Phenotypic and Genotypic Characterization of *Staphylococcus aureus* Isolated from Raw Camel Milk Samples from Libya and its Histopathological Effects on Mice Liver

A Thesis

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*All the praises and thanks be to **Allah**, the most beneficent and merciful of all.*

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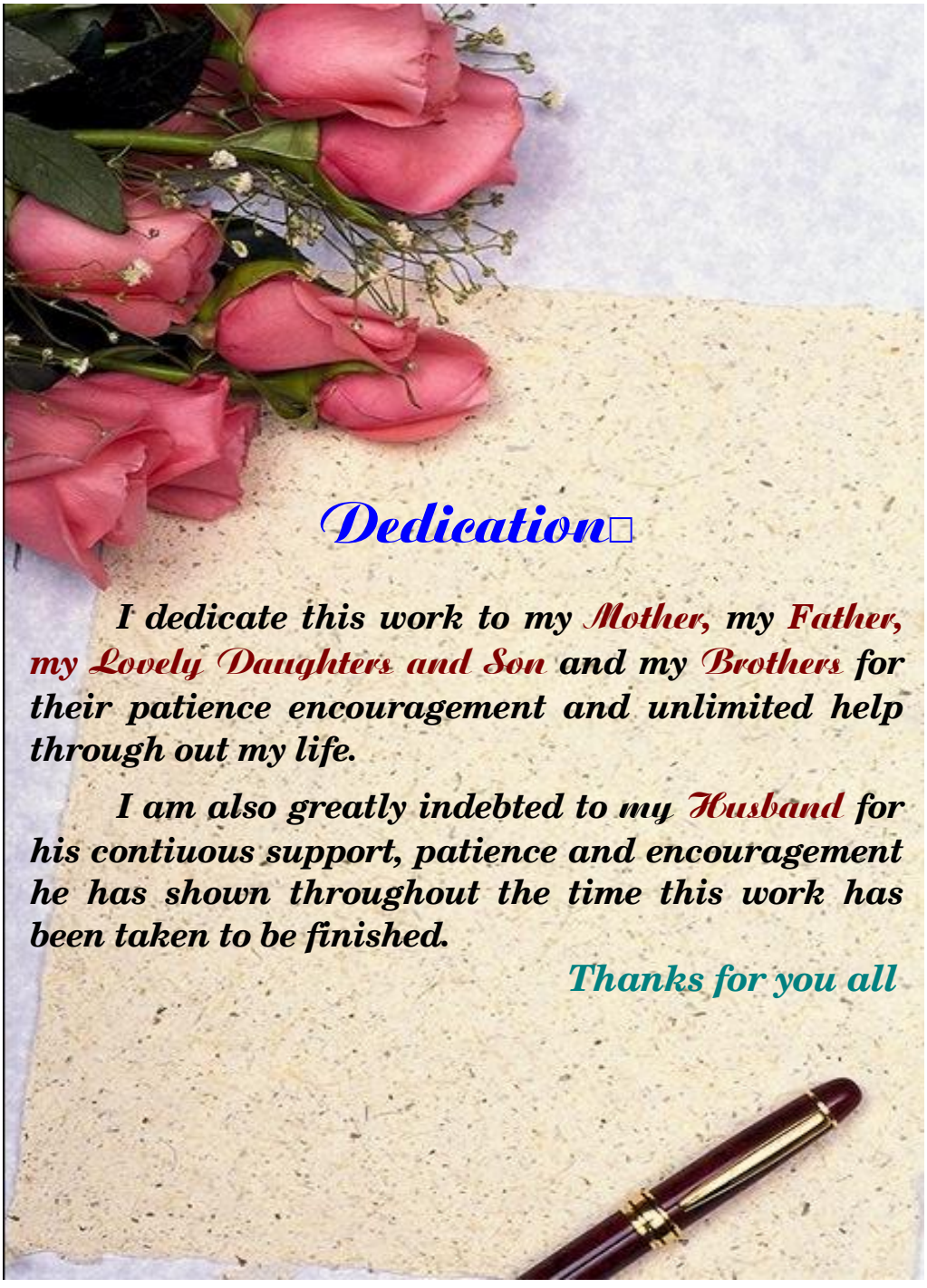

Dedication□

I dedicate this work to my Mother, my Father, my Lovely Daughters and Son and my Brothers for their patience encouragement and unlimited help through out my life.

