Assiut University web-site: www.aun.edu.eg

# PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF *STREPTOCOCCUS UBERIS* ISOLATED FROM MASTITIC COW'S MILK

HANAA A.E. ASFOUR[1], SAMAH F. DARWISH[2] and SAFAA A. EL-WAKEEL[1]
[1] Mastitis and Neonatal Diseases Department, Animal Reproduction Research Institute (ARRI), Giza, Egypt
[2] Biotechnology Research Unit, Animal Reproduction Research Institute (ARRI), Giza, Egypt

**Received:** 26 September 2016; **Accepted:** 17 October 2016

## ABSTRACT

A total number of 240 milk samples was collected from clinical (88 quarter milk samples; QMS), subclinical (108 QMS) and bulk tank (44 BTM) cow's milk selected from different dairy farms for detection of some phenotypic virulence factors and some putative virulence associated genes by polymerase chain reaction (PCR) in the isolated *S. uberis* strains. Also detection of antibiotic resistance for the isolated strains using conventional assay was applied. Using biochemical tests and molecular assay, the confirmed *S. uberis* strains was 48 out of 74 Streptococcus species (64.9%). The % of *S. uberis* isolation from the total examined milk samples was 20%. The higher *S. uberis* incidence was detected in bulk tank milk samples (45.5%) followed by clinical and subclinical milk samples (18.2 % and 11.1%, respectively). In studying the phenotypic virulence factors of the collected *S. uberis* isolates, it was found that β-haemolysis and positive CAMP factor like reaction were detected in only 6.25% of *S. uberis* isolates for each of them, while slime production as indicator for biofilm formation was detected in 75% of these isolates. A total of 48 isolates was tested for their in *vitro* antimicrobial sensitivity. Some of the isolates were highly sensitive to a limited number of antibiotics. On the other hand, the majority of the isolates were highly resistant to a large number of other antibiotics. In studying the genotypic virulence genes, *gapC* gene was detected in all the isolated strains of *S. uberis* while *oppF*, *cfu* and *sau* genes were detected in 93.8%, 68.8% and 62.5%, respectively. On the contrary, *lbp* gene couldn't be detected in any of the isolated strains of *S. uberis*. At least 2 of the five different virulence genes were detected in each isolate of *S. uberis*. There were some strains harboring 4 virulence genes and the higher rate of these strains was detected in that isolated from clinical mastitis. Moreover, the higher strains harboring 3 virulence genes were detected in that isolated from subclinical mastitis. In conclusion, it was observed that *S. uberis* should be given a great concern as a threat for the dairy cows. As it caused both clinical and subclinical mastitis as well as it was isolated with high percentage in BTM. Moreover, this pathogen nowadays emerges as resistance to different antimicrobial agents especially for those commonly utilized. Furthermore, *S. uberis* harbors different virulence factors and genes that capable it to persist in the mammary gland of the dairy animals for a long time and speeding of infection from cow to cow may occur resulting in higher prevalence rates of infection between different dairy farms.

***Key words:*** *S. uberis mastitis; haemolysis; CAMP; biofilm; antimicrobial sensitivity; PCR assays; virulence genes.*

## INTRODUCTION

Mastitis caused by *Streptococcus uberis* has been detected increasingly in dairy farms over the last decades. Infection with some strains can induce mild subclinical inflammation whilst others induce severe inflammation and clinical infections of the bovine udder. It represents the leading pathogen in a growing amount of dairy herds (Kromker *et al*., 2014 and Günther *et al*., 2016).

*Corresponding author:* Dr. HANAA A.E. ASFOUR
*E-mail address:* hanaaasfour@yahoo.com
*Present address:* Mastitis and Neonatal Diseases Department, Animal Reproduction Research Institute (ARRI), Giza, Egypt

Coagulase negative staphylococci, *S. uberis* and *S. dysgalactiae* are considered to be both contagious and environmental pathogens (Taponen and Pyorala, 2006). *S. uberis* pathogen is ubiquitous for which it is considered as environment-associated. Not only straw bedding and pasture, but also the bovine skin and digestive mucosae are typical localizations inhabited by *S. uberis*. Due to its capacity to persist within the mammary tissue, some infections may eventually turn cow-associated. In other cases, the infection was short, but in any case, there was a high risk of re-infection. Although many varieties remained susceptible to most antimicrobial agents, the problem for the dairy farm lied in the high rate of re-infection (Kromker *et al*., 2014). It should be concluded that *S. uberis* caused the increase in total bacteria count,

somatic cell count (SCC) and the decrease in κ-casein level, which significantly affects the technological quality of cows' milk (Pecka-Kiełb *et al*., 2016).

*S. uberis* is an important pathogen that has been implicated in bovine mastitis but the virulence factors associated with pathogenesis are not well understood (Reinoso *et al*., 2011). Others, however, have proposed numerous virulence traits that may be associated with the ability of *S. uberis* to cause mastitis as the ability to form biofilm (Varhimo *et al*., 2011).

Molecular diagnostic methods revealed that *S. uberis* may be subdivided into many different varieties with different epidemiological properties (Kromker *et al*., 2014). Despite the severe economic impact caused by the high prevalence of *S. uberis* in many well-managed dairy herds, virulence factors associated with pathogenesis were not well understood and constituted a major obstacle for the development of strategies to control this important mastitis pathogen (Oliver *et al*., 1998). Several putative virulence associated genes of *S. uberis* have been described. Among these, lactoferrin binding proteins (Moshynskyy *et al*., 2003), adherence to and invasion of epithelial cells mediated by *S. uberis* specific adhesion molecule (SUAM) (Almeida *et al*., 2006), CAMP factor (Jiang *et al*., 1996), a surface dehydrogenase protein *gapC* (Pancholi and Fischetti, 1993) and *opp* proteins involved in the active transport of solutes essential for growth in milk (Smith *et al*., 2002) have been found.

The aim of this work was to determine the incidence rate of *S. uberis* infection in both mastitic cows and bulk tank milk of different dairy farms based on both phenotypic and genotypic assays. Also, detection of some phenotypic virulence characteristics and some putative virulence associated genes in the isolated *S. uberis* strains were performed. Additionally, antibiotic susceptibility of the isolated *S. uberis* strains was investigated using disk diffusion method.

## MATERIALS AND METHODS

**A- Collection of milk samples:**
Total number of 240 milk samples; included 88 QMS collected from clinical mastitic cows, 108 QMS collected from subclinical mastitic cows and 44 BTM samples, were included in the present study. The quarter milk samples were collected from a single visit at milking time at the farms using physical examination and California mastitis test (CMT). Samples were subjected to somatic cell count (SCC) in order to confirm the subclinical status of mastitis (> 250,000 cells/ml) of the collected samples using the Nucleocounter SCC-100 (Chemometric Nucleocounter Family, Denmark) (Lasagno *et al*., 2011).

**B- Bacteriological isolation:** One standard loop of milk samples was streaked on 7% sheep blood agar, Edward's media, macConkey agar and mannitol salt agar (Himedia, Mumbai, India). The inoculated plates were incubated aerobically at 37°C. The plates were checked for growth after 24-48h. Primary identification of Streptococci especially *S. uberis* was based on colony size, shape, colour, haemolytic characteristics, Grams reaction and catalase test (Quinn *et al*., 2011).

**C- Phenotypic characterization of *S. uberis*:**
**1- Colony characteristic on Edward's media as selective medium for *S. uberis*:**
Colonies that were primary identified as Streptococci were streaked on Edward's media plates as a selective medium, incubated at 37$^{o}$C and examined after 24-48 h for growth and change in colour of the medium. The presence of growth, haemolysis and esculin hydrolysis (dark background) were indications of *S. uberis*. Then, colonies which grew on Edward's media were picked and streaked on macConkey agar. The absence of growth on macConkey agar was an indication of *S. uberis*. The isolates were initially identified using standard conventional biochemical tests according to Quinn *et al*. (2011). Since *S. uberis* is a fastidious bacterium, so it was sub-cultured on brain heart infusion agar for further PCR assays.

