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Research Article

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Phylogenetic and Gene Sequence Analyses of *Staphylococcus aureus hlg, E. coli TraT, Streptococcus uberis 16SrRNA* and *Staphylococcus haemolyticus Tuf Genes* Isolated from Cattle Mastitis

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Abstract

The main aim of this study is to investigate and understanding the molecular epidemiology of the most common and prevalent bacteria (*E. coli, Staphylococcus aureus, Streptococcus uberis and Staphylococcus haemolyticus*) that cause bovine mastitis in Egypt dairies that generate the routine and standard mastitis control measures. Out of 220 (160 sub-clinical and 60 active clinical mastitis), positive milk samples were collected from 20 cattle dairy farms. Locally field isolates were detected and confirmed phenotypic by culturing, gram staining, biochemical and molecular identification to be in overall cow level prevalence of *Staphylococcus aureus* 11 (5%), *Staphylococcus haemolyticus* 18 (8.1%), *E. coli* 40 (18.1%) and *Streptococcus uberis* 19 (8.6%). PCR identification of *hlg* gene of *Staphylococcus aureus*, *E. coli TraT, Streptococcus uberis 16SrRNA, Staphylococcus hemolyticus tuf* genes isolates revealed *TraT* gene was found in all forty (100%) *E. coli* isolates, (*tuf)* virulence gene was found in all (18) *Staphylococcus haemolyticus* isolates (100%), (*hlg*) gene was found in 11 (42.3%) *Staphylococcus aureus* isolates and (*16SrRNA*) gene was conserved in all *Streptococcus uberis* isolates. Phylogenetic analysis, showed clear clustering of isolated *E. coli, Staphylococcus haemolyticus and Streptococcus uberis* Egyptian strain and different strains uploaded from gene bank. In Conclusion: This study focuses and gave a clear vision on the role of human being in transmission of cattle mastitis pathogens in dairy cattle farms. Regular monotiring of *Staphylococcus aureus, E. coli, Staphylococcus haemolyticus and Streptococcus uberis* sequence changes is very important and essential for molecular epidemiological investigations and also can be used in vaccine development and evaluation studies.

Keywords: Bioinformatics; Epidemiology; Mastitis; PCR; Phylogenetics

Abbreviations

Hlg: Gamma Hemolysein; *TraT* gene: Serum Resistance Gene; *Tuf* gene: Translation Elongation Factor

Introduction

Mastitis is known as one of the foremost complicated and common disease among dairy cows and can be defined as inflammation of parenchyma of mammary glands caused by different infectious and none infectious agents [1]. The use of molecular techniques in pathogen diagnosis has improved and increased over the ultimate years. PCR-based techniques have been described and used for diagnosis of wide range of mastitis pathogens [2]. *TraT* plasmid encodes an outer membrane protein thought to block the membrane attack complex present in the serum of the host [3]. The *Staphylococcus aureus* gamma-hemolysins are β-barrel pore-forming toxins that are secreted from the bacteria as monomers.

The gamma-toxin monomers are comprised S and *F* class subunits, corresponding to slow and fast elution from an ion exchange column [4]. *Tuf* gene small size and its conserved location in the bacterial chromosome play a distinct role in its superiority in DNA sequencing for construction of phylogenetic trees on species and genus level in *Staphylococci* [5]. *16SrRNA* gene is regarded as one of the foremost effective tool for *Streptococcus uberis* identification [6]. Phylogenetics could be a powerful tool for microbial epidemiology. Phylogenetic methods are often wont to analyze nucleotide sequence data in such a way that the order of descent of related strains is often determined [7].

Objective of the Study

The main objective of this study is conducting molecular identification of *E. coli TraT, Staphylococcus aureus hlg, Streptococcus uberis 16SrRNA*, *Staphylococcus haemolyticus tuf* genes by convention PCR, applying Phylogenetics and gene sequence analysis to give insight to the source and origin, molecular epidemiology and disease pattern in Egypt dairies.

Materials and Methods

Bacterial isolates and bacteriological examination

Twenty six (26) *Staphylococcus aureus*, Forty (40) *E. coli*, Eighteen (18) *Staphylococcus haemolyticus* and Nineteen (19) *Streptococcus uberis* isolates were identified and isolated from 220 (160 sub-clinical and 60 active clinical mastitis) positive milk samples were collected from 20 cattle dairy farms milk samples from different governorates during the 2018 and 2020 years in Egypt**.** These isolates were identified by phenotypic, gram staining, biochemical and molecular identification as described by [8] and kept lyophilized till used in this study**.** Isolation, Morphological identification and Biochemical identification of *E. coli, Staphylococcus aureus and coagulase negative staphylococc*i, *Streptococci* was done according to methods described by [8].

Ethical approval

This study was applied in accordance to the regulations and ethics of the European Union for the protection of experimental animals (2010/63/EU) (http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:276:0033:0079:en:PDF).

