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Identification of bacteria isolated from bovine clinical mastitis using VITEK2 compact in Al-Qadisiyah province

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بـــــــــــسم الله الرحمن الرحيم

فَتَعَالَى اللَّهُ الْمَلِكُ الْحَقُّ وَلا تَعْجَلْ بِالْقُرْآنِ مِن قَبْلِ أَن يُقْضَى إِلَيْكَ وَحْيُهُ وَقُل رَّبِّ زِدْنِي عِلْمًا

صدق الله العلي العظيم

سورة طه

Dedication

- *My parents.*
- *All members of my family.*
- *all martyrs of Iraq.*
- *All my teachers.*
- *My dear doctor Dr. Hayder N. Ayyez*
- *Anyone who helped me in this research.*

Abstract

Mastitis is a major problem in the dairy cattle and the present study was conducted to identify the important bacteria which were responsible for cases of acute mastitis in livestock farm of dairy cattle in Al- Qadisiyah province. Twenty two cases of acute mastitis were involved in this study which revealed implication of different bacterial types which were rarely recorded in previous studies on bovine mastitis in Iraq and they were diagnosed using VITEK 2 compact system and included *Granulicatella elegans, Enterococcus columbae, Enterococcus cloac dissolvent* while other isolates were as common as other reported bacteria in previous studies which included *E. coli*, *Staph. Lentus*, *Staph. aureus* and Streptococus spp.

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Chapter One

Introduction

1. Introduction:

Mastitis is considered as one of the most costly important disease of dairy livestock resulting in major economic losses because of fall in milk production, milk quality decreased for dairy purposes in addition to other costs for treatment and laboratory identification of causative agents (**Halasa** el al, 2007).

There are two types of mastitis depending on severity; clinical and subclinical. The first is threatening to a farmer in a dairy herd and therapeutic is given immediately to control disease while the subclinical type mostly remain unnoticed by the owner, which cannot be diagnosed without field or laboratory test. The incidence of the subclinical type was more (10-50%) when compared to clinical form (1-10%). Losses due to mastitis in dairy industry was 526 million dollars from which subclinical form are subjected for 70% of these losses (Varshney el al,2004).

The major bacteria reported to cause mastitis in India in 2016 were *E. coli* and *Staph. aureus* (**Lakshmi** el al, 2016). *Staph. aureus* was identified using selective media in Mexico (2013) by (**Bautista** el al, 2013). VITEK 2 Compact system was used for fast identification of bacteria isolated from mastitis in cattle in the Kingdom of Saudi Arabia in 2016 (**Elbehiry el al , 2016**).

The aim of this study was to reveal the actual role of the diagnostic method used in investigation of different pathogens that cause mastitis for more investigation in treatment and control program.

Chapter Two

Literatures Review

2. Review Literatures

2.1 Mastitis:

Mastitis is the inflammation of mammary gland accompanied with pathological of udder and physical ,chemical , microbiological major changes characterized by an increase in somatic cells especially , leukocytes in the milk. mastitis is recognized as the most important and costly disease of dairy animals (Lighter *et al* ,1988). inflammation of the mammary gland lead to a different of structure changes in milk (Harmon, 1994). mastitis in bovine is caused primarily by bacteria that invade the udder, proliferated and produce toxins that are harmful to the mammary gland (Hansen *et al*, 2004). which from causing economic losses, mastitis also has the risk for the transmission of zoonotic diseases like, leptospirosis, tuberculosis and streptococcal sore throat to human (Radostits *et al* , 2000). it is useful to consider that three major factors are involved in mastitis: the microorganisms as the causative agent, the cow as host, and the environment, which can influence both the cow and the microorganisms (Schroeder, 1997).

2.2 **Predisposing Factor Of Mastitis:**

Stress, previous attack and fatigue have often been predisposed to mastitis, trauma to the mammary glandfrom any cause can damage gland tissue and ducts and this could lead to mastitis (Sordillo *et al*, 1987). Immune suppression as Immune factors of the animal due to infection with GIT disease or respiratory disease may be lead to mastitis. Age older animal became more susceptible for mastitis (Keefe and Leslie, 1997). Teat injuries and sores and incomplete emptying of mammary glands and hereditary factors all these factors were became more predisposing factors for mastitis (Jarret, 1981). Poor house hygiene and unhygienic milk practices (Karimuoibo *et al*, 2005).