**2- Detection of slime production by Congo red agar method.**
Slime production as an indicator for biofilm formation was evaluated by cultivation of *S. uberis* isolates on Congo red agar (CRA) plates as described by Mathur *et al*. (2006). Isolates were interpreted according to their colony phenotypes. Black colonies with dry consistency and rough surface and edges were considered a positive indication of slime production, while both black colonies with smooth, round and shiny surface and red colonies with dry consistency and rough edges and surface were considered as intermediate slime producers. Red colonies with smooth, round, and shiny surface were indicative of negative slime production.

**3- CAMP factor like reaction:**
Bacteria were screened for CAMP factor activity as previously described by Jiang *et al*. (1996). Briefly, *S. uberis* strains were streaked perpendicular to a streak of β-haemolytic *S. aureus* on blood agar plates and after 6-20 h incubation at 37°C, they were observed for haemolysis.

**4- Antibiotic susceptibility testing of the isolated *S. uberis*:**
Antimicrobial susceptibility of *S. uberis* strains to 14 antibiotics using Disk diffusion technique was performed according to the National Committee for Clinical Laboratory Standards (NCCLS, 2008) on Mueller Hinton agar (Himedia, Mumbai, India) using commercially available antimicrobial test discs [ciprofloxacin; CIP (5µg), norfloxacin; NOR (10µg),

florfenicol FFC (15µg), chloramphenicol; C (30µg), amoxicillin-clavulanic acid; AMC (30µg), amoxicillin; AMX (25 µg), ampicillin; AM (10µg), penicillin; P (10 U), tetracycline; TE (30µg), neomycin; N (30µg), erythromycin; E (15µg), streptomycin; S (10µg), cloxacillin; CX (1µg) and oxacillin; OX (1µg)]. Results were recorded by measuring the inhibition zones and scored as sensitive, intermediate susceptibility and resistant according to the NCCLS recommendations.

**C- Genotypic characterization of *S. uberis*:**

**1. DNA extraction from Streptococcus isolates:**

Crude DNA template was prepared by boiling followed by snap chilling into ice according to method previously reported by Asfour and Darwish (2011). Briefly, the colonies grown over the surface of brain heart agar plates were harvested and washed twice by phosphate buffer saline. A small quantity of bacterial pellets was dissolved in 200 µl TE buffer (10 mM Tris, 1mM EDTA pH 7.6) and boiled in a boiling water bath for about 10 min and then immediately snap chilled into ice. A centrifugation step was followed at 8000 rpm for 10 min. to sediment debris while the supernatant was aspirated and kept at -20°C until time for PCR. Five microliters of this lysate was used as a template in PCR assays.

**2. Primers:**

Different primers were used in this study. Primer sequences, their references, product sizes and annealing temperatures are listed in table 1.

**Table 1:** Primers used in the study, their nucleotide sequences, species specific, references, Annealing temperatures ($T_a$) and their PCR products sizes.

| Primer name | Sequence 5'-3' (reference) | Target taxon/gene | $T_a$ °C | Product size bp |
|---|---|---|---|---|
| St F<br>St R | 5' TTATGCTCGTCTTGCTCTTTACGG 3'<br>5' GCACACGTCCAAGTGATGTAGCTG 3'<br>(Almeida *et al*., 2013 ) | Genus Streptococcus | 58 | 281 bp |
| Hsp40 F<br>Hsp40 R | 5' AATTACGAGGTGCTGGACAA 3'<br>5' TTCTTGACCACTTGCCTCAG 3'<br>(Chiang *et al*., 2008) | *S. uberis* | 62 | 119 bp |
| *cfu* F<br>*cfu* R | 5' TATCCCGATTTGCAGCCTAC 3'<br>5' CCTGGTCAACTTGTGCAACTG 3'<br>(Reinoso *et al*., 2011) | CAMP factor coding gene | 56 | 205 bp |
| *gapC* F<br>*gapC* R | 5' GCTCCTGGTGGAGATGATGT 3'<br>5' GTCACCAGTGTAAGCGTGGA 3'<br>(Reinoso *et al*., 2011) | Glyceraldehydes 3- phosphate dehydrogenase protein gene (GAPDH) | 56 | 200 bp |
| *oppF* F<br>*oppF* R | 5' GGCCTAACCAAAACGAAACA 3'<br>5' GGCTCTGGAATTGCTGAAAG 3'<br>(Smith *et al*., 2002) | Oligopeptide permease gene | 53 | 419 bp |
| *lbp* F<br>*lbp* R | 5' CGACCCTTCAGATTGGACTC 3'<br>5' TAGCAGCATCACGTTCTTCG 3'<br>(Reinoso *et al*., 2011) | Lactoferrin-binding proteins gene | 53 | 698 bp |
| *sau* F<br>*sau* R | 5' ACGCAAGGTGCTCAAGAGTT 3'<br>5' TGAACAAGCGATTCGTCAAG 3'<br>(Reinoso *et al*., 2011) | *S.uberis* specific adhesion molecule gene | 63 | 776 bp |

**3. Molecular confirmation of presumptive Streptococcus isolates by PCR:**

All presumptive isolates were subjected to Streptococcus general specific PCR assay using the primer pair (St F and St R) that was specific to all Streptococcus species. PCR was performed in 25µl reaction volumes containing 5 µl of DNA template, 20 pmol of each primer and 1X of PCR master mix (Dream Taq Green PCR Master Mix, Fermentas Life Science). Amplification was carried out in a Nexus gradient Master cycler (Eppendorf, Germany) under the following conditions: one cycle of initial denaturing at 95°C for 5 min and 40 three-step cycles, which included denaturation at 94°C for 30s, annealing at 58°C for 30s, and extension at 72°C for 45s. PCR products were analyzed in 2% agarose gel stained with ethidium bromide. Amplification of 281 bp products confirmed the isolate to be Streptcoccus spp.

**4. Molecular confirmation of *S. uberis* amongst PCR confirmed Streptococcus isolates:**

All Streptococcus confirmed isolates were subjected to *S. uberis* specific PCR using Hsp40 F and Hsp40 R primer set using the above mentioned amplification condition except 62°C for annealing temperature. Amplification of 119 bp confirmed the isolate to be *S.uberis.*

**5. Detection of virulence genes amongst *S. uberis* confirmed isolates by PCR:**

Five different virulence markers were assayed by different PCR assays. Amplification conditions used for these PCR assays were as previously mentioned but with the specified annealing temperatures shown in table 1.

## RESULTS

From the total number of 240 milk samples under the current study, 74 Streptococcus spp. were isolated with a percentage of 30.8%. PCR using Streptococcus specific primer set confirmed all the isolates to be Streptococcus species. Based on both biochemical tests and *S. uberis* specific PCR assay, 48 out of 74 Streptococcus isolates were confirmed to be *S. uberis* with a percentage of 64.9%. Figure 1 (A & B) showed the specific PCR products of both Streptococcus specific and *S. uberis* specific PCR assays. Table (2) showed the incidence of *S. uberis* isolated from different types of milk samples. It was found that, the overall percentage of *S. uberis* isolation in the examined milk samples was 20%. Additionally, the higher incidence of *S. uberis* was found in bulk tank milk samples (45.5%) followed by clinical and subclinical milk samples (18.2 % and 11.1%, respectively).

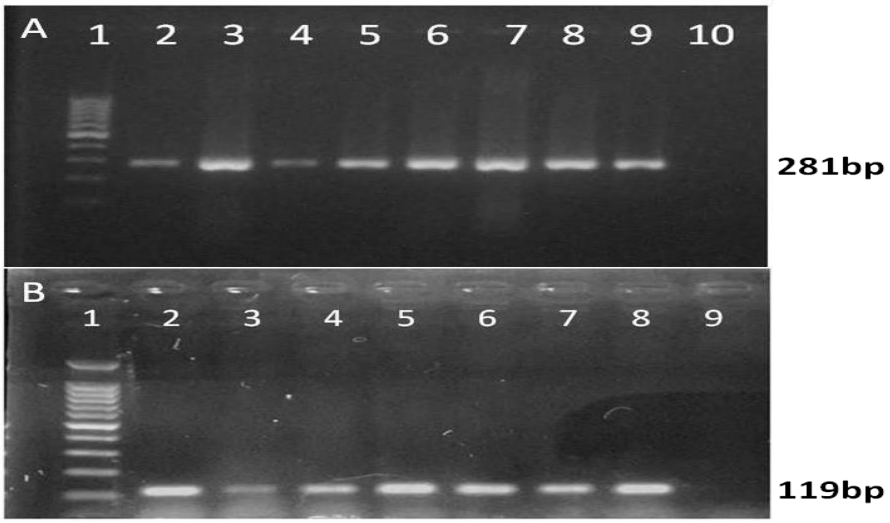


**Figure 1: (A)** Positive amplification of 281 bp PCR products of Streptococcus species specific PCR assay. Lane 1: 100 bp ladder DNA marker, lane 2-9: positive Streptococcus isolates and lane 10: negative control.
**(B)** Positive amplification of 119 bp PCR products of *S. uberis* specific PCR assay. Lane 1: 100 bp ladder DNA marker, lanes 2-8: positive *S. uberis* isolates, Lane 9: negative control.

