Comparative study on causes of clinical mastitis in cows

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Abstract
A total of one hundred random samples of mastitic milk were collected from dairy cows suffering from clinical mastitis in different dairy farms at Damietta Governorate. All collected mastitic milk samples were subjected to bacteriological and mycological examinations. The obtained results revealed that the most bacterial isolates were Staph. aureus, E.coli, Strept. dysagalactiae, coagulase-negative staplylococci, Strept.uberis, Corynebacterium spp. and *Pseudomonas aeruginosa* with incidence of 30%, 26%, 13%, 8%, 6%, 6% and 1.0%, respectively. While, the most frequently mycotic isolates were Candida albicans and Cryptococcus neoformans with incidence of 9% and 1.0%, respectively. In Vitro, antimicrobial susceptibility test of the bacterial isolates revealed that the most effective antibiotics were Ciprofloxacin, Cefotaxime, Amoxycillin/ Clavulanic acid, Ampicillin/Sulbactam and Neomycin. While, the most effective antimycotics against *Candida albicans* and *Cryptococcus neoformans* were Fluconazole and Nystatin. The obtained results, conclusion, recommendation and preventive measures of clinical mastitis were discussed.

Key words:
Mastitis, mastitic milk, lactating cows, antibiotics.

Introduction

Mastitis is the inflammatory change of the mammary glands characterized by an increase in somatic cells in the milk, changes in milk composition and pathological changes in the mammary tissues. Mastitis usually occurs primarily in response to intramammary bacterial infection, but also to intramammary mycoplasmal, fungal, or algal infections. Bacterial pathogens that commonly isolated from mastitic milk can be classified into two main complex topics. The first topic is the contagious organisms that colonize the mammary gland and can be spread by milking machines and men at milking time as the milking process affects the potency of the teat orifice. Contagious pathogens which commonly isolated from mastitic milk include Staphylococcus aureus and Streptococcus agalactiae. The second topic is the environmental pathogens that do not normally infect the mammary gland but do so when the cow's environment, the teats and udder or the milking machine is contaminated with these organisms and they gain access to the teat cistern **(Fox, 2009).** Environmental pathogens (noncontagious) include Streptococcus uberis, Streptococcus dysagalactiae, Escherichia coli, coagulase-negative Staphylococcus spp., Enterobacter spp. Archanobacterium pyogenes, Klebsiela spp., Serratia spp. and Pseudomonas spp. **(Zhao and Lacasse, 2008; Fox, 2009 and Vasie, 2009).** If the infection is not eliminated, bacterial levels in the mammary gland eventually rise to a level at which they begin to damage the mammary epithelium **(Zhao and Lacasse, 2008).** As infection persists, the number of somatic cells in milk continues to increase and concomitantly, tissue damage is worsened. The alveoli in the gland start to loss structural integrity and the

blood-milk barrier is breached. This allows extracellular fluid to enter the gland and mix with the milk. Visible changes in the milk and udder start to occur. These can include external swelling, reddening of the gland, and clotting and wateriness of the milk. By definition, this is the start of clinical symptoms **(Zhao and Lacasse, 2008).**

Owing to clinical mastitis having many adverse economic implications by decrease quantity and quality of milk components and shorten the productive life of affected animals, this study was planned to investigate the prevalence of the most common bacteria and yeasts isolated from clinically mastitic cows at Damietta Governorate.

Materials and Methods

1. Collection of samples

A total of 100 random samples of mastitic milk were collected from dairy cows suffering from clinical mastitis in different dairy farms at Damietta Governorate. All mastitic cows were subjected to clinical examination by visual inspection, palpation of the udder for the detection of swelling, redness and pain, beside the physical changes in the milk secreted from each quarter, while California mastitis test was not used for detection of clinical mastitic cases. All milk samples were collected from mastitic quarters under complete aseptic conditions according to the method described by **(Habashy *et al.*, 2012).** The first foremilk stream was discarded then the mastitic milk was drawn from each quarter into two sterile screws capped McCartney bottles and all milk samples were transported to the laboratory in an ice container for bacteriological and mycological examinations.

2. Bacteriological examination.

2.1. Isolation of causative bacteria.

For bacteriological examination, milk samples were incubated aerobically at 37 ^{0}C for 24 hours then centrifuged at 3000 r.p.m. for 20 minutes. The cream and supernatant fluid were discarded, while the milk sediments were cultured onto nutrient broth, nutrient agar, MacConkey's agar, Baird-parker agar, mannitol salt agar, pseudomonas isolation agar and blood agar plates. All tubes and plates incubated aerobically for 37 ^{0}C for 24 hours. The developed colonies were picked up and subcultured for purification.

