Haemolysin and hyaluronidase genes of *Streptococcus agalactiae* recovered from mastitic cow's milk

Gamal Abd Elgaber Mohamed Younis (Affiliation: Professor and head of the Department of Bacteriology, Mycology and Immunology), * Rasha Mohamed Elkenany, and Shaimaa Abd Elhaleem Nabih

Department of Bacteriology, Mycology and Immunology, Faculty of Veterinary medicine, Mansoura University, Mansoura 35516, Egypt
*Corresponding author: dr_rashavet22@mans.edu.eg
gamalyounis_1@hotmail.com, sh.abdelhaleem@yahoo.com.au; Telephone: 01090226696, 01000896736

Abstract: Streptococcal mastitis causes great economic losses in dairy industries all over the world; therefore the aim of the research is to investigate the prevalence of streptococci in mastitic cows, detection of titre of haemolysin as well as identification of two virulence genes in *S. agalactiae* (including *β*- hemolysin/ cytolysin (*cylE*) and *hyl* (hyaluronidase) genes). About 110 (64.7%) out of 170 milk samples from cows were mastitic either clinical (48.2%) or subclinical mastitis (51.8%) with streptococci positive. Identification of *S. agalactiae (*50, 45.45%), *S. uberis* (46, 41.82%) and *S. dysgalactiae* (14, 12.73%) were screened by biochemical methods. Six of 10 isolates of *S. agalactiae* produced haemolysin titre ranged from 1:16 to 1:64. By PCR amplification, 6 (60%) of 10 phenotypically beta (*β*) haemolysis on modified Edward's media and sheep blood agar were cylE gene positive and 3 (30%) of 10 isolates were *hyl* gene positive. The genotype of *β*-hemolysin of *S. agalactiae* seemed to be having correlation with the expression of their phenotypes and also correlating well with the result of titres of haemolysin. The high percentage of *S. agalactia*e cylE gene and hyl gene in the present study help in understanding of the distribution of *S. agalactiae* and contribute to the establishment of preventive approaches to reduce the spread of infection.

[Younis, G. A., Rasha, M.E., and Shaimaa, A. N. **Haemolysin and hyaluronidase genes of *Streptococcus agalactiae* recovered from mastitic cow's milk.** *Biomedicine and Nursing* 2017;3(4): 97-104]. ISSN 2379-8211 (print); ISSN 2379-8203 (online). http://www.nbmedicine.org. 10. doi:10.7537/marsbnj030417.10.

Keywords: *S. agalactiae*, mastitis, β-haemolysin, cylE gene, hyl gene.

Introduction

Mastitis is a multifactorial disease caused by several species of gram-negative and gram-positive bacteria, mycoplasmas, fungi, and algae (***Zadoks et al., 2011***). Mastitis leads to economic losses including reduction in milk yield or milk quality and early culling of severely affected animals. It leads to expensive antibiotic treatment, veterinary services and losses of the young ones ***(Sordiell et al., 2000 and Leitner et al., 2001)***. *Streptococcus* is isolated frequently from bovine mammary glands ***(Facklam. 2002 and Fortin et al., 2003)***. *Streptococcus agalactiae*, *S. dysgalatiae* and *S. uberis* have been reported as the three most common causative agents of mastitis ***(Leigh, 1999 and Khan et al., 2003)***.

Streptococcus agalactiae (group B Streptococcus, GBS) has been widely reported as an important pathogen of both animals and man ***(Keefe, 1997; Mosabi et al, 1997 and Ko et al., 2001)***. In cattle, it causes bovine clinical mastitis and subclinical mastitis, and in humans, it is associated with infections among neonates and adults ***(Pinto et al., 2013)***. Man may be a source of infection for cattle ***(Zadoks et al., 2011).***

GBS exhibits cytolytic toxin, the beta (***β***) haemolysin. GBS ***β***-haemolysin is primarily a broad-spectrum cytolysin capable of destroying many eukaryotic cells ***(Tapsall and Phillips, 1991 and Nizet et al., 1996)***. It is therefore referred to as the GBS *β*-hemolysin/ cytolysin ***(Doran et al., 2002)***. The first report of the GBS *β*- hemolysin/ cytolysin provided by ***Todd (1934)*** described an extracellular molecule that is oxygen stable, acid and heat labile, and non-immunogenic, only *cylE* was essential for *β*- hemolysin/ cytolysin expression ***(Pritzlaff et al., 2001).***

S. agalactiae hyl encodes hyauronate lyase (hyaluronidase), a putative virulence factor facilitates the spreading of bacteria in host tissues ***(Akhtar and Bhakuni, 2004).*** The hyluronidase activity in *S. agalactiae* is associated with host specificity ***(Lin et al., 1994).***

Little data is available on the role of *S. agalactiae* in disease exacerbation through the production of *β*-haemolysin toxin and hyaluronidase. Therefore, the objective of this study was to determine the role of *S. agalactiae* in bovine mastitis in Egypt, determine the titre of haemolysin and detect *β*-haemolysin gene as well as hyaluronidase gene by PCR.