**Table 2:** Incidence rate of *S. uberis* in different cow's milk samples.

| Cow's milk samples | No. of milk samples | Isolated *S.uberis* | |
|---|---|---|---|
| | | No | % |
| Subclinical mastitic milk | 108 | 12 | 11.1% |
| Clinical mastitic milk | 88 | 16 | 18.2 % |
| BTM | 44 | 20 | 45.5% |
| Total | 240 | 48 | 20% |

All confirmed 48 *S. uberis* isolates were examined for their virulence using three different tests including haemolysis type, CAMP factor reaction and slime production status. Figures (2 and 3) showed the positive CAMP factor reaction and slime production on Congo red agar plates of *S. uberis* isolates, respectively. Table (3) showed the haemolysis types, CAMP factor reactions and slime production status of *S. uberis* confirmed isolates. The results indicated high prevalence of *S. uberis* isolates with α haemolysis, negative CAMP factor reaction and slime production between (87.5%, 93.75% and 75%, respectively). On the other side, the percentages of *S. uberis* with β-haemolysis and positive CAMP factor reaction were 6.25% for each of them.

**Figure 2:** A synergistic haemolytic CAMP-factor like reaction of *S. uberis* isolates on sheep blood agar within the zone of β- haemolytic *S. aureus* represented by the head of an arrow haemolysis.

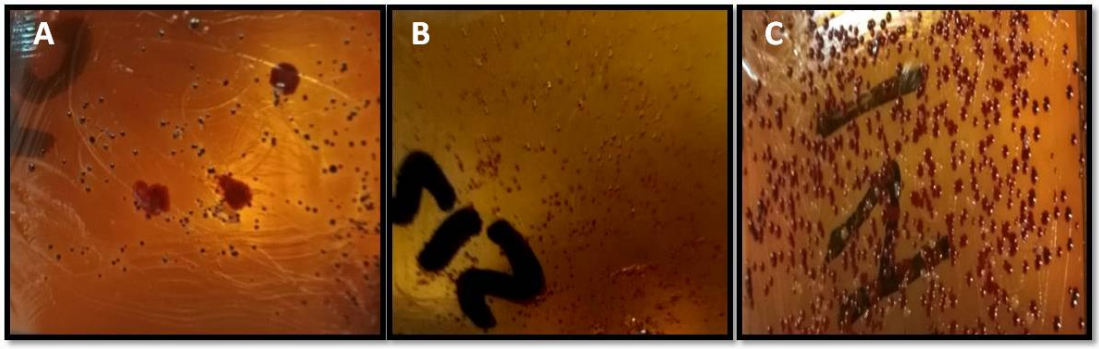


**Figure 3: (A)** Dry black crystalline strong biofilm producer *S. uberis* isolate. **(B)** Dry red intermediate biofilm producer *S. uberis* isolate. **(C)** Smooth red non biofilm producer *S. uberis* isolate.

**Table 3:** Prevalence of different haemolysis types, CAMP factor reaction and slime production status among *S. uberis* isolates.

| No. of *S. uberis* | Haemolysis types | | | CAMP factor reaction | | Slime production | |
|---|---|---|---|---|---|---|---|
| | α No. (%) | β No. (%) | ϒ No. (%) | Positive No. (%) | Negative No. (%) | Positive No. (%) | Negative No. (%) |
| 48 | 42 (87.5%) | 3 (6.25%) | 3 (6.25%) | 3 (6.25%) | 45 (93.75%) | 36 (75%) | 12 (25%) |

All 48 *S. uberis* isolates were tested for their in *vitro* antimicrobial sensitivity using disk diffusion method. Table (4) showed the numbers and percentages of both sensitive and resistant *S. uberis* isolates for each type of antibiotics. Figure (4) showed both a highly sensitive and a highly resistant *S. uberis* isolates on Muller Hinton agar plates. The results cleared that the majority of the isolates were highly sensitive to FFC, C, NOR and CIP (89.6%, 77.1%, 70.8% and 66.7%, respectively). More than half of *S. uberis* were susceptible to AMX and AMC (58.3% and 56.3%, respectively). On the other hand, most of the isolates (ranged between 77.1% and 95.8% of them) were highly resistant to E, S, TE, OX, P, AM, N and CX (Table 4).

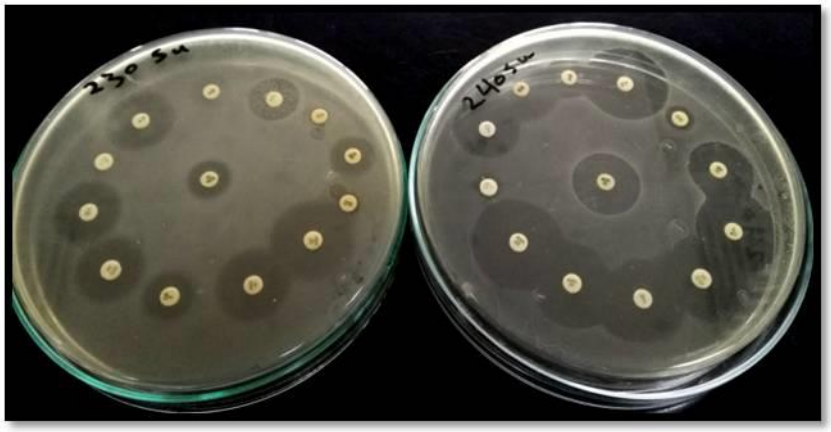

**Figure 4:** Highly resistant (left) and highly sensitive (right) *S. uberis* isolates to different antibiotics.

**Table 4:** Antimicrobial susceptibility patterns of *S. uberis* isolates.

| Antibiotic disks | Sensitive strains | | Resistant strains | |
|---|---|---|---|---|
| | No. | % | No. | % |
| FFC | 43 | 89.6% | 5 | 10.4% |
| C | 37 | 77.1% | 11 | 22.9% |
| NOR | 34 | 70.8% | 14 | 29.2% |
| CIP | 32 | 66.7% | 16 | 33.3% |
| AMX | 28 | 58.3% | 20 | 41.7% |
| AMC | 27 | 56.3% | 21 | 43.7% |
| E | 11 | 22.9% | 37 | 77.1% |
| S | 8 | 16.7% | 40 | 83.3% |
| TE | 5 | 10.4% | 43 | 89.6% |
| OX | 2 | 4.2% | 46 | 95.8% |
| P | 2 | 4.2% | 46 | 95.8% |
| AM | 2 | 4.2% | 46 | 95.8% |
| N | 2 | 4.2% | 46 | 95.8% |
| CX | 2 | 4.2% | 46 | 95.8% |

All 48 *S. uberis* isolates were also screened for the presence of five virulence associated genes using different PCR assays. Figures 5 (A-D) showed the positive amplification products of different PCR assays used for detection of *gapC*, *oppF*, *sau* and *cfu* genes, respectively.

