

Isolation and identification of bacteria strains that caused the infection of multiple abscesses in Bactrian camel

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Received: May 12, 2020; accepted: June 8, 2020.

Camel abscess is one of the important diseases that can be caused by pyogenic bacteria. Abscess were mainly reported on the dromedary camel of the Nile Delta of Egypt, but rarely reported on Bactrian camel. The purpose of this study is to identify the pathogens isolated from the samples of camel abscess. The scanning electron microscope was applied for pathogenic morphological study. The 16S rRNA genes amplification and sequencing, and Matrix Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) method were employed to identify the bacteria. In addition, drug sensitivity tests were performed for the potential prevention and control of the disease. The results showed that two bacterial strains of *Staphylococcus* were isolated. Amplification of 16S rRNA and construction of phylogenetic tree demonstrated that they were *Staphylococcus aureus* (*S. aureus*) and *Staphylococcus sciuri* (*S. sciuri*). The results of MALDI-TOF MS confirmed that the causative bacteria causing camel abscess were *S. aureus* and *S. sciuri*. Drug sensitivity tests showed that *S. aureus* was resistant to Amoxicillin, Penicillin G, Erythromycin, Linezolid, Clindamycin, and Chloramphenicol, and was moderately resistant to Rifampicin, Doxycycline, and Tetracycline, but was sensitive to seven other antibiotics. *S. sciuri* was resistant to Cefotaxime, but sensitive to fifteen other antibiotics. This study is the first report on *S. sciuri* infected camels and provides a scientific basis for selecting antibiotics that are effective for treating camel abscess.

Keywords: Bactrian camel; Isolation and Identification; *Staphylococcus sciuri*; *Staphylococcus aureus*.

Financial support: This work was funded by Ningxia Department of Science and technology for supporting the project funding (2019BBF02005).

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Introduction

The Bactrian camel is the species historically present in the colder part of Central and Eastern Asia [1, 2]. In China, native Bactrian camels are concentrated in temperate deserts and desert grasslands in western Inner Mongolia, Ningxia, Xinjiang, and Gansu [2]. Alxa League of Inner Mongolia is the area with the largest number of Bactrian camels in China and is known as the "Camel town". Bactrian camel was an indispensable mean of transport on the ancient "Silk Road" and played a vital role in trade

activities around the world, especially running through the entire Eurasia. At present, Bactrian camels are mainly used for meat, milk, and fur production [3], which are important pillar industries in Alxa League of Inner Mongolia.

In the past, researchers paid a little attention to camel disease. However, viral, bacterial, and parasitic diseases were all common in camels [4]. Camel abscess is one of the important diseases that can be caused by pyogenic bacteria [4, 5]. Camels with abscess suffer from anemia, weight loss, and even death due to toxins produced by

pathogenic bacteria [6], which leads to the reduction of reproduction and production efficiency, and therefore, severely affects the output of camel meat and fur [4, 7], causing huge economic losses to the camel industries.

Staphylococcus aureus (*S. aureus*) is a common and widely distributed bacterium associated with a variety of diseases ranging from skin and soft tissue infections to sepsis and toxic shock [8]. Camels are known to carry *S. aureus* that can cause mastitis, abscess, arthritis, and pneumonia. *S. aureus* is merging as multiple drug resistant pathogen in camel [9]. Camel abscess caused by *Staphylococcus aureus* was mainly reported on the dromedary camel of the Nile Delta of Egypt [4], but rarely reported on Bactrian camel.

Staphylococcus sciuri (*S. sciuri*) was first isolated in North Carolina, USA in 1976 [10] and is considered one of the most primitive species of the *Staphylococcus spp.* [11]. *S. sciuri* has been isolated from skin and skin structures from a variety of wildlife [12, 13] including marsupials, rodents, carnivores, monkeys, cetaceans, livestock such as cattle, sheep, horses, and dogs [14-19]. It has also been reported that *S. sciuri* was associated with various infections in human [12, 20-26]. However, *Staphylococcus sciuri* remains a rarely reported cause of camel infection.

At present, the methods used for microbial identification include identification media, Gram staining, biochemical analysis, 16S rRNA gene amplification and sequencing, etc. With the advanced technology, Matrix Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) technology has revolutionized the field of microbiological identification [27, 28]. The mechanism of this technology is based on the protein composition and molecular weight of various bacteria. MALDI-TOF MS is used to determine the unique protein fingerprints of various bacteria and compare them with the mass spectra of known bacteria to identify examined bacteria. MALDI-TOF MS is a

fast, sensitive, and accurate method with only one colony required for detection [28].