Materials and Methods

Samples collection

A total of 170 milk samples were collected from cows with clinical (n=70) or sub clinical (n=100) mastitis from 3 bovine dairy farms in Mansoura City, Egypt from May to September 2016. All milk samples were used for microbiological analysis.

Microbiological analysis

The milk samples were inoculated on modified Edwards's media **(Oxoid)** as a selective media for isolation of streptococci and incubated aerobically at 37°C for 24-72h. The suspected colonies yielding gram-positive cocci with catalase-negative were subcultured on 7% sheep blood agar. Colonies yielding *β*-haemolysis on blood agar were subjected to CAMP test and aesculin hydrolysis test as previously described (***Cruikshank et al., 1975 and Barrow and Feltham, 1993)***.

Determination of titre of β-hemolysin

Ten randomly selected isolates were inoculated in brain heart infusion (BHI) broth and incubated at 37°C under 20-25% Co_2 tension for 24h. The BHI broth was centrifuged and filtrated. The supernatant was collected to get a high yield of β-haemolysin. Two-fold serial dilutions were made in saline. One ml of saline was pipetted into dilution tubes. One ml of 1% sheep RBCs suspension was pipetted into all tubes. So, the final dilutions became 1:4, 1:8, 1:16, 1:32, 1:64 and 1:128. The tubes were incubated at 37°C for 30 min., and then over night at 4°C. The greatest dilution of the sample resulting in 50% hemolysis of 1 ml of sheep erythrocyte suspension was defined as 1 hemolytic unit ***(Marchlewics and Duncan, 1980)***.

Molecular detection of cylE and hyl genes of S. agalactiae

The polymerase chain reaction (PCR) was applied for the determination of *cylE* and *hyl* genes that were encoding β-haemolysin and hyaluronidase enzymes of *S. agalactiae.* DNA was extracted from *S. agalactiae* using QIAamp DNA Mini Kit **(Qiagen, Germany, GmbH)**. The DNA amplifications were performed using certain primers and under specific profiles, as shown in **Table (1) and (2)**. The amplified DNA products were separated by electrophoresis on 1.5% agarose gel **(Applichem, Germany, Gmbh)**. Gel pilot 100bp DNA ladder **(Qiagen, Germany, gmbh)** was used to determine the fragment sizes. The gel was photographed by a gel documentation system and the data was analyzed through computer software.

Results and discussion

Prevalence of streptococcal species

Bovine mastitis is a global problem responsible for many losses in growing dairy industry ***(Zeryehun and Abera, 2017)***. In the present study the overall prevalence of mastitis was 64.7% (110/ 170) where 48.2% (53/110) and 51.8% (57/110) cows with clinical and subclinical mastitis, respectively **(Table 3)**. This result was closely in agreement with the finding of 64.9% in AL-Diwanyia ***(Al-kuzaay and Kshash, 2013)*** and 64.3% in Eastern Ethiopia ***(Zeryehun and Abera, 2017)***. This study also showed the prevalence of 48.2% for clinical mastitis that was much higher than the findings of 10.7% in Ethiopia ***(Zeryehun and Abera, 2017)*** and 15.9% in AL-Diwanyia ***(Al-kuzaay and Kshash, 2013)***. In addition, the research revealed subclinical mastitis prevalence of 51.8% which was in agreement with the finding of 51.8% in Ethiopia ***(Zeryehun and Abera, 2017)***. The high prevalence of mastitis has been reported to be due to deficient dry cow therapy, unhygienic milking practices, poor udder hygiene, high yielders, and no grazing ***(Abrahamsen et al., 2014)***.

