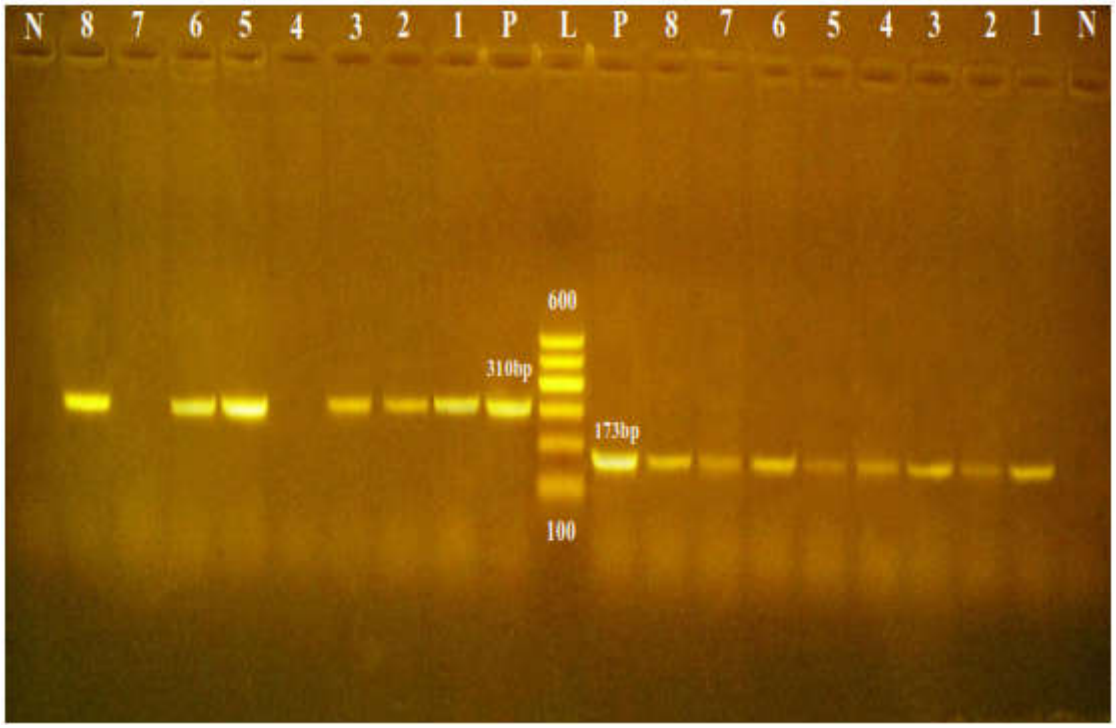

Fig. (4): Multiplex PCR amplification of *S. aureus* isolates extracted DNA for *mecA* and *blaZ* genes, L: represents the molecular size marker (100- 600bp DNA ladder), N: negative control, P: positive control of *mecA* (310bp) and 173bp for *blaZ*, samples 1, 2, 3, 5, 6 and 8 are positive for *mecA* gene and samples from 1 to 8 are positive for *blaZ* gene.

Discussion

Staphylococcus aureus is the most important bacterial microorganism causing highly economic losses in dairy herds. *Staphylococcus aureus* was isolated in the present study in an incidence of 50%, a result which nearly agreed with **Zecconi *et al.* (2006).**