The objective of this study was to isolate and identify the pathogens from the infected abscess of Bactrian camels and detect the drug sensitivity of each isolated pathogen to provide a scientific basis for prevention and control of the Camel abscess disease.

Materials and methods

Sample collection

The animal tissue samples were collected from one eight years old female Bactrian camel from Jingebi Camel Milk Professional Co. (Yingen Sumu, Zuoqi County, Alxa League, Inner Mongolia Autonomous Region, China) with 15 days of history of multiple abscesses and being diagnosed as abscess disease by a certified veterinary doctor (Figure 1). The skin crusts and hairs in the junction areas between healthy and affected skin of symptomatic animals were collected by using a sterile scalpel blade after atopic cleaning with 70% ethyl alcohol. Sterile swabs were used to collect pus and then being put into sterile sample tubes. All specimens were transported to the Clinical Veterinary Laboratory at School of Agriculture, Ningxia University, within 2–4 hours after collections.



Figure 1. The infection of multiple abscesses in Bactrian camel.

Microscopic examination

Preserved samples in sterile sealed bags were transferred to glass slides. 1 drop of 10% KOH solution was added to soften the dander for 5 minutes. The samples were then observed under an optical microscope [29].

Bacteria isolation and culture

0.1 ml of pus was inoculated on 5% sheep blood agar plates containing Lab-Lemco Powder 10 g/L, Peptone Neutralised 10.0 g/L, Sodium chloride 5.0 g/L, Agar 15.0 g/L, and Defibrinated Sheep Blood (Oxoid, Basingstoke, Hampshire, UK). The plates were incubated at 37°C for 24 hours [29]. The isolated bacterial colonies were inoculated into the Tryptic Soy Broth containing Tryptone 17.0 g/L, Soy peptone 3.0 g/L, Sodium chloride 5g/L, K₂HPO₄ 2.5 g/L, Glucose 2.5 g/L (Oxoid, Basingstoke, Hampshire, UK) according to the colony morphology and were incubated at 37°C, 220 rpm for 12 hours.

Scanning electron microscope observation

The bacterial isolates were observed under Scanning electron microscope. Firstly, 3% glutaraldehyde was added to suspend collected bacterial isolate and stored at 4°C for 10 min. Secondly, the isolates were washed twice with PBS for 5 min each and once with 4% (w/v) sucrose solution for 5 min. Thirdly, the isolates were passed through a gradual ethanol gradient (30, 50, 70, 80, 90, and 2× 100%, for 10min each) before being critical point dried with carbon dioxide using Critical Point Dryer (EM CPD300) (Leica, Wetzlar, Germany). Specimens were then mounted on aluminium stubs (diameter 12 mm) and coated with 5 nm gold by Auto-fine Coater (JFC-1200 Fine Coater) (JEOL, Tokyo, Japan). Images were obtained by using Inspect S50 Scanning Electron Microscope (FEI, Hillsboro, Oregon, USA) under high vacuum operated at 10-30 kV.

DNA extraction and polymerase chain reaction (PCR)

Bacterial genomic DNAs were extracted by using TaKaRa MiniBEST Bacteria Genomic DNA Extraction Kit (version 3.0) (Takara, Osaka,

Japan). 16S rRNA gene were amplified by using TaKaRa 16S rDNA Bacterial Identification PCR kit (Takara, Osaka, Japan) with forward primer of 5'-GAGCGGATAACAATTCACACAGG-3' and reverse primer of 5'-CGCCAGGGTTTTCCAGTCACGAC-3'. The PCR reaction mixture was 50 µL including 2 µL of DNA, 25 µL of PCR Premix, 1 µL each of forward and reverse primers, and 21 µL of 16S-free H₂O). The PCR reaction conditions were 94°C for 5 min followed by 30 cycles of 94°C for 1 min, 50°C for 1 min, 72°C for 1.5 min, and then a final incubation at 72°C for 5 mins. The PCR products were analyzed by 1% agarose gel electrophoresis, and then, were purified by using TaKaRa MiniBEST Agarose Gel DNA Extraction Kit (version 4.0) (Takara, Osaka, Japan) followed by DNA sequencing.