2.2. Identification of isolated bacteria.

All pure colonies were identified morphologically by gram staining and biochemically by catalase test, oxidase test, motility test, haemolysis on blood agar, nitrate reduction, urea hydrolysis, screening on triple sugar iron agar, indole test, cirate test, VP test, MR test, coagulase test, growth at 45°c and various biochemical tests were done according to **(Cruichshank *et al.*, 1975; Carter and Cole, 1990; Quinn *et al.*, 1994 and Winn *et al.*, 2006).**

3. Mycological examination.

3.1. Isolation of yeasts.

The sediment of each mastitic milk sample was cultured on to Sabouraud's dextrose broth, Sabouraud's dextrose agar containing 0.05 mg/ml chlorampenicol and malt extract agar acidified with lactic acid solution 10% at PH (3.5-4). The plates were incubated aerobically at 30 ^{0}C for 5 days and examined daily for yeast isolation according to **(Kwon-Chung and Bennett, 1992 and Fisher and Cook, 1998).**

3.2. Identification of isolated yeasts.

The identification of isolated yeasts from mastitic milk samples was based on macroscopical, microscopical and physical properties according to techniques recommended by **(Koneman *et al.*, 1978, Barnett *et al.*, 1983 and Kwon-Chung and Bennett, 1992).**

4. Antibiogram.

Antibiogram of the isolated bacterial strains and the isolated strains of yeast from mastitic milk in this study was performed by using disc diffusion standard technique. The isolated strains of bacteria were tested against different types of antibiotic discs according to **(Quinn *et al.*, 1994 and Winn *et al.*, 2006)** which include Amoxycillin/Clavulanic acid 20/10mcg, Ampicillin/ Sulbactam 10/10mcg, Ciprofloxacin 10 mcg, Cefotaxime 30mcg, Doxycycline HCL

30 mcg, Enrofloxacin 10mcg, Gentamycin 30mcg, Neomycin 30mcg and Erythromycin 15mcg. While, the recovered strains of yeast were tested against antifungal discs according to **(Lassa and Malinowski, 2007)** which include Clotrimazole 10 mcg, Fluconazole 10 mcg and Nystatin 50 mcg.

Results and Discussion

The results recorded in Table (1) revealed the incidence of the isolated strains of bacteria from the examined mastitic milk samples which collected from dairy cows suffering from clinical mastitis during this study at Damietta Governorate. The results showed that Staph.aureus was the main cause of clinical mastitis in dairy cows and isolated from 30 mastitic milk samples representing 30% of total examined samples. All isolated strains of Staph.aureus from the examined mastitic milk were found coagulase-positive. In addition, coagulase-negative staphylococci were isolated from 8 samples of mastitic milk representing 8% of total examined samples, Tables (1).

Higher incidence of Staph.aureus and coagulase-negative staphylococci in mastitic milk than our finding was recorded by **(Workineh *et al.,* 2002; Dego and Tareke, 2003; Nagahata *et al.,* 2007; Sinduh *et al.,* 2010; Ruban *et al.,* 2012 and Sharma *et al.,* 2012).** While, lower incidence of Staph.aureus in mastitic milk was obtained by **(Barbuddhe *et al.,* 2001; Kivaria and Noordhuizen, 2007; Sharma *et al.,* 2009; Vasie, 2009 and Anderson *et al.,* 2012).**

Staphylococcus aureus is the most important and prevalent contagious mammary pathogen. It causes clinical and subclinical intramammary infection with serious economic loss and herd management problems in dairy cows **(Dego *et al.,* 2002).** Prevalence of Staph.aureus infection may reach up to 82.22% in subclinical mastitis and up to 77.27% in clinical mastitis in dairy cows **(Sharma *et al.,* 2012).** Mastitis caused by Staph.aureus should be eliminated because of the severity of the clinical symptoms and also because of the risk of contamination of milk and milk products by thermostable toxins **(Sladek *et al.,* 2005).** Most strains of Staph.aureus which isolated from mastitic milk are enterotoxigenic strains and toxic shock syndrome toxin-1 (TSST-1) producers. Therefore, it represent hazard to consumer's health, particularly to children, immune compromised patients and to elders **(Lim *et al.,* 2004; Katsuda *et al.,* 2005; Moon *et al.,* 2007; Momtaz *et al.,* 2010 and Guimaraes *et al.,* 2011).**

Table (1). Prevalence of isolated bacteria from mastitic milk of cows having clinical mastitis.

