Diversity of Microorganisms Associated to She Camels' Subclinical and Clinical Mastitis in South Sinai, Egypt

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Abstract

The current study aimed to detect microbial causes of she camel's subclinical and clinical mastitis and antibiotic sensitivity test for some bacterial isolates. A total of 196 milk samples were collected from mastitic and apparent healthy she camels, milk samples of apparent healthy she camels were examined by California mastitis test for detection of subclinical mastitis. All samples were cultivated on different media for detection of bacteria and fungi causing mastitis. *S. aureus* and *Acholeplasma* were subjected to antibiotic sensitivity test to detect antibiotic of choice. Out of 40 apparently healthy she camels, subclinical mastitis was detected in 19 (47.5%). Out of 196 she camels milk samples, 40 samples were positive for bacterial isolation (20.4%). *E. coli* and *Staphylococcus epidermidis* were the most predominant isolated bacteria from apparently healthy she camel milk samples while *Pseudomonas aeruginosa*, *Staphylococcus epidermidis* and *Streptococcus agalactia* from mastitic she camel's milk samples. This is the first record for isolation of *Acholeplasma laidlawii* in South Sinai where 4 *Acholeplasma laidlawii* isolates were isolated from mastitic and apparent healthy she camel milk samples. *Aspergillus niger* was the most predominant fungi followed by *Candida albicans*. The most predominant mixed bacterial infection in apparent healthy she camels was *Proteus vulgaris* and *Staphylococcus epidermidis* while from mastitic she camels was *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. *S.*

aureus was sensitive to gentamycin, streptomycin, erythromycin, tetracycline, ciprofloxacin and nitrofurantion. *Acholeplasma laidlawii* was sensitive to gentamycin. From the obtained results, it was concluded that she camels' mastitis was caused by several bacteria and fungi either by single or mixed infection.

Key words: Mastitis, California mastitis test, Antibiotic sensitivity test.

Introduction

Mastitis is defined as an inflammation of the parenchymal tissue of the mammary gland. It is characterized by physical and chemical changes in the milk and pathological changes in glandular tissue of the udder which include swelling, heat, pain, and edema of mammary gland. The most important changes in the milk include discoloration, presence of clots and presence of a large number of Leukocytes ***(Hadef et al., 2018)***. Mastitis is a relatively infrequent disease in camels compared with cattle, but the incidence of mastitis may increase in dairy camels due to hand milking and teat malformation ***(Al-Tofaily and Al-Rodhan 2011).*** Many infective agents have been implicated as causes of mastitis in camel, however, bacterial infections are considered the primary cause of mastitis ***(Seifu and Bekele, 2010)*** either in the form of pure or mixed infection ***(Abdella and Mohammed, 2014).*** Various studies had been conducted worldwide on the isolation and identification of bacteria causing camel mastitis and their effect on quantity and quality of milk. *Staphylococcus aureus, Staphylococcus epidermidis, Micrococcus* spp., *Pasteurella haemolytica* and *Escherichia coli* were the main causes of she-camel mastitis ***(Al Juboori et al., 2013)***. Also, *Streptococcus agalactiae* was considered as one of the most important causes of mastitis in camel ***(Fischer et al., 2013).*** Yeasts caused mastitis as *Candida* spp., *Cryptococcus* spp., *Rhodoturulla* spp., *Sacharomyces* spp. and *Trichosporon* spp. Meanwhile, molds as *Aspergillus* spp., *Penicillium* spp., *Alternaria* spp., *Aerobasidium* spp., *Geotrichum* spp. and *Pichia* spp. ***(Ahmad and Gholib, 2016).*** Subclinical mastitis is costly disease due to no clinical sings and if not detected at time may progress to clinical mastitis and it may be caused by fungus such *as Aspergillus* spp., *Trichosporon* spp. and *Candida* spp. ***(Radostits et al., 2010).*** The

most common causative bacteria of subclinical mastitis were coagulase positive and coagulase negative Staphylococcus, Coliform bacteria ***(Eman et al., 2012)***. California mastitis test (CMT) was used to detect the subclinical mastitis of she camel compared to bacterial isolation, it was found that CMT is fast and effective, but less sensitive in diagnosis of subclinical mastitis than bacterial isolation ***(Hayder et al., 2018).*** Hence, this study aimed to detect microbial causes of clinical and subclinical mastitis in she camels.