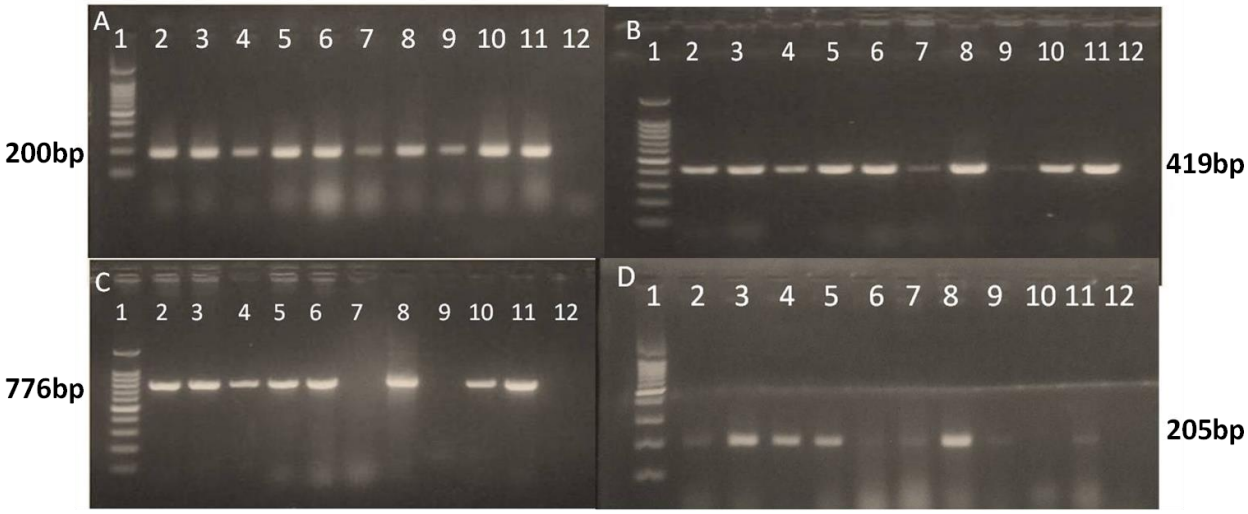


**Figure 5: (A)** Positive amplification of 200 bp PCR products of *gapC* gene. Lane 1: 100 bp ladder DNA marker, lanes 2-11: *gapC* positive *S. uberis* isolates and lane 12: negative control. **(B)** Positive amplification of 419 bp PCR products of *oppF* gene. Lane 1: 100 bp ladder DNA marker, lanes 2-11: *oppF* positive *S. uberis* isolates and lane 12: negative control. **(C)** Positive amplification of 776 bp PCR products of *sau* gene. Lane 1: 100 bp ladder DNA marker, lanes 2-6; 8; 10-11: *sau* gene positive *S. uberis* isolates; lanes 7& 9: *sau* gene negative *S. uberis* isolates and lane 12: negative control. **(D)** Positive amplification of 205 bp PCR products of *cfu* gene. Lane 1: 100 bp ladder DNA marker, lanes 2-9, 11: *cfu* gene positive isolates and lane 10: *cfu* gene negative isolate; Lane 12: negative control.

Table (5) showed the number and percent of *S. uberis* isolates positive for each type of virulence genes. As shown in table 5, *gapC* gene was detected in all the isolated strains of *S. uberis* while *oppF*, *cfu* and *sau* genes were detected in percentages of 93.8%, 68.8% and 62.5%, respectively. On the contrary, *lbp* gene couldn't be detected in any of the isolated *S. uberis*. The prevalence of different virulence genes among *S. uberis* isolates from different types of milk samples was shown in table (6). It showed that *S. uberis* isolates contained at least two types of virulence genes while some isolates carried three or four virulence genes. The higher rate of *S. uberis* harboring 4 virulence genes was detected in that isolated from clinical mastitis. Moreover, the higher strains harboring 3 virulence genes were detected in that isolated from subclinical mastitis.

**Table 5:** Prevalence of different virulence gene types in *S. uberis* isolates.

| Types of virulence genes | Positive isolates | |
|---|---|---|
| | Number | % |
| *gapC* | 48 | 100% |
| *oppF* | 45 | 93.8% |
| *cfu* | 33 | 68.8% |
| *sau* | 30 | 62.5% |
| *lbp* | Not detected | 0 |

**Table 6:** Prevalence of virulence genes among the *S. uberis* isolates from different milk samples.

| Cow's milk samples | No. of isolates | No. of detected genes/no. of *S. uberis* isolates (%) | | |
|---|---|---|---|---|
| | | 4 genes | 3 genes | 2 genes |
| Subclinical mastitic milk | 12 | 0 | 8 (66.7%) | 4 (33.3%) |
| Clinical mastitic milk | 16 | 9 (56. 25%) | 3 (18.75%) | 4 (25%) |
| BTM | 20 | 8 (40%) | 9 (45%) | 3 (15%) |
| Total | 48 | 17 (35.4%) | 20 (41.7%) | 11 (22.9%) |

## DISCUSSION

*Streptococcus uberis* is a worldwide pathogen that causes intra-mammary infections in dairy cattle. *S. uberis* has been described as an opportunistic pathogen that utilizes nutritional flexibility to adapt to a range of ecological niches, including the mammary gland (Ward *et al*., 2009 and Collado *et al*., 2016). It was suggested that cow-to-cow transmission of *S. uberis* potentially occurring in the majority of herds and may be the most important route of infection in many herds (Davies *et al*., 2016).

In this study, a total number of 240 different milk samples were collected from clinical, subclinical and bulk tank milk samples of different dairy cow farms aiming to isolate *S. uberis* that cause bovine mastitis to study its phenotypic and genotypic characteristics. Based on both phenotypic and genotypic identification, the number of Streptococcus spp. isolated from all tested milk samples was 74 (30.8%). Also, the confirmed *S. uberis* strains were 48 out of 74 Streptococcus spp. (64.9%). Previously, lower and higher percentages of *S. uberis* detection in mastitic milk samples, ranged from 39.9%, 55.38%, 55.38% and 18.48% of the isolated Streptococcal spp. were reported by Rossitto *et al*. (2002); Amosun *et al*. (2010); Adesola (2012) and Kia *et al*. (2014), respectively.

In contrast to the total examined milk samples, the incidence of *S. uberis* was 20%. Nearly similar, Ebrahimi *et al*. (2008) isolated *S. uberis* from normal, sub-acute and acute cow mastitic cases with a percentage of 18%. A higher incidence rate of Streptococcus spp. were isolated from mastitic cows (55 %) but a lower *S. uberis* was isolated with a percentage of 15.3% was detected by El-Jakee *et al*. (2013). Also, a higher incidence of *S. uberis* as the predominant pathogen was recorded by Steele *et al*. (2015) in cow's milk samples (46%). This variation in the results might be attributed to the difference in herd management between herds. Some practices can decrease the incidence as teat dipping before and after milking, washing milkers hands before and after milking, preparation of clean towel for each lactating cow, milking of infected cow lastly, using dry cow therapy method and treating clinical cases at early stage (Teklemariam *et al*., 2015).

In the current study, the higher *S. uberis* incidence rate was detected in bulk tank milk samples (45.5%). A higher incidence rate was detected by Zadoks *et al*. (2004) who cultured BTM samples from 48 dairy herds and found 81% positive for *S. uberis*. Very high incidence was reported by Katholm *et al*. (2012) who found *S. uberis* in 95% of BTM. Otherwise, Bi *et al*. (2016) isolated *S. uberis* in only 8.9% of BTM. Dogan and Boor (2004) suggested that high numbers of *S. uberis* in BTM were more likely to reflect high numbers of *S. uberis* shed by mastitic cows, rather than multiplication of these organisms under cooling conditions required for production of Grade A milk.

In clinical and subclinical mastitic milk samples, *S. uberis* was detected in 18.2 % and 11.1%, respectively. Higher incidence of *S. uberis* was recovered from milk of clinical mastitic cows with 26.3 %, while in subclinical mastitic milk samples, *S. uberis* was detected in 16.7% (El-Jakee *et al*., 2013). In contrary, Teklemariam *et al*. (2015) found that, the prevalence of *S. uberis* isolation in subclinical mastitis was higher than that of clinical mastitis (88.9 % and 11.1%, respectively).