Isolated bacteria	Positive samples	
	No.	%
Staph.aureus	30	30
Escherichia coli	26	26
Streptococcus dysagalactiae	13	13
Coagulase-negative staphylococci	8	8
Streptococcus uberis	6	6
Corynebacterium spp.	6	6
Pseudomonas aeruginosa	1	1

n= 100

The results recorded in Table (1) revealed that Escherichia coli was isolated from 26 mastitic milk samples which representing 26% of total examined milk samples of cows with clinical mastitis. Higher incidence of E.coli in mastitic milk of dairy cows was recorded by **(Jones and Ward, 1989 and Habashy *et al.,* 2012).** While, lower incidence of E.coli in mastitic milk samples than our finding was reported by **(Barbuddhe *et al.,* 2001; Willayat *et al.,* 2004; Kivaria and Noorduizen, 2007; Vasie, 2009 and Kalmus *et al.,* 2011).** This high obtained incidence of E.coli isolation from mastitic milk of cows may be the result of the poor hygienic measures where the muddy bedding materials cause a consequent udder infection from fecal contamination on such bedding glands.

Escherichia coli is one of the most important environmental pathogens causing mastitis in dairy cows resulting in inflammation that ranges from subacute to peracute. Necrosis of the mammary epithelium occurs during severe, naturally occurring clinical E.coli mastitis **(Zhao and Lacasse, 2008).** Acute Escherichia coli mastitis is one of the major sources of economic loss in the dairy industry, due to reduced milk production, treatment costs, discarded milk and occasional fatal disease **(Vangroenwghe *et al.,* 2005).** In addition, Shiga toxin-producing Escherichia coli (STEC) isolates from mastitic milk were potentially pathogenic for human in that they belonged to serogroups associated with diarrhea and haemolytic-uraemic syndrome **(Lira *et al.,* 2004 and Osman *et al.,* 2012).**

The results recorded in Table (1) showed that Streptococcus dysagalactiae was isolated from 13 mastitic milk samples, while Streptococcus uberis isolated from 6 mastitic milk samples, and representing 13% and 6% of total examined milk samples of cows with clinical mastitis, respectively. Higher results than our finding were recorded by **(Dego and Tareke, 2003; Vasie, 2009; Kalmus *et al.,* 2011 and Habashy *et al.,* 2012).** While, lower incidence of streptococci than our result was reported by **(Barbuddhe *et al.,* 2001; Kivaria and Noordhuizen, 2007; Amsaveni *et al.,* 2010 and Sharma *et al.,* 2012).**

Streptococcus dysagalactiae and *Streptococcus uberis* are noncontagious (environmental) bacteria which may escape the natural defence mechanisms by multiplication along the streak canal (especially after milking), or by propulsion into the teat cistern by vaccum fluctuations at the teat end during milking. The infection occurs after bacteria gain entrance to the mammary gland via the teat canal, **(Zhao and Lacasse, 2008).**

The results recorded in Table (1) revealed that Corynebacterium spp. was isolated from 6 mastitic milk samples and representing 6% of total examined milk samples of dairy cows with clinical mastitis. Higher incidence of Corynebacterium spp. in mastitic milk than this result was recorded by **(Dego and Tareke, 2003),** while lower incidence was reported by **(Workineh *et al.,* 2002 and Sharma *et al.,* 2012).**

Corynebacterium bovis primarily colonize the teat canal and are capable of causing occasional udder infections with mild increase in SCC and slight reduction in milk production. C.bovis is isolated frequently from milk samples collected aseptically from dairy cows and generally considered a minor mammary gland pathogen or a commensal, **(Pankey *et al.,* 1985).**

Pseudomonas aeruginosa was isolated from only one mastitic milk sample which representing 1.0% of total examined milk samples of cows with clinical mastitis, Table (1). Higher incidence of Ps.aeruginosa in mastitic milk samples was recorded by **(Seddek *et al.,* 2000; Barbuddhe *et al.,* 2001 and Kivaria and Noordhuizen, 2007).** Pseudomonas aeruginosa was detected in raw milk and in the environment of dairy cows. This organism was isolated from milker's hands, moist areas of the cow's body (udder & teats), drinking water, feeds, feces, milking equipments and walls and floors of the barn **(Otte *et al.,* 1978).** *Ps. aeruginosa* was implicated in many cases of

both subclinical and clinical mastitis. The incidence *Ps.aeruginosa* in bovin mastitis may reach up to 60.46% **(Barbuddhe *et al.*, 2001).** In Egypt, several cases of food poisoning due to Ps.aeruginosa were traced to the consumption of contaminated raw milk and its products **(Ahmed *et al.*, 1989).**

Table (2). Prevalence of isolated yeasts from mastitic milk of cows having clinical mastitis.