PCR amplification and Sequencing of *E. coli TraT gene, Staphylococcus aureus hlg gene, Staphylococcus haemolyticus tuf gene* and *Streptococcus uberis 16SrRNA* gene

All the identified *E. coli*, *Staphylococcus aureus*, *Staphylococcus haemolyticus*, *Streptococcus uberis* isolates were examined by PCR for the presence of *E. coli TraT*, *Staphylococcus aureus hlg*, *Streptococcus uberis 16SrRNA*, *Staphylococcus haemolyticus tuf* genes. The primers sequences and PCR product sizes are shown in table 1. DNA extraction from samples was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Primers were utilized in a 25- µl reaction containing 12.5 µl of Emerald Amp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentrations, 4.5 µl of water, and 6 µl of DNA template. The reaction was performed in an applied biosystem 2720 thermal cycler. Electrophoresis grade agarose (1.5 g) was prepared in 100 ml TBE buffer in a sterile flask, it was heated in microwave to dissolve all granules with agitation, and allowed to cool down at 70˚C, then 0.5 µg/ml ethedium bromide was added and mixed thoroughly. The warm agarose was poured directly in gel casting apparatus with desired comb in apposition and left at room temperature for polymerization. The comb was then removed, and also the electrophoresis tank was full of TBE buffer. 20 µl of each PCR product samples, negative control and positive control were loaded to the gel. Gene ruler 100 bp ladder (Fermentas, Thermo, Germany) was wanted to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and also the data was analyzed through computer software.

Gene	Sequence	Amplified product	Reference
Trat	GATGGCTGAACCGTGGTTATG	307 bp	[9]
	CACACGGGTCTGGTATTTATGC		
hlg	GCCAATCCGTTATTAGAAAATGC	937 bp	[10]
	CCATAGACGTAGCAACGGAT		
16S rRNA	CGGGGGATAACTATTGGAAACGATA	912 bp	[11]
	ACCTGTCACCCGATGTACCGAAGTA		
tuf	GCCAGTTGAGGACGTATTCT	412 bp	[5]
	CCATTTCAGTACCTTCTGGTAA		

Table 1: Primers sequences, target genes, amplicon sizes.

Deduced amino acids sequences analysis was performed using the CLUSTAL W multiple sequence alignment program, version 1.83 of Mega Align module of Laser gene DNA Star software which was applied according to the standard methods described by [12]. Phylogenetic analysis of *E. coli TraT*, *Staphylococcus haemolyticus tuf* and *Streptococcus uberis 16SrRNA* genes were performed using, neighbor joining in MEGA6 [13]. Phylogenetic and Sequence analysis of *hlg* of *Staphylococcus aureus* were analyzed by using BLAST Web tool of the Gene Bank (NCBI) https://blast.ncbi.nlm.nih.gov/Blast.cgi. The sequences were aligned using pair wise alignments. The phylogenetic tree was generated using neighbor joining method.

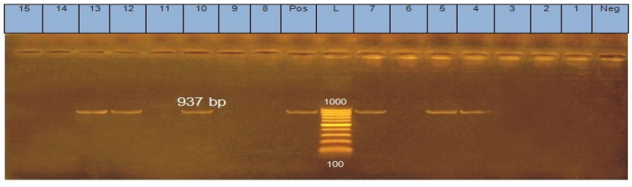


Figure 1: Agarose gel showing polymerase chain reaction (PCR) amplified product of 937 bp of gamma hemolysin (*hlg*) gene for *Staphylococcus aureus*, lanes (4,5,7,10,12,13): samples positive for *hlg* gene, lane (pos.): positive control, lane (Neg.): Negative control, Lane (L): MW 100bp ladder (DNA marker).

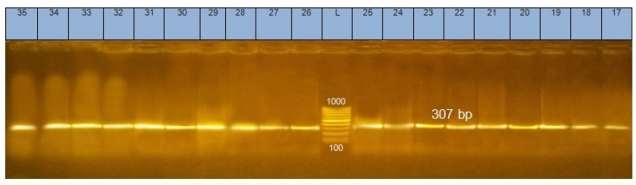


Figure 4: Agarose gel showing polymerase chain reaction (PCR) amplified product of 307 bp of *TraT* gene for *E. coli*, lanes 17 to 35: samples positive for, *TraT* gene lane (pos.): positive control, lane (Neg.): Negative control, Lane (L): MW 100bp ladder (DNA marker).

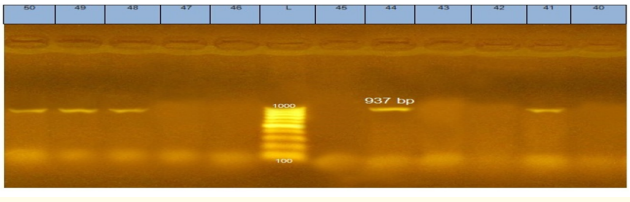


Figure 2: Agarose gel showing polymerase chain reaction (PCR) amplified product of 937 bp of gamma hemolysin (*hlg*) gene for *Staphylococcus aureus*, lanes (41, 44, 48, 49, 50): samples positive for *hlg* gene, lane (pos.): positive control, lane (Neg.): Negative control, Lane (L): MW 100bp ladder (DNA marker).

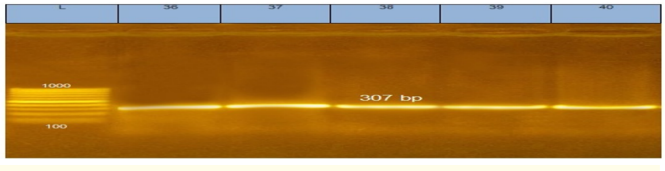


Figure 5: Agarose gel showing polymerase chain reaction (PCR) amplified product of 307 bp of *TraT* gene for *E. coli*, lanes 36 to 40: samples positive for, *TraT* gene lane (pos.): positive control, lane (Neg.): Negative control, Lane (L): MW 100bp ladder (DNA marker).