2.3 **Forms of Mastitis:** The mastitis classify according to :

1. Source of infection:

A. Contagious mastitis:

The udder and teat are the reservoir of infection. In which infected quarter is the main source of infection that transmitted into healthy quarter by contaminated milking equipments, milker hands ,water and suckling calves (Andrews *et al* , 2004).

B. Environmental mastitis:

Primary source of environmental pathogens were the surroundings or environment of the cow[manure, bedding, soil, contaminated water , ect](William *et al* ,1997).

2. Signs:

A. Clinical mastitis: when there are systemic or local signs or milk component change.

The clinical mastitis classify into following :

1- Peracute mastitis: Sever inflammation with swelling, pain and heat of the quarter associated with marked systemic reaction.

2- Acute mastitis: Sever inflammation but without a marked systemic reaction.

3- Sub acute mastitis: There are no systemic signs, mild inflammation with persistent abnormality of the milk like flakes, the udder may became painful on palpation (Radostits *et al* ,2000).

4- Chronic mastitis: Continuous or interrupted changes in the physical features of the foremilk include colts, flakes, pus, or water appearance are the most typical feature of chronic mastitis (Kelly,1974).

5- Gangrenous mastitis:

The lesion are histopathologically changes as progressive swelling, vascular degeneration, focal erosion and ulceration occur throughout the ductal system (Carlton and McGavin,1995).

B. Subclinical mastitis:

There are no visible signs of the disease somatic cell count (scc)of milk will be increased, culturing of milk will detect microbial agent in milk (Schroeder,1997). This form difficult to detect, adversely affects milk quality and production and constitutes a reservoir of pathogenes that lead to infection of other animals within the herd(Shearer and Harris, 2003). The PH was significantly higher in subclinical mastitis milk than the normal one (Batavani *et al*, 2007).

2.4 **Etiology** :

Bacteria, fungal, viral, yeast and algae are different microorganism cause mastitis. The bacteria include:

A. Major pathogens those that cause mastitis ,and can be classification into two types: Contagious pathogens (*Streptococcus agalactiae* , *Staphylococcus aureus* , *Mycoplasma bovis*) and Environmental pathogen ,*Streptococcus* uberis ,and the gram – bacteria like *E. coli*, Klebsiella spp, Enterobacter spp, (Radostits *et al*, 2007).

Some bacteria like *S. epidermidis* is part of the normal flora of teat skin and considered as teat opportunistic pathogens (Radostits *et al*, 2007). *S.haemolyticus* has been isolated from udder skin of cattle (Devriese and DeKeyser,1980). CNS typically isolated from milk *S.saprophyticus* were also common in the cows' environment(Devriese,1979).

2.5 Epidemiology:

Various microorganisms more than 135 have been isolated from cattle mammary infection ,but the majority of infections are caused by Staphylococci ,Streptococci and G-bacteria (Bradley, 2002). Incidence rate mastitis of heifers is higher than that of older cows shortly after calving . in studies at Canada and Netherlands heifers have an IMI prevalence of up to 50%in the third trimester of gestation and high IMI prevalence of coagulase negative staphylococci and *S. aureus* shortly after calving (Green *et al*, 2007). Coliforms mastitis bacteria were the main isolated mastitis pathogens and accounted for 21.1%of total clinical mastitis in England (Pankey *et al*, 1991). *S. aureus* is a predominant etiological agent of both subclinical &clinical form of udder inflammation (Myllys *et al*, 1998 &Roberson,1999). The most bacteria isolated from dairy herd in the Ahavaz and Iran were *streptococcus agalactiae* , *S. dysgalactiae* , *S. uberis* (20%, 12.5%, 0,83%)respectively (Moatamedi *et al* , 2007). A study in Turkey was isolated bacteria aerobic from 235 CMT positive milk samples , the most bacteria were isolated include ,*S. aureus* , CNS Streptococcus spp , *Eschericha coli* and *Arcanobacterium pyogenes* (88 isolates , 108 isolates ,18 isolates ,19 isolatesan and 2isolates) respectively were isolated (Hulya et al,2005). Hidden mastitis were registered in 90.5% and clinical mastitis in 9.5% of total cases (klimiene *et al* ,2005).