In this study *Staphylococcus aureus* isolates were bacteriological examined and identified using the culturing on Baired parker medium, coagulase tube test and DNase test. This was in agreement with **Brown and Ngeno. (2007),** who reported that all *S. aureus* strains were positive for the coagulase test and give a typical morphology on Baird Parker agar. The prevalence of coagulase positive *S. aureus* was 72% in our study which agreed with **Bedane *et al.* (2012), and Hamza *et al.* (2015**). This high prevalence of *S. aureus* in this study may be explained that transmission of infection occurs during the milking process by milker's hands, contaminated equipments and milking machine **Scherrer *et al.* (2004).** Several virulence factors were produced by *S. aureus* including coagulase protein which encoded by coa gene which is important in the pathogenicity **Hassan *et al.* (2010) and Hamza *et al.* (2015).** Through turn fibrinogen to fibrin which lead to abscessiation and persistence of microorganism in host tissue **McAdow *et al.* (2011**). Furthermore, **Bhati *et al.* (2014**) concluded that coagulase is considered to be virulence factor in intrammmary infection. Coagulase gene can be used as a simple and effective method for typing of *S. aureus* isolates from cow's milk. The antibiotic sensitivity results showed that the isolates were resistant to oxacillin, rifampicin, and tetracycline. While these isolates were sensitive to ciprofloxacin, clindamycin, doxycycline, erythromycin, gentamicin, levofloxacin, moxifloxacin, nitrofurantion, tigecycline, trimethoprim / sulfamethoxazle and vancomycin, these results are in accordance with **(Rushdy *et al.*, 2007; Omar *et al.*, 2014; Pu *et al.*, 2014 and Ashraf *et al.*, 2016)**. In this research, *coa* gene was detected in five isolates (62.5%) and give a single amplicon of 630bp as shown in Fig (1). This seem to be agreed with **Enany *et al.* (2013) and Abdeen *et al.* (2015)** who recorded a single amplicon of *coa* genes at 600bp isolated from bovine milk, with no size polymorphisms of this gene. Moreover, the findings reported by **Cabral *et al.* (2004)** suggesting that the amplicon of about 600bp are predominant in bovine strains. Epidemiological studies indicate that *S. aureus* strains agents of milk produce a group of virulence factor and which believed that there is a relationship between severity of infection and the virulence factors produced by *Staphylococcus aureus*. Presence of *clfA, hlg* genes and protein A (*spa*) considered as the *Staphylococcus* species' virulence genes in development and severity of infection **Mohammed *et al.* (2015) and Ahmed *et al.* (2016).**

Staphylococcus aureus protein A (*spa*) which encoded by the *spa* gene is a major important surface proteins of bacterial cell wall product which binds with FC region of immunoglobulin G and impairs the opsonisation of serum complement and phagocytosis by polymorphonuclear leukocytes of the host immune system. So, the decrease in *spa* on cell surface of *S. aureus* resulted in increasing number of free receptor sites for complement C3b and phagocytosis (**Gao and Stewart, 2004, and Gharib *et al.*, 2013**). In this study amplification of *spa* gene of *Staphylococcus aureus* was detected at 229bp in 6 isolates (75%). (**Mohammad *et al.*, 2015**) achieved that *spa* gene can be used for typing the isolates of *S. aureus*. Detection of genetic polymorphisms in the X region of the *spa* gene can be used for typing of *S. aureus* **Gao and Stewart. (2004**). Also, **Karahan *et al.* (2011**) concluded that detection of *spa* gene polymorphisms with PCR proposed as good diagnostic methods for typing of *S. aureus* isolates which provide important results for the assessment of effective strategies against *Staphylococcal* control. Recently, the prevalence of antibiotic resistance in *S. aureus* strains become a serious problem in a dairy herds (**Wang *et al.*, 2008).**

Also, the *mecA* gene was detected at 310bp in 6 isolates (75%). The *mecA* gene on *S. aureus* isolates was the main gene responsible for resistance to methicillin through the production of Penicillin binding protein (PBP2a) as mentioned by **Ito *et al*. (2003).** Application of PCR for detection of *blaZ* gene can be recommended for routine clinical use in veterinary laboratories, *S. aureus* resistance to B-lactam antibiotics via hydrolyzing the 13-iactam ring and convert to inactive form (**Kernodle, 2000**). **Rich and Roberts (2004),** reported that the isolation of *S. aureus* strains methicillin resistant (MRSA) were recently increased. In our results all tested isolates of *S. aureus* subjected to PCR were positive for *blaZ* gene and give a single amplicon at 173bp. The same result obtained by **Chandrasekaran *et al.* (2014)** who recorded a single amplicon of 173bp of *blaZ* gene. Our results showed that all tested isolates for *mecA* gene were positive for *blaZ* genes and this come in agree with **Soares *et al.* (2012)** who noticed that all positive strains to *mecA* were also positive for *blaZ* gene and the presence of both genes was correlated to phenotypic beta-lactamic resistance.

