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Risk Factor Analysis of *Salmonella* Typhimurium, *Staphylococcus aureus,* Standard Plate Count and Somatic Cell Count in Bulk Tank Milk in Cattle Dairies

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ABSTRACT

Bulk tank milk analysis was referred to as a useful and appropriate diagnostic tool to evaluate milk quality and mastitis pathogens in cattle dairy herds. Out of the total number of 150 pooled tank milk samples collected from 150 cattle dairy farms, 13 locally field *Staphylococcus aureus* isolates were detected and confirmed phenotypically by culturing, gram staining, biochemical, and molecular identification to be *Staphylococcus aureus* in the overall herd by the prevalence of 8.6%. Isolation and identification of *Salmonella* Typhimurium field isolates from bulk tank milk samples revealed that 20 locally field isolates were detected and confirmed phenotypically by culturing, gram staining, biochemical and molecular identification to be *Salmonella* Typhimurium in the overall herd by the prevalence of 13.3%. The results of total bacterial plate count (cfu/ml) revealed that the geometric mean of 150 dairy farms was 3.2×10^8 cfu/ml. The geometric mean of somatic cell count (SCC)/ml in Bulk tank milk samples of 150 cattle dairy farms were 556.7×10^3. The geometric mean value of *Staphylococcus aureus* count in this study was 3.7×10^3 cfu/ml. Serological identification of the 20 isolates revealed that they were *Salmonella* Typhimurium. The study provided various risk factors that had a clear and effective role in determining the level of *Salmonella* Typhimurium, *Staphylococcus aureus*, Standard plate count, and Somatic cell count in bulk tank milk. The PCR amplification with (*hlg*) gene-specific primers revealed a product with an approximate size of 937 bp. (*hlg*) gene found in 13 (54%) *Staphylococcus aureus* isolates. The PCR identification of *sopB* (*SigD*) virulence gene for *Salmonella* Typhimuriym revealed a product with an approximate size of 517 bp. *SopB* gene found in all *Salmonella* Typhimurium isolates (100%). Phylogenetic and partial gene sequence analysis of (*hlg*) *Staphylococcus aureus* gene of Egyptian isolated strain showed a great identity with the different *Staphylococcus aureus* strains uploaded from the gene bank. Phylogenetic analysis of *Salmonella* Typhimurium (*sopB*) virulence gene of Egyptian isolated strain indicated a great homology with the different *Salmonella* Typhimurium strains uploaded from the gene banks. The results of the present study emphasize the importance of more efficacious preventive programs for controlling the mastitis and bacteriological quality of bulk tank milk and monitoring mastitis economic losses.

Keywords: Mastitis, PCR, Phylogenetic analysis, Risk factors, *Salmonella* and *Staphylococcus aureus*

INTRODUCTION

The using of continuous and regular methods for monitoring and assessing udder health is extremely useful for improvements of udder health status, milk quality premiums programs. The regular assessment of bulk tank milk (BTM) analysis is regarded as one of the foremost significant tools to give insight and perception from the udder health status, and also the proper application of sanitary and hygienic measures in dairy herds, (Jayarao and Wolfgang, 2003). Bulk tank milk analysis is one of the most important diagnostic method for assessment of milk quality and mastitis pathogens (Godkin et al., 1993; Riekerink et al. 2006). There are numerous diagnostic tests commonly used to quantify and assess the quality of BTM, such as Somatic cell count (SCC) and standard plate count (SPC) (Cicconi-Hogan et al., 2012). The SPC assesses the bacterial count in the milk, and estimates the number of aerobic bacteria present per milliliter of milk. SPC is considered one of the foremost important tools to assess management and milk quality, as reported and described in previous studies and milk quality management recommendations (Schroeder, 2009). A high bulk tank SPC can be a consequence of bacteria from dirty milking equipment, milk from cows with subclinical or clinical mastitis, or contamination from dirty udders (Murphy and Boor, 2000). The regulatory cut-off for SPC was 100,000 cfu/mL (Cicconi-Hogan et al., 2012). Quantifying and determining of bulk milk SCC is an internationally recognized diagnostic tool to determine the quality of the milk and also the udder health status of the cattle within the herd. Many management practices were related to higher bulk milk SCC (BMSCC) (Schukken et al. 2003). Milk borne pathogens including *Salmonella* and *Staphylococcus aureus* were identified by several researches in BTM with various prevalence rates from dairy farms (Ruzante et al., 2010; Cicconi-Hogan et al., 2012). *Salmonella* was thought to be one of the main important

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and significant food borne bacterial diseases worldwide. Risk factors related to incidence of *Salmonella* in BTM was not previously identified. Fecal contamination related to poor milking hygiene was considered one of the most important causes of bulk tank contamination with *Salmonella* (Van Kessel et al. 2004). The effector protein genes *sopB* were located in numerous regions of the *Salmonella* chromosome, and were present in a wide range of *Salmonella* serotypes, supposing that this effector protein may play vital virulence functions (Mirold et al., 2001). *Staphylococcus aureus* was regarded as one in all of the most important contagious mastitis pathogens in dairy cattle, and was related to large economic losses (Halasa et al., 2007; Hogeveen et al., 2011; Keefe, 2012). Hemolysins produced by *Staphylococcus aureus* are very vital virulence factor, with cytotoxic action responsible and answerable for lysing erythrocytes, and it results in worsening of clinical signs during blood stream infections (Duan et al., 2018). The *Staphylococcus aureus* gamma-hemolysin contains two polypeptides, whereas the gamma-hemolysin locus (*hlg*) has three open reading frames (Cooney et al., 1993). Phylogenetic methods can be used to analyze nucleotide sequence data in such a way that the order of descent of related strains can be determined (Hall and Barlow, 2006). So the aim of this study is to quantify and determine the prevalence of *Salmonella* Typhimurium and *Staphylococcus aureus* in the BTM in cattle dairies, identifying the most important risk factors affecting the level of SPC, SCC, in BTM in cattle Dairies, identifying the most important risk factors affecting prevalence of *Salmonella* Typhimurium and *Staphylococcus aureus* in bulk tank milk in cattle dairies, molecular identification of *sopB* gene of *Salmonella* Typhimurium and *hlg* gene of *Staphylococcus aureus*, phylogenetic and gene sequence analysis of *sopB* gene of *Salmonella* Typhimurium and *hlg* gene of *Staphylococcus* aureus to give insight to the source and origin, molecular epidemiology and disease pattern of *Salmonella* Typhimurium and *Staphylococcus aureus* in Egypt's dairies.

MATERIALS AND METHODS

Ethical approval

The approval from the Institutional Animal Ethics Committee to hold out this study wasn't required as not contact and no invasive procedure on the animals were performed. However, current study was applied in accordance to the Institutional Animal Ethics of Animal Reproduction Research Institute (ARRI), Haram, Giza, Agricultural Research Center (ARC), and in accordance to the regulations and ethics of the European Union for the protection of experimental animals (2010/63/EU).

Study animals, study area and study design

A cross-sectional study was carried out from 2017 to 2019 in Delta region, Alexandria Road and upper and lower Egypt Districts from total number of one hundred and fifty (150) private dairy cattle farms with total population number of 8667 lactating cows belonging to Cairo, Giza, Qaluiobia, Sharkia, Monofia, Alexandria, Behera, Dakahlia, Benisuif, Fayoum, Sohag, Aswan and Asuit governorates.

Bulk tank milk sample collection and testing

A total number of 150 BTM samples were collected. The BTM samples were intended for checking the hygienic quality of raw milk collected in accordance to the standard methods described according to ISO707 (2008). After turning on the agitator for at least 10 minutes, 60ml BTM samples were collected from the top of the bulk tank using clean sanitized dipper. Samples were immediately refrigerated and transported to the laboratory in ice box, and examined within 24 hours after collection.

Microbiological examination of bulk tank milk samples

Preparation of serial dilution was carried out in accordance to methods described by APHA (1992) and APHA (2004). One ml completely mixed milk sample was transferred under aseptic conditions to 9ml of sterile ¼ strength Ringer's solution, and well mixed to urge 1/10 dilution. One ml from the first dilution was added to 9 ml of sterilized diluents to obtain tenth fold serial dilutions. The previous prepared dilutions were subjected to the subsequent microbiological examinations: The Total Bacterial Plate Count (TBPC) (cfu/ml) was assessed according to the methods descried by APHA (1992) and BAM on line (2009). Two ml of every previously prepared decimal dilution were inoculated into duplicate plates (one ml each), then 12 to 15 ml of Standard Plate Count Agar medium (Oxoid, CM0463) was added (cooled to 45 ± 1 °C) into each plate as well as control one. The plates were thoroughly mixed and incubated for 48 ±2 hours at 35 °C. Colony Forming Unit (CFU) per ml was calculated and recorded. The standard cut-off for SPC is 100,000 cfu/mL, in accordance with the European Union standards **(**Cicconi-Hogan et al., 2012). Analysis of BTM samples for SCC was performed according to the methods described by Zecconi et al. (2002). The SCC was quantified and measured using the electronic soma count 150, from Bentley (Chaska, MN 55318, United States). The standard cut off point of BSCC was 400, 000 cell/ml according to the regulations described by USDA (2011).

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Staphylococcus aureus count (cfu/ml) was assessed as reported by the methods illustrated by ISO 6888-1(1999) and ICMF (1986)**.** From each dilution, 0.1 ml was inoculated onto Baird parker agar plate. Inoculated plates were then incubated at 37 °C for 48 hours. Typical colonies of *Staphylococcus aureus* were enumerated, and therefore the average number per ml was calculated. Isolation and identification of *Staphylococcus aureus* was carried as stated in Quinn et al. (2002) and BAM on line (2009). Pure separated suspected colonies of *Staphylococcus aureus* were picked up from Baird-Parker agar plates, transferred to nutrient agar slants, and incubated at 37 °C for 24 hours. The isolated *Staphylococcus aureus* isolates were identified by Microscopic examination and Biochemical reactions. Biochemical tests used to confirm *Staphylococcus aureus* were coagulase test, catalase test, indole production, methyl red test, Voges-proskauer reaction, urease production, citrate utilization and sugar fermentation as stated in Toply and Wilsons (1993), colle et al. (1996), Harrigan (1998) and Quinn et al. (2002). Phenotypic characterization of some virulence factors was carried out to detect coagulase test according to methods described by Quinn et al. (2002), and hemolysis assay according to methods described by Koneman et al. (1997). Isolation and identification of *Salmonella* Typhimurium was carried out according to methods described by ISO-6579 (2002). Five ml of BTM samples were aseptically inoculated into 50 ml (1:10) of Preenrichment media (Buffer Pep-tone Water (BPW), and thoroughly mixed before being incubated at 37 °C ± 1 °C for 18 ± 2 hours. A volume of 0.1 ml was transferred to a tube containing 10 ml of the Rappaport Vassiliadis broth and then incubated at 41.5 ºC for 24 hours. From the enrichment culture, 10 µl were inoculated onto the surface of Xylose Lysine Deoxycholate (XLD), Hektoen Enteric and *Salmonella Shigella* agar plates, then incubated at 37ºC for 24 hours. *Salmonella* typical colonies were isolated, and further tested by standard biochemical methods and also serotyped using specific commercial sera. Microscopic identification of *salmonella* isolates films from suspected purified colonies were prepared, fixed and stained with gram's stain (Cruickshank et al., 1975). Biochemical identification of *Salmonella* isolates was carried out according to the methods described by ISO-6579 (2002). Purified isolates were examined by different biochemical reactions as oxidase, urea hydrolysis, H2S production on TSI, lysine decarboxylation, indole, methyl red test, Voges Proskauer, and citrate utilization tests.

Serotyping of *Salmonella*

Salmonella isolates were subjected to serological identification according to the methods described by Kauffman-White Scheme (Kauffmann, 1973) for determination of somatic (O) and flagellar (H) antigens.

Questionnaire and data collection

The study questionnaire sheet was designed according to the standard frame prepared by Dufour et al. (2010). The questionnaire sheets included questions related to hygiene of animals, environment and mastitis management. The questions were designed to be as closed as possible to avoid different interpretation between farmers.

