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ISOLATION AND IDENTIFICATION OF GBS BACTERIA FROM MASTITIS BY CAMP TEST AND LANCEFIELD'S SEROLOGICAL GROUPING

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ABSTRACT

Mastitis is an udder tissue inflammation which has infected various species of animals. It happens through several types of pathogenic bacteria, particularly *Streptococcus agalactiae*. GBS is a leading cause of cow mastitis. In our sample, 9.52% of *Streptococcus agalactiae* were isolated which were collected from bovine mastitic milk and identified by biochemical tests such as catalase, oxidase, Production of indole, fermentation of sugar, an examination of antibiotic sensitivity, CAMP test and group kits of Lancefield. The results showed that all *Streptococcus agalactiae* isolate was diagnosed by CAMP test by the appearance of the arrowhead in blood agar and by the appearance of visible agglutination on a card in the serological grouping kit of Lancefield.

Keyword: *Streptococcus agalactiae*, CAMP test, Lancefield's serological grouping

Introduction

In dairy farms, bovine mastitis has been described as the most important disease due to decreased milk production, reduced farm productivity, discarded milk, healthcare costs and culling (Gröhn *et al.*, 2005). Clinical and subclinical are types of mastitis, udder inflammation is clinical mastitis that can be identified by changes in the characteristics of milk and normal udder structure, subclinical mastitis is udder inflammation that shows no visible changes in the milk or udder (Blowey and Edmondson, 2010).

Streptococcus agalactiae, B *Streptococcus* group (GBS), is a Gram-positive encapsulated bacterium belonging to the pyogenic *Streptococci* group. This is the only *Streptococcus* species that harbour the cell-wall-specific polysaccharide antigen of Lancefield group B, common to all GBS strains (Edwards *et al.*, 2016).

Is a circular bacterium with a propensity to form chains (as expressed in the name of the genus *Streptococcus*). It is a beta-hemolytic, catalase-negative, and possible anaerobic (Whiley & Hardie, 2009). GBS can be subdivided by type-specific capsular polysaccharides into 10 separate serotypes (Ia, Ib, and II to IX) (Edwards *et al.*, 2016).

Streptococcus agalactiae is one of the world's most common pathogens which contributes to mastitis in dairy herds (Carvalho-Castro *et al.*, 2017). It for the dairy industry is an important economic cause of failure (Ruegg, 2017). GBS may cause an acute febrile condition in cows or a more chronic subacute disorder, which lead to a reduction in milk production, hence (agalactiae) which means "no milk" (Ruegg, 2017).

It considered an udder-specific pathogen and a common cause of cattle mastitis. It's mandatory intramammary and purely infectious pathogen in dairy cattle (Sørensen *et al.*,

2019; Cobo-Ángel *et al.*, 2018). Numerous origins of bovine GBS are still unknown in animals; however, GBS has recently been isolated from various body sites and from milk cow habitats (Cobo-Ángel *et al.*, 2018).

Materials and Methods

Isolation and Identification

Milk samples were collected, in the microbiology laboratory during the period from October 2019 to January 2020 under sterile conditions, labeled and transported by cooler boxes for culture. One hundred and five (105) samples were obtained from different areas of Iraq to differentiate the *Streptococcus agalactiae* from cases of acute mastitis. Using a sterilized loop, cow milk samples were grown on nutrient broth and then blood agar and incubated at 37°C for 24 hours. Then, to get pure growth, the suspected colonies were selected and subcultured on selective Edward media. Biochemical tests such as catalase, oxidase, indol growth, sugar fermentation, camp testing, antibiotic sensitivity test and Lancefield group kits were used to identify these.

CAMP test

CAMP testing was performed by *Staphylococcus aureus* bacteria, with a vast area of partial haemolysis (beta – haemolysin) extending through the middle of the agar plate of sheep or ox blood. A line of suspected Group B *Streptococcus* is produced at right angles and taken within 1 to 1.5 mm of the *Staphylococcal* line. The plate is incubated between 18 to 24 hours at 37 ° C. An Arrowhead of full haemolysis indicates a positive CAMP test (Markey *et al.*, 2014).