Phylogenetic analysis

The sequencing results were evaluated to determine the closest relatives by using BLASTn (<https://blast.ncbi.nlm.nih.gov>). The similarity analysis was performed by using the Sequence Distances method of MegAlign software (DNASar, Madison, Wisconsin, USA). A phylogenetic analysis of sequences together with sequences of the closest relatives available in the GenBank database was conducted by using the neighbor-joining (NJ) method and Kimura 2-parameter model in the MEGA 6.0 software (DNASar, Madison, Wisconsin, USA). Bootstrap confidence values (1,000 replications) were given at the respective nodes.

MALDI-TOF MS identification

The bacterial isolates were identified by using Autof MS 1000 (Autobio Diagnostics, Zhengzhou, Henan, China). For Autof MS 1000, the target slide is a metal reusable slide with ninety-six sample sites. The bacterial spots were treated by 1 ml of Autobio sample pretreatment reagent (10 mg/mL of HCCA in acetonitrile and trifluoroacetic acid). Spectra analysis was collected by using software Autof Acquirer (version 1.0.123) and was analyzed by using software Autof Analyser (version 1.0.50). The interpretation criteria of manufacturer were applied with a score >9.0 for

Table 1. Zone of inhibition diameter interpretive criteria for *Staphylococcus spp.*

Antibiotic family	Antibiotics	Abbreviation	Disc content	R (mm)	I (mm)	S (mm)
β-lactams	Cefoxitin	FOX	30µg	≤24	–	≥25
	Amoxicillin	AMC	30µg	≤28	–	≥29
	Cefotaxime	CTX	30µg	≤28	–	≥29
	Penicillin G	P	10U	≤28	–	≥29
Ansacycins	Rifampicin	RD	5µg	≤16	17–19	≥20
Aminoglycosides	Kanamycin	K	30µg	≤13	14–17	≥18
	Gentamicin	CN	10µg	≤12	13–14	≥15
Macrolides	Erythromycin	E	15µg	≤13	14–22	≥23
Oxazolidinones	Linezolid	LZD	30µg	≤20	–	≥21
Sulfonamides	Sulphamethoxazole	SXT	25µg	≤12	13–16	≥17
Fluoroquinolones	Ciprofloxacin	CIP	5µg	≤15	16–20	≥21
	Levofloxacin	LEV	5µg	≤15	16–18	≥19
Lincosamides	Clindamycin	DA	2µg	≤14	15–20	≥21
Phenicols	Chloramphenicol	C	30µg	≤12	13–17	≥18
Tetracyclines	Doxycycline	DO	30µg	≤12	13–15	≥16
	Tetracycline	TE	30µg	≤14	15–18	≥19

Notes: R-Resistant; S-Susceptible; I-Intermediate.

species-level identification, a score between 6.0 and 9.0 for genus-level identification, and a score <6.0 for unidentified [27].

Antimicrobial susceptibility testing

Antimicrobial susceptibilities for *S. aureus* and *S. sciuri* against 16 antimicrobial agents (Oxoid, Basingstoke, Hampshire, UK) were evaluated by applying disc diffusion method on Mueller-Hinton agar (Oxoid, Basingstoke, Hampshire, UK) according to the criteria recommended by the Clinical and Laboratory Standards Institute (CLSI, 2018) who set the testing standards for quality guidelines in medical laboratory around the world. The antimicrobial agents used in this study included Cefoxitin, Amoxicillin, Cefotaxime, Penicillin G, Rifampicin, Kanamycin, Gentamicin, Erythromycin, Linezolid, Sulphamethoxazole, Ciprofloxacin, Levofloxacin, Clindamycin, Chloramphenicol, Doxycycline, and Tetracycline. Three repeats for each bacterial strain had been done for each antibiotic. *Staphylococcus aureus* ATCC25923 and *Staphylococcus sciuri* ATCC29062 (Microbiologics, St. Cloud, Minnesota, USA) were used as the quality control reference strains. The criteria for the interpretation of zone diameter used in this study were described in Table 1.

Results and discussion

Bacterial isolation and scanning electron microscope observation

No fungal spores were observed under a light microscope, which indicated that the abscess disease was not caused by fungi. There were 2 different types of colonies on blood agar medium. One of them showed a gray-white round, neat edge, smooth surface, opaque, and hemolytic colony and was named as LT-1. The other one was a yellow round, neat edge, smooth surface, opaque, and non-hemolytic colony and was named as LT -2. The colony morphology observation under the scanning electron microscope at 40,000× demonstrated that both types of colonies were non-flagellate cocci and were similar to *Staphylococcus* (Figure 2). The results suggested that the abscess was caused by the mixed infection of 2 different types of *Staphylococcus*.