Statistical analysis

All data analysis was carried out using the statistical studies. Association between the occurrence of infection and therefore the potential risk factors were studied using Chi-square $\chi 2$ and odds ratio (OR). A database and statistics system for epidemiology on microcomputers were used for performing $\chi 2$ Chi-square tests and odds ratio analysis. Answers to the questionnaire were transferred to Microsoft Excel, and grouped by their categorical response (e.g., Yes, No). Odds ratio was computed according to methods described by Thrusfield (2005). A P-value of < 0.05 and odds ratio of >1 and $\chi 2 > 3.82$ were regarded a significant association between the response and a category of the count.

Molecular identification of *Staphylococcus aureus* and *Salmonella* Typhimurium

All the identified *Staphylococcus aureus* and *Salmonella* positive isolates were examined by PCR for the presence of, *Staphylococcus aureus hlg* gene and *sopB* gene of *Salmonella*. The primers sequence and PCR product sizes are shown in table 1.

DNA extraction

DNA extraction from samples was done using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) accord to the manufacturer's instructions. Briefly, 200 µl of the sample suspension was used to be incubated with 10 µl of proteinase K and 200 µl of lysis buffer at 56^{O}C for 10 minutes. After incubation, 200 µl of one hundred percent (100%) ethanol was once introduced to the lysate. The sample was then washed and centrifuged. Nucleic acid was eluted with a hundred (100) µl of elution buffer.

Oligonucleotide primer

Primers used were provided from Metabion (Germany) which are listed in table 1.

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PCR Design and amplification

Primers were employed in a 25 µl reaction containing 12.5 µl of Emerald Amp Max PCR Master Mix (Takara, Japan), 1µl of every primer of 20 pmol concentrations, 4.5 µl of water, and 6 µl of DNA template. The reaction was done in biosystem 2720 thermal cycler.

Analysis of the PCR products

1.5% agarose gel was used for separating PCR products. For gel analysis, 20 µl of the products was loaded in each gel slot. Generuler 100 bp ladder (Fermentas, Thermo, Germany) was used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra), and therefore the data was analyzed by using computer software.

Phylogenetics and gene sequence analysis of, *sopB* gene of *Salmonella* Typhimurium and *hlg* Gene of *Staphylococcus aureus*.

It was performed in Elim biopharmaceuticals, Germany. Amino acids sequence analysis was done using the CLUSTAL W multiple sequence alignment program, version 1.83 of MegAlign module of Lasergene DNAStar software Pairwise, which was designed by Thompson et al. (1994) and Phylogenetic analysis was performed using neighbor joining and in MEGA6 (Tamura et al. 2013).

Table 1. Primer sequences, target genes, amplicon sizes and cycling conditions

Microorganism	Gene	Sequence(5'-3')	Amplified product	Reference
Salmonella	*sopB*	F-5' tcagaagRcgtctaaccactc-3'	517 bp	Huehn*et al.* 2010
		R-5'- taccgtcct cat gcacactc-3'		
S. aureus	*hlg*	F-5'- GCCAATCCGTTATTAGAAAATGC-3'	937 bp	Kumar *et al.*, 2009
		F-5'- CCATAGACGTAGCAACGGAT-3'		

S. aureus: *Staphylococcus aureus*

RESULTS AND DISCUSSION

Out of a total number of 150 pooled tank milk samples which were collected from 150 dairy cattle farms, 13 locally field isolates were detected and confirmed phenotypically by culturing, gram staining, biochemical and molecular identification to be *Staphylococcus aureus* in overall herd prevalence of (8.6%) as shown in table 2. These results nearly agreed with Rysanek et al. (2007) (12.3%) and Neder et al. (2011) (11.7%), but disagreed with Sischo et al. (1993) (45%), Keefe et al. (1997) (70%), Stephan et al. (2001) (32.4%), Phuektes et al. (2003) (33%), Yagoub et al. (2005) Howard (2006) (57.1%), Miranda-Morales et al. (2008) (30%) (30%), USDA (2008) (43%), Olde Riekerink et al. (2010) (74%), Katholm et al. (2012) (97%), Khudor et al. (2012) (28.5%), Lee et al. (2012) (21.7%), Amal (2014) (40%), El-Gedawy et al. (2014) (17%) and Zecconi et al. (2020) (42 %).

Isolation and identification of *Salmonella* Typhimurium field isolates from bulk tank milk samples revealed that 20 locally field isolates were detected and confirmed phenotypically by culturing, gram staining, biochemical and molecular identification to be *Salmonella* Typhimurium in overall herd prevalence of (13.3%) as shown in table 2, this finding agreed with Rohrbach et al. (1992) (8.9%), O'Donnell (1995) (0.36%), Steele et al. (1997) (0.17%), Karns et al. (2005) (11.8%), Van Kessel et al. (2008) (11%) and Abo-shama (2013) (14%) but disagreed with Hassan et al. (2000) (1.5%), Jayarao and Henning (2001) (6.1%), Murinda et al. (2002) (2.24%), Warnick et al. (2003) (1.1%),Van Kessel et al. (2004) (2.6%), Jayarao et al. (2006) (6%), Pangloli et al. (2008)(7%), Addis et al. (2011) (28.6%), Van Kessel et al. (2011) (48%), Tajbakhsh et al. (2013) (3.63%), El-Gedawy et al. (2014) (9%) and Sonnier et al. (2017) (18%). Serological identification of the 20 isolates revealed that they were *Salmonella* Typhimurium. The results of TBPC (cfu/ml) as shown in table 3, revealed that the geometric mean of 150 dairy farms was 3.2×10^8 cfu/ml.This finding agreed with (1.9×10^8 cfu /ml) Godefay and Molla (2000), (2.67×10^8 cfu/ml), Khin Zar Lin (2015), but disagreed with Abdallah (2002) (2.7×10^7 cfu/ml), Bonfoh (2003) (1.1×10^7 cfu/ml), Chye et al. (2004)(1.2×10^6 cfu/ml), Axelsson (2004) (2.4×10^4 cfu/ml) , El-Diasty and El-Kaseh (2009) (6.1×10^5 cfu/ml), Pantoja et al. (2009) (1.2×10^4), Uddin et al. (2011) (1.28×10^9cfu/ml), Hakem et al. (2012) (minimum count 3.7×10^5 and maximum count 4.9×10^5cfu/ml) ,Beli

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(2015) (3.89 × 10^6 cfu/ml), Meshref (2013), 3.62×10^7 ±1.37×10^7 cfu /ml) and Tasci (2011)(3.95×10^6 cfu /ml). *Staphylococcus aureus* is known as one of the foremost important causes of cattle mastitis, and it causes great economic losses in the dairy industry (Dufour et al., 2012). In term of *Staphylococcus aureus* count (SAC), the geometric mean value of SAC in present study was 3.7×10^3 cfu/ml as shown in table 3. These findings agreed with Stephan et al. (2001) (3×10^3 cfu/ml) but disagreed with Peles et al. (2007) (6.0 ×10^3 cfu/ml). The results in table 3 show that the geometric mean of SCCs/ml in BTM samples of 150 cattle dairy farms were 556.7×10^3 ; this result agreed with Erskine et al. (1987) (SCC 700×10^3 cells/ml), Fox et al. (2003) (533×10^3 cells/ml) and Pantoja (2009) (600×10^3 cells/ml), and however disagreed with Desmasures et al. (1997) (176×10^3 cells/ml), Secretaríade Agricultura Ganadería Pescay Alimentos (SAGPyA) (2005) (384×10^3 cells/ml), Rysanek et al. (2007) (240×10^3 cells/ml) in milk without pathogen, (330.8×10^3 cells/ml) with single pathogen and (367.6×10^3 cell/ml)with double pathogens, Vissio et al. (2008) (250×10^3 cells/ml). Bulk tank SCC could be a general indicator of the udder health in a herd, and it was also considered as an indirect index of milk quality (Schukken et al., 2003). Bulk tank SCC (BTSCC) was mainly known as one of the important tools to define the national and international regulative standards that control the hygienic milk production. The national standards for BTSCC differ from < 400,000 cells/ml (EU, Australia, New Zealand and Canada) to < 1,000,000 cells/ml (Brazil) (USDA, 2013). The USDA introduced a program that permits dairy processors to get an export certificate that confirm farm-level consent with the (400,000 cell/ml) limit adopted within the EU (USDA 2011).

Table 2. Point prevalence of *Staphylococcus aureus* and *Salmonella* Typhimurium in bulk tank milk in different localities

	Governorates													
	cairo	Giza	Qulobia	Sharkia	Monofia	Alexandria	Behira	Dkhalia	Benisuef	Fayoum	sohag	Aswan	Asuet	total
Poulation at risk	1520	644	671	729	841	718	284	502	620	618	549	510	461	8667
S.aureus	1	1	1	2	2	0	0	3	1	0	1	0	1	13
S.typhimurium	2	1	1	3	3	1	1	4	0	1	1	1	1	20

S. aureus: *Staphylococcus aureus: S.* Typhimurium: *Salmonella* Typhimurium

Table 3. Geometric mean value of Standard plate count, Somatic cell count and *Staphylococcus aureus* in bulk tank milk in different localities

Governorates	Geometric mean of SPC cfu /ml	Geometric mean of SCC ×10^3	Geometric mean of *S. aureus* cfu/ ml×10^3
Cairo	7.9×10^8	455	3.2
Giza	6.3×10^8	372	4.7
Qulobia	1.7×10^7	438	5.7
Sharkia	2.4×10^8	669	3.8
Monofia	6.9×10^8	352	7.2
Alexandria	1.7×10^8	552	3.4
Behira	1.2×10^8	620	5.6
Dkahlia	2.9×10^7	559	3.7
Benisuef	9.7×10^7	679	2.1
Fayoum	3.7×10^8	597	2.3
Sohag	2.4×10^7	567	3.1
Aswan	7.2×10^8	812	4.7
Asuet	3.7×10^8	778	2.7
Geometric mean value	3.2×10^8	556.7	3.7

SPC: Standard plate count; SCC: Somatic cell count

As shown in tables 4, 5 and 6 risk factors that were found to be associated with *Staphylococcus aureus*, *Salmonella* Typhimurium, SPC and SCC in bulk tank milk samples were Purchasing cattle or heifers as a risk factor has a significant association with *Staphylococcus aureus, Salmonella* Typhimurium, SPC and SCC in bulk tank milk as shown in table 4 (OR: 7.2, $\chi 2$: 92 and P < 0.00001, OR: 4.2, $\chi 2$: 44 and P < 0.0014, OR: 6.22, $\chi 2$: 78.7 and P < 0.000012 and OR: 3.14, $\chi 2$: 23.3 and P < 0.001 respectively). In spite of the actual fact that non lactating heifers have not yet been in connection with the milking equipment, they would still be infected with *Staphylococcus aureus* (Fox, 2009). Therefore, the purchase of *Staphylococcus aureus*-positive heifers was considered as one of the foremost vital risk factors for the introduction and spread of *Staphylococcus aureus* within the herd after they start lactation. The introduction of cattle was previously related to an increased risk of clinical salmonellosis. These animals were also carrier of *Salmonella*, and should contribute to extend *Salmonella* infection in the farm. These findings agreed with Nielsen et al. (2007). Cows purchased into the dairy herd were also infected with mastitis pathogens, and were a possible risk to other cows within the herd, and therefore affecting the level of bulk tank milk SCC and SPC. To prevent the introduction of mastitis pathogens when purchasing cows, a balance need is required to be found between minimizing the probability of buying

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an infected cow and maintaining sufficient choice within the population from which to pick replacements (Gunn et al., 2008). Quarantine and test all purchased incoming heifers for contagious mastitis as a risk factor recorded a significant association with *Staphylococcus aureus*, Salmonella Typhimurium SPC and SCC in bulk tank milk as shown in table 4 (OR: 4.92, χ2. 24 and P<0.00028, OR.: 3.92, χ2: 31.4 and P<0.0028, OR: 4.12, χ2. 42.8 and P<0.00024 and OR: 3.92, χ2:34.4 and P<0.00128 respectively).