Streptococcus is a common pathogen of clinical or sub-clinical mastitis resulting in great economic losses in dairy farms in Egypt ***(Benić et al., 2012)***. In the current study, a total of 110 (64.7%) streptococci recovered from milk samples were identified by biochemical methods as *S. agalactia*e (50, 45.45%), *S. uberis* (46, 41.82%) and *S. dysgalactiae* (14, 12.73%) **(Table 3)**. The organisms were isolated from cases of mastitis by other investigators ***(Kerro-Dego et al., 2003 and Seyoum et al., 2003)***. The biochemical characteristics of recovered *S. agalactiae* were catalase negative, *β*-haemolysis on sheep blood agar **(photo. 1)**, CAMP test positive, lactose positive and aesculin hydrolysis negative ***(Watt, 1988)***. Our results revealed that *S. agalactiae* is the predominant species of streptococci. This result is consistent with previous researchers ***(Prabhu et al., 2012)***. Also, another study recorded the prevalence of infection with group B streptococci (GBS) up to 44% in infected herds ***(Keefe, 1997)***. ***Ekin and Gurturk (2006)*** isolated 44.7% *S. agalactiae* from bovine mammary glands. Recently, other researchers ***(Kia et al., 2014 and Ding et al., 2015)*** recovered 52.95% and 70.4% *S. agalactiae* from mastitic cow's milk. However, the result in this study was higher than those obtained by ***Elhaig et al. (2014)*** who recorded that 20% 0f bacterial isolates were *S. agalactiae* in Egypt.

Phenotypic characterization of β-haemolysin of S. agalactiae

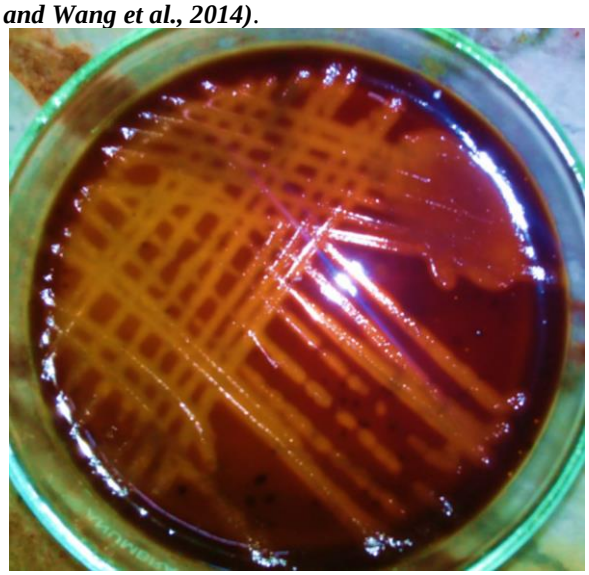
Most GBS strains as *S. agalactiae* produce a surface-associated beta haemolysin/cytolysin (β-h/c), which plays a key role in GBS pathogenesis. It can target a wide spectrum of cells, and hyper production of this haemolysin is associated with fulminant disease in clinical GBS cases as well as severe cases of infection in animal models ***(Rosa- Fraile et al., 2014)***. *S. agalactiae* invasion and disease pathogenesis is a complex process that is achieved through numerous virulence factors. The *S. agalactiae* β-hemolysin is

considered as one of the most important virulence factors. Invasive *S. agalactiae* infections are almost exclusively caused by β-hemolytic strains and absence of *S. agalactiae* β-hemolysin prevents the bacteria to survive inside the phagocytic cell ***(Sagar et al., 2013)***. In the current study, ten *S. agalactiae* isolates, which showed β-hemolysis on sheep blood agar plates, were titrated for β-haemolysin. Six (60%) out of 10 isolates of *S. agalactiae* revealed titre of β-haemolysin ranged from 1:16 to 1:64 **(Photo. 2,3,4)**, while 4 (40%) of 10 isolates were negative (**Table 4**). The highest titre of beta toxins was 1/64 (3 isolates, 50%), followed by 1/32 (1 isolate, 16.67%), whereas 1/16 (2 isolates, 33.33%) was the lowest titre. This result was close to the results of ***Nizet et al. (1996)*** who found that the titre of haemolysin ranged from 1:4 to 1:64.

Genotypic characterization of cylE and hyl genes of S. agalactiae

PCR assay is a rapid, accurate, sensitive and specific method for identification of the virulence genes (*cylE* and *hyl* genes) of *S. agalactiae*. Thus, the present work detected the *cylE* gene encoding β-hemolysin in 6 (60%) of 10 *S. agalactiae* isolates **(photo. 5).** This result was close to the result of ***Ding et al. (2015)*** who recorded 50% of *cylE* genes in isolates. On the other hand, this result was higher than those obtained by ***Spellerberg et al.* (2000)** and ***Bergseng et al. (2007),*** who found 34.3% and 23% of *cylE* genes in isolates, and lower than those obtained by ***Dmitriev et al. (2002)*** who recorded 100% of *cylE* genes. By PCR test, 6 isolates harbored *cylE* genes were phenotypically expressed β- haemolysin with variable titres, while other 4 isolates were negative for *cylE* gene without expression of beta haemolysin. The genotypes of *β*-haemolysin of *S. agalactiae* in this study seemed to be having a correlation with the expression of their phenotypes and also correlating well with the result of the titre of haemolysin as illustrated in **Table (4)**.