The differences in the incidence rates of *S. uberis* clinical and subclinical mastitis in the previous researches was explained by Günther *et al*. (2016) who demonstrated that all *S. uberis* isolates from clinical and subclinical mastitis evaded the immune surveillance of the mammary epithelial cells (MEC), representing by far the most abundant first line sentinels of the udder. Failure to activating their immune alert early after infection explained the commonly observed belated and weak onset of udder inflammation during *S. uberis* mastitis. On the other hand they proved that macrophages can indeed mount a vigorous immune response against *S. uberis*.

In this work we studied some of phenotypic characteristics of the isolated strains of *S. uberis* that indicated to virulence factors. The 1st step on detecting phenotypic virulence factors of *S. uberis* isolates was their haemolytic effect on sheep blood agar. The higher percentage of *S. uberis* isolates showed α haemolysis (87.5%), while β or ϒ haemolysis was recorded in only 6.25% (for each of them) of the isolates. In this side of work, Kia *et al*. (2014) reported that all *S. uberis* in their study were α haemolysic strains.

The role of CAMP factor in pathogenicity is unclear, although it can't be ruled out as a putative virulence factor (Lasagno *et al*., 2011). Considering CAMP factor like reaction only 6.25% of the tested *S. uberis* isolates were positive for CAMP factor reaction in our study. While, Christ *et al*. (1988); Lämmler (1991); Khan *et al*. (2003) and Lasagno *et al*. (2011) found 10%, 25%, 3.9% and 28% CAMP positive *S. uberis* strains, respectively.

Biofilms provide a sheltered and protected area for bacterial growth allowing them to be resistant to antibiotics; disinfectants and host defenses, thus the difficulties of treating recurrent infections may be related to the ability of the infecting pathogens to produce biofilms (Melchior *et al*, 2005). Therefore, the ability of *S. uberis* to produce slime might be a desirable virulence factor during colonization of the udder. It has been shown that slime production is important; allowing the bacteria to aggregate and form biofilms (Arciola *et al*., 2002).

Slime production indicating biofilm formation was detected in 75% *S. uberis* isolates in this study. Moore (2009) detected strong *S. uberis* biofilm former in 78% of the tested strains isolated from mastitic cows and when evaluated for slime (polysaccharide) production, all 27 strains were positive by the Congo red agar method. Recently, Collado *et al*. (2016) reported that different *S. uberis* strains have the ability to form biofilm in *vitro*. The high incidence of biofilm formation among the isolated strains may be due to that milk or its components could contribute to the pathogenesis of *S. uberis* mastitis by assisting in biofilm production as the indigenous flora of raw milk appears to contribute to biofilm formation by *S. uberis* since limited amounts of biofilm were produced when indigenous flora were removed from milk (Almeida *et al.*, 2015a).

Recent increase in antibiotics resistance of bacterial strains isolated from cow milk with mastitis represented a strong motivation to study the most efficient antibiotic for treatment (Nadăş *et al*., 2014). In studying the antimicrobial susceptibility of the isolated *S. uberis* strains, it was noticed that they were highly susceptible to FFC and C. Guérin-Faublée *et al*. (2002) and Moges *et al*. (2011) recorded that all *S. uberis* strains isolated from mastitic milk were susceptible to C. On the other hand most of the isolates (ranged between 77.1% and 95.8% of them) were highly resistant to E, S, TE, OX, P, AM, N and CX. In accordance with our results, Ebrahimi *et al*. (2008) also observed a high resistance rate among *S. uberis* isolates against S, P, AM and CX. According to Piepers *et al*. (2007) *S. uberis* was more frequently resistant to the penicillin within the class of penicillins. Adesola (2012) illustrated that, all their studied *S. uberis* isolates were resistant to AM, N and TE. Recently, Petrovski *et al*. (2015) reported that all streptococcal isolates demonstrated resistance to aminoglycosides (N and S). Discordant isolates of *S. uberis* that were susceptible to penicillin, but resistant to OX, were also found demonstrated cross-resistance to the cephalosporins tested. So they recommended that the treatment of bovine mastitis caused by Streptococci, particularly *S. uberis*, with isoxazolyl penicillins should be discouraged nationally and internationally.

*S. uberis* is an important pathogen that has been implicated in bovine mastitis but the virulence factors associated with pathogenesis are not well understood (Reinoso *et al*. 2011). Our study aimed to detect 5 putative and known virulence-associated genes by PCR assays in 48 *S. uberis* strains isolated from different cow's milk samples of different dairy farms. The results revealed that *gapC* gene was detected in all the isolated strains of *S. uberis*. While *oppF*, *cfu* and *sau* genes were detected in 93.8%, 68.8% and 62.5%, respectively. On the contrary, *lbp* gene couldn't be detected in any of the isolated strains of *S. uberis*.

*GapC* was included because in several pathogenic bacteria GAPDH protein has been described as being associated with virulence (Maeda *et al*., 2004) due to its ability to bind several host proteins (Pancholi and Fischetti, 1993) or to confer resistance against reactive oxygen species produced by host phagocytic cells (Holzmuller *et al*., 2006).

Our result was higher than that recorded by Reinoso *et al*. (2011) who found *gapC* only in 62 (79.4%) of *S. uberis* isolated from bovine mastitis. But in another recent work of Reinoso *et al*. (2015) they recorded the presence of *sua*, *cfu*, and *gapC* genes in the most of *S. uberis* strains.

Another gene included in this study was *oppF*, which is another important factor playing a significant role during growth of *S. uberis* in milk. The essential amino acids can be taken up by *S. uberis* through the expressed oligopeptide binding protein encoded by the *oppF* gene (Smith *et al.*, 2002 and Taylor *et al*., 2003). The *oppF* gene was successfully detected in 93.8% of *S. uberis* isolates. On the contrary, it was reported to be absent by Zadoks *et al.* (2005) while Reinoso *et al*. (2011) found it in 64.1% of the strains.

The gene *cfu*, coding for CAMP factor in *S. uberis*, is a further putative virulence factor homologous to Fc binding (Reinoso *et al*., 2011). *cfu* gene was detected in 68.8% in this study, however, a positive CAMP reaction was observed only in 6.25% using phenotypic method. This difference was also supported by (Reinoso *et al*., 2011) who found *cfu* gene in 76.9% of the strains examined although a positive CAMP reaction was observed in only 23% of *S. uberis* strains. Our result was in contrast to those of Khan *et al*. (2003), who reported positive *cfu* gene in 3.8% of *S. uberis* strains corresponding to a phenotypically positive CAMP-reaction only. These conflicted results suggested that the presence of this gene might not be related to expression of the CAMP factor (Reinoso *et al*., 2011). This may explain the difference observed here and by Khan *et al*. (2003). On the other hand, Ward *et al*. (2009) showed that a coding sequence for CAMP factor was not identified in *S. uberis* 0140J that is pathogenic for both the lactating and non-lactating bovine mammary gland.

Adherence to and internalization into MEC are central mechanisms in the pathogenesis of *S. uberis* mastitis. The ability to adhere to and invade into bovine mammary epithelial cells (BMEC) was potentially mediated by the *S. uberis* adhesion molecule (SUAM). Through these pathogenic strategies, *S. uberis* reaches an intracellular environment where humoral host defenses and antimicrobials in milk are essentially ineffective, thus allowing persistence of this pathogen in the mammary gland (Prado *et al*., 2011 and Almeida *et al*., 2015b). In our study the presence of *sua* gene was declared in 62.5% of the tested *S. uberis* isolates however many previous works reported higher prevalence of the *sua* gene. Reinoso *et al*. (2011) reported a prevalence of the *sua* gene of 83.3 % in their study. Shome *et al.* (2012) and Yuan *et al*. (2014) detected *sua* gene in 100 % of the examined *S. uberis* strains. Recenty, Perrig *et al*. (2015) illustrated that the prevalence of the *sua* was 97.8 % of 137 *S. uberis* isolates from bovine milk with subclinical or clinical mastitis. Our lower prevalence of *sua* gene in the tested *S. uberis* isolates may be attributed to an intact *sua* gene does not appear necessary for adherence (Tassi *et al*., 2015).