Isolated yeasts	Positive samples	
	No.	%
C. albicans	9	9
Cr. neoformans	1	1

n = 100

The results recorded in Table (2) revealed the incidence of isolated yeasts from the examined mastitic milk of dairy cattle suffering from clinical mastitis. The obtained results showed that Candida albicans was isolated from 9 mastitic milk samples which representing 9% of total examined milk samples of dairy cows with clinical mastitis. Higher incidence of C.albicans in mastitic milk was recorded by **(Kivaria and Noordhuizen, 2007 and Asfour *et al.*, 2009),** while lower incidence of C.albicans in mastitic milk was obtained by **(Kumar and Thakur, 2000 and Senthilvelan *et al.*, 2006).** In addition, the results recorded in Table (2) showed that Cryptococcus neoformans was isolated from only one mastitic milk samples which representing 1.0% of total examined mastitic milk of cows with clinical mastitis. Higher incidence of Cr.neoformans in mastitic milk was recorded by **(Asfour *et al.*, 2009).**

Yeasts or their spores are very common in the dairy environment. Most animals show variable degrees of susceptibility to yeast infection. A common way for yeast to gain access into the udder endogenously is via the blood stream or exogenously by infusion of contaminated treatment preparations or using contaminated infusion equipment into the udder. Milking machine malfunction or poor milking technique are considered as possible routes that allow intramammary infection by yeasts. Yeasts do not respond to antibiotics and the abuse of antibiotics may worsen the signs of mastitis. Infection of the mammary glands by yeast should be suspected when there was a history of unsuccessful antibiotic treatment which might aggrevatemycotic mastitis such as infection with Candida spp. which utilizes penicillin and tetracycline as a source of nitrogen **(Tarfarosh and Purohit, 2008).** Candida spp. are normal commensals of oral mucosa in human beings but increased exposure to proximity to affected animal and/or consumption of contaminated milk could be the cause for development of thrush in milkers. Candida albicans and its spores have the ability to survive pasteurization which is assumed to be of public health significance and has been indicated in cases of thrush in human beings **(Schmitt, 1971 and Tarfarosh and Purohit, 2008).**

Table (3). In Vitro susceptibility pattern of the isolated bacteria against different antibiotics.

Isolated bacteria / Antibiotics	Staph.aureus	Coaguluse-negative staphy-lococci	Escherichia coli	Strept. dysa-galactiae	Strept.uberis	Corynebacte-rium spp.	Pseudomonas aeruginosa
	"n.=30"	"n.=8"	"n.=26"	"n.=13"	"n.=6"	"n.=6"	"n.=1"
Amoxycillin/ Clavulanic acid 20/10mcg	18/30 (60%)	5/8 (62.5%)	10/26 (38.5%)	13/13 (100%)	6/6 (100%)	4/6 (66.7%)	-
Ampicillin/ Sulbac-tam 10/10mcg	18/30 (60%)	5/8 (62.5%)	10/26 (38.5%)	13/13 (100%)	6/6 (100%)	4/6 (66.7%)	-
Ciprofloxacin 10mcg	28/30 (93.3%)	6/8 (75%)	22/26 (84.6%)	13/13 (100%)	6/6 (100%)	4/6 (66.7%)	1/1 (100%)
Cefotaxime 30mcg	20/30 (66.7%)	6/8 (75%)	26/26 (100%)	7/13 (53.8%)	3/6 (50%)	4/6 (66.7%)	1/1 (100%)
Doxycycline HCL 30mcg	15/30 (50%)	4/8 (50%)	-	9/13 (69.2%)	4/6 (66.7%)	4/6 (66.7%)	-
Enrofloxacin 10mcg	10/30 (33.3%)	-	22/26 (84.6%)	-	-	-	1/1 (100%)
Gentamycin 30mcg	10/30 (33.3%)	2/8 (25%)	26/26 (100%)	-	-	-	1/1 (100%)
Neomycin 30mcg	15/30 (50%)	4/8 (50%)	26/26 (100%)	7/13 (53.8%)	3/6 (50%)	3/6 (50%)	-
Erythromycin 15mcg	10/30 (33.3%)	-	-	9/13 (69.2%)	4/6 (66.7%)	3/6 (50%)	-