Conclusion and Recommendation

Staphylococcus aureus considered to be the main cause of cows' mastitis. Vitek 2 compact system is a power full tool for the rapid and accurate diagnosis and identification of *Staphylococci* isolated from milk. The severity of mastitis in cows is greatly affected by the presence of antibiotic resistance and virulence genes in the causative *Staphylococcus* strains and this is easily detected by the application of PCR which is rapid and accurate technique for the detection of these genes mainly coa, spa, *hlg*, *clfA*, *mecA* and *blaZ* genes. Detection of subclinical mastitis test in dairy farms. Hygienic measures should be applied in dairy farms to minimize occurrence of mastitis, periodical application of disinfectants should be applied to kill pathogens causing mastitis and avoidance the miss use of antibiotics and use according to results of laboratory examination.

References

A.P.H.A, American Public Health Association (1992). Standard methods for the examination of dairy products 15th. American Public Health Association Washington, DC, USA.

Abdeen, E.; Walid, M.; Hussien, H. and Roshdy, S. (2015). PCR for detection of virulence and resistance genes of coagulase positive *Staphylococcus aureus* from clinical mastitis in Egypt. Int. J. Basic Appl. Sci., 4 (3): 315-319.

Ahmed, A.F.; Karsten, F.; Jehan, A.; Sameh, A.I. and Mohammed, A. (2016). Genotypes and virulence factors of *Staphylococcus aureus* isolated from bovine subclinical mastitis. Global Vet., 7 (5): 476-481.

Ashraf, A.; Ahmad, M.; Mokhtar, A.; Fatma, I.; Hofy, H. and Salem, S. (2016). Molecular characterization for some virulence and antibiotic resistance genes of *Staphylococcus aureus* isolated from dairy cattle's subclinical mastitis in EL-Sharkia Governorate. Benha Uni, Med. J., 30 (1): 219 -230.

Bedane, A.; Kasim, G.; Yohannis, T.; Habtamu, T.; Asseged, B. and Demelash, b. (2012). Study on prevalence and risk factors of bovine mastitis in Borona Pastoral and Agro-Pastoral Settings of Yabello District, Borana Zone , Southern Ethiopia, J. Agri. Environ. Sci., 12 (10): 1274-1281.

Bhati, T.; Nathawat, P.; Mir, I.A.; Sharma, S.; Yadav, R. and Kataria, A. (2014). PCR-RFLP of *Staphylococcus aureus* coagulase gene isolated from bovine subclinical mastitis, J. Pure Appl. Microbiol., 8 (6): 4711-4714.

Biomerioux (2000). Vitek2 product information, document 510769-4EN1. Biomerieux, Inc., Durham, NC, USA.

Brown, P.D. and Ngeno, C. (2007). Antimicrobial resistance in clinical isolates of *Staphylococcus aureus* from hospital and community sources in Southern Jamica. Int. J. Infect. Dis., 11(3): 220-225.

Brown, R.W.; Barnum, D.A.; Jasper, D.B.; McDonald, J. S. and Schultze, W.D. (1981). Microbiological procedures used in the diagnosis of bovine mastitis, 2nd ed. National Mastitis Council, Washington, DC, USA.

Carbal, K.G.; Lammler, C.; Zschoc, M.; Langoni, H.; Victoria, C. and DaSilva, A. (2004). Pheno- and genotyping of *Staphylococcus aureus*, isolated from bovine milk samples from Sao Paulo State, Brazil. Can. J. Microbiol., 50 (11): 901-909.

Chandrasekaran, D.; Venkastesan, P.; Tirummurugaan, K.G.; Subapriya, S.; Nambi, A.P.; Thirunavukkarasu, P.S. and Vairamuthu, S. (2014). Comparison of tests for detection of lactamase *Staphylococcus au*reus in mastitis cows: Int. J. Sci. Environ. Technol., 3: 1525-1527.

Chatzigeorgiou, K.S.; Sergentanis, T.N.; Tsiodras, S.; Hamodrakas, S.J. and Bagos, P.G. (2011). Phoenix 100 versus Vitek 2 in the identification of gram-positive and gram-negative bacteria: a comprehensive meta-analysis. J. Clin. Microbiol., 49: 3284-3329.