In the current work, *lbp* can't be detected in any isolate of *S. uberis* under the study, while Reinoso *et al*. (2011) found *lbp* in 11.5% and this was a very low prevalence when compared with other genes they detected. This may be attributed to that the presence of *lbp* gene isn't necessary for virulence of *S. uberis*. As Almeida *et al*. (2015b) reported that *S. uberis* expresses SUAM that has affinity for lactoferrin (Lf) and a central role adherence to and internalization of *S. uberis* into BMEC. Mechanisms underlying the pathogenic involvement of SUAM rely partially on its affinity for Lf, which together with a putative receptor on the surface of BMEC creates a molecular bridge which facilitates adherence to and internalization of *S. uberis* into MEC (Almeida *et al*., 2006 and Patel *et al*., 2009). Since adhesion is the first step in biofilm formation, it is possible that Lf contributes to that process.

Finally, we noticed that at least 2 of the five different virulence genes were detected in each isolate of *S. uberis* under the study. There were some strains harboring 4 virulence genes the higher level of these strains was detected in that isolated from clinical mastitis (56. 25%). Moreover the higher strains harboring 3 virulence genes were detected in that isolated from subclinical mastitis (66.7%). Notcovich *et al*. (2016) reported that, there were significant differences between the strains in the proportion of quarters developing clinical mastitis. These results illustrated the difference in the ability of *S. uberis* strains to cause mastitis and the severity of the infections caused. In agreement with the present results, Reinoso *et al*. (2011) found that not all genes were present in the strains but all of the detected virulence-associated genes were present in

combination. Also, they found 60.3% isolates carried seven to 10 virulence-associated genes and detection of virulence-associated genes in individual *S. uberis* strains isolated from infected animals revealed one to 10 virulence genes. Reinoso *et al*. (2015) recorded the presence of 3 genes in most of *S. uberis* strains.

## CONCLUSION

*S. uberis* should be given a great concern as a threat for the dairy cows. It was isolated from milk of both clinincal and subclinical mastitis as well as it was isolated with high percentage in BTM. So, *S. uberis* is becoming a major health problem of dairy cows and undoubtedly will have an adverse effect on productivity of dairy industry. Moreover, this pathogen nowadays emerges as resistance to different antimicrobial agents especially for those commonly utilized. Furthermore, *S. uberis* harbors different virulence factors and genes that allow it to persist in the mammary gland of the dairy animals for a long time and speeding of infection from cow to cow may occur resulting in higher prevalence rates of infection between different dairy farms.

## REFRENCES

*Adesola, A.E. (2012):* Antimicrobial resistance pattern of Streptococci and Staphylococci isolated from cases of bovine clinical mastitis in Nigeria. Nat. Sci., 10 (11): 96-101.

*Almeida, A.; Albuquerque, P.; Araujo, R.; Ribeiro, N. and Tavares, F. (2013):* Detection and discrimination of common bovine mastitis causing Streptococci. Vet. Microbiol., 164: 370-377.

*Almeida, R.A.; Kerro-Dego, O.; Prado, M.E.; Headrick, S.I.; Lewis, M.J.; Siebert, L.J.; Pighetti, G.M. and Oliver, S.P. (2015a):* Protective effect of anti-SUAM antibodies on *Streptococcus uberis* mastitis. Vet. Res., 46:133.

*Almeida, R.A.; Kerro-Dego, O.; Headrick, S.I.; Lewis, M.J. and Oliver, S.P. (2015b):* Role of *Streptococcus uberis* adhesion molecule in the pathogenesis of *Streptococcus uberis mastitis*. Vet. Microbiol., 30;179 (3-4): 332-335.

*Almeida, R.A.; Luther, D.A.; Park, H.M. and Oliver, S.P. (2006):* Identification, isolation, and partial characterization of a novel *Streptococcus uberis* adhesion molecule (SUAM). Vet. Microbiol., 115:183-191.

*Amosun, E.A.; Ajuwape, A.T.P. and Adetosoye, A.I. (2010):* Bovine Streptococcal mastitis in Southwest and Northern States of Nigeria. Afr. J. Biomed. Res., 13(1): 33-37.

*Arciola, C.R.; Campoccia, D. and Montanaro, L. (2002):* Detection of biofilm-forming strains of *Staphylococcus epidermidis* and *S. aureus*. Expert Rev. Mol. Diag., 2(5):478-484.

*Asfour A.E. Hanaa and Darwish F. Samah (2011):* Phenotypic and genotypic detection of both *mecA*- and *blaZ* genes mediated β-lactam resistance in Staphylococcus strains isolated from bovine mastitis. Global Veterinaria. 6 (1): 39-50.

*Bi, Y.; Wang, Y.J.; Qin, Y.; Guix Vallverdú, R.; Maldonado García, J. and Sun, W. (2016):* Prevalence of bovine mastitis pathogens in bulk tank milk in China. PLoS ONE. 11(5): 13 pages.

*Chiang, YC.; Pai, WY.; Chen, C.Y. and Tsen, HY. (2008):* Use of primers based on the heat shock protein genes hsp 70, hsp 40, and hsp 10, for the detection of bovine mastitis pathogens *Streptococcus agalactiae*, *Streptococcus uberis* and *Streptococcus bovis*. Molecular and Cellula probes. 22: 262-266.

*Christ, D.; Schwarz, S. and Lämmler, C. (1988):* DNA finger printing of *Streptococcus uberis.* Med. Sci. Res., 16: 1297-1298.

*Collado, R.; Prenafeta , A.; González-González, L.; Pérez-Pons, J.A. and Sitjà, M. (2016):* Probing vaccine antigens against bovine mastitis caused by *Streptococcus uberis.* Vaccine. 34(33): 3848-3854.

*Davies, P.L.; Leigh, J.A.; Bradley, A.J.; Archer, S.C.; Emes, R.D. and Green, M.J. (2016):* Molecular epidemiology of *Streptococcus uberis* clinical mastitis in dairy herds: Strain heterogeneity and transmission. J. Clin. Microbiol., 54 (1): 68-74.

*Dogan, B. and Boor, K.J. (2004):* Short communication: growth characteristics of *Streptococcus uberis* in UHT-treated milk. J. Dairy Sci., 87(4):813-815.

*Ebrahimi, A.; Nikookhah, F.; Nikpour, S.; Majiian, F. and Gholami, M. (2008):* Isolation of Streptococci from milk samples of normal, acute and subclinical mastitis cows and determination of their antibiotic susceptibility patterns. Pak. J. Biol. Sci., 11(1):148-50.

*El-Jakee, J.; Hableel, H.S.; Kandil, M.; Hassan, O.F.; Khairy, E.A. and Marouf, S.A. (2013):* Antibiotic resistance patterns of *Streptococcus agalactiae* isolated from mastitic cows and ewes in Egypt. Global Veterinaria 10 (3): 264-270.

*Guérin-Faublée, V.; Tardy, F.; Bouveron, C. and Carret, G. (2002):* Antimicrobial susceptibility of Streptococcus species isolated from clinical mastitis in dairy cows. Int. J. Antimicrob. Agents. 19(3): 219-226.

*Günther, J.; Czabanska, A.; Bauer, I.; Leigh, J.A.; Holst, O. and Seyfert, H.M. (2016): Streptococcus uberis* strains isolated from the bovine mammary gland evade immune recognition by mammary epithelial cells, but not of macrophages. Vet. Res., 47:13.

*Holzmuller, P.M.; Hide, D.; Sereno, D. and Lemesre, J.L. (2006): Leishmania infantum* amastigotes resistant to nitric oxide cytotoxicity: impact on

*in vitro* parasite developmental cycle and metabolic enzyme activities. Infect. Genet. Evol., 6: 187-197.

*Jiang, M.; Babiuk, L. and Potter, A. (1996):* Cloning, sequencing and expression of the CAMP factor gene of *Streptococcus uberis*. Microbiol. Path., 20: 297-307.

*Katholm, J.; Bennedsgaard, T.W.; Koskinen, M.T. and Rattenborg, E. (2012):* Quality of bulk tank milk samples from Danish dairy herds based on real-time polymerase chain reaction identification of mastitis pathogens. J. Dairy Sci., 95(10):5702-5708.

*Khan, I.U.; Hassan, A.A.; Abdulmawjood, A.; Lämmler, C.; Wolter, W. and Zschöck, M. (2003):* Identification and epidemiological characterization of *Streptococcus uberis* isolated from bovine mastitis using conventional and molecular methods. J. Vet. Sci., 4(3): 213-223.