I am also greatly indebted to my Husband for his contiuous support, patience and encouragement he has shown throughout the time this work has been taken to be finished.

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List of Abbreviations

Abb.	Meaning
C.S.	Cross Section
CFU	Colony forming units
Chisam	Chloroform-iso-amyl alchol
CMT	California mastitis test
CNS	Cogulase nagtive staphylococci
CPS	Coagulase positive staphylococci
DNA	Deoxyribonucleic acid
Dnase	Deoxyribouuclease
EDTA	Etheline diamine tetra acetic acid
GST	Glutathione-S-transferase
Hx. & E.	Hematoxylene and Eosin
LAB	Lactic acid bacteria
LB Medium	Lauria-Bertani medium
LF	Left-fore
LH	Left-hind
MSA	Mannitol salt agar
PBS	Phosphate buffer saline
PCR	Polymerase chain reaction
PTS Ags	Pyrogenic toxin superantigens
RF	Right-fore
RH	Right-hind
RNA	Ribonucleic acid
RNase A	Ribonuclease A
rRNA	Ribosomal RNA
S. aureus	***Staphylococcus aureus***

List of Abbreviations Cont...

Abb.	Meaning
SCC	Somatic cell count
sec	***S. aureus*** gene that produces SEC
SEC	***Staphylococus*** enterotoxin C
ß-toxin	beta toxin
Tris	Tris (hydroxymethly) aminomethane
TSST	Toxic shook syndrome toxin
tst	***S. aureus*** gene that produces TSST
U.A.E	United Arab Emirates
UV	Ultraviolet light
Y-toxin	Gamma toxin
α - toxin	Alpha toxin

ABSTRACT

The present study was conducted to determine the phenotypic and hereditary description of ***Staphyllococcus aureus*** (***S. aureus***) isolated from raw camel milk from different regions in Libya (Tubrak, Shahat, Alsafsaf, Omar Al Mokhtar, Makailie, Labrag, and Gardas) as well as to identify the different toxin genes of these bacteria. Out of the total 220 milk samples collected from 55 teats of apparently healthy lactating she-camels, 6 coagulase positive ***Staphylococcus spp*** were obtained. These 6 (2.7 %) isolates were identified as ***S. aureus*** based on cultural and biochemical properties. All of the 6 isolates showed β-hemolysis on blood agar media enriched with 5% sheep blood. Gram-stained smears of the pure cultures exhibited clusters of Gram-positive cocci. The isolates also fermented mannitol with the color change of MSA and production of small yellow colonies. Isolates were positive for catalase and coagulase tests. The species identity of all 6 isolates could be confirmed by PCR amplification of the ***S. aureus***-specific chromosomal DNA fragment using ***23s rRNA*** primer for ***23s rRNA*** gene. The ability to synthesize classical enterotoxins was found in 3 of 6 (50%) isolates by using the qualitative PCR technique. The enterotoxin gene (*sec*) was identified in two isolates (33.3%), while the enterotoxin gene (*tst*) was identified in only one isolate (16.7%).

The study was also planned to investigate the histopathological changes of the liver of two groups of mice after intraperitoneal injection of one dose (0.1ml) of ***S. aureus*** (*sec*) and ***S. aureus*** (*tst*) aqueous solutions at a concentration of $5x10^8$ / 0.1ml. The relative weight of the liver of ***S. aureus*** (*tst*)-infected mice was significantly increased as compared with the control group and the ***S. aureus*** (*sec*)-infected. Liquid-filled abscesses appeared on the liver surfaces of the infected groups. Histopathological studies showed several microscopic changes in the liver of infected groups including inflammatory cells infiltration, hepatic cell degeneration, preiductal fibrosis, and the appearance of black spots thought to be colonies of ***S. aureus*** bacteria in association with inflammatory cells.