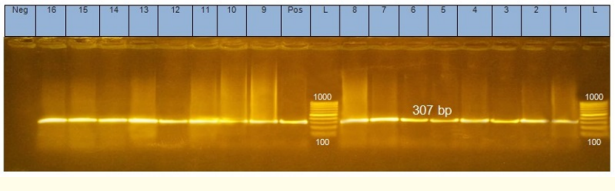


Figure 3: Agarose gel showing polymerase chain reaction (PCR) amplified product of 307 bp of *TraT* gene for *E. coli*, lanes 1 to 16: samples positive for, *TraT* gene lane (pos.): positive control, lane (Neg.): Negative control, Lane (L): MW 100bp ladder (DNA marker).

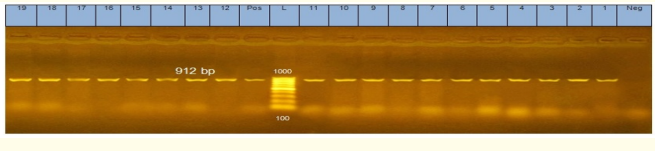


Figure 6: Agarose gel showing polymerase chain reaction (PCR) amplified product of 912 bp of *Streptococcus uberis 16SrRNA*, lanes 1 to 19: samples positive for, *16SrRNA* gene lane (pos.): positive control, lane (Neg.): Negative control, Lane (L): MW 100bp ladder (DNA marker).

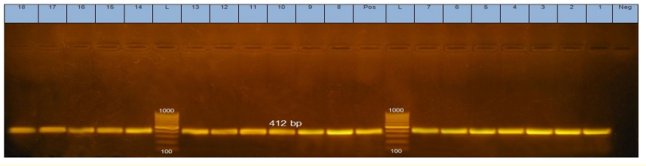


Figure 7: Agarose gel showing polymerase chain reaction (PCR) amplified product of 412 bp of *Staphylococcus haemolyticus tuf* gene, lanes 1 to 18: samples positive for, *tuf* gene lane (pos.): positive control, lane (Neg.): Negative control, Lane (L): MW 100bp ladder (DNA marker).

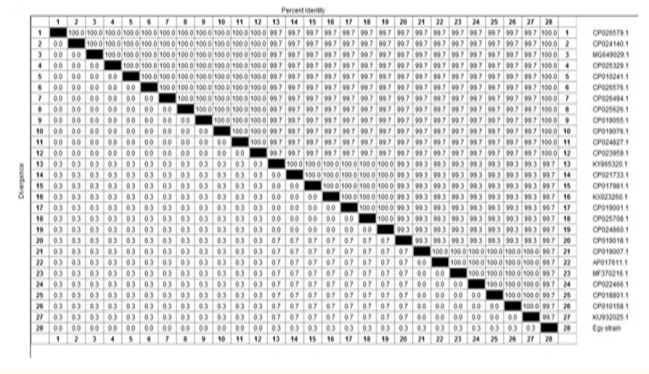


Figure 8: Nuclotide Sequence distance analysis of *E. coli TraT* virulence gene between the Egyptian isolated strain and different *E. coli* strains uploaded from gene bank.

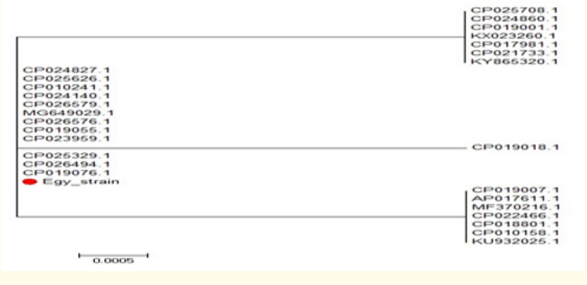


Figure 9: Phylogenetic tree for *E. coli TraT* virulence gene partial sequences that was generated using, neighbor joining in MEGA6. It showed clear clustering of the Egyptian isolated strain and different *E. coli* strains uploaded from gene bank.

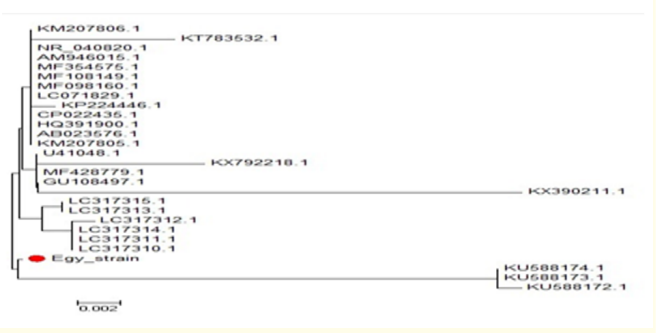


Figure 10: Phylogenetic tree for *Streptococcus uberis 16SrRNA* gene partial sequences that was generated using neighbor joining in MEGA6. It showed clear clustering of the Egyptian isolated strain and different *Streptococcus uberis* strains uploaded from gene bank.