Hyaluronidase enzyme is an essential factor in enabling the spread of the pathogens from an initial site of infection ***(Girish and Kemparajuk, 2007***). It has been assumed to facilitate the spread of *S. agalactiae* through the tissues of the infected host ***(Pritchard et al., 1994***). Also, it has a strong influence on intracellular survival of *S. agalactiae* and proinflammatory cytokine expression ***(Wang et al., 2014)***. Therefore, the *hyl* gene encoding hyaluronidase was another gene identified in 3 (30%) of 10 *S. agalactiae* isolates by PCR amplification in the present study **(Photo. 6).** This result was close to the result of ***Aprini et al (2016)*** who recorded 38.8% of *hyl* genes in isolates. On the other hand, this result was lower than those obtained by ***Krishnaveni et al. (2014).*** Both *cylE* and *hyl* genes could observe for 3 (30%) of 10 isolates (**Table 5**). These two genes are responsible for the intracellular survival of *S. agalactiae* inside macrophage ***(Sagar et al., 2013 and Wang et al., 2014)***.



Photograph 1. β-haemolysis of *S. agalactiae* on Modified Edward's media

Table 1. Oligonucleotide primer sequences used in this study

Target gene	Sequence	Amplified product	Reference
S. agalactiae cylE	TGACATTTACAAGTGACGAAG	248 bp	Bergseng et al. (2007)
	TTGCCAGGAGGAGAATAGGA		
S. agalactiae Hyl	CATACCTTAACAAAGATATATAACAA	950bp	Krishnaveni et al. (2014)
	AGATTTTTTAGAGAATGAGAAGTTTTTT		

Table 2. Cycling conditions during PCR

Target gene	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension
S. agalactiae cylE	94˚C 5 min.	94˚C 30 sec.	55˚C 45 sec.	72˚C 30 sec.	35	72˚C 7 min.
S. agalactiae Hyl	94˚C 5 min.	94˚C 30 sec.	52˚C 30 sec.	72˚C 50 sec.	35	72˚C 10 min.

Table 3. Incidence of *Streptococcus species* in mastitic milk

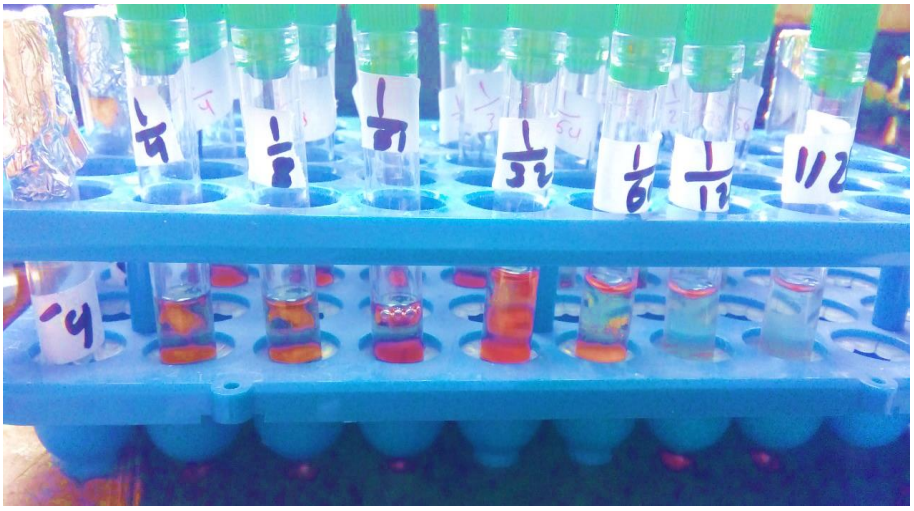
Isolates	Clinical mastitis	Subclinical mastitis	Total
S.agalactiae	19 (35.8%)	31 (54.4%)	50 (45.45%)
S.uberis	26 (49%)	20 (35%)	46 (41.82%)
S.dysgalactiae	8 (15%)	6 (10.5%)	14 (12.73%)
Total	53 (48.2%)	57 (51.8%)	110 (64.7%)