Lancefield's serological grouping

According to HiMedia (2019) instruction leaflet manual Lancefield LK06 – HiStrep™ Latex Test Kit is a fast latex

agglutination slide test to group Lancefield groups A, B, C, D, F and G *Streptococci* from cultivation plates. Latex particles in the HiStrep™ Latex Test Kit are sensitized individually with rabbit antibodies unique to one of the groups' *Streptococcal* carbohydrate antigens A, B, C, D, F or G. *Streptococcal* colonies are incubated in an enzyme solution to remove the antigen from culture plates. The extract/antigen preparation is tested against six suspensions of antibody-coated latex particles each belonging to one of the groups A, B, C, D, F and G on a reaction sheet. Particles in one of the suspensions will accumulate in the presence of homologous antigens to give clear agglutination, as opposed to the other suspensions that will remain unagglutinated.

Results

The current study involved collecting 105 milk samples from cows infected with mastitis from different regions of Iraq. Only 10 isolates suspected bacteria were detected at a 9.52% percentage. The results of phenotypic diagnosis on blood agar showed that colonies of these bacteria are small, smooth, grey to white color grow at the center of blood agar with β hemolysis. The suspected bacteria were sub-cultured again on selective medium under the anaerobic condition at 37°C for 24 hours modified Edwards media. The colonies on modified Edwards media appears colorless with complete hemolysis on the surface of blood agar. *S.agalactiae* appears under the microscope as gram-positive, spherical, and arranged in single or pairs, long or short chains. The result of biochemical tests appear in Table (1)

Table 1 : Biochemical tests result for *Streptococcus agalactiae*

NO.	Test	Results
1	Oxidase production	-
2	Catalase production	-
3	Indol production	-
4	CAMP test	+
5	Haemolysin production	+
6	Glucose	+
7	Maltose	+
8	Sucrose	+
9	Lactose	+
10	Arabinose	+
11	Galactose	+
12	Sorbitol	+
13	D-Mannose	+
14	Manitol,	-
15	Inositol	-

(+) positive ,(-) negative.

All isolates of *Streptococcus agalactiae* were diagnosis by CAMP test and shown positive result by appearance arrowhead of complete haemolysis (Figure 1). Suspected bacteria to be *Streptococcus* (group B) were confirmed by Lancefield grouping test. Positive samples were indicated by the occurrence of agglutination, which observed as a clear visible clumping of the latex particles. By contrast, in the negative result, the latex did not agglutinate and the milky appearance persisted without change during the one-minute test (Figure 2), and this detected to *S.agalactiae*.

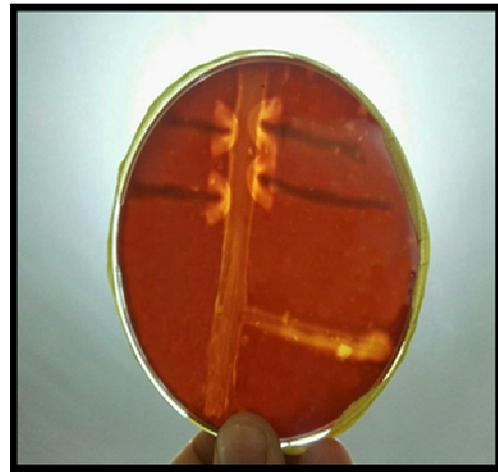


Fig. 1 : CAMP test for *Streptococcus agalactiae*



Fig. 2 : Lancefield's group show *Streptococcus agalactiae* positive (circle no.2)

A sensitivity test was performed to *Streptococcus agalactiae* isolated from cow's milk with mastitis towards 10 antibiotics by using the diffusion method. Out of the 10 bacterial isolates *Streptococcus agalactiae*, (100%) showed resistance to Cloxacillin, Erythromycin. Furthermore, (80 %) of the isolates were resistant to and Ceftriaxone, Cefixime, (60%) to Trimethoprim/Sulphamethoxazole. On other hand, most isolates (90%) were susceptible to Chloramphenicol followed by (80%) of bacteria sensitive to Penicillin and Ampicillin, (70%) to Gentamicin and also (60%) to Streptomycin.