Materials and methods

1. Sampling

One hundred ninety-six raw camel's milk samples were collected from 67 she camels (diseased and apparently healthy) from different farms in Ras Sudr of south Sinai (South Sinai research station of the desert research Center and different farms of Bedouins). Samples were collected under aseptic conditions according to ***National Mastitis Council (1999).***

2. California mastitis test (CMT):

Forty milk samples were collected from apparently healthy and tested by CMT to detect the prevalence of subclinical mastitis. CMT was done according to ***Schalm et al. (1971).***

3. Bacteriological examination of milk samples:

A loopful of each raw milk sample was inoculated into nutrient broth (Oxoid) and selenite cystine broth (Oxoid) then incubation at 37°C for 24 hours then take loopful from nutrient broth culture and cultivate on nutrient agar (Oxoid), MacConkey's agar (Oxoid), brain heart infusion agar (Oxoid), mannitol salt agar (Oxoid) and litmus milk media (Oxoid) for isolation of different bacteria. A loopful from selenite cystine broth culture is cultivated on S.S agar for isolation of *Salmonella* and other *Enterococcus* species then incubate at 37°C for 24 hours, then morphological and biochemical identification was done according to ***Quinn et al. (1994)***. Isolation of *Acholeplasma* was done according to ***Hazelton et al. (2018).*** *Acholeplasma* isolation from milk samples was done by using *Mycoplasma* agar (Oxoid CM0401) and broth (Oxoid CM0403) supplemented with *Mycoplasma* selective supplement G (Oxoid SR0059)

4. Isolation and identification of yeast and mold

Milk samples were cultivated on Sabouraud Dextrose Agar (Oxoid), Examine plates for fungal colonies exhibiting typical color and morphology. Yeasts will grow as creamy to

white colonies. Molds will grow as filamentous colonies of various colors.

5. Antimicrobial susceptibility testing of isolated bacteria

S. aureus as an important bacteria causing mastitis and globally showing high level of antimicrobial resistance was tested against 9 antibacterial agents (penicillin 10 µg, ampicillin 10 µg, vancomycin 30 µg, gentamycin 10 µg, streptomycin 100 µg, erythromycin 15 µg, tetracycline 30 µg, ciprofloxacin 5 µg and nitrofurantion 300 µg) and *Acholeplasma laidlawii* was tested against 6 antibacterial agents (tobramycin 10 µg, spirumycin 100 µg, enrofloxacin 5 µg, erythromycin 10 µg, amikacin 20 µg and gentamycin 200 µg). Antibiogram was performed using disk diffusion method as described by ***Ewing (1986)*** *and* ***Cruickshank (1975)***. The results were interpreted according to guidelines of ***Clinical and Laboratory Standards Institute (2015)***.

Results

1. Prevalence of subclinical she camel mastitis using CMT:

Out of 40 apparent healthy she camel's subclinical mastitis was detected in 19 she camels by CMT with a percentage of 47.5%.

2. Bacteriological examination of she camel's milk samples:

Out of 196 she camel's milk samples (134 sample from apparently healthy she camels, 62 from mastitic she camel's) from South Sinai Research Station and Bedouin farms, 40 samples (8 samples from apparently healthy she camels, 32 from mastitic she camel's) were positive for bacterial isolation with a percentage of 20.4% (4.08% from apparently healthy she camels, 16.32% from mastitic she camels).

3. Prevalence of Gram-negative bacteria in apparently healthy and clinical mastitic she camels

Concerning bacterial isolates obtained from apparently healthy she camel's milk samples, *E. coli* was the most predominant isolated bacteria with a prevalence of 35.4% followed by *Pseudomonas aeruginosa, Klebsiella pneumonia, Proteus vulgaris, Shigella flexneri, Enterobacter aerogenes,* and *Serratia marcescens* with a prevalence of 20.8%, 20.8%, 10.4%, 8.3%, 2.1% and 2.1% respectively. On the other hand, the most predominant bacteria isolated from mastitic she camel's milk samples were *Pseudomonas aeruginosa* and *E. coli* with a prevalence of 28.4% and 27% followed by *Klebsiella*

pneumonia, *Shigella flexneri*, *Enterobacter aerogenes*, *Yersinia enterococci*, *Proteus valgaris*, *Serratia marcescens* and *Salmonella typhimurium* with a prevalence of 16.2%, 14.9%, 6.8%, 2.7%, 1.4%, 1.4% and 1.4% respectively.