Table 4. Correlation of the titre of hemolysin and *cylE* gene detection by PCR

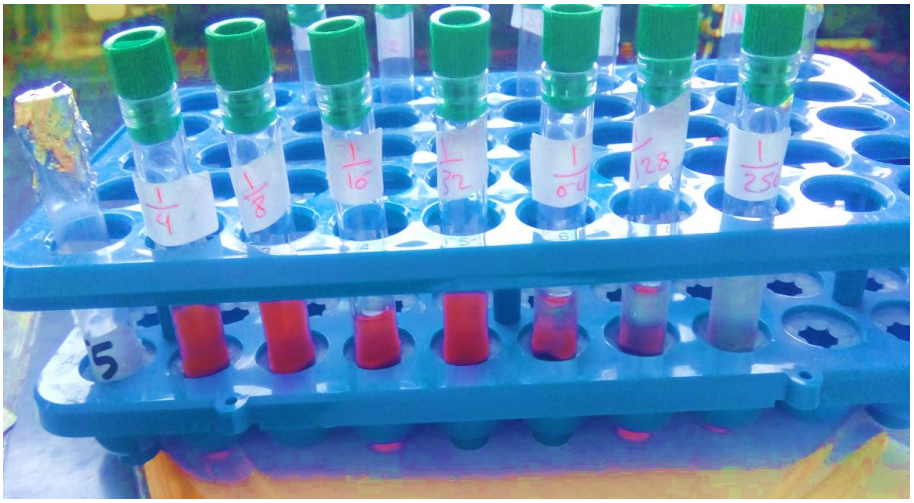
Code No. of tested isolates	Results	
	β-Haemolysin titre	***CylE***
1	1\64	+
2	-	-
3	-	-
4	1\16	+
5	-	-
6	1\64	+
7	1\32	+
8	1\64	+
9	-	-
10	1\16	+

Table 5. PCR amplification of *cylE* and *hyl* genes of *S. agalactiae*

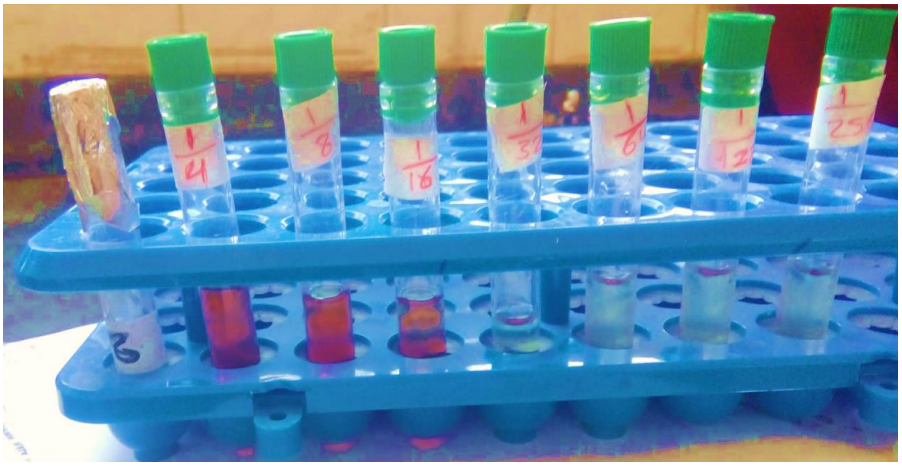
Code No. of tested isolates	Results	
	CylE	***Hyl***
1	+	-
2	-	-
3	-	-
4	+	+
5	-	-
6	+	+
7	+	-
8	+	-
9	-	-
10	+	+



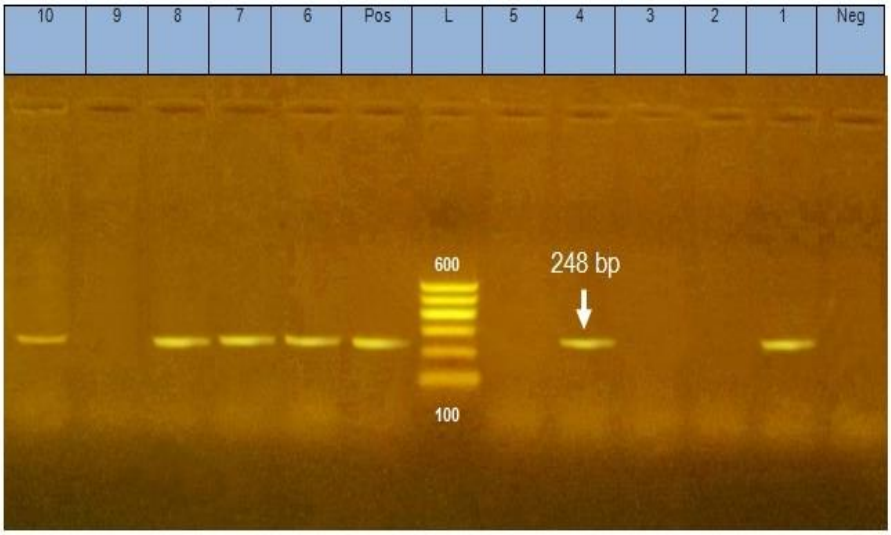
Photograph 2. Haemolysin titre with washed sheep RBCs showed 50% haemolysis at the dilution 1\32