Cremieux, A.C.; Dumitrescu, O.; Lina, G.; Valle, C. and Cote, J.F. (2009). Panton-valentine leukocidin enhances the severity of community-associated methicillin-resistant *Staphlococcus aureus* rabbit osteomyelitis. PLoS ONE 4: e7204.

Duran, N.; Ozer, B.; Duran, G.G.; Onlen, Y. and Demir, C. (2012). Antibiotic resistance genes & susceptibility patterns in *Staphylococci*. Indian J. Med. Res., *135: 389-396.*

Enany, M.E.; Younes, S.; AL-gammal, A. M.; Salem and El Dieb, H.A. (2013). Prevalence of coagulase (*coa*) gene and *mecA* gene of *S. aureus* isolated from bovine clinical mastitis. Seuz Canal Vet. Med. J., XVIII (1): 147-157.

Funke, G. and Funke, K. (2005). Performance of the new Vitek2 GP card for identification of gram positive *Cocci* in a routine clinical laboratory. J. Clin. Microbiol., 43: 84 -88.

Gao, J. and Stewart, G.C. (2004). Regulatory elements of the *Staphylococcus aureus* protein A (*spa*) promoter. J. Bacteriol., 186: 3738-3748.

Gharib, A.A.; Adel, M.A. and Bendary, M. (2013). Detection of the *coa* gene in *Staphylococcus aureus* from different sources by Polymerase Chain Reaction. Int. J. Microbiol. Res., 4 (1): 37-42.

Hamza, D.A.; Dorgham, S.M. and Arafa, A. (2015). Coagulase gene typing with emphasis on methicillin-resistance *Staphylococci*: emergence to public health. Advanc. Infect. Dis., 5: 196-203.

Hassan, M.; Ebrahim, R. and Elaha, T. (2010). Detection of some virulence factors in *Staphylococcus aureus* isolated from clinical and subclinical bovine Mastitis, African J. Bacteriol., 9 (25): 3753-3758.

Ito, T.; Okuma, k.; Ma, X.; Yuzawa, H. and Hiramatsu, K. (2003). Insights on antibiotic resistance of *S. aureus* from its whole genome genomic island SCC. Microb. Drug. Resist., 6: 41-52.

Iyer, A.P. and Kumonsani, T.A. (2011). PCR based detection of nosocomial infection causing MRSA (Methicillin resistant *Staphylococcus aureus*). 2nd International Confer-

ence on Biotechnology and Food science IPCBEE vol.7, IACSIT press, Singapore.

Karahan, M.; Acik, M.N. and Cetinkaya, B. (2011). Investigation of virulence genes by PCR in *Staphylococcus aureus* isolates originated from subclinical bovine mastitis in Turkey. Pak. Vet. J., 31 (3): 249-253.

Katayama, Y.; Robinson, D.A.; ENRIGHT, M.C. and Chambers, H. (2005). Genetic background affects stability of *mecA* in *Staphlococcus aureus*. J. Clin. Microbiol., 43: 2380-2383.

Kernodle, D.S. (2000). Mechanism of resistance to Beta-Lactam antibiotics in Gram - positive pathogenes. American Society of Microbiology, Washington, DC, USA.

Kumar, J.D.; Negi, Y.K.; Gaur, A. and Khanna, D. (2009). Detection of virulence genes in *Staphylococcus aureus* isolated from paper currency. Int. J. infect. Dis.,13: e450-e455.

Mcadow, M.; Kim, H.K.; Dedent, A.C.; Hendrickx, A.P.; Schneewind, O. and Missiakas, M. (2011). Preventing *Staphylococcus aureus* species through the inhibition of its agglutination in blood. PLoS Pathog., 7: e1002307.

McClure, J.A.; Conly, J.M.; Lau, V.; Elsayed, S.; Louie, T.; Hutchins, W. and Zhang, K. (2006). Novel multiplex PCR assay for detection of *Staphylococcal* virulence marker panton-valentine leukocidin genes and simultaneous discrimination of methicillin-susceptible from resistant *Staphylococci*. J. Clin. Microbiol., 44:1141-1144.