Herds that were not quarantined bought animals which have been generally observed to be positive for *Staphylococcus aureus* in BTM than herds where quarantine was once applied. Intramammary infections brought by *Staphylococcus aureus* were usually sub-clinical; thus, *Staphylococcus aureus* can continue to be unnoticed and disregarded by producers if no testing was done. Moreover, testing newly bought heifers previous to introducing them into the farm would be critical and necessary for forbidding and diminishing the spread of *Staphylococcus aureus* and retaining the occurrence of recent infections to an occasional degree (DaCosta et al., 2016). Non applying quarantine policy in cattle dairy farms as a risk factor encompassed a significant association with *Salmonella* prevalence in bulk tank milk. Approximately half of the dairy herds purchased animals, and about half of the buying herds did not quarantine incoming animals (Nöremark et al., 2016). If purchase could not be avoided, quarantine is often unable to reduce the risks (Vanselow et al., 2007). Not applying fly control as a risk factor as shown in table 4 was clearly related to *Staphylococcus aureus, Salmonella* Typhimurium, SPC and SCC in bulk tank milk (OR:3.2, χ2. 22 and P < 0.0016, OR: 2.29, χ2: 25.4 and P < 0.0029, OR: 4.2, χ2. 47.2 and P < 0.00032 and OR: 3.2, χ2. 39 and P < 0.006 respectively).

Flies played a vital role in the transmission of *Staphylococcus aureus* between infected and uninfected heifers, and not applying fly control in cattle dairy farms was considered one of the most important risk factors for heifer's intramammary infection caused by *Staphylococcus aureus* (Capurro et al., 2010; Piepers et al., 2011; Anderson et al., 2012). Presence of biting flies that purpose teat lesions was related to a high level of *Staphylococcus aureus* mastitis suggesting that fly control should be included during a mastitis management plan (Ryman et al., 2013). Flies carry a variety of mastitis-causing organisms that may colonize in teat lesions, and affect SCC and SPC in bulk tank milk. One among the foremost probable suggested causes for the increased incidence of mastitis, and also the increased bulk tank SCC and SPC level was that the irritation of udders by flies, and also the spreading of microorganisms by these insects. Another report showed that presence of biting flies causing teat lesions was related to a high level of mastitis, and suggested that fly control should be included during a mastitis management plan (Ryman et al., 2013). Heifers from herds using fly control had a lower prevalence of mastitis in comparison with herds where no fly control was applied (Nickerson et al., 1995). Heavy fly infestation was known as one of the foremost important risk factors for *salmonella* in dairy cattle farms.Flies that feed on cattle manure played a critical role in *Salmonella* shedding in dairy farm environments (Holt et al., 2007; Thomson et al., 2017). Not milking cows with a high SCC/CM grouped separately as shown in table 4, were significantly related to *Staphylococcus aureus*, SPC and SCC in bulk tank milk (OR :2.72, χ2: 17 and P< 0.0058, OR.: 1.73, χ2: 12.7 and P < 0.03, OR: 5.93, χ2 : 46.9 and P < 0.00023, OR: 4.73, χ2; 42.8 and P < 0.00021 respectively) and not milking all incoming cattle separately or last as shown in table 4 (OR: 6.34, χ2 : 62 and P < 0.00002, OR : 3.22, χ2 : 42.5 and P < 0.0021 and OR: 4.34, χ2: 42.8 and P < 0.00045 respectively) as shown in table 4.

It is still preferable to milk cows with a high SCC/CM last and grouping separately (Barnouin et al., 2004). Cows in herds that did not milk cows with mastitis last were significantly more likely to have mastitis than those who did that. Failure to milk cows with mastitis last played a vital role in spreading of mastitis pathogens between cows by milker's hands leading to contagious mastitis, and also played a great role in affecting bulk tank SPC and SCC (FAO, 2014). High SCC cows and clinical mastitis cases should be milked last, employing a specific milking unit for these cows or rinsing, cleaning, or disinfecting the unit after these cows are milked, and before first-lactation cows are milked were significantly related to low bulk tank SCC (Dufour et al., 2011). Milking cows with mastitis in no specific order was another practice that significantly affected the *Staphylococcus aureus* presence in BTM. Riekerink et al. (2006) also observed lower incidence rates for *Staphylococcus aureus* in BTM belonging to herds with mastitis-affected cows milked separately from healthy cows. Teat cups were known as the foremost bacterial transmission tool (Benić et al., 2012), and therefore, cows affected with mastitis should be milked separately or after healthy animals (NMC, 2001; Zecconi, 2006; Arnold and Bewley, 2011; Middleton 2013). Not cleaning and disinfecting equipment and milking unit after milk infected cattle as a risk factor for *Staphylococcus aureus, Salmonella* Typhimurium SPC and SCC in bulk tank milk (OR: 6.98, χ2. 75 and P<0.000016, OR: 2.78, χ2. 29.4 and P<0.016, OR: 5.68, χ2: 59.4 and P < 0.000012 and OR: 4.72, χ2: 55.2 and P< 0.00013 respectively) as shown in table 4. Poorly cleaned and sanitized milking utensils were regarded as a very important source of the many microorganisms in bulk tank milk. Cleaning and sanitation of the milking equipment may well be considered as a critical point in the milking procedure, because a cleaning failure could influence the level of bacterial contamination of bulk tank milk (Bava et al., 2009). Cleaning and sanitation of the milking equipment were also identified as a risk factor related to high bacterial counts (Elmoselmany et al., 2009a; Jayarao et al., 2004).

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Table 4. Odds Ratio, Chi-square and p value for risk factors associated with *Staphylococcus aureus*, *Salmonella* Typhimurium, Standard plate count and Somatic cell count in bulk tank milk in Egypt dairies

Risk factors	*S.aureus*			*S.* Typhimurium			SPC			SCC		
	OR	$\chi 2$	P value	OR	$\chi 2$	P value	OR	$\chi 2$	P value	OR	$\chi 2$	P value
Purchasing cattle or heifers	7.2	92	< 0.00001	4.2	44	< 0.0014	6.22	78.7	P<0.000012	3.14	23.3	P<0.001
Not quarantining and Test all purchased incoming heifers for contagious mastitis	4.92	24	< 0.00028	3.92	31.4	< 0.0028	4.12	42.8	P<0.00024	3.92	34.4	P<0.00128
missed fly control	3.2	22	< 0.0016	2.29	25.4	0.0029	4.2	47.2	P<0.00032	3.2	39	P<0.006
Not milking Cows with a high SCC/CM grouped separately and milked last at each milking	2.72	17	< 0.0058	1.73	12.7	<0.03	5.93	56.9	P<0.00023	4.73	42.8	P<0.00021
Not milking all incoming cattle separately or last	6.34	62	<0.00002	2.12	22.3	< 0.023	3.22	42.5	P<0.0021	4.34	42.8	P<0.00045
Not cleaning and disinfect equipments and milking unit after milk infected cattle	6.98	75	<0.000016	2.78	29.4	< 0.016	5.68	59.4	P<0.000012	4.72	55.2	P<0.00013
7- Sanitary condition of the Barn, access alleys and Cubicle passages	2.72	20	< 0.014	3.22	30.5	< 0.002	3.12	32.5	P<0.0014	2.82	27.5	P<0.001
Not using Separate cleaned and sanitized calving paddocks or pens for cows and heifers	2.34	16	< 0.028	2.5	17.5	< 0.013	4.6	47.5	P<0.00023	3.16	37.6	P<0.0013
Not housing pregnant heifers with dry cows	4.35	32	< 0.00028	3.15	27.2	< 0.0018	2.25	22.3	P<0.028	2.21	20.5	P<0.024
Herds don't practice checking SCC records	4.27	59	< 0.000012	2.47	19.4	< 0.0032	5.12	79.1	P<0.000002	7.23	82	P<0.00000012

OR: odds ratio, $\chi 2$: chi square, SPC: Standard plate count, SCC: Somatic cell count, p value: probaplity value

Table 5. Odds Ratio, Chi-square and p value for risk factors significantly associated with *Staphylococcus aureus*, *Salmonella* Typhimurium, Standard plate count, Somatic cell count in bulk tank milk in Egypt dairies

Risk factors	*S.aureus*			*S.* Typhimurium			SPC			SCC		
	OR	$\chi 2$	P value	OR	$\chi 2$	P value	OR	$\chi 2$	P value	OR	$\chi 2$	P value
Cleanliness of the cows (udder and teats)	2.65	19	< 0.023	4.25	49	<0.00023	5.15	69.7	<0.000001	4.21	45.9	< 0.0012
Muddy bedding materials	2.25	16	< 0.013	3.15	36	<0.00015	5	66	< 0.000023	6.5	86	<0.0000003
Storage manure indoor	3.27	59	< 0.00123	4.17	62.7	<0.00002	6.21	89.7	< 0.000013	2.17	29.1	< 0.0021
Herd size	3	39	< 0.0003	4.21	49	<0.00001	3.2	37	< 0.0003	2.2	18.4	< 0.013
Not t applying pre and post-milking teat dipping	2.79	16	< 0.026	1.87	17.5	< 0.012	3.69	31.4	< 0.00013	2.69	21.4	< 0.0023
Not stripping milk regularly into strip cup to detect mastitis	2.27	19	< 0.015	1.37	9.2	< 0. 04	2.37	29.5	< 0.001	6.2	78	<0.0000001
Milking wet teats	2.42	24	< 0.0021	2.27	22	< 0.0021	5.1	72	<0.000002	7.1	92	< 0.0000001
Not using single paper towels or suitable woven cloths for teats drying	2.58	18	< 0.013	2.78	15	< 0.03	5.58	67	< 0.00004	7.4	98	< 0.0000013
Not replacing and sanitize teat cup Liners according to manufactures instructions	5.79	55	< 0.0006	2.9	20.82	< 0.003	6.26	86.4	< 0.00037	6.79	76.9	< 0.0000032
Not testing and service milking machine every 6 months or annually	6.24	45	< 0.0004	4.12	41.2	< 0.0002	5.26	68.2	<0.000001	4.24	48.9	< 0.00002
Not Wearing latex Gloves	2.32	18.9	< 0.038	7.1	90	<0.0000003	7.1	90	<0.0000003	7.4	93	<0.000000123

OR: odds ratio, $\chi 2$: chi square, SPC: Standard plate count. SCC: Somatic cell count. p value: probaplity value

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Table 6. Odds Ratio, Chi-square and p value for risk factors significantly associated with *Staphylococcus aureus*, *Salmonella* Typhimurium, Standard plate count, Somatic cell count in bulk tank milk in Egypt dairies

Risk factors	*S.aureus*			*S.* Typhimurium			SPC			SCC		
	OR	$\chi 2$	P value	OR	$\chi 2$	P value	OR	$\chi 2$	P value	OR	$\chi 2$	P value
Not washing automatic Clusters with hot disinfectant after milking a cow with CM or a high SCC	2.43	18	< 0.024	2.12	15.7	< 0.021	4.23	48	< 0.00012	5	68.7	<0.00000024
Not applying Back flushing	5.98	59	< 0.00001	3.28	39.7	< 0.0012	7.28	98.4	< 0.0000021	6.12	88.2	< 0.000001
Dirty Bulk tank surface	7.5	85	< 0.000002	5.23	68.2	<0.0000001	6.7	88.3	< 0.000003	7.2	95.3	< 0.0000003
Claw pieces don't thoroughly brushed after each milking	3.5	41	< 0.0001	4.2	58	< 0.000002	6.7	78	<0.0000001	7	93	< 0.0000002
Gaskets and milk valves don't removed cleaned and brushed daily	5.7	75	<0.000001	5.2	72	< 0.000001	6	82	< 0.0000023	7	93	< 0.00000004
Specific cleaning and sanitary program to milk pipelines	4.5	45	< 0.000021	4.7	42	< 0.0001	5.4	62	< 0.000023	6.8	73	< 0.000005
Not cleaning inflation and all other rubber plastic like parts and free from cracks	3.5	35	< 0.0021	3.7	32	< 0.001	4.4	62	< 0.0003	5	53	< 0.00002
Summer season	3.21	39	< 0.00012	2.15	19.7	< 0.02	4.24	42.7	< 0.00014	3.27	47.8	< 0.0002
Region variation	2.31	19	< 0.0014	2.46	20.7	< 0.002	3.24	41.4	< 0.0012	2.17	27.8	< 0.0031