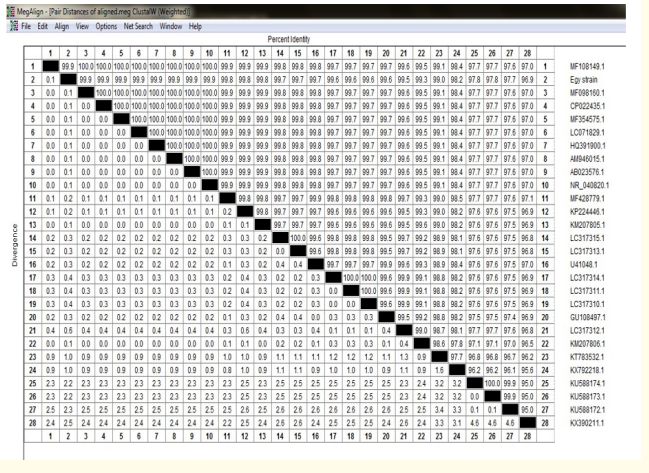


Figure 11: Nueclotide Sequence distance analysis of *Streptococcus uberis 16SrRNA* gene between the Egyptian isolated strain and different *Streptococcus uberis* strains uploaded from gene bank.

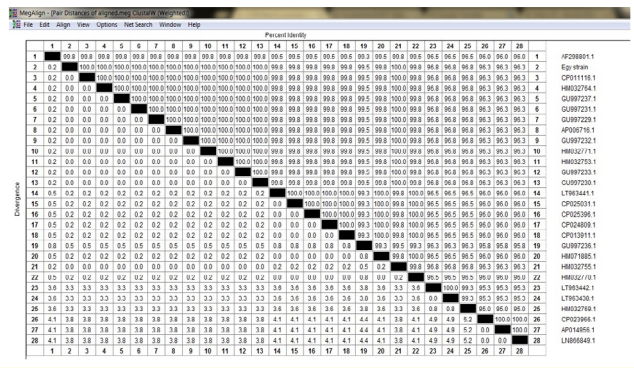


Figure 12: Nueclotide Sequence distance analysis of *Staphylococcus haemolyticus tuf* gene between the Egyptian isolated strain and different *Staphylococcus hemolyticus* strains uploaded from gene bank.

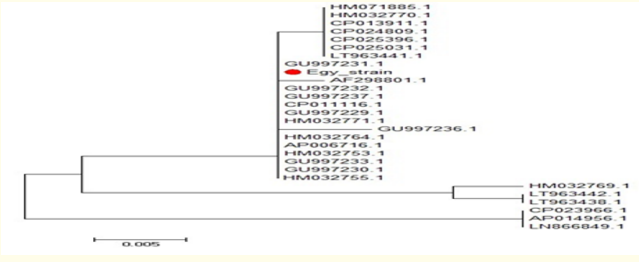
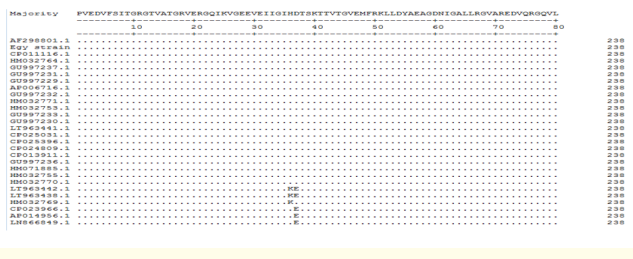


Figure 13: Phylogenetic tree for *Staphylococcus haemolyticus tuf* gene partial sequences that was generated neighbor joining in MEGA6. It showed clear clustering of the Egyptian isolated strain and different *Staphylococcus hemolyticus* strains uploaded from gene bank.



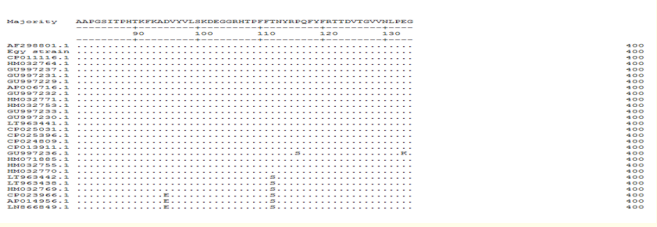
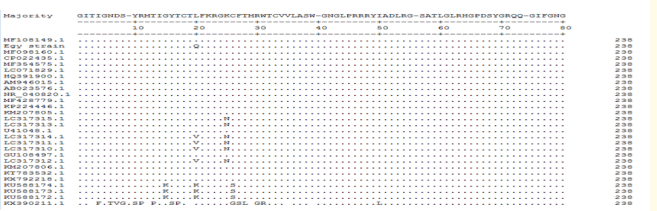


Figure 14: Deduced amino acids alignment of *Staphylococcus haemolyticus tuf* gene *of* Egyptian isolated strain (using CLUSTALW multiple sequence alignment program version 1.83 of Mega Align module of laser gene DNA star) and different *Staphylococcus haemolyticus* strains uploaded from gene bank.