Discussion

The results of this study showed that the percentage of *Streptococcus agalactiae* isolated from mastic milk was (9.52%), which was an agreement with the result by Alkuzaay & Kshash (2013) as 13.2% in the province of AL-Diwaniya, as well as Ammar *et al.* (2018), which found the percentage of 4.79% in Egypt, While Lakew *et al.* (2019) reported 10.3 per cent of Eastern Ethiopia's milk samples of cows. The result confirms the fact that *Streptococcus agalactiae* is a major Bovine mastitis pathogen which, according to Shan *et al.* (2020) causes significant losses in the dairy industry.

The specimens being examined were diagnosed based on of the phenotypic properties of colonies growing on blood agar, Edward agar, as well as gram staining, microscopic

examination features, and biochemical tests, were identical to those described (Brown, 2007), (Goldman and Lorrence, 2009), (Markey *et al.*, 2014).

One of the important ways to diagnose *Streptococcus agalactiae* is by biochemical tests. All isolate produce β haemolysis (complete lysis of Rbc) on blood and Edward agars (Ammar *et al.*, 2018; Najum and Hameed, 2015). GBS gave negative results for oxidase, catalase, and indole production test. The current results of this study were in agreement with Lucia *et al.* (2017).

The result revealed that the positive isolates find positive CAMP testing by a hemolytic "arrowhead" formation between the *S.aureus* and *S.agalactiae* line that was grown in blood agar (Abbas, 2020). According to Krishnaveni *et al.* (2014), who appears to be the first stage of the reaction by prominent half-moon hemolysin region, In RBCs of sheep, there is a substance named sphingomyelin considering converting this substance into ceramide and this transition by the sphingomyelinase enzyme that secreted from *S.aureus*. After that, the CAMP factor interacts with the pretreated cell membrane and through this process contributes to cell lysis.

The carbohydrate fermentation test results gave positive results because the changed from red to yellow colour due to phenol red indicator that changed the colour in the acidic pH, this is in agreement with Belhadj *et al.* (2014). β hemolytic *Streptococcus* could ferment several sugars and gave positive results for Glucose, Maltose, Sucrose, Lactose, Arabinose, Galactose, Sorbitol, D-Mannose while could not fermentation (negative results) of Mannitol, Inositol, this result was an agreement with Bwalya *et al.* (2020), Lucia *et al.* (2017), Adikesavalu *et al.* (2017), Hardi *et al.* (2011), Pourgholam *et al.* (2011).

While the results of Lancefield's serological grouping according to the instructions of the manufacturer company were interpreted.

Strep.agalactiae isolates from mastitic milk showed a variation in the percentage of resistance to Cloxacillin, Erythromycin (100%); Ceftriaxone, Cefixime (80%); Trimethoprim/ Sulphamethoxazole (60%).

These results agree with Ammar *et al.* (2018) who isolated GBS bacteria from milk sample which collected from mastitic dairy farms of cattle recorded high resistance to Cloxacillin, Ceftriaxone (100%), Erythromycin (79%), Trimethoprim/ Sulphamethoxazole (54.7%). In the other hand, the results disagree with Meloska *et al.* (2012) who found the *Streptococcus agalactiae* was susceptible to cefixime (100%).

while (90%) were susceptible to Chloramphenicol; (80%) of bacteria sensitive to Penicillin and Ampicillin, Gentamicin (70%) and also Streptomycin (60%), these results agree with Pirzada *et al.* (2016) who isolated *Strep.agalactiae* from the milk of mastitis which found GBS sensitive to Chloramphenicol (80%), penicillin (63.66%), Streptomycin (60%), Singh *et al.* (2018) who found (95.34%) of GBS sensitive to Gentamicin furthermore, Ebrahimi *et al.* (2013) who found (96.7%) sensitive to Ampicillin.

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