OR: Odds Ratio, $\chi 2$: Chi Square, SPC: Standard Plate Count, SCC: Somatic Cell Count, p value: probability value

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Milk residues left on the milking equipment contact surface; support the growth of a variety of microorganisms (Murphy and Boor, 2007: Holm et al., 2004). The main aim of sanitizing is to kill residual microorganisms existing on these surfaces immediately prior to milking. Inadequate or improper cleaning and sanitizing permitted the bacteria to remain on the equipment's surfaces, and to grow and multiply these results in elevated count of *Staphylococcus aureus*, *Salmonella* Typhimurium, SPC and SCC in bulk tank milk. Satisfactory parlor sanitization and disinfection were very important and essential after each and every milking to help and assist to control the bulk tank total bacterial counts, and to limit the risk of cross infection of mastitis pathogens among cows from contaminated milking equipment (Ian ohnstad, 2013). Not being replaced and sanitized the teat cup liners according to manufactures instructions were significantly associated with *Staphylococcus aureus*, *Salmonella* Typhimurium, SPC and SCC in bulk tank (OR: 5.79, $\chi 2$: 55 and $P < 0.0006$, OR: 2.9, $\chi 2$: 20.82 and $P < 0.003$, OR: 6.26, $\chi 2$: 86.4 and $P < 0.00037$ and OR: 6.79, $\chi 2$: 76.9 and $P < 0.0000032$, respectively) as shown in table 4.

The teat cup liner is that the only piece of the milking machine that comes into direct contact with the cow. Worn and rough liners are difficult to be cleaned, and will harbor mastitis bacteria, and also, they affect significantly on the bulk tank SPC and SCC level (Roger Blowey and Peter Edmondson, 2010). The milking equipment consists mainly of various rubber parts. Among these rubber parts, the foremost important part could be a teat cup liner which provided direct contact force to teat tissue. Properties of teat cup liners directly affected the technical and technological process of milking, udder health condition and quality of obtained milk, and seriously was that the finding that cracks on the inner surface of teat cup liners were increasing proportionally with exposition time within the primary production, and that they were a source of pathogenic microorganisms (Gálik et al., 2015). The rubbers parts of milking equipment were taken into an account of one of the foremost potential sources of milk contamination (Krzyś et al., 2011). Sanitary condition of the barn, access alleys and cubicle passages as a risk factor was significantly associated with *Staphylococcus aureus*, *Salmonella* Typhimurium, SPC and SCC in bulk tank (OR: 2.72, $\chi 2$: 20 and $P < 0.014$, OR: 3.22, $\chi 2$. 30.5 and $P<0.002$, OR. 3.12, $\chi 2$. 32.5 and $P< 0.0014$ and OR. 2.82, $\chi 2$. 27.5 and $P < 0.001$ respectively) as shown in table 4.

Facility hygiene included the cleanliness of the barn, access alleys and milking parlor, thought as an essential and vital part of hygienic milk production and quality control program. Cleaning and renewing the bedding of the cubicles and yards should ideally be performed during milking, so as cows exit from the parlor, they were able to walk back along clean passage ways, pasted fresh food, and then lay down in clean cubicles (Blowey and Edmondson, 2010). This kept the teats as clean as possible during the primary critical 20 to 30 minutes after milking, when the cow was more susceptible to mastitis, because the teat sphincter did not fully close (Blowey and Edmondson, 2010). It also reduced the quantity of feces carried back on to the cubicle beds by soiled feet. Clean farms and houses were strongly related to lower SPC and SCS (Chassagne et al., 2005). Implementing and maintaining few and straight forward hygienic practices in terms of barn cleaning can significantly improve microbiological quality of cow milk, and reduce somatic cell count (Zucali et al., 2011). Surveys were performed to determine relations among measures associated with facility hygiene and microbial counts in bulk tank milk. A correlation between the dirtiness of the access alley to the milking parlor, and therefore the total bacterial count in bulk tank milk were reported by Christiansson et al. (1999). A powerful association was observed among increased bacterial content in bulk tank milk and cleanliness score of the farm facilities and milking cows (Elmoslemany et al. 2009a; Zucali et al. 2011). There was a distinct association amongst cleanliness of housing areas, and therefore the rate of clinical mastitis and high somatic cell counts in bulk tank milk were also established (Barkema et al., 1998). The poor hygienic condition of the farm facilities was a striking feature, which was along with inefficient sanitation of the facilities favored the proliferation and dissemination of *salmonella* among animals and environmental contamination. Proper and regular cleaning and ordinary barn disinfection routines in dairy cattle farms played an important role in controlling salmonellosis in dairy cattle farms. These agreed with Tarazi and Abo-shehada (2015). Sanitation and hygienic measures of dairy cattle farms had a great impact on reduction and lowering *Staphylococcus aureus* levels in dairy cattle farms (Sartori et al., 2017). Not testing and servicing the milking machine every 6 months or annually were considered as risk factor for *Staphylococcus aureus*, *Salmonella* Typhimurium, SPC and SCC in bulk tank milk (OR: 6.24, $\chi 2$: 45 and $P < 0.0004$, OR: 4.12, $\chi 2$: 41.2 and $P < 0.0002$, OR. 5.26, $\chi 2$: 68.2 and $P < 0.000001$ and OR: 4.24, $\chi 2$: 48.9 and $P < 0.00002$ respectively) as shown in table 4.

There were a wide variety of milking machine efficiency faults. These usually resulted from gradual changes in milking machine performance due to continued use, wear and age. The efficiency and performance of milking machine components affected mastitis incidence, mainly by their effects on the new mastitis infection rate. It is very important and critical that milking machines should be tested by a qualified technician or adviser on a regular basis. Regular testing, service and maintenance of milking machine is extremely necessary to maintain proper mechanical performance, to boost the speed and completeness of milking, and to improve bulk tank SPC and SCC level. These findings agreed with Rodrigues et al. (2005). Milking machines without proper maintenance in general became the main reason of high bacteriological counts in milk (Reinemann et al., 1997; Murphy and Boor, 2000). The milking parlor should be kept clean, and proper performance of the milking system should be ensured by having the system inspected at the minimum

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annually (Dufour et al., 2011). Not applying pre and post-milking teat dipping was as a risk factor for *Staphylococcus aureus, Salmonella* Typhimurium, SPC and SCC in bulk tank milk (OR:2.79, $\chi 2$: 16 and $P < 0.026$, OR: 1.87, $\chi 2$: 17.5 and $P < 0.012$, OR: 3.69, $\chi 2$: 31.4 and $P < 0.00013$ and OR: 2.69, $\chi 2$:21.4 and $P < 0.0023$, respectively) as shown in table 4.

The practice of pre- and post-milking teat dipping was one of the main important and critical components for controlling and preventing of mastitis and a control program in a dairy herd. Teat dipping is now a widely and universally accepted practice for reducing the bacterial population around the teat end, thus for reducing the risk of mastitis. The proper application of an accurate post-milking teat disinfection product to cow teats after milking could be the most significant and important task. After milking, bacteria multiply on the teat skin, and will extend into the teat canal. If the entire surface of every teat is disinfected immediately after milking, this establishment of bacteria would be minimized. Teat disinfection is on was in every of the foremost effective sub-clinical and clinical mastitis control measures available. Failure to cover the entire teat of each cow at every milking was that the most typical error in teat disinfection. Teat dipping was very important in reduction of *Staphylococcus aureus* teat colonization (Da Costa et al., 2014). Number of the advantages of those practices was related to reducing and lowering of the microbial contamination of the teats (Zucali et al., 2011). Post-milking teat sanitization reduced BTSCC, and was clearly and significantly related to individual-cow somatic cell count (ICSCC) (Tadich et al., 2003; Barnouin et al., 2004a; Dufour et al., 2011). Dipping all teats after each milking had the greatest effect on reduction of bulk milk somatic cell counts, and it increased milk yields more than any other milking practice. Pre-milking teat dipping played an important and significant role in reduction of BTSCC. Proper and correct teat-end disinfection before the cluster attached can reduce teat surface bacteria by 75% (Reinemann et al., 2008), and was effective in preventing and controlling mastitis caused by environmental and contagious pathogens (Ruegg and Dohoo, 1997). Not stripping milk regularly into strip cup to detect mastitis as a risk factor was significantly associated with *Staphylococcus aureus*, SPC and SCC in bulk tamk milk (OR: 2.27, $\chi 2$: 19 and $P < 0.015$, OR: 1.37, $\chi 2$: 9.2 and $P < 0.04$ and OR.:2.37, $\chi 2$: 29.5 and $P < 0.001$, OR: 6.2, $\chi 2$:78 and $P < 0.0000001$ respectively) as shown in table 4.

In the herds in which fore-milking was practiced using the fore-stripper, lower SCC was observed. These findings agreed with Skrzypek (2002), Wagner and Ruegg (2002) and Down (2016). Fore-milking and therefore the visual appraisal of fore-milk were widely recommended to be performed before each milking as they eased accurate identification of the clinical mastitis in individual quarters and immediate treatment of affected cows, like wise as their milking using separate equipment. Fore-stripping was extremely useful for checking milk quality, and had a great role in milk let down. Not utilizing separate cleaned and sanitized calving paddocks or pens for cows and heifers was significantly associated with *Staphylococcus aureus, Salmonella* Typhimurium, SPC and SCC in bulk tank milk (OR: 2.34, $\chi 2$:16 and $P<0.028$, OR: 2.5, $\chi 2$; 17.5 and $P < 0.013$, OR: 4.6, $\chi 2$: 47.5 and $P < 0.00023$ and OR : 3.16, $\chi 2$:37.6 and $P < 0.0013$, respectively) as shown in table 4. The present study provided evidence for the importance of using separate calving pens for cows and heifers, and its disinfection for the successful control of *Staphylococcus aureus, Salmonella* Typhimurium, SPC and SCC in bulk tank milk. the current results agreed with Down (2016), Barnouin et al. (2004) and O'Reilly et al. (2006). This effect may could be due to a reduction in pathogen exposure, but additionally may reflect, the negative impact of cross-suckling calves indirectly which was related to mastitis incidence (Green et al., 2007). Cows are susceptible to infection before calving because their natural defense mechanisms are low, new infections occur, and subclinical infections which persisted through the dry period may flare into clinical cases (Down 2016). Housing pregnant heifers with dry cows as a risk factor was significantly associated with *Staphylococcus aureus, Salmonella* Typhimurium, SPC and SCC in bulk tank (OR:4.35, $\chi 2$.:32 and $P < 0.00028$, OR:3.15, $\chi 2$. 27.2 and $P < 0.0018$, OR: 2.2, $\chi 2$: 22.3 and $P < 0.028$ and OR: 2.21, $\chi 2$:20.5 and $P< 0.024$, respectively) as shown in table 4.