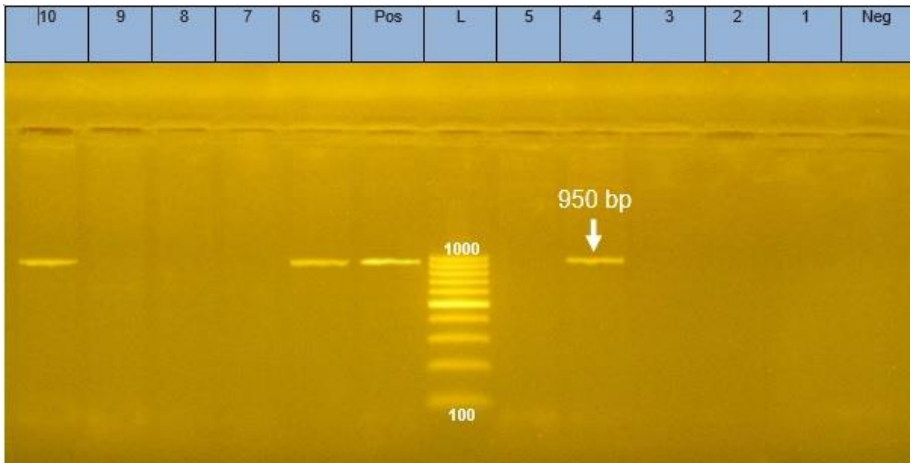
Photograph 3. Haemolysin titre with washed RBCs showed 50% haemolsis at the dilution 1\64



Photograph 4. Haemolysin titre with washed sheep RBCs showed 50% haemolysis at the dilution 1\16



Photograph 5. Agarose gel electrophoresis of PCR products showing amplification of *cylE* gene of *S. agalactiae*; Lane L: DNA molecular weight marker (100bp), lane Pos: positive control, lane Neg: negative control, lanes 1,4, 6,7,8,10: positive for *cylE* gene (248bp), lanes 2,3,5,9: negative for *cylE* gene.



Photograph 6. Agarose gel electrophoresis of PCR products showing amplification of *hyl* gene of *S. agalactiae*; Lane L: 100:1000 bp DNA ladder; lane pos: positive control, lane Neg: negative control, lanes 4,6,10: positive for *hyl* gene (950bp), lanes 1,2,3,5,7,9 negative for *hyl* gene.

Conclusions

The current study reported an overall prevalence of streptococcal species, especially *S. agalactiae*, associated with mastitis that was a major health problem of dairy cows and will have a drawback on the production of dairy industry and hence warrants serious attention in Egypt. Particularly, the prevalence of *cylE* gene encoding β-hemolysin and *hyl* gene encoding hyaluronidase in the present study help in the understanding of the distribution of *S. agalactiae* and contribute to the establishment of preventive approaches to reduce the spread of infection.

Acknowledgements

We would like to thank Dr. Essam Mesbah, head of the department of Surgery, for helping in collections of samples.

Author's information:

1. Gamal Abd Elgaber Mohamed Younis

Affiliation: Professor and head of the Department of Bacteriology, Mycology and Immunology, Faculty of Veterinary medicine, Mansoura University, Mansoura 35516, Egypt

Email: gamalyounis_1@hotmail.com

Telephone: 01001918140

2. Rasha Mohamed Elkenany

Lecturer in the Department of Bacteriology, Mycology and Immunology, Faculty of Veterinary medicine, Mansoura University, Mansoura 35516, Egypt

Email: dr_rashavet22@yahoo.com

Telephone: 01090226696

3. Shaimaa Abd Elhaleem Nabih

Veterinarian, Mansoura 35516, Egypt

Email: sh.abdelhaleem@yahoo.com.au

Telephone: 01000896736

Title of the manuscript: **Haemolysin and hyaluronidase genes of *Streptococcus agalactiae* recovered from mastitic cow's milk**