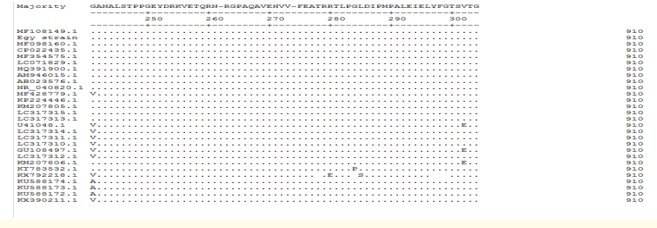
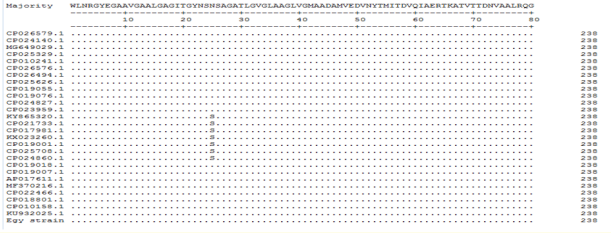


Figure 15: Deduced amino acids alignment of *Streptcoccus uberis 16SrRNA* gene of Egyptian isolated strain (using CLUSTALW multiple sequence alignment program versions 1.83 of Mega Align module of laser gene DNA star) and different *Streptcoccus uberis* strains uploaded from gene bank.



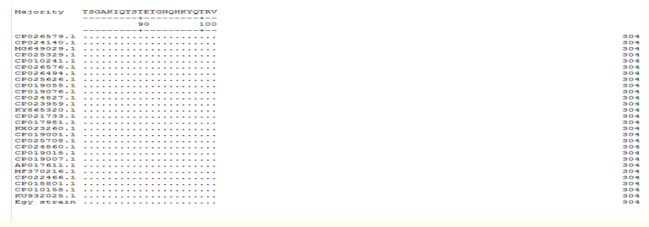


Figure 16: Deduced amino acids alignment of *E. coli TraT* virulence gene of Egyptian isolated strain (using CLUSTALW multiple sequence alignment program version 1.83 of Mega Align module of laser gene DNA star) and different *E. coli* strains uploaded from gene bank.

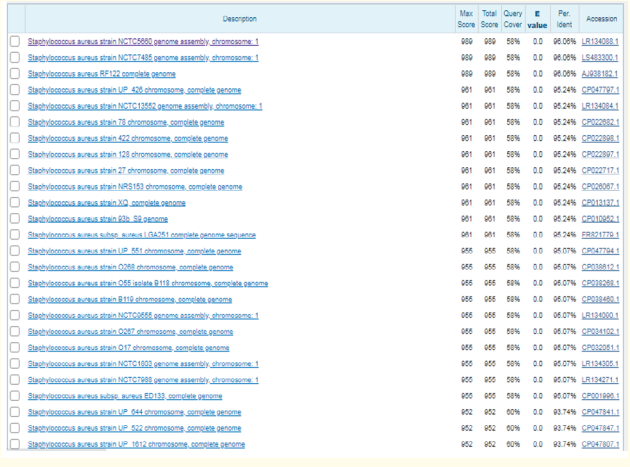


Figure 17: Nucleotide Sequence analyses of *hlg* gene *of Staphylococcus aureus* isolated from cattle mastitis between the Egyptian isolated strain and different *Staphylococcus aureus* strains uploaded from gene bank.

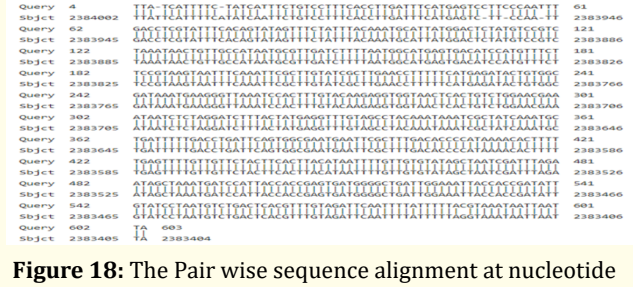


Figure 18: The Pair wise sequence alignment at nucleotide level *hlg* gene.

Results

PCR amplification and Sequencing of *E. coli TraT gene, Staphylococcus aureus hlg gene, Staphylococcus haemolyticus tuf gene* and *Streptococcus uberis 16SrRNA* gene

Molecular identification of *hlg* gene of *Staphylococcus aureus*, *E. coli TraT, Streptococcus uberis 16SrRNA, Staphylococcus haemolyticus tuf* genes isolates revealed in products with approximate size 937 bp (Figure 1 and 2), 307 bp (Figure 3-5), 912 bp (Figure 6) and 412 bp (Figure 7) respectively. The *TraT* gene was found in all forty (100%) *E. coli* isolates. Translation elongation factor (*tuf)* virulence gene was detected in all (18) *Staphylococcus hemolyticus* isolates (100%). (*hlg*) gene was found in (11/26) (42.3%) *Staphylococcus aureus* isolates. (*16SrRNA*) gene was conserved in all [19] *Streptococcus uberis* isolates (100%).

Phylogenetic and partial gene sequence analysis of *E. coli TraT* gene (Figure 8 and 9), *Staphylococcus haemolyticus tuf* gene (Figures 12 and 13), *Streptococcus uberis 16SrRNA* gene [10,11], *Staphylococcus aureus hlg* gene [17] revealed clar clustering of isolated Egyptian strains and different strains uploaded from gene bank.

Deduced amino acids alignment report of the sequenced 400 amino acids of *Staphylococcus haemolyticus tuf* gene (Figure 14) showed great homology between the Egyptian *Staphylococcus haemolyticus* strain and the different *Staphylococcus haemolyticus* strains uploaded from gene bank.

Deduced amino acids alignment report of the sequenced 910 amino acids of *Streptococcus uberis 16SrRNA* gene (Figure 15) showed great homology between the Egyptian *Streptococcus uberis* strain and the different *Streptococcus uberis* strains uploaded from gene bank.