2.6 Transmission :

Cleanness of the udder is thought to influence the quantity and type of bacteria present on teat surfaces , dirty teats and udder are considered to be source environmental bacteria in milk (Galton *et al*, 1982; Guterbock,1982). Various mechanisms of transmission have been identified, including flies (Owens *et al* ,1988; Gillespie *et al* , 1999). Fomites found in the milking place, such as milking equipment, milker hands common udder cloths and strip cups (Fox *et al* , 1991). The environmental

contain on pathogens in particular have been isolated from bedding materials , soil, rumen , feces , vulva , lips ,mammary gland and teats(Cullen ,1966;Bramley ,1982). Also can through the use of bulk mastitis treatment , administrated through a common syringe and cannula (Radostits *et al* ,2007). Condition which contribute to trauma of mammary gland include incorrect use of udder washes , wet teats and failure to use teat dips failure to prepare milking animals or pre milking stimulation for milk ejection , over milking , insertion of mastitis tubes or teat cannula (Khan and Khan, 2006).

2.7 **Pathogenesis :**

keratin derived from stratified squamous epithelium is lined teat canal. Break down to keratin has been reported to cause increased susceptibility of teat canal to bacteria invasion and colonization (Bramley and Dodd, 1984). Fibrous proteins of keratin in teat canal bind electrostatically to mastitis pathogens, which alter the bacteria cell well ,rendering it more susceptible to osmotic pressure . inability to maintain osmotic pressure cause lysis and death of invading pathogens (Murpphy and Stuart, 1953). During milking , bacteria present near opening of teat find opportunity to enter the teat canal , causing trauma and damage to the keratin or mucous membranes lining the teat sinus (Capuco *et al*,1992). Bacteria multiply and produce toxins, enzymes and cell wall components which stimulate the production of inflammatory mediators attracting phagocytes . Neutrophils are the predominant cells stage of mastitis and constitute >90% of total leukocytes (Sordillo et al 1987). Phagocytes move from the bone marrow toward the invading bacteria in large numbers attracted by chemical messengers or chemotactic agents such as cytokines ,complement and prostaglandins (Persson *et al* ,1992 ; banumaann and Graudie, 1994). Increased number of leukocytes in milk causes increase in number of somatic cells . clots are formed by aggregation of leukocytes and blood clotting factors which may be block the

ducts and prevent complete milk removal ,resulting in scar formation with proliferation of connective tissue . Alveoli begin to shrink and replaced by scar tissue . helps in formation of small pockets making difficult for antibiotics to reach there and also prevent complete removal of milk (Jones ,2006).

2.8 **Diagnosis :**

Field examination of the udder :

A. Inspection : enlargement one or more quarter in acute cases, while in chronic cases often lead to smaller size due to atrophy and fibrosis. Color change like redness because inflammation or bluish due to cyanosis.

B. Palpation :

It will reveal the presence of heat ,swelling and pain in acute or subacute forms. supramammary lymph nodes and consistency of glandular tissue are screened through deep palpation.

Laboratory examination of milk :

A. Physical examination:

Color and consistency of the normal milk are tasted , alteration in color can be due to presence of blood or pus . clots is always abnormal . the smell of secretion may also be changed as a result of mastitis (Quinn et al,1994).

B. Chemical examination:

There are different test used in chemical examination of milk include:

-California mastitis test
-Negretti field test
-Modified white side test
-Card field test
-PH determination test
-Determination of chloride test
-Catalase test
-Concentration of sodium , potassium and lactose
-Somatic cell count test

C. Other examination to milk include:

-Microscopic examination of incubated milk
-Milk NAGase test (N-acetyl-β-D-gluscoaminidase)
-Direct ELASA test
-Electrical conductivity test

California mastitis test (CMT)

This test was developed to check milk form individual quarters but has also been used on bulk milk samples (Schalm and Noorland ,1957). Positive result based on CMT may be good indicator of the presence of bacteria that cause udder infection . The reagent of CMT is simply a detergent plus bromcresol purple (used as an indicator of PH). The result is record according to table (4) (Schalm *et al* , 1971).

Table 4 .Description of the score of the CMT and its results. (Schalm *et al* , 1971).

Score	Interpretation	description	Total cell count(cell/MLmilk)
N	Negative	Mixture remains liquid	0-200 000
T	Trace	A slight slime develops which disappears	150 000-500 000

		with continued swirling	
1	Weak positive	A distinct slime develops which may or may not persist no gel formation	400 000-1.500 000
2	Distinct positive	Immediate gel formation, causing a thickening and sticking together of the milk upon swirling	800 000–5000 000
3	Strong positive	Strong gelling and congealing of milk it tends to become viscous and adhere to plastic surfaces of the paddle	>5 000 000