4. Prevalence of Gram positive bacteria in apparently healthy and clinical mastitic she camels

Concerning bacterial isolates obtained from apparently healthy she camel's milk samples, *Staphylococcus epidermidis* was the most predominant isolated bacteria with a prevalance of 70.7% followed by *Streptococcus agalactiae*, *Bacillus cereus* and *Enterococcus faecalis* with a prevalence of 22% , 4.9% and 2.4% respectively. On the other hand, the most predominant bacteria isolated from mastitic she camel's milk samples were *Staphylococcus epidermidis* and *Streptococcus agalactiae* with a prevalence of 33.3% for each followed by *Bacillus cereus*, *Staphylococcus aureus* and *Enterococcus faecalis* with a prevalence of 18.2%, 9.1% and 6.1% respectively.

5. Prevalence of *Acholeplasma*

Bacteriological examination of 196 raw camel's milk samples of apparently healthy and mastitic she camels to detect the prevalence of *Acholeplasma*, the results showed that, 4 isolates were characterized by fried egg appearance *Acholeplasma laidlawii* was detected with a prevalence of 2.04% (4/196).

6. Prevalence of fungi in apparently healthy and clinical mastitic she camels

Aspergillus niger (*A. niger*) was the most predominant mold isolated from apparently healthy she camel's milk samples with a prevalence of 93.3%. Also, *Candida albicans* (*C. albicans*) yeast was isolated from apparent healthy she camel's milk samples with a prevalence of 6.7%. Concerning mastitic she camel's milk samples, the most predominant isolated mold was *A. niger* with a prevalence of 64.9% followed by *C. albicans* yeast with prevalence of 29.7%. Also, *Aspregillus flavus* (*A. flavus*) and *Aspergillus fumigatus* (*A. fumigatus*) molds were isolated by 2.7 % for each.

7. Prevalence of mixed bacterial infection:

Mixed bacterial infection was detected in apparently healthy and mastitic she camels where the most predominant mixed bacterial infection in case of apparently healthy was *Proteus valgaris* and *Staphylococcus epidermidis* with a prevalence of 36.4% while, the most predominant mixed bacterial infection in case mastitic she camels was *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* with a prevalence of 25%.

8. Antibiotic sensitivity test of *S. aureus* and *Acholeplasma*. Antibiotic sensitivity test by disk diffusion method revealed that *S. aureus* was resistant to penicillin, ampicillin and vancomycin while was sensitive to gentamycin, streptomycin, erythromycin, tetracycline, ciprofloxacin and nitrofurantion. *Acholeplasma laidlawii* was resistant to tobramycin, spirumycin, enrofloxacin, erythromycin, amikacin while, sensitive to gentamycin.

Discussion

Mastitis is a global problem as it adversely affects animal health, economics of milk production and quality of milk. It affecting every country, including developed ones and causes huge financial losses ***(Sharma et al., 2007)***. In this study, the prevalence of subclinical mastitis using California mastitis test was 47.5% and these results were higher than those obtained by ***Memon et al. (2019) and Mogeh et al. (2019)*** where the prevalence rate of subclinical mastitis was 22.75% and 25.8% respectively. The high prevalence of subclinical mastitis may be attributed to poor hygienic condition of milking area and tick infestations act as rick factor for incidence of subclinical mastitis. The most Gram-negative pathogen recovered from apparent healthy and mastitic she camels was *E. coli* with a prevalence of 30.3%, these results were similar to those obtained by ***Yam et al. (2015)*** but were higher than those reported by ***Hadef et al. (2018) and Mogeh et al. (2019)*** who detected *E coli* by 10.72 % and 21% respectively**.** *E. coli* is one of the most common causes of mastitis ***(Abdella and Mohammed 2014).*** The high prevalence of *E. coli* may be attributed to coliform environmental mastitis with poor hygienic condition in milking area. *Pseudomonas aeruginosa* was isolated in a prevalence of 25.4 % and this was higher than those obtained by ***Al-Juboori et al. (2013) and Mogeh et al. (2019)*** where the prevalence of *Pseudomonas aeruginosa* isolates was 1.66% and 6.25% respectively. The study revealed that the most common Gram-positive isolates from mastitic and apparent healthy she camels was *Staphylococcus epidermidis* with a prevalence of 54.1%, these results were agreed with those obtained by ***Sundhan and Sharma (2010) and Memon et al. (2019)*** who stated that, *Staphylococcus epidermidis* is the one of the most predominant isolates in camel herds also caused mastitis. The prevalence of *Staphylococcus aureus* was 4.05%, these results were similar to those obtained by ***Hussein et***