Deduced amino acids alignment report of the sequenced 304 amino acids of *E. coli TraT* gene (Figure 16) showed great homology between the Egyptian *E. coli* strain and the different *E. coli* strains uploaded from gene bank.

The Pair wise sequence alignment at nucleotide level *hlg* gene (Figure 18) demonstrated clear similarity and clustering of the Egyptian *Staphylococcus aureus strain* with other *Staphylococcus aureus* strains in the gene Bank.

Discussion

Molecular identification of *TraT* virulence gene of *E. coli* isolates revealed that the PCR amplification with *TraT* gene specific prim-

ers was conducted with genomic DNA, which revealed in a product of approximate size 307 bp. Also *TraT* gene was found in all forty (100%) *E. coli* isolates these results disagree with [14] (72%). *TraT*, the serum resistance-associated gene, was the foremost prevalent virulence determinant [15]. *TraT* is meant to be a surface exclusion lipoprotein and facilitates extracellular protease activity. It absolutely was therefore the foremost prevalent virulence factor identified within *E. coli* isolates from cattle clinical mastitis.

On the other hand Molecular identification of translation elongation factor (*tuf)* virulence gene of *Staphylococcus haemolyticus* isolates revealed that the PCR amplification with (*tuf*) gene specific primers was conducted with genomic DNA, which revealed in a product of approximate size 412 bp. Translation elongation factor (*tuf)* virulence gene was found in all (18) *Staphylococcus haemolyticus* isolates (100%). The *tuf* gene cluster, which is found within the short tandem repeat region on bacterial chromosome, shows a clear diversity among members of *Staphylococci*. *Tuf* gene small size and its conserved location in bacterial chromosome play a distinct role in its superiority in DNA sequencing for construction of phylogenetic tree on species and genus level in *Staphylococci* [5]. Analysis of the *tuf* gene had great role for discrimination of *Staphylococcus haemolyticus* clinical isolates, and provides a reference methodology with high accuracy for recognizing clinical infections associated with *Staphylococcus haemolyticus* [16].

Molecular identification of (*hlg*) virulence gene of *Staphylococcus aureus* isolates revealed that the PCR amplification with (*hlg*) gene specific primers was conducted with genomic DNA, which revealed in a product of approximate size 937 bp. (*hlg*) gene was found in (11/26) (42.3%) *Staphylococcus aureus* isolates. *Staphylococcal* γ-hemolysins are bicomponent toxins forming a protein family with leucocidins and α -toxin. Two active toxins (AB and CB) can be formed combining one in every of the class-S components, *HlgA* or *HlgC*, with the class-F component *HlgB*. These two γ-hemolysins form pores with marked similarities to α-toxin [4].

Molecular identification of (*16SrRNA)* gene of *Streptococcus uberis* isolates revealed that the PCR amplification with (*16SrRNA*) gene specific primers was conducted with genomic DNA, which revealed in a product of approximate size 912 bp. (*16SrRNA*) gene was conserved in all *Streptococcus uberis* isolates. *16SrRNA* genes databases more cost effective expensive and more attractive as future technique in mastitis diagnostics [6].

Phylogenetic and partial gene sequence analysis of *E. coli TraT* virulence gene *that* was generated using, neighbor joining in MEGA6 showed clear clustering of isolated *E. coli* Egyptian strain and different *E. coli* strains uploaded from gene bank. Sequence distance of *E. coli TraT* virulence gene was created by the Mega Align module of Laser gene DNA Star. Sequence identities between the isolated Egyptian strain and different *E. coli* strains uploaded from gene bank revealed that 99.7% to 100% homology. When analyzing nucleotide sequence of *TraT* virulence gene of the *E. coli* Egyptian isolated strain in the current study showed 100% nucleotide identity with the Chinese *E. coli* strains WCHEC005237 plasmid pRMTB1-005237 (accession No. CP026579), the Chinese *E. coli* strain 14EC020 plasmid pEC020b (accession No. CP024140), the French *E. coli* strain plasmid PCOV28B clone COV28B–c2 (accession No. MG6949029), the Chinese *E. coli* strain C7 plasmid A (accession No. CP010241), the Chinese *E. coli* strain ExPEC XM (accession No. CP025329), the Hong Kong *E. coli* strain HS13-1 plasmid pHS 13-1IncF (accession No. CP026494), the Chinese *E. coli* strain SCEC020007 plasmid pNDM5-020007 (accession No. CP025626), the Hong Kong *E. coli* strain CRE1540 plasmid p1540-4 (accession No. CP019055) and 99.7% nucleotide identity with the Chinese *E. coli* strain SH21G plasmid pEC295cfr (accession No. KY865320), the American *E. coli* strain AR – 0114 (accession No.CP021773), the Chinese *E. coli* strain C611-eco (accession No. CP017981), the British *E. coli* strain ECO1-AZ155 (accession No. CP019001), the American *E. coli* strain YDC107 (accession No. CP025708). In this study the Egyptian *E. coli* isolate were distributed into common sequence types isolated from humans and pigs and ducks all over the world especially in France, China and USA. Most of the cattle farms from which we isolated the Egyptian *E. coli* isolates were located in the same geographical area of duck farms in addition to the fact that the tremor of the farms lacked the biosecurity measures, which facilitates the circulatory transmission of *E. coli* strains between the human beings and other animal farms to cattle dairy farms.

