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 Streptococcusagalactia 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.
Staphylococcus 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., *Rhodotorula* 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).
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 camels) from South Sinai Research Station and Bedouin farms, 40 samples (8 samples from apparently healthy she camels, 32 from mastitic she camels) 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 vulgaris, 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 prevalence 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 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, Aspergillus 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 vulgaris 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 nitrofurantoin. *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 risk 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 *Hadeef 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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كانت العدوى البكتيرية المختلطة الأكثر انتشارًا في الإبل السليمة ظاهريًا هي البروتيس فلجارز و ستافيلوكوكس إيدرميدس بينما كانت البكتيريا المختلطة في التهاب الضرع هي سودوموناس أيروجونوزا و كليسيلا نيموني. ستافيلوكوكس أوريس كانت حساسة للجنتاميسين والميكلارايغين والإريثروميسين والسيبروفلوكساسين والنيتروفوارتنين بينما كانت أكوليبلازما لاو حساسة للجنتاميسين. يمكن استنتاج أن التهاب الضرع في الإبل سببه عدة بكتيريا وفطريات إما عن طريق عدوى مفردة أو مختلطة.