Subtitle: *Streptococcus agalactiae* in mastitic cow's milk

Reference

1. Abrahamsen M, Persson Y, Kanyima B, Bage R. Prevalence of subclinical mastitis in dairy farms in urban and peri-urban areas of Kampala, Uganda, Tropical Animal Health and Production 2014; 46(1): 99-105.
2. Akhtar MS, Bhakuni V. S. pneumonia hyaluronate lyase: An over view. Gurr. Sci. 2004; 86: 285-9.
3. Al-kuzaay Gh, Kshash Q. Streptococcus agalactiae mastitis of bovine detection by Polymerase Chain Reaction (PCR) test in AL-Diwanyia province AL-Qadisiya. Journal of Veterinary Medicine Science 2013; 12(2).
4. Aprini CM, Cardoso PG, Paiva IM, Costa GM. Virulence Genes of the Streptococcus agalactiae Associated with Bovine Mastitis in Minas Gerais Livestock Herds, Brazil. Applied Microbiology 2016; 2:1-7.
5. Barrow GT, Feltham R K. Cowan and steel's manual for the identification of medical bacteria, third edition Cambridge University Press. Cambridge.1993: 33.
6. Benić M, Habrun B, Kompes G. Clinical and Epidemiological Aspects of Cow Mastitis Caused by Staphylococcus aureus and its Methicillin-Resistant Strains. Rad Hrvatske akademije znanosti i umjetnosti. Medicinske znanosti 2012; 37:113-21.
7. Bergseng H, Bevanger L, Rygg M, Bergh K. Real-time PCR targeting the sip gene for

detection of group B Streptococcus colonization in pregnant women at delivery. Journal of Medical Microbiology 2007; 56:223-8.
8. Cruickshank R, Duguid JP, Marmion BP, Swan HA. Medical microbiology. 12th ed. Churchill Livinstone. Edinburgh London. 1975: 587.
9. Ding Y, Zhao J, He X, Li M, Guan H, Zhang Z, Li P. Antimicrobial resistance and virulence-related genes of Streptococcus obtained from dairy cows with mastitis in Inner Mongolia, China. Pharmceutical Biology 2015; 54(1): 162-7.
10. Dmitriev A, Shakleina E, Tkacikova L, Mikula I, Totolian A. Genetic heterogeneity of the pathogenic potentials of human and bovine group B streptococci. Folia Microbiology (Praha) 2002; 47(3):291-5.
11. Doran KS, Chang JC, Benoit VM, Eckmann L, Nizet, V. Group B streptococcal beta-hemolysin/cytolysin promotes invasion of human lung epithelial cells and the release of interleukin-8. Journal of infectious Disease 2002; 185:196-203.
12. Ekin IH, Gurturk K. Characterization of bovine and human group B streptococci isolated in Turkey. Journal of Medical Microbiology 2006; 55:517-21.
13. Elhaig M, Selim A. Molecular and bacteriological investigation of subclinical mastitis caused by Staphylococcus aureus and Streptococcus agalactiae in domestic bovins from Ismailia, Egypt. Tropical Animal Health and Production 2014.
14. Facklam R. What happened to the streptococci: Overview of taxonomic and nomenclature changes. Clinical Microbiology Review 2002; 15:613-30.
15. Fortin MS, Messier J, Higgins R. Identification of catalase-negative, non- β-haemolytic, gram-positive cocci isolated from milk sample. Clinical Microbiology 2003; 41:106-9.
16. Girish KS, Kemparaju K. The magic glue hyaluronan and its eraser hyaluronidase: a biological overview. Life Science 2007; 80:1921-43.
17. Keefe GP.Streptococcus agalactiae mastitis: a review. Canadian Veterinary Journal 1997; 38: 429-37.
18. Kerro-Dego O, Tareke F. Bovine mastitis in selected areas in southern Ethiopia. Tropical Animal Health and Production 2003; 35(3): 197-205.
19. Khan IU, Hassan AA, Abdulmawjood A, Lanimler C, Wolter W, Zschock M. Identification and epidemiological characterization of Streptococcus uberis isolated from bovine mastitis using conventional methods. Journal of Veterinary Science 2003; 4:213-23.
20. Kia G, Mehdi GH, Keyvan R. Prevelance and antibiotic susceptibility of Streptococcus Spp. in Germi, Iran: Animal and Veterinary Sciences 2014; 2(2):31-5.
21. Ko WC, Lee HC, Wang LR, Lee CT, Liu AJ, Wu JJ. Serotyping and antimicrobial susceptibility of group B streptococcus over an eight-year period in southern Taiwan. Eur. Journal of Clinical Microbiology Infectious Disease 2001; 20: 334-9.
22. Krishnaveni N, Isloor SK, Hegde R, Suryanarayanan VVS, Rathnma D, Veeregowda BM, Nagaraja CS, Sundareshan S. Rapid detection of virulence associated genes in Streptococcal isolates from bovine mastitis. African journal of microbiology research 2014; 8(22):2245-54.
23. Leigh JA. Streptococcus uberis: A permanent barrier to the control of bovine mastitis. Veterinary Journal 1999; 157: 225-38.
24. Leitner G, Chaffer M, Zamirs MT, Glickman A, Winkler M, Weisblit, L. Saran A. Udder disease etiology, milk somatic cell and NAGase activity in Israel Assaf Sheep throughout lactation Small Ruminant Research 2001; 39:107-12.
25. Lin Sk, Coligan JE, Pritchard DG. Cloning and expression of the gene of group B streptococcal hyaluronate lyase. Journal of Biology and chemistry 1994; 269:30113-6.
26. Marchlewicz BA, Duncan JL. Properties of a hemolysin produced by group B streptococci. Infection and Immunity 1980; 30: 805-13.
27. Mosabi JM, Arimi SM, Kangethe EK. Isolation and characterization of group B streptococci from human and bovine sources within and around Nairobi. Epidemiology Infection 1997; 118: 215-20.
28. Nizet W, Gibson RL, Chi EM, Framson PE, Hulse M, Ribens CE. Group B streptococcal beta-hemolysin expression is associated with injury of lung epithelial cells. Infection and Immunity 1996; 64:3818-26.
29. Pinto TCA, Costa NS, Souza ARV, Silva LGD, Corrêa ABD, Fernandes FG, Oliveira IC, Mattos MC, Rosado AS, Benchetrit LC. Distribution of serotypes and evaluation of antimicrobial susceptibility among human and bovine Streptococcus agalactiae strains isolated in Brazil between 1980 and 2006. Brazilian Journal of Infectious Diseases 2013; 17(2): 131-6.
30. Prabhu KN, Isloor S, Hegde R, Rathnamma D, Veeregowda BM, Narasimha Murthy HM, Shome R, Suryanarayana VVS. Development of