These findings agreed with Down (2016). Understanding the mastitis risk factors for dry cows and heifers could also be considered as a critical and vital step to reduce mastitis prevalence. At the time of dry off, cows are not given the protection from post-dip, and their udders are not observed closely for signs of infection. All of those factors contribute and play a key role in elevation the bulk tank level of microbial count and SCC. Herds don't keep SCC records was believed as a risk factor for *Staphylococcus aureus, Salmonella* Typhimurium, SPC and SCC in bulk tank milk (OR: 4.27, $\chi 2$. 59.2 and $P < 0.000012$, OR: 2.47, $\chi 2$: 19.4 and $P < 0.0032$, OR: 5.12, $\chi 2$: 79.1 and $P < 0.000002$, OR. 7.23, $\chi 2$. 82 and $P < 0.00000012$, respectively) as shown in table 4. The lowest SCC observed in the herds that was practiced milk recording was possibly because of increased farmer knowledge on individual cows, and its importance as a factor associated with SCC. These results agreed with Hutton et al. (1990) and Down (2016). If dairy producers do not record milk, then it would be difficult to identify cows with constantly elevated SCC, thereby may cause an increasing in the overall bulk SCC. Barkema et al. (1999) found that farmers of herds with a low bulk tank SCC kept better records and were extra acquainted and familiar with every cow in their herds. Milking wet teats was a risk factor for *Staphylococcus aureus, Salmonella* Typhimurium, SPC and SCC in bulk tank milk (OR: 2.42, $\chi 2$. 24 and $P < 0.0021$, OR: 2.27, $\chi 2$: 22 and $P < 0.0021$, OR: 5.1, χ: 72 and $P < 0.000002$, OR: 7.1, $\chi 2$. 92 and $P < 0.0000001$, respectively) as shown in table 5.

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Milking wet teats is unacceptable, both for risk of mastitis and elevation of bulk tank SCC and all over total microbial count and subsequent milk quality issues. Water plays an important and vital role in fostering mastitis pathogens on the udder and teats. Water laden with bacteria on udder and teat surfaces can enter teat cup liners, and may increase bacterial contamination of milk, and also increase mastitis incidence, and then subsequently elevate bulk tank SCC (Blowey and Edmondson, 2010). Not using single paper towels or suitable woven cloths for teats' drying was believed as a risk factor for *Staphylococcus aureus, Salmonella* Typhimurium, SPC and SCC in bulk tank milk (OR: 2.58, χ2.18 and P<0.013, OR. 2.78, χ2. 15 and P<0.03, OR. 5.58, χ2. 67 and P<0.00004 and OR: 7.4, χ2 .98 and P<0.0000013, respectively) as shown in table 5.

Effective drying had a great impact on the level of SCC in bulk tank milk, and was achieved by using single paper towels or suitable woven cloths. Each cloth must only be used for one cow per milking. Cloths should then be placed in disinfectant solution, washed before the next milking (Blowey and Edmondson, 2010). Not wearing latex gloves was taken as risk factor for *Staphylococcus aureus, Salmonella* Typhimurium, SPC and SCC in bulk tank milk (OR: 2.92, χ2:20.4 and P < 0.028, OR. 2.32, χ2. 18.9 and P < 0.038, OR: 7.1, χ2. 90 and P < 0.0000003, OR: 7.4, χ2. 93 and P < 0.000000123, respectively) as shown in table 5.

These findings agreed with Bach et al. (2008) and Signorin et al. (2008). The milker can spread contagious mastitis as he handles each cow. The use of gloves was one of the foremost important factors to prevent mastitis caused by *Staphylococcus aureus* agents in dairy cows (Petersson-Wolfe et al., 2010; Arnold and Bewley, 2011), and was included in the National Mastitis Council mastitis control plan (NMC 2001). The operator's hands can be a mean for bacterial dissemination, and gloves have a great role in reducing total bacteria count in bulk tank milk (Nickerson, 2014); Wearing latex gloves decreased *Staphylococcus aureus* prevalence, and incidence in BTM (Dufour et al., 2012). Not washing automatic clusters with hot disinfectant after milking a cow with CM or a high SCC was considered as a risk factor for *Staphylococcus aureus,* Salmonella Typhimurium, SPC and SCC in bulk tank milk (OR: 2.43, χ2:18 and P < 0.024, OR: 2.12, χ2. 15.7 and P < 0.021, OR.:4.23, χ2: 48 and P < 0.00012, OR: 5, χ2: 68.7 and P < 0.00000024, respectively) as shown in table 5.

These findings agreed with Wenz et al. (2007) and Down (2016). At the end of milking, a small quantity of milk is held inside the mouth piece of the line. When the cluster is connected to the next cow, the milk from the previous cow will run down the inner part of the liner, and contaminate the teat of the next cow to which is milking. This represented a risk of infection transferring (Blowey and Edmondson, 2010). Teat contamination occurred via the cluster which was previously contaminated during milking of an infected cow was considered as a great vital point for cross-contamination between cows. Clusters are a usual origin and source of bacteria for dairy cows. Infected cow (clinical case or sub-clinical case with excessive bacterial numbers) had the potential to infect the subsequent 5 to 6 cows milked on that cluster. It was not ideal to wash the cluster (either back-flushing or through-flushing) with cold water. Not applying back-flushing as a risk factor significantly associated with *Staphylococcus aureus, Salmonella* typhimurium, SPC and SCC in bulk tank (OR :5.98, χ2. 59 and P < 0.00001, OR. 3.28, χ2. 39.7 and P < 0.0012, OR. 7.28, χ2. 98.4 and P < 0.0000021, OR. 6.12, χ2. 88.2 and P < 0.000001, respectively) as shown in table 6. Dirty bulk tank surface as a risk factor was significantly associated with *Staphylococcus aureus, Salmonella* Typhimurium, SPC and SCC in bulk tank (OR :7.5, χ2: 85 and P < 0.000002, OR: 5.23, χ2: 68.2 and P < 0.0000001, OR: 6.7, χ2: 88.3 and P < 0.000003 and OR: 7.2, χ2: 95.3 and P <0.0000003, respectively) as shown in table 6.

Claw pieces were not thoroughly brushed after each milking considered as a risk factor was significantly associated with *Staphylococcus aureus, Salmonella* Typhimurium, SPC and SCC in bulk tank (OR: 3.5, χ2: 41 and P<0.0001, OR. 4.2, χ2: 58 and P<0.000002, OR: 6.7, χ2: 78 and P<0.0000001 and OR. 7, χ2. 93 and P<0.0000002, respectively) as shown in table 6. Gaskets and milk valves were not removed cleaned and brushed daily, it as a risk factor was significantly associated with *Staphylococcus aureus, Salmonella* Typhimurium, SPC and SCC in bulk tank (OR: 5.7, χ2.:75 and P < 0.000001, OR: 5.2, χ2:72 and P <0.000001, OR: 6, χ2: 82 and P < 0.0000023 and OR. 7, χ2. 93 and P < 0.00000004, respectively) as shown in table 6. Specific cleaning and sanitary program to milk pipelines as a risk factor was significantly associated with *Staphylococcus aureus, Salmonella* Typhimurium, SPC and SCC in bulk tank (OR: 4.5, χ2: 45 and P < 0.000021, OR: 4.7, χ2: 42 and P < 0.0001, OR: 5.4, χ2: 62 and P < 0.000023 and OR: 6.8, χ2: 73 and P < 0.000005, respectively) as shown in table 6. Not cleaning inflation and all other rubber plastic like parts and free from cracks as a risk factor were significantly associated with *Staphylococcus aureus, Salmonella* Typhimurium, SPC and SCC in bulk tank (OR: 3.5, χ2. 35 and P < 0.0021, OR: 3.7, χ2: 32 and P < 0.001, OR. 4.4, χ2: 62 and P < 0.0003 and OR.; 5, χ2:53 and P < 0.00002, respectively) as shown in table 6.

Back-flushing units will help to get rid of both environmental and contagious bacteria, and this may only be of benefit on reducing the bulk tank SCC and SPC level (Blowey and Edmondson, 2010). All internal parts of the tank that can come into contact with milk must be cleaned and disinfected. It was very necessary that the worker checks the internal parts of the tank to be cleaned and disinfected before milking (Blowey and Edmondson, 2010). Dirty bulk tanks played a definite role, and have a great impact on bulk tank SCC and total bacterial level. It is very important to regularly

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check and replace the rubber parts. The rubber plastic like parts was thought to be one of the foremost important sources for bulk tank microbial contamination. Therefore, most standard cleaning program should be beginning with hot water at 35°C to 40°C, flowed by 8 to 10 minutes cleaning with alkaline detergent and disinfectant, and at last cold water rinse (Reinemann et al., 2003). Herd size more than 50 as a risk factor was significantly associated with *Staphylococcus aureus, Salmonella* Typhimurium, SPC and SCC in bulk tank (OR: 3, $\chi 2$: 39 and $P < 0.0003$, OR: 4.21, $\chi 2$: 49 and $P< 0.00001$, OR: 3.2, $\chi 2$: 37 and $P < 0.0003$, OR: 2.2, $\chi 2$: 18.4 and $P< 0.013$, respectively) as shown in table 5.

These findings agreed with Green et al. (2008). Higher bulk tank SCC was found in larger herds than in the smaller ones (Skrzypek, 2002; Skrzypek et al., 2003; Danko´w et al., 2004; Schewe et al., 2015). The present results disagreed with Norman et al. (2000), Oleggini et al. (2001) and Van Schaik et al. (2002). It would seem that the significant relationship between herd size, and SCC can be described by the fact that the increase in herd size played an important role in elaboration, and magnified the risk of infectious diseases, including mastitis. Another cause may be the fact that in small herds fewer cows were handled by one person, as a result of which animals were treated more individually than in larger herds (Skrzypek et al., 2004). Herd size more than 50 as a risk factor was significantly associated with total bacterial count in bulk tank milk. These results agreed with Goldberg et al. 1992, Gran et al. (2002); Jayarao et al (2004). Herd size (50 cows or more) as a risk factor was significantly associated with Salmonella in bulk tank milk based on the present results which agreed with Ruzante et al. (2010). Large herds may have a greater possibility of buying calves from more than one sources outside with the accompanying the risk of introducing *Salmonella* via subclinical shedders that was stressed by transportation. High cattle density can also additionally be a characteristic of large herds, and may promote *Salmonella* transmission (cummings et al., 2010). Herd size was frequently related to incidence of *Salmonella* in cattle dairy farms (Vaessen et al., 1998; Kabagambe et al., 2000; Warnick et al., 2001; Huston et al., 2002; Blau et al., 2005). Herd size had a great impact on bacterial counts in bulk tank milk (Goldberg et al., 1992; Gran et al., 2002; Jayarao et al., 2004; Elmoslemany et al., 2010). Storage manure indoor as a risk factor was significantly associated with *Staphylococcus aureus, Salmonella* Typhimurium, SPC and SCC in bulk tank (OR :3.27, $\chi 2$: 59 and $P < 0.00123$, OR: 4.17, $\chi 2$: 62.7 and $P < 0.00002$, OR: 6.21, $\chi 2$: 89.7 and $P < 0.000013$, OR: 2.17, $\chi 2$: 29.1 and $P < 0.0021$, respectively) as shown in table 5. Cattle manure was recognized to contain and carry extensive range of microorganisms which can be pathogenic or non-pathogenic to both animals and humans (Godwin, 1997).

It is really worth citing that vectors, e.g., flies and vermin, which might also additionally spread and cause subsequent infections to other animals with pathogens from stored manure and flies, carry a number of mastitis-causing organisms that can colonize teat lesions, and consequently affect the SCC and SPC in bulk tank milk (Holt et al., 2007; Thomson et al., 2017). This requires frequent waste removal, once to several times per day depending on the housing, climate conditions (hot climate leads to drinking, and more frequent urination) and animal density. Animal manure should then be transported to a designated storage or disposal area, out of contact with animal (Manyi-Loh et al., 2016). Muddy bedding materials as a risk factor was significantly related to *Staphylococcus aureus, Salmonella* Typhimurium, SPC and SCC in bulk tank (OR :2.25, $\chi 2$: 16 and $P< 0.013$, OR: 3.15, $\chi 2$: 36 and $P < 0.00015$, OR: 5, $\chi 2$: 66 and $P < 0.000023$, OR: 6.5, $\chi 2$: 86 and $P < 0.0000003$, respectively) as shown in table 5.