Deduced amino acids alignment report of the sequenced 304 amino acids of *E. coli TraT* gene showed great homology between the Egyptian *E. coli* strain and the different *E. coli* strains uploaded from gene bank.

Phylogenetic and partial gene sequence analysis of *Staphylococcus haemolyticus tuf* virulence gene that was generated using neighbor joining in MEGA6, showed clear clustering of isolated *Staphylococcus haemolyticus* Egyptian strain and different *Staphy-*

lococcus haemolyticus strains uploaded from gene bank. Sequence distance of *Staphylococcus haemolyticus tuf* virulence gene was created by the Mega Align module of Laser gene DNA Star. Sequence identities between the isolated Egyptian strain and different *E. coli* strains uploaded from gene bank revealed that 99.8% to 100% homology. When analyzing nucleotide sequence of *tuf* virulence gene of the *Staphylococcus hemolyticus* Egyptian isolated strain in the current study showed 100% nucleotide identity with the Russian *Staphylococcus hemolyticus* strain ShlMCV14 translation elongation factor Tu (*tuf*) gene (accession No. HM032764), *Staphylococcus haemolyticus* strain ShlNN878 translation elongation factor Tu gene (accession N0. GU997237), *Staphylococcus haemolyticus* strain ShlNN892 translation elongation factor *Tuf* gene (accession No. GU997231), *Staphylococcus haemolyticus* strain ShlNN708 translation elongation factor *Tuf* gene (accession N0. GU997229), *Staphylococcus haemolyticus* strain ShlNN8893 elongation factor *Tuf* gene (accession N0. GU997232), *Staphylococcus haemolyticus* strain ShlMCV28 translation elongation factor *Tuf* gene (accession No. HM032771), *Staphylococcus haemolyticus* strain ShlNN996 translation elongation factor *Tuf* gene (accession No. HM032753), *Staphylococcus haemolyticus* strain ShlNN894 translation elongation factor *Tuf* gene (accession No. GU997233), *Staphylococcus haemolyticus* strain ShlNN784 translation elongation factor *Tuf* gene (accession No. GU997230) and *Staphylococcus haemolyticus* strain ShlMCV2 translation elongation factor *Tu* gene (accession No. HM032755) and the Japanese *Staphylococcus haemolyticus* strain JCSC1435 DNA (accession No. AP006716).

Deduced amino acids alignment report of the sequenced 400 amino acids of *Staphylococcus hemolyticus tuf* gene showed great homology between the Egyptian *Staphylococcus haemolyticus* strain and the different *Staphylococcus haemolyticus* strains uploaded from gene bank.

Phylogenetic and partial gene sequence analysis of *Streptococcus uberis 16SrRNA* gene *that* was generated using, neighbor joining in MEGA6 showed clear clustering of isolated Egyptian *Streptococcus uberis* strain and different *Streptococcus uberis* strains uploaded from gene bank.

Nucleotide Sequence distance of *streptococcus uberis16SrRNA* gene was carried out by the MegAlign module of Laser gene DNA Star. Sequence identities between the isolated Egyptian strain and different *Streptococcus uberis* strains uploaded from gene bank revealed that revealed that 99.6% to 99.9% homology. When analyzing nucleotide sequence of *16SrRNA* gene of the *Streptococcus uberis* Egyptian isolated strain in the current study showed 99.9% nucleotide identity with the Chinese *Streptococcus uberis* strain CAU10062 16S ribosomal RNA gene (accession N0.MF098160), the Chinese *Streptococcus uberis* strain CAU:2621 16S ribosomal RNA gene (accession N0.MF354575), the New Zealand *Streptococcus uberis* strain NZ01 (accession N0.CP022435), the Japanese *Streptococcus uberis* gene for 16S ribosomal RNA, partial sequence, strain: JCM 5709 (accession N0.LC071829), the Chinese *Streptococcus uberis* strain TRMSU001 16S ribosomal RNA gene (accession N0. HQ391900), the English *Streptococcus uberis* strain 0140J (accession N0. AM946015).

The nucleotide sequence identities between the isolated *Staphylococcus aureus* strain and different *Staphylococcus aureus* strains located in gene bank revealed in 96.06% homology with English *Staphylococcus aureus* strain LR134088.1, English *Staphylococcus aureus* strain LS483300.1 and American *Staphylococcus aureus* strain AJ938182.1. In this study the Egyptian *Staphylococcus aureus* strains were clustered with various *Staphylococcus aureus* strains isolated from humans from Asian countries. This suggests the transmission of *Staphylococcus aureus* isolates between humans and cows in addition to the fact that the cattle dairy farms lacked the biosecurity measures, which facilitates the circulatory transmission of *Staphylococcus aureus* strains between the human beings to cattle dairy farms.

Deduced amino acids alignment report of the sequenced 910 amino acids of *Streptococcus uberis 16SrRNA* gene showed great homology between the Egyptian *Streptococcus uberis* strain and the different *Streptococcus uberis* strains uploaded from gene bank.

Nucleotide Sequence alignment of *hlg* gene of *Staphylococcus aureus* was performed by BLAST tool of NCBI to determine the similar homologous nucleotide sequence uploaded located in gene bank. The Pair wise sequence alignment at nucleotide level *hlg* gene (Figure 18) showed clear similarity and clustering of the Egyptian *Staphylococcus aureus strain* with other *Staphylococcus aureus* strains in the gene Bank. A substitution was found at: T 19 A, C 589 G and showed deletions at 4bp in Tyrosine base and 13 bp in Adenine base.