al. (2013) and Hanna and Abeer (2015) where the prevalence of *S. aureus* was 4.2% and 3.33% respectively**.** Meanwhile, ***Mogeh et al. (2019)*** found that the prevalence of *S. aureus* was 24.2%**.** The prevalence of *Streptococcus agalactiae* isolates was 27%, these results were higher than those obtained by ***Memon et al. (2019)*** where the prevalence of *Streptococcus* isolates was 13.2%. Meanwhile, ***Saleh and Faye (2011)*** detected high prevalence of *Streptococcus* isolates (42.9%)**.** In the current study, *Acholeplasma laidlawii* was isolated from mastitic she camel in South Sinai and this is the first record for isolation of *Acholeplasma laidlawii* in this area, where *Acholeplasma laidlawii* was isolated in a percentage of 2.04%. ***Mederos et al. (2014)*** isolated *Acholeplasma laidlawii* and *Acholeplasma oculi* from camel species. Meanwhile, ***Ebtesam (2016) and Al-Farha et al. (2017)*** isolated *Acholeplasma laidlawii* from clinical and subclinical mastitis cases of cattle. ***Al-Farha et al. (2017)*** isolated *A. laidlawii* in a percentage of 10.8%. ***Ebtesam (2016)*** isolated *Acholeplasma* spp. from clinical and subclinical mastitis milk samples of dairy cows in percentage of 10.3% and 5.6% respectively. The lower detection of *Acholeplasma laidlawii* as a cause of mastitis in camels than cattles may be attributed to species of animals and genetic factors.

She camel mastitis may be caused by fungus such *as Aspergillus* spp. and *Candida* spp. ***(Radostits et al., 2010)***. In the present study, the prevalence of fungi isolated from apparent healthy and mastitic she camel was 20.6 % and the most predominant fungi was *Aspergillus niger* (73.1%) followed by *Candida albicans* (23.07%). Meanwhile, *Aspergillus flavus* and *Aspergillus fumigatus* present with low percentage 1.9% for each one. ***Hanaa et al. (2011)*** detected prevalence of *Candida albicans, Aspergillus fumigatus* and *Aspergillus niger* by 10.5%, 9% and 2% respectively. ***Mosaad et al. (2011)*** found that the prevalence of *Candida albicans, Aspergillus niger* and *Aspergillus fumigatus* was 10%, 2.5% and 2.5% respectively. ***Al Dughaym and Fadlelmula (2015)*** detected that the prevalence of *Aspergillus fumigatus, Candida albicans* and *Aspergillus niger* was 10%, 7.5% and 5.7% respectively. The high prevalence of fungi in the current data may be attributed to the udder infestation by ticks which act as a predisposing factor for mastitis. Mixed bacterial infection from mastitic and apparent healthy she camels was 13.8%, these results were

lower than those obtained by ***Hadef et al. (2018)*** where mixed bacterial infection was 54.9%.