A wide variety of bedding materials are used in cattle barns as straw saw dust, wood and shavings. Bacterial concentrations in fresh and clean bedding were usually much lower than in concentrations in used bedding (Slaghuis et al., 1997). Schreiner and Ruegg (2003) reported that the main and common sources of exposure of environmental mastitis pathogens to the cow were the presence of moisture, mud and manure in the environment of the cow. One of the most vital and important steps affecting bulk tank microbial count and SCC was the transmission of dirt from dirt muddy bedding and soil to milk. Dirt basically was transmitted to milk when it was attached to the exterior of the teats and rinsed off all through milking operation (Murphy and Boor, 2000). The contamination of the teat with muddy soil was regarded one of the foremost important reasons for of elevated concentrations of microbial count in bulk tank milk, and consequently the level of *Salmonella* in bulk tank milk. Practices that expose the teat end to organic bedding sources, moist and muddy pens extended the risk of mastitis prevalence and milk contamination (Ruegg, 2006). Cleanliness of the cows (udder, teats and hocks) as a risk factor was significantly associated with *Staphylococcus aureus, Salmonella* Typhimurium, SPC and SCC in bulk tank (OR: 2.65, $\chi 2$. 19 and $P < 0.023$, OR: 4.25, $\chi 2$: 49 and $P < 0.00023$, OR. 5.15, χ: 69.7 and $P < 0.000001$ and OR: 4.21, $\chi 2$: 45.9 and $P < 0.0012$, respectively) as shown in table 5.

Cleanliness of the cows had a great and vital role in microbial contamination of the bulk tank milk, and had a great impact on the level of *Staphylococcus aureus* SPC and SCC level in bulk tank milk. These results agreed with Ruegg (2003), Schreiner and Ruegg (2003), Elmoslemany et al. (2009b), Piccinini et al. (2009), Zucali et al. (2011) and Cicconi-Hogan et al. (2013). Milking cows should be kept clean, groomed daily and therefore the udders and teats thoroughly should be washed before every milking because the coat and skin were always dirty as this might act as a source of spoilage bacteria. . Cleaning and removal of soil particles, bedding material and manure from the udder and flanks was very essential to prevent the entry of many types of bacteria into the bulk tank milk (Zelalem et al., 2011).

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Summer season as a risk factor was significantly associated with *Staphylococcus aureus*, Salmonella Typhimurium, SPC and SCC in bulk tank (OR: 3.21, $\chi 2$: 39 and P< 0.00012, OR: 2.15, $\chi 2$: 19.7 and P < 0.02, OR: 4.24, $\chi 2$: 42.7 and P < 0.00014 and OR. 3.27, $\chi 2$. 47.8 and P < 0.0002, respectively) as shown in table 6.

The results agreed with Leslie (1996), Sargeant et al. (1998), Norman et al. (2000), Ruegg and Tabone (2000), Zadnik et al. (2001), Berry et al. (2006), Lievaart et al. (2007), Olde Riekerink et al. (2007), Summer et al. (2007), Elmoslemany et al. (2010) and Zucali et al. (2011), Cicconi-Hogan et al. (2013). They reported that bulk tank milk SCC counts were higher in summer, and was perhaps related to the influence and impact of higher temperature and humidity on mastitis risk. Green et al. (2006) suggested that the rise in BMSCC during this period was that the consequences of arise in chronic high individual cow SCC (ICSCC), and disagreed with Berry et al. (2006) who reported that BMSCC was commonly lowest during April, and highest in November. It was additionally recognized that favorable temperature and humidity in the summer played a critical role in favoring growth and quantity of environmental bacteria in bedding material (Harmon 1994). Heat stress may additionally amplify the cow's susceptibility to infection via both lowering host resistance or by increasing host exposure to pathogens (Morse et al., 1988). Variations and fluctuation in temperature and humidity can have a strong impact on bacterial counts in milk. Summer season had a great impact on bulk tank total aerobic count as shown by Izsler (2010). Prevalence of *Staphylococcus aureus* in bulk tank was higher in summer. These results disagreed with findings of Makovec and Ruegg (2003) and Olde Riekerink et al. (2007) who observed a high incidence of *Staphylococcus aureus* in bulk tank milk during December and January. *Salmonella* incidence in bulk tank milk used to be higher in summer season. The present results which agreed with Holley et al. (2006) and Semenov et al. (2007). They mentioned that survival of *Salmonella* in the environment ought to additionally increase in the summer months, although soil composition, moisture and temperature fluctuation appeared to play a role. Region variation as a risk factor was significantly associated with *Staphylococcus aureus, Salmonella* Typhimurium, SPC and SCC in bulk tank (OR.2.31, $\chi 2$: 19 and P < 0.0014, OR: 2.46, $\chi 2$:20.7 and P < 0.002, OR: 3.24, $\chi 2$: 41.4 and P < 0.0012 and OR: 2.17, $\chi 2$: 27.8 and P < 0.0031, respectively) as shown in table 6.

Delta region was significantly associated with *Staphylococcus aureus, Salmonella* Typhimurium, SPC and SCC in bulk tank. These regional variations ought to be variant in climate and agricultural tradition. The climate and geographical aspects and features of region had a great impact on the degree of bacterial contamination and SCC level in bulk tank milk. The results agreed with Raghib et al. (2004). A possible reason for the elevation of TBC in Delta region ought to be due to poor hygienic condition of the collection tanks distributed by milk collectors. This finding agreed with Reinemann et al. (2000). PCR identification of virulence gene (*hlg*) of *Staphylococcus aureus* isolates revealed a product of approximate size 937 bp. The gene (*hlg*) which was found in 13 (54%) *Staphylococcus aureus* isolates (figure 3); these results disagreed with El-baz et al. (2016) (81.1%), Maeda et al. (2016) and Ali (2017) (90%). Hemolysins were produced by *Staphylococcus aureus* isolates, and played a vital role in the pathogenesis of the disease caused by *Staphylococcus aureus* (Lo et al., 2011). γ-hemolysin (*hlg*) gene was found within a 4.5 kb ScaI fragment of *Staphylococcus aureus* chromosome. It was another form of pore forming toxin, unlike α-hemolysin. The *hlg* gene consisted of two transcription units (the first one encodes γ-hemolysin-A (*HlgA*) like protein, a class S (slow) component, and the second one codes γ-hemolysin C (*hlgC*) and γ-hemolysin B (*hlgB*), a category of F(fast) and S(slow) component (Divyakolu et al.2019). Staphylococcal γ-hemolysin (*hlg*) consisted of polypeptides nominated as S (Slow), (*hlgA* or *hlgC*) and F (Fast), (*hlgB*), which coordinated in lysis of target cells, where the S components were proposed to affect cell type susceptibilities to those toxins Meyer et al. (2019). These proteins after determining of their cell targets undertook conformational changes and formed oligomeric complexes. This process resulted in transmembrane-pore formation, and also the cell death (Meyer et al. 2019). This toxin targeted polymorph nuclear cells, monocytes, macrophages and erythrocytes (Vandenesch et al. 2012). γ-hemolysin, belonging to a group of genes that coded for both *hlgA* and *hlgC* as the S (slow) component, or *hlgB* as the F (fast) component which was found within the core genome (Du Mont et al. 2014). On the other hand, molecular identification of *sopB* (*SigD*) virulence gene for *Salmonella* Typhimuriym isolates revealed that the PCR amplification with (*sopB*) gene specific primers was conducted with genomic DNA, which revealed in a product of approximate size 517 bp. *sopB* gene, was found in all *Salmonella* Typhimurium isolates (100%) figures 1 and 2. The *Salmonella* outer protein *(sopB*) gene well was conserved in all *Salmonella* Typhimurium strains. These results agreed with Mirold et al. (2001) who stated that the *sopB* gene was found in the SPI-5 pathogenicity island, and was detected in all sequenced *Salmonella* Typhimurium strains. The outer proteins (Sops) of *Salmonella* were playing a definite role in the manipulation of various stages of polymorphonuclear leukocyte influx, and rearrangement of the cytoskeleton (Boyle et al., 2006). *SopB*/*SigD*, an inositol phosphatase, was needed for fluid and chloride secretion, neutrophil recruitment, and played an important role in causing deformation to epithelial barrier function during the invasion (Bertelsen et al., 2004). It mediated the virulence by inhibiting inositol phosphate signaling pathways (Marcus et al. 2001; Marcus et al., 2002). *SopB*/*SigD* also had a great antiapoptotic activity, and so on they played an important and distinct role in the intracellular replication because of the up keeping and maintain of Akt activation (García-Gil et al., 2018).

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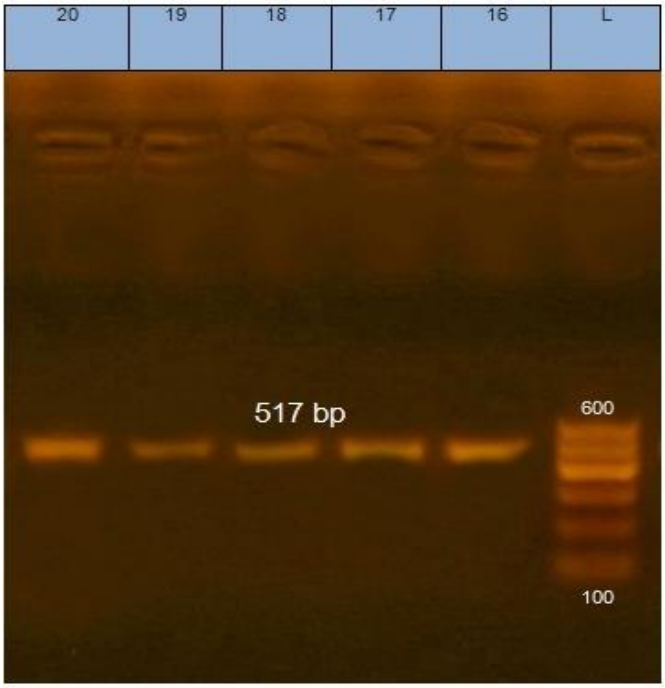

Figure 1. Agarose gel shows a PCR amplified product of 517 bp. of (*sopB*) virulence gene for *Salmonella* Typhimurium, lanes (16) to (20): samples positive for *sopB* gene, Lane (L): MW 100bp ladder (DNA marker)**.**

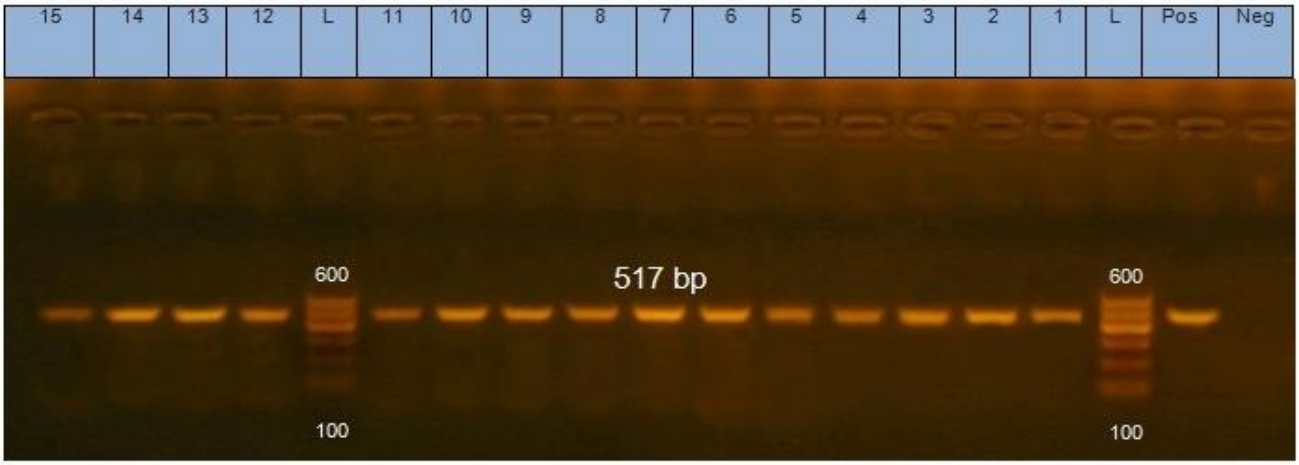

Figure 2. Agarose gel shows a polymerase chain reaction (PCR) amplified product of 517 bp of (*sopB*) virulence gene for *Salmonella* Typhimurium, lanes (1) to (15): samples positive for *sopB* gene, Lane (positive): positive control, Lane (Negative): Negative control, Lane (L): MW 100bp ladder (DNA marker).