Results recorded in Table (3) showed susceptibility pattern (In vitro) of the isolated bacteria from mastitic milk against different antibiotics. The obtained results revealed that the isolated strains of coagulase-positive Staph.aureus were sensitive to Amoxycillin/ Clavulanic acid 20/10 mcg, Ampicillin/Sulbactam 10/10 mcg, Ciprofloxacin 10 mcg and Cefotaxime 30mcg at rate 18/30 (60%), 18/30 (60%), 28/30 (93.3%), and 20/30 (66.7%), respectively. Also, the isolated Staph.aureus were susceptible at a rate 15/30 (50%), to each of Doxycycline HCL 30 mcg and Neomycin 30 mcg and susceptible at a rate 10/30 (33.3%), to each of Enrofloxacin 10 mcg, Gentamycin 30 mcg and Erythromycin 15 mcg. While, the isolated strains of coagulase-negative staphylococci were susceptible at rates 5/8 (62.5%) to each of Amoxycillin/ Clavulanic acid 20/10 mcg and Ampicillin/Sulbactam 10/10 mcg, 6/8 (75%) to each of Ciprofloxacin 10 mcg and Cefotaxime 10mcg, 4/8 (50%) to each of Doxycycline HCL 30 mcg and Neomycin 30 mcg and susceptible to Gentamycin 30 mcg at rate 2/8 (25%). While, the isolated strains of coagulase-negative staphylococci were resistant to both Enrofloxacin 10 mcg and Erythromycin 15 mcg, Table (3).

The obtained results showed that all isolated strains of Escherichia coli from mastitic milk samples were more sensitive to each of Cefotaxime 30 mcg, Gentamycin 30 mcg and Neomycin 30 mcg at a rate 26/26 (100%). Also, the isolated strains of E.coli were susceptible to both Ciprofloxacin 10 mcg and Enrofloxacin 10 mcg at a rate 22/26 (84.6%) and susceptible

to each of Amoxycillin/Clavulanic acid 20/10 mcg and Ampicillin/Sulbactam 10/10 mcg at a rate 10/26 (38.5%). On the other side, the isolated strains of E.coli were resistant to each of Doxycycline HCL 30 mcg and Erythromycin 15 mcg, Table(3).

The obtained results revealed that all isolated strains of Strept. dysagalactiae from mastitic milk samples were more sensitive at a rate 13/13 (100%) to each of Amoxycillin/ Clavulanic acid 20/10 mcg, Ampicillin/Sulbactam 10/10 mcg and Ciprofloxacin 10 mcg. Also, the isolated strains of Strept.dysagalactiae were susceptible to both Doxycycline HCL 30mcg and Erythromycin 15 mcg at a rate 9/13 (69.2%) and susceptible to each of Cefotaxime 30 mcg and Neomycin 30 mcg at a rate 7/13 (53.8%). Also, the obtained results revealed that all strains of Strept.uberis were more sensitive at a rate 6/6 (100%) to each of Amoxycillin/Clavulanic acid 20/10 mcg, Ampicillin/ Sulbactam 10/10 mcg and Ciprofloxacin 10 mcg. In addition, the isolated strains of Strept.uberis were susceptible `to both Doxycycline HCL 30 mcg and Erythromycin 15 mcg at a rate 4/6 (66.7%) and susceptible to each of Cefotaxime 30 mcg and Neomycin 30 mcg at a rate 3/6 (50%). On the other side, all isolated strains of both Strept.dysagalactiae and Strept.uberis were resistant to each of Enrofloxacin 10 mcg and Gentamycin 30 mcg, Table (3).

The obtained results showed that the isolated Corynebacterium spp. were susceptible at a rate 4/6 (66.7%) to each of Amoxycillin/ Clavulanic acid 20/10 mcg, Ampicillin/ Sulbactam 10/10 mcg, Ciprofloxacin 10 mcg, Cefotaxime 30 mcg and Doxycycline HCL 30 mcg. Also, the isolated Corynebacterium spp. were susceptible at a rate 3/6 (50%) to both Neomycin 30 mcg and Erythromycin 15 mcg. While it were resistant to each of Enrofloxacin 10 mcg and Gentamycin 30 mcg, Table (3).