PCR results and phylogenetic analysis

The 16S rRNA gene amplification and sequencing of LT-1 and LT-2 strains generated approximately 550 bp PCR products and sequences. The sequence analysis results using the Sequence Distances method of MegAlign software showed

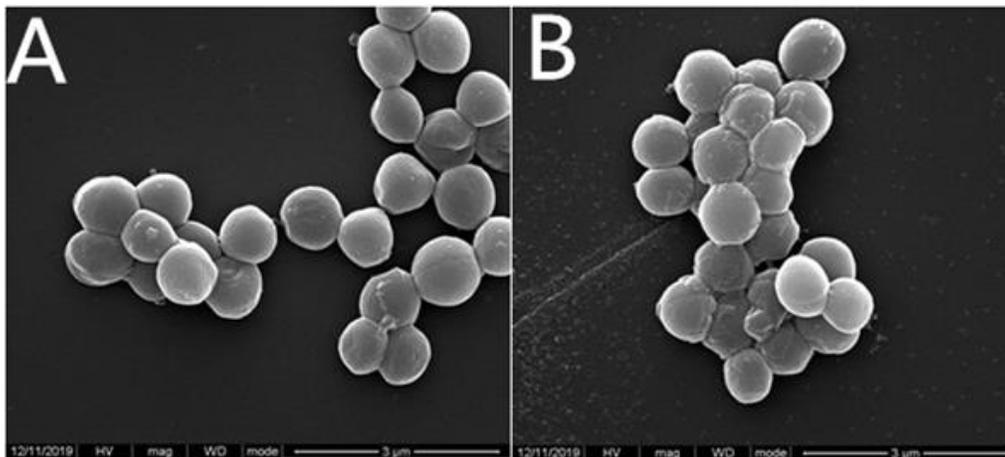


Figure 2. Morphological observation of isolated strains. A: LT-1 strain. B: LT-2 strain.

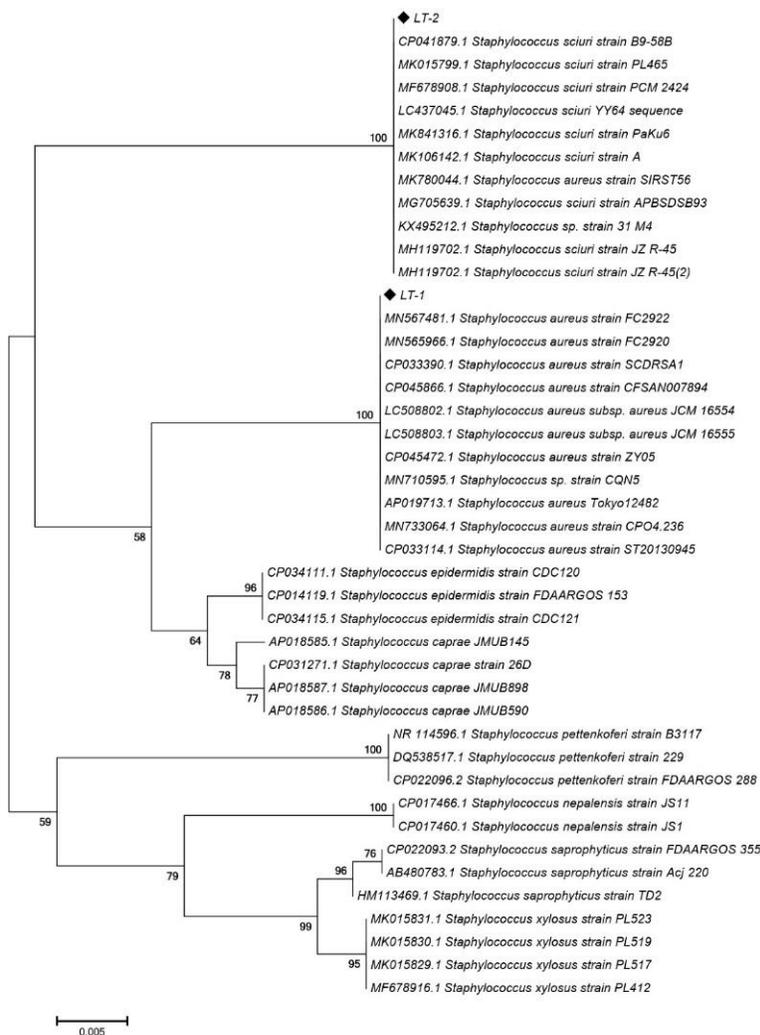


Figure 3. Phylogenetic construction of LT-1 and LT-2 using neighbor-joining and Kimura 2-parameter model in the MEGA software. Bootstrap confidence values (1,000 replications) are given at the respective nodes. The scale bar indicates the length representing 0.005 nucleotide substitutions per site.