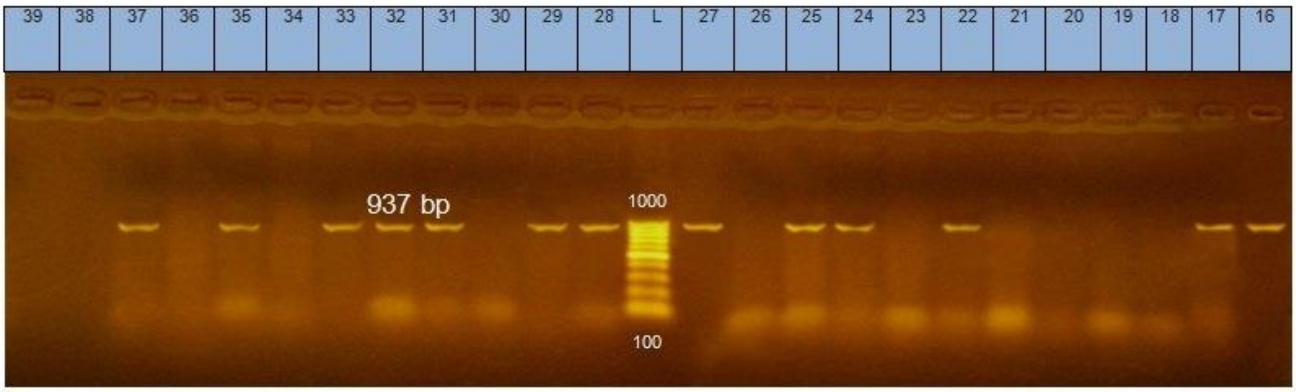

Figure 3. Agarose gel shows a PCR amplified product of 937 bp. of gamma hemolysin (*hlg*) gene for *Staphylococcus aureus*, lanes (16, 17, 22, 24, 25, 27, 28, 29, 31, 32, 33, 35, 37): samples positive for *hlg* gene, Lane (L): MW 100bp. ladder (DNA marker).

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Phylogenetic and partial gene sequence analysis of *hlg* gene of *Staphylococcus aureus* that was generated using neighbor joining in MEGA6 showed a clear clustering of isolated Egyptian *Staphylococcus aureus* strain, and different *Staphylococcus aureus* strains uploaded from gene bank as shown in figures 6 and 7. Sequence distance of *Staphylococcus aureus hlg* virulence gene was created by the MegAlign module of Laser gene DNA Star. Sequence identities between the isolated Egyptian strain and different *Staphylococcus aureus* strains were uploaded from gene bank revealing a 95.8% to 98.9% homology as shown in figure 7. Analyzing of nucleotide sequence of *hlg* virulence gene of the Egyptian isolated strain in the current study showed a 98.9% nucleotide identity with the American *Staphylococcus aureus* strain FDAARGOS-412 (accession no. CP023500) by Minogue et al. (2017), the Belgium *Staphylococcus aureus* strain NMR08 (accession no. CP023560) by Coppens et al. (2017), the Korean *Staphylococcus aureus* strain CN1 (accession no. CP003979) by Chen et al. (2013), the Japanese *Staphylococcus aureus* strains TMUS2126 and TMUS2134 (accession no. AP014652 and AP014563, respectively) by Yamaguchi et al. (2015) and a 98.1% nucleotide identity with the Taiwan *Staphylococcus aureus* strain M013 (accession no. CP003166) by Huang et al. (2012), the Taiwan (MRSA) methicillin resistant *Staphylococcus aureus* strains SA967 and SA40 (accession no. CP003603 and CP003604) by Chen et al. (2013). Amino acids alignment report of the sequenced 934 amino acids of (*hlg*) *Staphylococcus aureus* virulence gene of Egyptian isolated strain (using CLUSTALW) multiple sequence alignment program version 1.83 of MegaAlign module of laser gene DNA star as shown in figure 8 showed a great homology between the *Staphylococcus aureus* Egyptian strain, and the different *Staphylococcus aureus* strains uploaded from gene bank. Phylogenetic and partial gene sequence analysis of *sopB* gene of *Salmonella* that was generated using a neighbor joining in MEGA6 as shown in figures 4 and 5 showed a clear clustering of isolated Egyptian *Staphylococcus aureus* strain and different *S. aureus* strains uploaded from gene bank. Nucleotide sequence distance of *Salmonella* Typhimurium *sopB* virulence gene was created by the MegaAlign module of Laser gene DNA Star. Nucleotide Sequence identities between the isolated Egyptian strain and different *Salmonella* strains uploaded from gene bank revealed a 98.6% to 100% homology as shown in figure 4. Nucleotide sequence analysis of *sopB* virulence gene of the Egyptian isolated strain showed a 100% nucleotide identity with, the Taiwan *Salmonella* Typhimurium strain BL10 (accession no. CP024619) by Hong et al. (2018), the English *Salmonella* Typhimurium strain VNP151-sc-2315230 (accession no. LT795114) by pathogen informatics (2017), the American *Salmonella* Typhimurium RM1160 strain (accession no. CP022658) by Parker et al. (2017), the English *Salmonella* Typhimurium strain DT2 (accession no. HG326213) by Kingsley et al. (2013), the English *Salmonella* Typhimurium strain DT104 (accession no. HF937208) by Mather et al. (2013), *Salmonella* Typhimurium var.5 strain CFSAN001921 (accession no. CP006048) by Hoffman et al. (2013), the English *Salmonella* Typhimurium strain U288 (accession no. CP003836) by Hotton et al. (2012), the American *Salmonella* Typhimurium strain 798 (accession no. CP003386) by Petersson et al. (2012), the American *Salmonella* Typhimurium strain Uk1 (accession no. CP002614) by (Luo et al. (2011), the American *Salmonella* Typhimurium strain ST4/74 (accession no. CP002487) by Richardson et al. (2011), the Japanese *Salmonella* Typhimurium strain T000240 (accession no. AP011957) by Izumiyia et al. (2011), the *Salmonella* Typhimurium strain SL1344 (accession no. FQ312003) by Kroger et al. (2012), the American *Salmonella* Typhimurium strain 14028S (accession no. CP001363) by Jarvik et al. (2010). Amino acids alignment reported of the sequenced 514 amino acids of *Salmonella* Typhimurium (*sopB*) virulence gene of Egyptian isolated strain (using CLUSTALW) multiple sequence alignment program version 1.83 of MegaAlign module of laser gene DNA star as shown in figure 9 showed a great homology between the Egyptian strain *Salmonella* Typhimurium and the different *Salmonella* Typhimurium strains uploaded from gene bank.

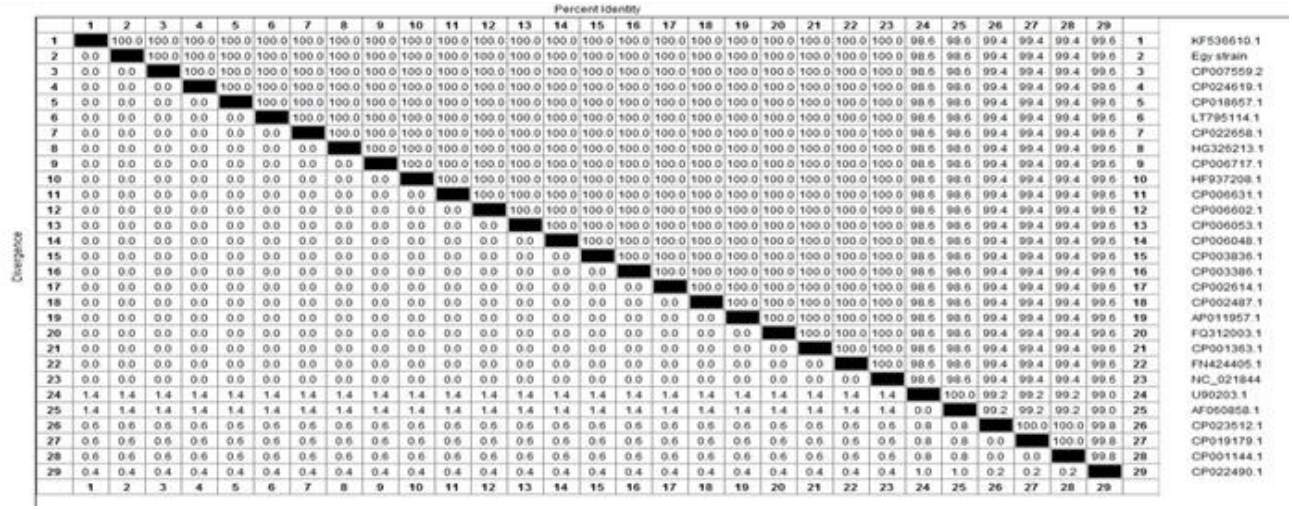

Figure 4. Nucleotide Sequence distance analysis of *Salmonella* Typhimurium (*sopB*) virulence gene between the Egyptian isolated strain and different *Salmonella* Typhimurium strains uploaded from gene bank.

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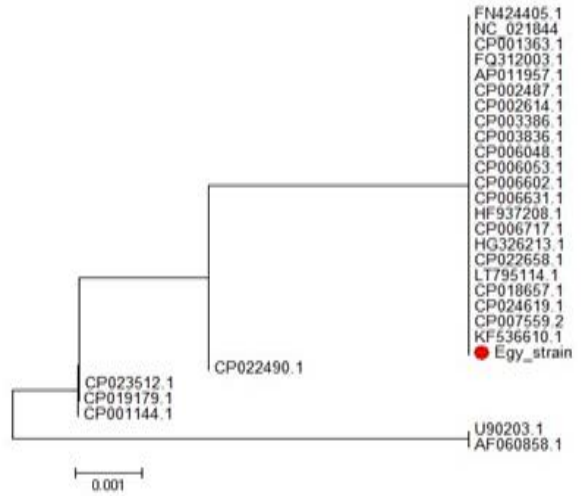

Figure 5. Phylogenetic tree for *Salmonella* Typhimurium (*sopB*) virulence gene partial nucleotide sequences that was generated using a neighbor joining in MEGA6. It shows a clear clustering of the Egyptian isolated strain and different *Salmonella* Typhimurium strains uploaded from gene bank**.**

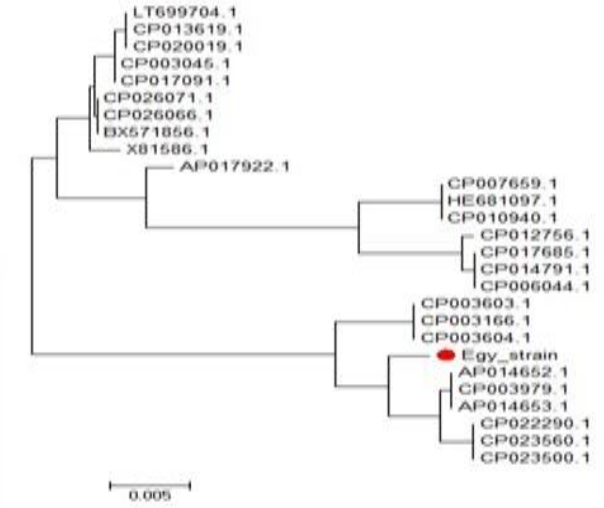

Figure 6. Phylogenetic tree for *Staphylococcus aureus* (*hlg*) virulence gene partial nucleotide sequences that was generated using a neighbor joining in MEGA6. It shows a clear clustering of the Egyptian isolated strain and different *S. aureus* strains uploaded from gene bank**.**