Mohammed, S.; Abdelrahman, M. and Abdulla, D. (2015). Phenotypic and genotypic detection of virulence factors of *Staphylococcus aureus* isolated from clinical and subclinical mastitis in cattle, Vet. World J.,: 2231 -0916.

Murray, P.R.; Baron, E.J. and Jorgensen, J.H. (2003). Manual of clinical microbiology, 8thed, American Society for Microbiology , Washington, DC, USA.

Omar, N.Y.; Ali, H.A.; Harfoush, R.A. and El Khayat, E.H. (2014). Molecular typing of methicillin resistant *Staph. aureus* clinical isolates on the basis of protein a and coagulase gene polymorphisms. Int. J. Microbiol., 11: 112-123.

Pu, W.; Su, Y.; Li, J.; Li, C. and Yang, Z. (2014). High incidence of oxacillin-susceptible *mecA*-positive *Staph. aureus* (OS -MRSA) associated with bovine mastitis in China. PLoS ONE, 9 (2): 134-139.

Quinn, P.G.; Carter, M.E. and Markey, B.K. (2002). Clinical veterinary microbiology. Garfos: Mosby Ltd., St. Louis, USA.

Rich, M. and Roberts, L. (2004). Methicillin-resistant *Staphylococcus aureus* isolates from companion animals. Vet. Rec., 154: 310-315.

Rubin, R.J.; Harrington, C.A.; Poon, A.; Di etrich, K.; Greene J.A. and Molduddin, A. (1999). The economic impact of *Staphylococcus aureus* infection in New York city hospitals, Emerg. Infect. Dis., 5: 9-17.

Rushdy, A.A.; Salama, M.S. and Othman, A.S. (2007). Detection of methicillin/ oxacillin resistant *Staphylococcus aureus* isolated from some clinical hospitals in Cairo using Meca/Nuc genes and antibiotic susceptibility profile. Int. J. Agric. Biol., 9: 800-806.

Saleh, E.A.; Abd El-Mohsen, R.G. and Ibrahim, M.S. (2016). Molecular identification of *Staphylococcus aureus* in imported frozen and locally slaughtered meat. Alex. J. Vet. Sci., 51 (1): 162-169.

Sambrook, J.; Fritschi, E.F. and Maniatis,

T. (1989). Molecular cloning: A laboratory manual, Cold Spring Harbor Laboratory Press, New York, USA.

Scherrer, D.; Coti, S.; Muehlberr, J.E.; Zweife, C. and Stephan, R. (2004). Phenotypic and genotypic characteristics of *S. aureus* isolates from raw bulk-tank milk samples. Vet. Microbiol., 101: 101-107.

Shorr, A.F. (2007). Epidemiology and economic impact of methicillin-resistant *Staph-ylococcus aureus*. Pharmacoeconomics, 25 (9): 751-768.

Soares, L.C.; Pereira, I.A.; Pribul, B.R.; Oliva, M.S.; Coelho, S.M.O. and Souza, M.M.S. (2012). Antimicrobial resistance and detection of *mecA* and *blaZ* genes in coagulase-negative *Staphylococcus* isolated from bovine mastitis. Presq. Vet. BRAS., 32 (8): 692-696.

Wada, M.; Lkhagvadorj, E.; Bian, L.; Wang, C.; Chiba, Y.; Nagata, S.; Shimizu, T.; Yamashiro, Y.; Asahara, T. and Nomoto, K. (2010). Quantitative reverse transcription-PCR assay for the rapid detection of methicillin-resistant *Staphylococcus aureus*. J. Appl. Microbiol., 108: 779-788.

Wang, Y.; Wu, C.M.; Lu, L.M.; Ren, G.W.; Cao, X.Y. and Shen, J.Z. (2008). Macrolide-lincosamide-resistant phenotypes and genotypes of *Staphylococcus aureus* isolated from bovine clinical mastitis. Vet. Microbiol., 130: 118-125.

Zecconi, A.; Calvinho, L.F. and Fox, K.L. (2006). *Staphylococcus aureus* intramammary infections. IDF Bulletin, 408: 1-42.