polymerase chain reaction for detection of predominant Streptococcal isolates causing subclinical bovine mastitis. Indian Journal of Biotechnology 2012; 12: 208-12.
31. Pritchard DG, Lin B, Willingham TR, Baker J R. Characterization of the group B streptococcal hyaluronate lyase. Archives of Biochemistry and Biophysics 1994; 315:431-7.
32. Pritzlaff CA, Chang JC, Kuo SP, Tamura GS, Rubens CE, Nizet V. Genetic basis for the β-hemolytic/cytolytic activity of group B Streptococcus. Molecular Microbiology 2001; 39:236-47.
33. Rosa-Fraile M, Dramsi S, Spellerberg B. Group B Streptococcal haemolysin and pigment, atale of twins. FEMS Microbiology Review 2014; 38: 932-46.
34. Sagar A, Klemm C, Hartjes L, Mauerer S, van Zandbergen G, Spellerberg B. The β-hemolysin and intracellular survival of Streptococcus agalactiae in human macrophages. Puplic library of science One 2013; 8: e60160.
35. Seyoum T, Ameni G, Ashenafi M. The prevalence of bovine mastitis, bacterial isolation and their susceptibility to antibiotics in Central Africa 2003; 52(4):182-189.
36. Sordielli DO, Buzzola FR, Gomez MI, Steele Moore L, Berg D, Gentilini E, Catalanno M, Reitz AJ, Toller ST, Denamiel G, Jeric P, Lee JC. Capsule expression by bovine isolates of Staphylococcus aureus from Argentina: Generic and Epidemiologic analyses. Journal of Clinical Microbiology 2000; 38:846-50.
37. Spellerberg B, Martin S, Brandt C, Lutticken R. The cyl genes of Streptococcus agalactiae are involved in the production of pigment. FEMS Microbiology Letters 2000; 188: 125-8.
38. Tapsall JW, Phillips EA. The hemolytic and cytolytic activity of group B streptococcal hemolysin and its possible role in early onset group B streptococcal disease. Pathology 1991; 23:139-44.
39. Todd EW. A comparative serological study of streptolysins derived from human and animal infections with note on pneumococcal hemolysin, tetanolysin, and streptococcal toxin. Journal of Pathology and Bacteriology 1934; 39: 299-321.
40. Wang Z, Guo C, Xu Y, Liu G, Lu Ch, Liu Y. Two novel functions of hyaluronidase from Streptococcus agalactiae are enhanced intracellular survival and inhibition of proinflammatory cytokine expression. Infection and Immunity 2014; 82(6): 2615-25.
41. Watts JL. Characterization and identification of streptococci isolated from mammary gland. Journal of Dairy Science 1988; 71:1616-24.
42. Zadoks RN, Middleton JR, McDougall S, Katholm J, Schukken YH. Molecular epidemiology of mastitis pathogens of dairy cattle and comparative relevance to humans. Journal of Mammary Gland Biology and Neoplasia 2011; 16(4): 357-72.
43. Zeryehun T, Abera G. Prevalence and Bacterial Isolates of Mastitis in Dairy Farms in Selected Districts of Eastern Harrarghe Zone, Eastern Ethiopia. Journal of Veterinary Medicine 2017:1-7.

12/25/2017