*Kia, G.; Mehdi, G. and Keyvan, R. (2014):* Prevalence and antibiotic susceptibility of *Streptococcus spp.* in cows with mastitis in Germi, Iran. Anim. Vet. Sci., 2 (2): 31-35.

*Kromker, V.; Reinecke, F.; Paduch, J.H. and Grabowski, N. (2014):* Bovine *Streptococcus uberis* intramammary infections and mastitis. Clin. Microbial., 3,4: 1-7.

*Lämmler, C. (1991):* Biochemical and serological properties of *Streptococcus uberis*. J. Vet. Med., 38:737-742.

*Lasagno, M.C.; Reinoso, E.B.; Dieser, S.A.; Calvinho, L.F.; Buzzola, F.; Vissio, C.; Bogni, C. and Odierno, L.M. (2011):* Phenotypic and genotypic characterization of *Streptococcus uberis* isolated from bovine subclinical mastitis in Argentinean dairy farms. Rev. Argent. Microbiol., 43: 212-217.

*Maeda, K.; Nagata, H.; Kuboniwa, M.; Kataoka, K.; Nishida, N.; Tanaka, M. and Shizukuishi, S. (2004*): Characterization of binding of *Streptococcus oralis* glyceraldehyde-3 phosphate dehydrogenase to Porphyromonas gingivalis major fimbriae. Infect. Immun., 72: 5475-5477.

*Mathur, T.; Singhal, S.; Khan, S.; Upadhyay, D.J.; Fatma, T. and Rattan, A. (2006):* Detection of biofilm formation among the clinical isolates of Staphylococci: and evaluation of three different screening methods. Ind. J. Med. Microbiol., 24: 25-29.

*Melchior, M.B.; Vaarkamp, H. and Fink-Gremmels, J. (2005):* Biofilms: A role in recurrent mastitis infections. Vet. J., 171: 398-407.

*Moges, N.; Asfaw, Y.; Belihu, K. and Tadesse, A. (2011):* Antimicrobial susceptibility of mastitis pathogens from small holder dairy herds in and around Gondar, Ethiopia. J. anim. Vet. Adv., 10 (12): 1616-1622.

*Moore, G.E. (2009):* Biofilm production by *Streptococcus uberis* associated with intramammary infections. University of Tennessee Honors Thesis Projects. Trace: Tennessee Research and Creative Exchange.

*Moshynskyy, I.; Jiang, M.; Fontaine, M.; Perez-Casal, J.; Babiuk, L. and Potter, A. (2003):* Characterization of a bovine lactoferrin binding protein of *Streptococcus uberis*. Microb. Pathogenesis. 35: 203-215.

*Nadăş, G.C.; Fiţ, N.; Bouari, C.; Chirilă, F.; Răpuntean, S. and Rus, V. (2014):* The susceptibility to antibiotics of some bacterial strains isolated from cow milk with mastitis. Bull. UASVM Vet. Med., 71(2): 495-497.

*National Committee for Clinical Laboratory Standards (NCCLS) (2008):* Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals; Approved Standard, 2nd Edn. NCCLS Document M31- A3.Wayne, PA: National Committee for Clinical Laboratory Standards.

*Notcovich, S.; deNicolo, G.; Williamson, N.B.; Grinberg, A.; Lopez-Villalobos, N. and Petrovski, K.R. (2016):* The ability of four strains of *Streptococcus uberis* to induce clinical mastitis after intramammary inoculation in lactating cows. N. Z. Vet. J., 64(4):218-223.

*Oliver, S.; Almeida, R. and Calvinho, L. (1998):* Virulence factors of *Streptococcus uberis* isolated from cows with mastitis. Zbl. Vet. Med. B., 45: 461-471.

*Pancholi, V. and Fischetti, V.A. (1993):* Glyceraldehyde-3-phosphate dehydrogenase on the surface of group A Streptococci is also an ADP-ribosylating enzyme. P. Natl. Acad. Sci., 90: 8154-8158.

*Patel, D.; Almeida, R.A.; Dunlap, J.R and Oliver, S.P. (2009):* Bovine lactoferrin serves as a molecular bridge for internalization of *Streptococcus uberis* into bovine mammary epithelial cells. Vet. Microbiol., 137:297-301.

*Pecka-Kiełb, E.; Vasil, M.; Zachwieja, A. Zawadzki, W. Elečko, J.; Zigo, F.; Illek, J. and Farkasŏvá, Z. (2016):* An effect of mammary gland infection caused by *Streptococcus uberis* on composition and physicochemical changes of cows' milk. Pol. J. Vet. Sci., 19(1): 49-55.

*Perrig, M.S.; Ambroggio, M.B.; Buzzola, F.R.; Marcipar, I.S.; Calvinhod, L.F.; Veaute, C.M. and Barbagelata, M.S. (2015):* Genotyping and study of the *pauA* and *sua* genes of *Streptococcus uberis* isolates from bovine mastitis. Melina S. Rev. Argent. Microbiol., 47(4):282-294.

*Petrovski, K.R.; Grinberg, A.; Williamson, N.B.; Abdalla, M.E.; Lopez-Villalobos, N.; Parkinson, T.J.; Tucker, I.G. and Rapnicki, P. (2015):* Susceptibility to antimicrobials of mastitis-causing *Staphylococcus aureus*, *Streptococcus uberis* and *Streptococcus dysgalactiae* from New Zealand and the USA

as assessed by the disk diffusion test. Aust. Vet. J., 93(7): 227-233.

*Piepers, S.; De Meulemeester, L.; de Kruif, A.; Opsomer, G.; Barkema, H.W. and De Vliegher, S. (2007):* Prevalence and distribution of mastitis pathogens in subclinically infected dairy cows in Flanders, Belgium. Dairy Res. J., 74: 478-483.

*Prado, M,E.; Almeida, R.A.; Ozen, C.; Luther, D.A.; Lewis, M.J.; Headrick, S.J. and Oliver, S.P. (2011):* Vaccination of dairy cows with recombinant *Streptococcus uberis* adhesion molecule induces antibodies that reduce adherence to and internalization of *S. uberis* into bovine mammary epithelial cells. Vet. Immunol. Immunopathol., 141:201-208.

*Quinn, P.J.; Markey, B.K.; Leonard, F.C.; FitzPatrick, E.S.; Fanning, S. and Hartigan, P.J. (2011):* Veterinary Microbiology and Microbial Disease. 2nd ed., Wiley-Blackwell, J Wiley and Sons Ltd Publication, UK.

*Reinoso, E.B.; Lasagno, M.C.; Dieser, S.A. and Odierno, L.M. (2011):* Distribution of virulence-associated genes in *Streptococcus uberis* isolated from bovine mastitis. FEMS Microbiol. Lett., 318: 183-188.

*Reinoso, E.B.; Lasagno, M.C. and Odierno, L.M. (2015):* Genetic patterns of *Streptococcus uberis* isolated from bovine mastitis. Rev. Argent. Microbiol., 47(2):108-111.

*Rossitto, P.V.; Ruiz, L.; Kikuchi, Y.; Glenn, K.; Luiz, K.; Watts, J.L. and Cullor, J.S. (2002):* Antibiotic susceptibility patterns for environmental streptococci isolated from bovine mastitis in Central California Dairies, J. Dairy Sci., 85 (1): 132-138.

*Shome, B.R.; Bhuvana, M.; Mitra, S.D.; Krithiga, N.; Shome, R.; Velu, D.; Banerjee, A.; Barbuddhe, S.B.; Prabhudas, K. and Rahman, H. (2012):* Molecular characterization of *Streptococcus agalactiae* and *Streptococcus uberis* isolates from bovine milk. Trop. Anim. Health Prod., 44: 1981-92.

*Smith, A.J.; Kitt, A.J.; Ward, P.N. and Leigh, J.A. (2002):* Isolation and characterization of a mutant strain of *Streptococcus uberis*, which fails to utilize a plasmin derived beta-casein peptide for the acquisition of methionine. J. Appl. Microbiol., 93: 631- 639.