Concerning, antibiotic sensitivity test of *S. aureus* the obtained results detected that it was sensitive to gentamycin, streptomycin, erythromycin, tetracycline, ciprofloxacin and nitrofurantion. Meanwhile, it was resistant to penicillin, ampicillin and vancomycin. ***Mogeh et al. (2019)*** and ***Memon et al. (2019)*** reported that *S. aureus* was sensitive to gentamycin but **Ali *et al.* (2019)** reported that *S. aureus* was resistant to gentamycin. On the other hand, the study revealed that *S. aureus* was sensitive to streptomycin and this agreed with ***Al-Tofaily and Rodhan (2011)*** and ***Badria et al. (2016)*** who reported that *S. aureus* was sensitive to streptomycin, however ***Ali et al. (2019)*** found that *S. aureus* was resistant to streptomycin. In this work, *S. aureus* was sensitive to erythromycin and this was similar to those obtained by ***Badria et al. (2016)*** but disagreed with those obtained by ***Al-Tofaily and Rodhan (2011)*** where they reported that *S. aureus* was resistant to erythromycin. From the current study, *S. aureus* was sensitive to tetracycline this agreed with **Ismail (2015)**. On the other hand, ***Mogeh et al. (2019)*** found that *S. aureus* was resistant to tetracycline. The present work stated that *S. aureus* was sensitive to ciprofloxacin and this agreed with ***Yam et al. (2015)*** but ***Ali et al. (2019)*** found that *S. aureus* was resistant to ciprofloxacin. Also, in the present study *S. aureus* was sensitive to nitrofurantion and this agreed with ***Tuteja et al. (2003).*** *S. aureus* was resistant to ampicillin, penicillin and vancomycin, and these results were similar to those obtained by ***Ali et al. (2019)*** but ***Subramaniyan et al. (2016)*** reported that *S. aureus* was sensitive to ampicillin.

The study detected that *Acholeplasma laidlawii* was sensitive to gentamycin, meanwhile it was resistant to tobramycin, spirumycin, enrofloxacin, erythromycin and amikacin and these results were disagreed with those obtained by ***Tomar et al. (2017)*** who reported that *Acholeplasma laidlawii* was sensitive to amikacin, enrofloxacin and spirumycin.

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الأسباب الميكروبية لالتهاب الضرع تحت الإكلينيكي والإكلينيكي للإبل في جنوب سيناء ، مصر

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هدفت الدراسة الحالية إلى الكشف عن الأسباب البكتيرية والفطرية لالتهاب الضرع تحت الإكلينيكي والإكلينيكي للجمال واختبار حساسية المضادات الحيوية لبعض العزلات البكتيرية. تم جمع 196 عينة حليب من الإبل السلمية ظاهريا والمصابة بالتهاب لضرع وفحصت عينات اللبن من الإبل السلمية ظاهريا بواسطة اختبار كاليفورنيا للكشف عن التهاب الضرع تحت الإكلينيكي. تمت زراعة جميع العينات على أوساط مختلفة للكشف عن البكتيريا والفطريات المسببة لالتهاب الضرع. تم إخضاع بعض العزلات البكتيرية لاختبار حساسية المضادات الحيوية للكشف عن المضاد الحيوي المختار. من بين 40 من الإبل التي تبدو بصحة جيدة ، تم اكتشاف التهاب الضرع تحت الإكلينيكي في 19 (47.5٪). من أصل 196 عينة لبن نوق كانت 40 عينة موجبة للعزل البكتيري (20.4٪). كانت الايشيريشيا كولاى وستافيلوكوكس ابيدرميدس اكثر البكتيريا انتشارًا فى لبن الإبل السلميه ظاهريا بينما كانت سودوموناس ايروجونوزا وستافيلوكوكس ابيدرميدس اكثر البكتيريا انتشارًا فى حاله التهاب الضرع. تم عزل أربع عزلات من بكتيريا أكوليبلازما لاوي من عينات لبن النوق المصابة بالتهاب الضرع والسليمة ظاهريا. كانت الاسبيرجيلس نيجر أكثر الفطريات انتشارًا تليها

كانديدا البيكانز. كانت العدوى البكتيرية المختلطة الأكثر انتشارًا في الإبل السليمة ظاهريا هى البروتيس فلجارز و وستافيلوكوكس ابيدرميدس بينما كانت البكتريا المختلطه فى التهاب الضرع هى سودوموناس ايروجونوزا و كلبسيلا نيمونى . ستافيلوكوكس اوريس كانت حساسة للجنتامايسين والستربتومايسين والإريثروميسين والتتراسيكلين والسيبروفلوكساسين والنيتروفوارنتوئين بينما كانت أكولييلازما لاوي حساسة للجنتامايسين. يمكن استنتاج أن التهاب الضرع في الإبل سببه عدة بكتيريا وفطريات إما عن طريق عدوى مفردة أو مختلطة.