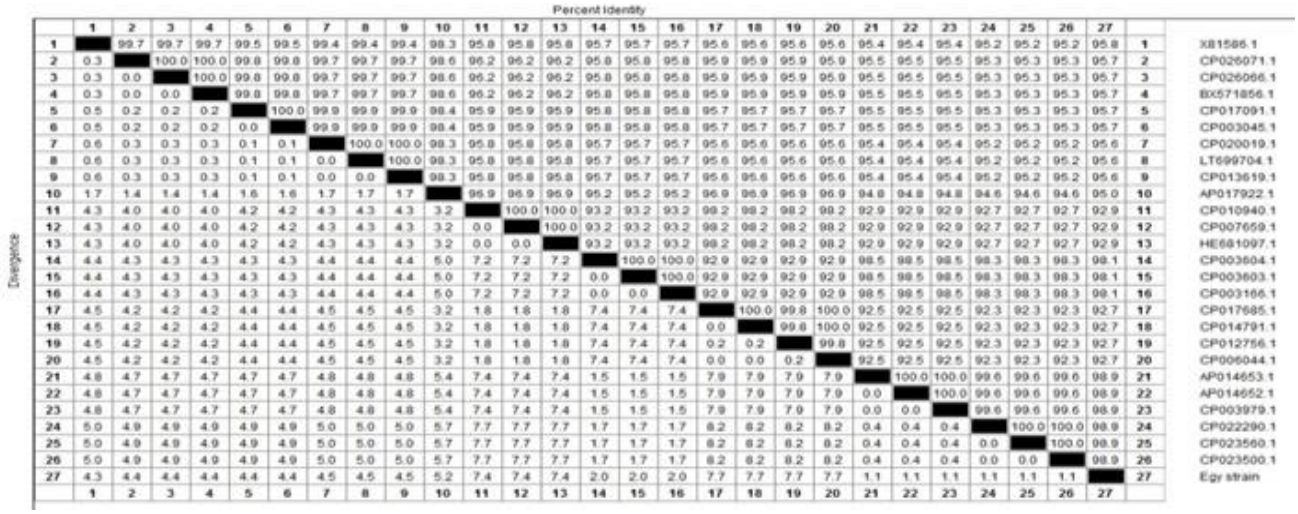

Figure 7. Nucleotide sequence distance analysis of (*hlg*) *Staphylococcus aureus* virulence gene between the Egyptian isolated strain and different *Staphylococcus aureus* strains uploaded from gene bank

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Majority   ANPLLENAKAANDTEDIGKGNDVEIIKRTEDKTSNKWGVTQNIQFDFVKDKKYNKDALILKMQGFISSRTTYYNYKNTNH
           ---------+---------+---------+---------+---------+---------+---------+---------+
                    10        20        30        40        50        60        70        80
           ---------+---------+---------+---------+---------+---------+---------+---------+
X81586.1   ..........................................G.........V.............................. 238
CP026071.1 ..........................................................................  238
CP026066.1 ..........................................................................  238
BX571856.1 ..........................................................................  238
CP017091.1 ..........................................................................  238
CP003045.1 ..........................................................................  238
CP020019.1 ..........................................................................  238
LT699704.1 ..........................................................................  238
CP013619.1 ..........................................................................  238
AP017922.1 ..........................................................................  238
CP010940.1 ..........................................................................  238
CP007659.1 ..........................................................................  238
HE681097.1 ..........................................................................  238
CP003604.1 ....................S.I.................................................  238
CP003603.1 ....................S.I.................................................  238
CP003166.1 ....................S.I.................................................  238
CP017685.1 ......K...................................................................  238
CP014791.1 ......K...................................................................  238
CP012756.1 ........R.................................................................  238
CP006044.1 ......K...................................................................  238
AP014653.1 ....................S.I.................................................K...  238
AP014652.1 ....................S.I.................................................K...  238
CP003979.1 ....................S.I.................................................K...  238
CP022290.1 ....................S.I.................................................K...  238
CP023560.1 ....................S.I.................................................K...  238
CP023500.1 ....................S.I.................................................K...  238
Egy strain ....................S.I.....................G...........................K...  238
```


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```
Majority   TYGRNMDVTHAIKRSTHYGNSYLDGHRVHNAFKNRNYTVKYEVNWKTHEIKVKGQN-YEHE-ISQIIRCYVY
           ---------+---------+---------+---------+---------+---------+---------+--
                    250       260       270       280       290       300       310
           ---------+---------+---------+---------+---------+---------+---------+--
X81586.1   ..................................................................................  934
CP026071.1 ..................................................................................  934
CP026066.1 ..................................................................................  934
BX571856.1 ..................................................................................  934
CP017091.1 ..................................................................................  934
CP003045.1 ..................................................................................  934
CP020019.1 ..................................................................................  934
LT699704.1 ..................................................................................  934
CP013619.1 ..................................................................................  934
AP017922.1 ..................................................................................  934
CP010940.1 .................................................................................I.  934
CP007659.1 .................................................................................I.  934
HE681097.1 .................................................................................I.  934
CP003604.1 ............................I....V.........................N.........I.  934
CP003603.1 ............................I....V.........................N.........I.  934
CP003166.1 ............................I....V.........................N.........I.  934
CP017685.1 ............................I.....................................  934
CP014791.1 ............................I.....................................  934
CP012756.1 ............................I.....................................  934
CP006044.1 ............................I.....................................  934
AP014653.1 ..............................V.........................N.........I.  934
AP014652.1 ..............................V.........................N.........I.  934
CP003979.1 ..............................V.........................N.........I.  934
CP022290.1 ..............................V.........................N.........I.  934
CP023560.1 ..............................V.........................N.........I.  934
CP023500.1 ..............................V.........................N.........I.  934
Egy strain ..............................V.........................N.............  934
```

Figure 8. Deduced amino acids alignment of (*hlg*) *Staphylococcus aureus* virulence gene of Egyptian isolated strain using (CLUSTALW) multiple sequence alignment program version 1.83 of MegaAlign module of laser gene DNA star and different *Staphylococcus aureus* strains uploaded from gene bank

```
Majority   HPQIIGVMNILGIPTAYALSLISTGKDIFCADFHLCCRELRVGIVMPVMRECIFYGLPGLVIKLLDLSISKRYCRKAMSF
           ---------+---------+---------+---------+---------+---------+---------+---------+
                    10        20        30        40        50        60        70        80
           ---------+---------+---------+---------+---------+---------+---------+---------+
KF536610.1 ..................................................................................  238
Egy strain ..................................................................................  238
CP007559.2 ..................................................................................  238
CP024619.1 ..................................................................................  238
CP018657.1 ..................................................................................  238
LT795114.1 ..................................................................................  238
CP022658.1 ..................................................................................  238
HG326213.1 ..................................................................................  238
CP006717.1 ..................................................................................  238
HF937208.1 ..................................................................................  238
CP006631.1 ..................................................................................  238
CP006602.1 ..................................................................................  238
CP006053.1 ..................................................................................  238
CP006048.1 ..................................................................................  238
CP003836.1 ..................................................................................  238
CP003386.1 ..................................................................................  238
CP002614.1 ..................................................................................  238
CP002487.1 ..................................................................................  238
AP011957.1 ..................................................................................  238
FQ312003.1 ..................................................................................  238
CP001363.1 ..................................................................................  238
FN424405.1 ..................................................................................  238
NC_021844  ..................................................................................  238
U90203.1   ..................................G.........................V..V.........  238
AF060858.1 ..................................G.........................V..V.........  238
CP023512.1 ..................................G............................V.........  238
CP019179.1 ..................................G............................V.........  238
CP001144.1 ..................................G............................V.........  238
CP022490.1 ..................................G.....................................  238
```


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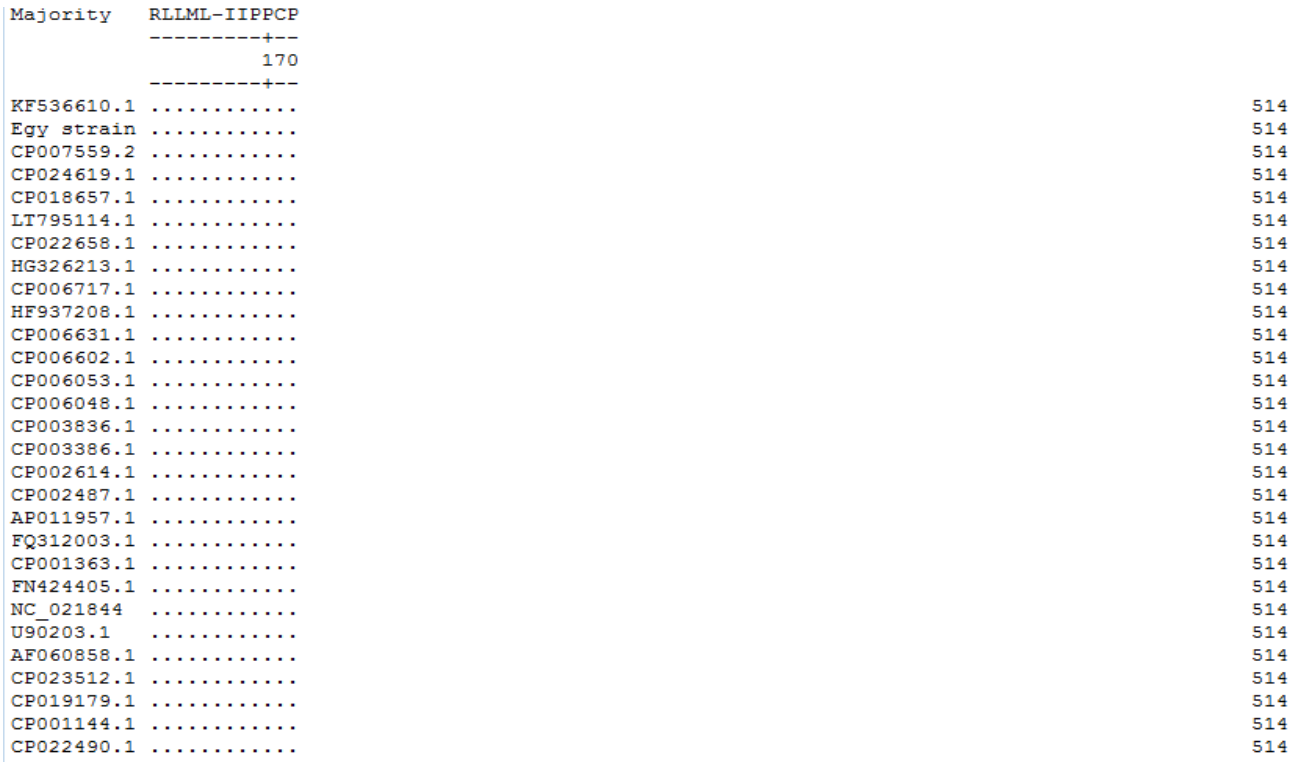

Figure 9. Deduced Amino acids alignment of *Salmonella* Typhimurium (*sopB*) virulence gene of Egyptian isolated strain using (CLUSTALW) multiple sequence alignment program version 1.83 of MegaAlign module of laser gene DNA star and different *Salmonella* Typhimurium strains uploaded from gene bank

CONCLUSION

The results of present study provided an allocation for the elaborateness of more efficacious preventive programs for controlling the mastitis and bacteriological quality of bulk tank milk, enhancement of raw milk quality, decreasing and monitoring the economic losses caused by the disease to dairy industry. Analyzing of nucleotide sequence of *hlg* virulence gene of the *Staphylococcus aureus* Egyptian isolated strain in the current study showed a 95.8% to 98.9% nucleotide identity with different *Staphylococcus aureus* strains uploaded from gene bank. Nucleotide sequence identities between the isolated Egyptian *Salmonella* strain and different *Salmonella* strains uploaded from gene bank revealed a 98.6% to 100% homology.

DECLERATIONS

Author`s contributions

M.F and H.M designed the concept of the review article, and M.F and H.M designed and performed study design and the economic frame work .All authors shared in writing, reviewed and approved the manuscript. All authors read and approved the final version.

Competing interests

The authors declared that they have no competing interests.

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