Table 2. Antimicrobial susceptibility results.

Antibiotic family	Antibiotics	<i>S. aureus</i> (LT-1)	Susceptibility	<i>S. sciuri</i> (LT-2)	Susceptibility
β-lactams	FOX	25.33±0.47	S	32.00±0.00	S
	AMC	28.67±0.47	R	38.33±0.47	S
	CTX	37.67±0.47	S	28.00±0.00	R
	P	22.00±0.00	R	32.33±0.47	S
Ansacycins	RD	19.00±0.00	I	34.00±0.00	S
Aminoglycosides	K	27.00±0.00	S	32.00±0.00	S
	CN	27.33±0.47	S	36.00±0.00	S
Macrolides	E	7.00±0.00	R	33.67±0.47	S
Oxazolidinones	LZD	13.00±0.00	R	34.33±0.47	S
Sulfonamides	SXT	28.67±0.94	S	30.00±1.63	S
Fluoroquinolones	CIP	41.00±0.82	S	33.67±0.47	S
	LEV	41.00±0.82	S	32.67±1.25	S
Lincosamides	DA	7.00±0.00	R	24.33±0.47	S
Phenicols	C	7.00±0.00	R	30.00±0.00	S
Tetracyclines	DO	12.67±0.94	I	29.33±0.47	S
	TE	16.33±0.94	I	32.00±0.00	S

that the percentage identities of LT-1 were 100% with *Staphylococcus aureus* and 100% with *Staphylococcus sciuri* for LT-2. The phylogenetic tree was constructed based on the amplification sequences of 2 strains. All sequences were compared to the other *staphylococcus spp.* The phylogenetic tree results showed that LT-1 was on the same branch as *Staphylococcus aureus* and LT-2 was on the same branch as *Staphylococcus sciuri* (Figure 3). These results confirmed that those 2 bacterial strains were *S. aureus* and *S. sciuri*, which showed that camel abscess was a mixed infection of *S. aureus* and *S. sciuri*. *S. sciuri* has long been considered as a non-pathogenic commensal bacterium [14]. However, it has been reported that *S. sciuri* is associate with bovine mastitis [30], canine dermatitis [31], fatal exudative epidermitis in piglets [32], and wound infection of horse [14]. This study suggested that *S. sciuri* can also be colonized in camel and be associated with multiple abscesses in Bactrian camel.

MALDI-TOF MS analysis

Camel abscess disease often has multiple abscesses on affected animals' eyes, cheeks, neck, legs, and hump, which were highly contagious. Camels, especially with eye

infections, would die within 10-15 days if not being treated promptly. MALDI-TOF MS technology is currently rarely used in the veterinary field. However, this technology can greatly short the time of diagnosis, and therefore, to promote early treatment of the disease and reduce the mortality. Our results showed that LT-1 was *S. aureus* with a score of 9.704 while LT-2 was *S. sciuri* with a score of 9.618. This result was consistent with the 16S rRNA identification results.

Antimicrobial susceptibility analysis

Both *S. aureus* and *S. sciuri* can cause wound infections and purulent symptoms. Severely infected animals can develop sepsis, which ultimately causes death of animals. Therefore, the drug sensitivity study of infectious bacterial strains is the key to select effective drugs for the treatment of abscess disease, which can provide a scientific basis for treating camel abscess effectively. The results of the drug sensitivity studies of *S. aureus* and *S. sciuri* to 16 antibiotics were shown in Table 2. *S. aureus* was resistant to Amoxicillin, Penicillin G, Erythromycin, Linezolid, Clindamycin, Chloramphenicol; and moderately resistant to Rifampicin, Doxycycline, Tetracycline, while it was sensitive to seven other

antibiotics. These results indicated that *S. aureus* has multiple drug resistance which may be caused by the abuse of antibiotics. *S. sciuri* was resistant to Cefotaxime and sensitive to fifteen other antibiotics. The resistance to Cefotaxime categorized this bacterial strain as methicillin-resistant *Staphylococcus*, which demonstrated the resistance to beta-lactam. Our results are in accordance with the results reported by Coimbra *et al.* [25].

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