Results recorded in Table (3) revealed that the isolated strain of Pseudomonas aeruginosa was sensitive to each of Ciprofloxacin 10mcg, Cefotaxime 30 mcg, Enrofloxcin 10 mcg and Gentamycin 30 mcg, while it was resistant to Amoxycillin/Clavulanic acid 20/10 mcg, Ampicillin/Sulbactam 10/10 mcg, Doxycycline HCL 30 mcg, Neomycin 30 mcg and Erythromycin15 mcg.

Table (4). In Vitro susceptibility pattern of the isolated yeasts against antimycotic drugs.

Isolated yeasts / Antimycotic drugs	Candida albicans "n.=9"	Cryptococcus neoformans "n.=1"
Clotrimazole 10 mcg	5/9 (55.6%)	-
Fluconazole 10 mcg	9/9 (100%)	1/1 (100%)
Nystatin 50 mcg	6/9 (66.7%)	1/1 (100%)

The results recorded in Table (4) revealed that all isolated strains of Candida albicans from mastitic milk samples were more sensitive to Fluconazole 10 mcg at a rate9/9 (100%), while it was susceptible to Clotrimazole 10 mcg and Nystatin 50 mcg at a rate5/9 (55.6%) and6/9 (66.7%), respectively. The isolated strain of Cryptococcus neoformans from mastitic milk samples was sensitive to both Fluconazole 10 mcg and Nystatin 50 mcg, while it resistant to Clotrimazole 10 mcg, Table(4).

Conclusion and Recommendations

Data given by the obtained results from this work point out that the main causes of clinical mastitis which isolated from the examined mastitic milk of cows were contagious pathogen such as Staph.aureus and environmental

pathogens such as *E.coli*, Coagulase-negative staphylococci, Strept. dysagalactiae, Strept. uberis, Corynebacterium spp., Pseudomonas aeruginosa, Candida albicans and *Cr.neoformans*. Some of these organisms have public health significance and susceptible to antimicrobial drugs as well as it more resistence to other drugs. Therefore, to prevent and control of mastitis in dairy cows, the following suggestions should be applied: routine examination of dairy animals and application of mastitis control programme for early detection of subclinical and clinical mastitis, isolation of its causative organisms and its treatment with the effective drug of choise. Prevention of appearance of new mastitic cases as well as elemination of the cases with sever clinical symptoms. Strict hygienic measures should be applied for healthy udder, mastitic udder, teat dip, milking machine, dairy utensils and worker's hands. Educational programmes should be done for milk producers specially about the personal hygiene, environmental hygiene and proper feeding with balanced rations containing antioxidants and trace elements for dairy animals.

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دراسة مقارنة عن مسببات إلتهاب الضرع الظاهري في الأبقار

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الملخص العربي

أجريت هذه الدراسة علي عدد مائة عينة عشوائية من ألبان الأبقار المصابة بالتهاب الضرع الظاهري والموجودة بالمزارع المختلفة المنتشرة في محافظة دمياط وذلك للمقارنة بين الميكروبات المسببة لالتهاب الضرع الظاهري ومدي استجابة الميكروبات المعزولة لكل من المضادات الحيوية والفطرية معمليا .وقد أسفرت الدراسة عن عزل الميكروبات بالمعدلات الموضحة كالآتي: المكور العنقودي الذهبي الإيجابي لإنزيم التجلط (30%) المكورات العنقودية السالبة لإنزيم التجلط (8%) ، الميكروب القولوني (26%) المكورالسبحي (13%) dysagalactiae، المكورالسبحي (6%) Uberis، كورينبكتريوم (6%)، سدمونس إيروجينوزا (1%)، كانديدا ألبيكانز (9%) كريبتوكوس نيوفورمانس (1%) . كما أوضحت الدراسة المعملية أن معظم البكتريا المعزولة من عينات ألبان الأبقار المصابة بإلتهاب الضرع الظاهري حساسة للمضادات الحيوية التالية :سيبروفلوكاسين , سيفوتاكسيم ,أموكسسيللين /حمض كلافيولنك ,أمبسيللين /سلبكتام ,نيومايسين .أما الفطريات المعزولة من العينات حساسة للمضادات الفطرية :فلوكونازول, نيستاتين .هذا وقد تم مناقشة النتائج ومناقشة تأثير وجود الميكروبات المعزولة من الناحية الصحية والاقتصادية ومناقشة التوصيات اللازمة للوقاية والعلاج والحصول علي لبن صحي آمن للاستهلاك الآدمي.