Conclusion

In conclusion the presence *TraT* gene in a high proportion of mastitis isolates indicate the vital role of *traT gene* in the patho-

genesis of *E. coli* mastitis. *TraT* is necessary for virulence in the mammary gland. *Tuf* gene was present in 100% of *Staphylococcus haemolyticus* isolates. *Tuf* gene has a highly conserved genomic location and amino acid sequence, and has been used in the construction of phylogenetic trees for *Staphylococcus haemolyticus* discrimination. (*hlg*) gene was found in 11 (42.3%) *Staphylococcus aureus* isolates. (*16SrRNA*) gene was conserved in all *Streptococcus uberis* isolates. The sequence variability of the *16SrRNA* allows a differentiation of the bacteria genus *Streptococcus*, including the species *Streptococcus uberis*. Phylogenetic analysis, showed clear clustering of isolated *E. coli, Staphylococcus haemolyticus and Streptococcus uberis* Egyptian strain sand different strains uploaded from gene bank. The Egyptian *E. coli* isolate were distributed into common sequence types isolated from humans and pigs and ducks all over the world especially in France, China and USA. Most of the cattle farms from which we isolated the Egyptian *E. coli* isolates were located in the same geographical area of duck farms in addition to the fact that the tremor of the farms lacked the biosecurity measures, which facilitates the circulatory transmission of *E. coli* strains between the human beings and other animal farms to cattle dairy farms. In this study the Egyptian *Staphylococcus aureus* strains were clustered with various *Staphylococcus aureus* strains isolated from humans from Asian countries. This suggests the transmission of *Staphylococcus aureus* isolates between humans and cows in addition to the fact that the cattle dairy farms lacked the biosecurity measures, which facilitates the circulatory transmission of *Staphylococcus aureus* strains between the human beings to cattle dairy farms. Regular monitoring of *Staphylococcus aureus, E. coli, Staphylococcus haemolyticus and Streptococcus uberis* sequence changes is very important and essential for molecular epidemiological investigations and also can be used in vaccine development and evaluation studies.

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Conflict of Interest

All authors declare no conflict of interest.

Bibliography

1. Constable PD., *et al*. "A textbook of the diseases of cattle, horses, sheep, pigs and goats". 11th ed. USA: Saunders Ltd. (2017).
2. Amr E., *et al*. "Molecular biological tools applied for identification of mastitis causing pathogens". *International Journal of Veterinary Science and Medicine* (2017).
3. Miajlovic H and Smith SG. "Bacterial self-defence: how Escherichia coli evades serum killing". *FEMS Microbiology Letter* 354 (2014): 1-9.
4. Alonzo F., *et al*. "The bicomponent pore-forming leucocidins of Staphylococcus aureus". *Microbiology and Molecular Biology Reviews* 78 (2014): 199-230.
5. Hwang SM., *et al*. "Tuf gene sequence analysis has greater discriminatory power than 16SrRNA sequence analysis in identification of clinical isolates of coagulase-negative staphylococci". *Journal of Clinical Microbiology* 49 (2011): 4142-4149.
6. Oultram WH., *et al*. "Ametataxonimic approach could be considered for cattle clinical mastitis diagnostics". *Frontiers in Veterinary Science* 4 (2017): 1-8.
7. Losada MP., *et al*. "Microbial Sequence Typing in the Genomic Era". *Infection, Genetics and Evolution* 63 (2019) 346-359.
8. Quinn PJ. "Clinical Veterinary Microbiology". 4th ed., London: Mosby, Edinburg (2002): 287-292.
9. Kaipainen T., *et al*. "Virulence factors of E. coli isolated from bovine clinical mastitis". *Veterinary Microbiology* 85.1 (2002): 37-46.
10. Kumar J D., *et al*. "Detection of virulence genes in Staphylococcus aureus isolated from paper currency". *International Journal of Infectious Diseases* 13 (2009) 450-455.
11. Osakabe YC., *et al*. "Detection of Streptococcus Species by Polymerase Chain Reaction in Infectious Crystalline Keratopathy". *Cornea* 25.10 (2006) 1227-1230.
12. Thompson JD., *et al*. "CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice" *Nucleic Acids Research* 22 (1994): 4673-4680.
13. Tamura K., *et al*. "MEGA6: "molecular evolutionary genetics analysis version 6.0". *Molecular Biology and Evolution* 30 (2013): 2725-2729.
14. Cengiz S., *et al*. "Detection of several virulence properties, antibiotic resistance and phylogenetic relationship in E. coli iso-

lates originated from cow mastitis". *Acta Veterinaria-Beograd* 64 (2014): 13-425.

15. Oliveira FA., *et al*. "Virulence characteristics and antimicrobial susceptibility of uropathogenic Escherichia coli strains". *Genetics and Molecular Research* 10 (2011): 4114-4125.

16. Capurro A., *et al*. "Comparison of a commercialized phenotyping system, antimicrobial susceptibility testing, and tuf gene sequence-based genotyping for species-level identification of coagulase-negative staphylococci isolated from cases of bovine mastitis". *Veterinary Microbiology* 134 (2009): 327-333.