2.9 **Treatment of mastitis:**

A large range of causative agents which caused mastitis with inherent differences in antibiotic susceptibility, so the deciding of therapeutic strategy is often difficult(Schultze,1986). Development of resistance and to increased stringency regarding

residues (Erskine *et al* ,1993). Generally ,two forms of therapy are practiced lactation therapy and dry cow therapy (Erskine et al,1993). Treatment is frequently instituted without the causative agent being identified , so that a broad spectrum antibiotic is used. Cure rate with antibiotic therapy during lactation is very low . many infection animals become chronic cases and have to be culled (Khan&Khan,2006).the dosage , dose interval , formulation and route of administration and any supportive treatment necessary such as fluid and electrolyte therapy (Ersine et al, 1993;Andrews et al ,2004). Compound containing one or more antimicrobial agents and corticosteroid ,intrammary infusion mainly in lactating cows (O Rourke,1994). In dry cow therapy has been an efficacious and economically effective method of reducing the frequency of intrammary infections (Heald *et al* , 1977). Antibiotic is not flushed from the gland , so that it is concentration is higher for a longer period (Schultze , 1986; Andrews *et al* , 2004).

2.10 **Prevention and control:**

Using a suitable disinfectant to teat skin surface after milking , before milking teat disinfection (Andrews *et al* , 2004). proper antibiotic therapy is recommended for all quarters of all animals at drying off, it helps to control environmental Streptococci during the early dry period (Khan and Khan, 2006). Closed herd concept should also be maintained with respect to replacement heifers and milking cows (Kirk *et al* , 1994). In addition the advent of post milking teat antiseptic has been important in contributing to decreasing contagious IMI such as *S. aureus* (Natzke *et al*,1972).

Chapter Three

Materials and Methods

3-Materials and methods:

Milk samples of cattle diagnosed with mastitis were collected from a farm of dairy cattle in Al-Qadisiyah province during November 2017 to February 2018 . Twenty two milk samples were collected aseptically from clinical cases using sterile tubes and stored at 4C° until processed.

Samples were mixed and streaked on blood agar plates and incubated for 24-48hrs. The growing colonies were subjected for staining and preliminary identified Gram stains, bacteria were subjected for identification by VITEK 2 compact system according to the instruction provided by the company.

Three to Five well isolated colonies from each isolates were transferred to glass tube contain 4 ml distal water to measure and adjust turbidity that represent bacterial cells number per 1ml which must be equal to 0.5 Macfarlane standard.

Bacterial suspension transferred to cassette by negative pressure, then loaded cassette with bacterial suspension entered to VITEK 2 compact system machine to complete biochemical reaction within 12 hours.

Interpretation of results were performed according VITEK 2 compact system special software to identify bacterial species and strains.

Chapter four

Results & Discussions

4-1Results:

Bacterial colonies after subculture on blood agar showed 13 pure colonies from total of 22 cases of acute mastitis from which only two were Gram negative while other were Gram positive.

According to VITEK 2 compact system results; the isolated bacteria listed in following table.

Table one: Bacteria identified by VITEK 2 compact system

Isolated Bacteria	Number of cases
E. coli	2
Staph. lentus	3
Staph. aureus	2
Granulicatella elegans	2
Enterococcus columbae	1
Enterococcus cloac dissolvent	1
Streptococus spp.	2
Sum	13

4-2Discussion:

Conventional biochemical tests that used to identify Mastitis in cattle was previously widely studied in Iraq but there were new isolates have been diagnosed in this study; *Granulicatella elegans, Enterococcus columba* and *Enterococcus cloac dissolvent*. Others were reported in the previous studies which may indicate that this disease has new causative agents within the progress of the bacterial variations during the last decades especially regarded to the mutations and the antibiotic resistance.

The ordinary isolates of mastitis *E. coli* and *Staph. aureus* came similar to that isolated by (5). Other similar articles had reported the same isolates like in 3,4,5 and 6. But the others; *Granulicatella elegans, Enterococcus columba* and *Enterococcus cloac dissolvent;* were recorded for the first time in Iraq.

Chapter five

Conclusions and Recommendations

5-1 Conclusion:

1- **VITEK 2 compact** system is useful technique for rapid diagnosis and identification of causative agents causes mastitis result in prevent spread of bacterial infectious diseases within herd compared with conventional biochemical methods which need more time and cost for bacterial identification.
2- **Using VITEK2 compact** system result in identification of bacterial isolates not commonly known as causes of mastitis in cattle**.**

5-2 Recommendations:

1- Using new techniques that give perfect and rapid result for deals with mastitis to avoid highly economical losses that result from mastitis.
2- Determination of drugs of choice depend on VITEK2compact system is very useful because of it prepared according standard laboratory methods.

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