*Steele, N.M.; Williamson, J.H.; Laven, R.A. and Hillerton, J.E. (2015):* Using polymerase chain reaction to identify *Streptococcus uberis* in bovine milk: how does it compare with bacterial culture? Proceedings of the New Zealand Society of Animal Production. 75:20-23.

*Taponen, S. and Pyorala, S. (2006):* Coagulase negative staphylococci as cause of bovine mastitis-Not so different from *Staphylococcus aureus*? Vet. Microbiol., 134: 29-36.

*Tassi, R.; McNeilly, T.N.; Sipka, A. and Zadoks, R.N. (2015):* Correlation of hypothetical virulence traits of two *Streptococcus uberis* strains with the clinical manifestation of bovine mastitis. Vet Res., 46:123.

*Taylor, D.L.; Ward, P.N.; Rapier, C.D.; Leigh, J.A. and Bowler, L.D. (2003):* Identification of a differentially expressed oligopeptide binding protein (*OppA2*) in *Streptococcus uberis* by representational difference analysis of cDNA. J. Bacteriol., 185:5210-5219.

*Teklemariam, A.D.; Nigussie, H.; Tassew, A.; Tesfaye, B.; Feleke, A. and Sisay, T. (2015):* Isolation and phenotypic characterization of *Streptococcus uberis* from mastitic cows in and around Batu town, Ethiopia. J. Animal &Plant Sci., 26 (3): 4124-4137.

*Varhimo, E.; Varmanen, P.; Fallarero, A.; Skogman, M.; Pyorala, S.; Livanainen, A.; Sukura, A.; Vuorela, P. and Savijoki, K. (2011):* Alpha- and beta-casein components of host milk induce biofilm formation in the mastitis bacterium *Streptococcus uberis*. Vet. Microbiol., 149: 381-389.

*Ward, P.N.; Holden, M.T.; Leigh, J.A.; Lennard, N.; Bignell, A.; Barron, A.; Clark, L.; Quail, M.A.; Woodward, J.; Barrell, B.G.; Egan, S.A.; Fiel, T.R.; Maskell, D.; Kehoe, M.; Dowson, C.G.; Chanter, N.; Whatmore, A.M.; Bentley, S.D. and Parkhill, J. (2009):* Evidence for niche adaptation in the genome of the bovine pathogen *Streptococcus uberis*. BMC Genomics. 28: 10-54.

*Yuan, Y.; Dego, O.K.; Chen, X.; Abadin, E.; Chan, S.; Jory, L.; Kovacevic, S.; Almeida, R.A. and Oliver, S.P. (2014):* Conservation of *Streptococcus uberis* adhesion molecule and the *sua* gene in strains of *Streptococcus uberis* isolated from geographically diverse areas. J. Dairy Sci., 97: 7668-7673.

*Zadoks, R.N.; González, R.N.; Boor, K.J. and Schukken, Y.H. (2004):* Mastitis-causing Streptococci are important contributors to bacterial counts in raw bulk tank milk. J. Food Prot., 67(12): 2644-2650.

*Zadoks, R.N.; Schukken, H.Y. and Wiedmann, M. (2005):* Multilocus sequence typing of *Streptococcus uberis* provides sensitive and epidemiologically relevant subtype information and reveals positive selection in the virulence gene *pauA*. J. Clin. Microbiol., 43: 2407–2417.

# التوصيف المظهرى والجينى للمكور السبحى يوبرس المعزول من لبن الأبقار المصابة بإلتهاب الضرع

*هناء عبد المنعم عبد الفتاح عصفور ، سماح فكرى درويش ، صفاء أبو العنين الوكيل*

Email: hanaaasfour@yahoo.com Assiut University web-site: www.aun.edu.eg

تم تجميع عدد ٢٤٠ عينة لبن من أبقار مصابه بإلتهاب الضرع الظاهرى (٨٨ عينة من أرباع الضرع) والغير ظاهرى (١٠٨عينة من أرباع الضرع) وأيضا (٤٤عينة) من خزانات تجميع اللبن من مزارع الألبان المختلفة للكشف عن بعض المواصفات الظاهرية والجينيه المتعلقه بالضراوه فى ميكروب المكور السبحى يوبرس المعزول من هذه العينات وكذلك للكشف عن مقاومة هذه العترات للمضادات الحيوية المختلفه بإستخدام الطرق التقليديه. وبإستخدام كل من الإختبارات البيوكيميائية التقليديه والفحص الجزيئي بواسطة إختبار تفاعل البلمره المتسلسل تم التأكد من عزل ٤٨ عتره من المكور السبحى يوبرس من أصل ٧٤ عتره من المكورات السبحيه (٦٤,٩٪) وكانت نسبة عزل هذا الميكروب من إجمالى عينات اللبن تحت الدراسه بوجه عام ٢٠٪. وقد لوحظ أن أعلى نسبة عزل لهذا الميكروب كانت من خزانات تجميع اللبن (٤٥,٥٪) يليها عزله من العينات اللبن المجمعه من الحيوانات المصابه بإلتهاب الضرع الظاهرى ثم تلك المصابه بإلتهاب الضرع الغير ظاهرى (بنسب ١٨,٢٪ و ١١,١٪ على التوالي). عند دراسة عوامل الضراوه الظاهرية لمعزولات المكور السبحى يوبرس تم الكشف عن وجود إنحلال الدم من النوع (بيتا) وكذلك عامل كامب بنسبه ٦,٢٥ ٪ لكل منهما فى حين كان تكوين الغشاء الحيوى أو اللزوجه كمؤشر لتشكيل البيوفيلم قد وجد بنسبة ٧٥٪ فى هذه المعزولات. تم إجراء إختبارالحساسيه لمعزولات المكور السبحى يوبرس لعدد من المضادات الحيويه وأظهرت النتائج أن بعض المعزولات كانت حساسه لعدد محدود من المضادات الحيويه فى حين أن معظمها كان مقاوم لعدد كبير من المضادات الحيويه الأخرى فى كل عترات المكور السبحى يوبرس. عند دراسة بعض الجينات الوراثية المسئوله عن الضراوه فى معزولات المكور السبحى يوبرس بإستخدام إختبار تفاعل البلمره المتسلسل تم الكشف عن وجود جين *gapC* فى كل العترات وأيضا وجد كل من الجينات *sau, cfu, oppF* بنسب ٩٣,٨٪ و ٦٨,٨٪ و ٦٢,٥٪ على التوالى بينما لم يتم الكشف عن وجود الجين المسئول عن إرتباط هذا الميكروب باللاكتوفيرين فى أى من العترات المعزوله (*lbp*). تم الكشف عن وجود إثنين على الأقل من جينات الضراوه من أصل الخمس جينات المختلفة في كل عتره من عترات المكور السبحى يوبرس تحت الدراسة. وكانت هناك بعض العترات منها تحتوى على أربعه من هذه الجينات والتى لوحظ وجودها بالنسبة الأكبر فى تلك التى تم عزلها من حالات إلتهاب الضرع الظاهرى. علاوة على ذلك تم الكشف عن عترات أخرى تحوي ٣ جينات ضراوه وقد لوحظ بأنها موجوده بالنسبة الأكبر فى تلك المعزولة من حالات إلتهاب الضرع الغير ظاهرى. وقد خلصت هذه الدراسه إلى أن المكور السبحى يوبرس ينبغي إعطاؤه إهتمام أكبير بإعتباره من الميكروبات الخطيره التى تهدد الأبقار الحلابه حيث تم عزله بنسب لا يستهان بها فى كل من الخزانات المجمعه للبن وأيضا من حالات إلتهاب الضرع الظاهرى والغير ظاهرى والذى أظهر مقاومة للعديد من المضادات الحيويه شائعة الإستخدام فى المزارع الحلابه لعلاج حالات إلتهاب الضرع. علاوة على ذلك فإنه يحوى من مقومات الضراوه الظاهريه والجينيه التى تمكنه من المكوث داخل ضرع الحيوانات الحلابه لفترات طويله مما يترتب عليه زيادة معدل إنتشار العدوى من حيوان إلى أخر وربما زيادة إنتشاره بين المزارع المختلفه مما يؤدى إلى خسائر إقتصاديه كبيره فى صناعه الألبان.