1. INTRODUCTION

Camels are important source for milk production in nomadic societies. Camel milk is supposed to have medicinal properties, as it contains insulin-like protein, so it has hypoglycemic effect. Milk is an excellent source of nutrients for human ***(Abrhaley and Leta, 2018)*** and, yet in a different context, it provides a suitable medium for microbial growth and metabolism. In raw milk, bacteria can affect the quality, safety and consumer acceptance of dairy products. Nonpathogenic bacteria may affect milk and milk products quality ***(Samaržija et al., 2012)***. Thus, many countries have milk quality regulations, including limits on the total number of bacteria in raw milk, to ensure the quality and safety of the final product. The number and types of microorganisms in milk, immediately after milking, are affected by several factors such as animal health, equipment cleanliness, season, and food. It is hypothesized that differences in feeding and housing strategies of cows may influence the microbial quality of milk ***(Swai and Schoonman, 2011)***.

Staphylococcus spp (***S. spp***) are microorganisms that are naturally present in milk and dairy products. ***Gabriela et al. (2009)*** reported that they are often associated with food-borne disease outbreaks due to the ability of some strains to produce a thermostable enterotoxins. Diseases are usually associated with coagulase and thermonuclease positive ***Staphylococcus aureus***

(S. aureus). Milk is a good substrate for ***S. aureus*** growth and enterotoxin production. Enterotoxins are thermostable to heat retaining some biological activity even after 28 minutes at 121^{o}C. The bacterium is also capable of producing several pathological conditions in human. Pathogens can invade the teat canal ascending toward the mammary parenchyma, then colonize, multiply and produce their toxins, and finally predispose to mastitis. So, teat skin should be free from microbial contamination lesions to maintain the health of animals ***(Ahmed et al., 2010)***. A high percentage of subclinical mastitis in camels is reported by several authors ***(Barbour et al., 1985; Abdurahman et al., 1995; Obeid et al., 1996; Almaw and Molla, 2000)***. The pathogenic bacteria reported by different scientific groups in camels are similar to those associated with mastitis in cows or other animals kept in traditional nomadic environments or camel farms ***(Barbour et al., 1985; Almaw and Molla, 2000)***.

In 1880, ***S. aureus*** was first discovered by a surgeon named Sir Clifton Smith in pus from surgical abscesses in Aberdeen, Scotland ***(Ogston, 1984)***. It is a nonmotile, non-sporeforming, Gram positive, aerobic facultative anaerobic coccus, with the appearance of grape-like clusters when viewed through a microscope. ***S. aureus*** cells are spherical and are about 1µm in diameter. They form a cluster arrangement because of their special division way. These cells divide in

three dimensional axis, and the new cells remain attached to each other followed by each division successively.

S. aureus is catalase positive and oxidase negative. The catalase test is an important, yet simple, method to distinguish staphylococci from streptococci, which are catalase negative ***(Raus and Love, 1983)***. Typical ***S. aureus*** has large, round, creamy smooth colonies with golden yellow color. Most strains have beta or alpha hemolysis when growing on blood agar plates ***(Kloos and Schleifer, 1975)***. ***S. aureus*** can survive for several hours on dry environmental surfaces and grow at a temperature range of 7 to 48^{O}C ***(Neely and Maley, 2000)***. There are about 32 described species of ***Staphylococcus (Kloos and Bannerman, 1994),*** but ***S. aureus*** is the only pathogen of increasing importance due to the rise in antibiotic resistance ***(Lowy, 1998)***.

Infectious agents have caused epidemic and endemic diseases involved in the deaths of hundreds of millions of humans, as well as significant animal morbidity and mortality throughout history ***(Musser and DeLeo, 2015)***. ***S. aureus*** is a widespread Gram-positive coccus that is both a human commensal bacterium and pathogen. Approximately 50% to 60% of individuals are intermittently or permanently colonized with ***S. aureus*** and, thus, there is relatively high potential for infections ***(Wertheim et al., 2005; Gorwitz et al., 2008)***. A relatively high percentage of healthy people are asymptomatically colonized with ***S. aureus*** in the anterior nares