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Phenotypic, Molecular Identification and Risk Factors Evaluation of *Staphylococcus aureus* Isolated from Skin Lesions in Sheep and Their Breeders

A Thesis

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

(قُلْ لَوْ كَانَ الْبَحْرُ مِدَادًا
لِكَلِمَاتِ رَبِّي لَنَفِدَ الْبَحْرُ قَبْلَ أَنْ
تَنْفَدَ كَلِمَاتُ رَبِّي
وَلَوْ جِئْنَا بِمِثْلِهِ مَدَدًا.)

صدق الله العظيم

سورة الكهف

الآية (109)

Dedication

To the one who carried the niche of science and flooded the universe with the light of the Holy Prophet Muhammad (peace be upon him)

To who spent the rest of jihad to reach desired and his soul covers from its attic with blessing what has been accomplished my father,

May God have mercy on his soul

To my kindergarten when my chest narrows the spring that Patent has shown me and drowned me with kindness and helped me to pray my loving mother

To candles that lit my life ... my children and who support me... my brothers and sisters to all those

I dedicate the fruit of my effort.

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ABSTRACT

Staphylococcus aureus is one of the dominant pathogenic bacteria among skin infections in human and animals.

The current study designed for Isolation and identification of *S. aureus* from sheep breeders and sheep by conventional method &Automated Vitek 2 system, Molecular identification of *S. aureus* using 23rRNA (staur4 and 6), Detection of methicillin resistant gene (*mec*A), Beta- lactamase gene (*blaZ*), Beta-hemolysin factor gene (*Hib*) and Identification of *S. aureus* biofilm producing gene (Inter Cellular Adhesions A & D) by conventional PCR; Studying antimicrobial susceptibility pattern for *S. aureus* by Kirby Bauer disk diffusion method, Evaluation of relationship between *S. aureus*, MRSA infections and possible risk factors in sheep breeders and sheep.

A total of 119 skins lesion samples collected from sheep breeders (44) and sheep (75) in the period since Oct. 2021 up to Feb. 2022, of Baladruze Distract – Kannan Regions and Baqubah Distract-Buhruz Regions. Swabs were cultured on mannitol salt agar, Blood agar, verified by Gram stain, Nigrosin stain, biochemical tests (Catalase, Coagulase, and DNase), confirming by VITEK2 technique and conventional PCR for (staur4 and 6) and methicillin resistant gene (*mec*A). Evaluation of antibiotic sensitivity for Methicillin, Oxacillin, Levofloxacin, Ofloxacin, Ciprofloxacin, Erythromycin, Gentamycin, Tetracycline, Rifampicin, Imipenem, Vancomycin and Chloramphenicol, Molecular detection by conventional PCR for beta hemolysin gene *(Hib)*, Inter Cellular Adhesions (*IcaA* & *IcaD*); Beta-lactamase gene (*blaZ*).

Staphylococcus aureus was isolated from 15/44, (34.09%) from Sheep breeders skin lesions, whereas MRSA was 6/15, (40%), *S. aureus* was isolated from 46/75, (61.33%) from sheep skin lesions and MRSA was 14/46, (30.43%).

Male sheep breeders are more infected (29.54%) with MRSA (11.36%),more than female breeders (4.54%) and MRSA (2.27%), the main age group infected with *S. aureus* among sheep breeders (51-60) years, by (13.63%), have the highest incidence of MRSA (11.36%).

A significant positive association was observed through direct contact with sheep skin lesions and hospitalization during the last 4 weeks and recent visits to Outpatient clinics.

It has also been observed that (40%) of sheep at the <age of 2.8 years are infected with skin lesions caused by *S. aureus*, while *S. aureus* infection was higher among male sheep (29.54%), MRSA (11.36%), versus in female (4.54%), MRSA (2.27%). *S. aureus* infection was higher among sheep female (41.33%), MRSA (12%).

Ewes have greater risk for infection with *S. aureus*, MRSA, among rams. Significant correlations were reported between infection with MRSA and breed Awassi have greater risk for infection with *S. aureus* for mixed breed, While Significant positive association to Sheep Abscess, Abrasions have risk to get infection with *S. aureus* versus sheep with intact skin

Intra cellular adhesion gene (*Ica*A), was detected (5/5), 100% of sheep breeders *S. aureus* isolates versus 4/5, (80%) of sheep isolates by Conventional PCR (*Ica*D) gene was not detected in all 10 isolates from sheep breeders and sheep. Beta hemolysin gene (*Hib*) was not detected in sheep breeder isolates versus 40% of sheep isolates. Methicillin resistant gene (*mec*A) detected among (40%) of sheep breeder of *S. aureus*, and in (30.43%) isolate of sheep. Beta-Lactamase gene (*blaZ*) in *S. aureus* isolate of sheep breeders and sheep was detected in 100% of selected isolates by Conventional PCR.

Staphylococcus aureus isolated from sheep breeders have resistance for members of antibiotics classes;' penicillins, polypeptides, and fluoroquinolones. Macrolide which include the following: methicillin, levofloxacin, Ofloxacin, erythromycin and vancomycin. A total of (40%) of *S. aureus* from sheep

breeders have multidrug resistant trait for Penicillins, Polypeptides, Fluoroquinolones, Macrolide antibiotics. While a Non multidrug resistant *S. aureus* from sheep breeders was reported for Penicillins (oxacillin, 4/15, 26%) and Polypeptides antibiotics (vancomycin 8/15, 53.33%). Absolute sensitivity was reported for gentamycin, tetracycline, rifampicin, Imipenem and chloramphenicol.

Levofloxacin and Ofloxacin resistance was reported in (34.78%) of sheep isolates. A total of (30%) of *S. aureus* have resistance for methicillin that was confirmed early by detection of *mec*A gene. Oxacillin and erythromycin resistance was reported in (17.39%). Vancomycin resistance was reported in (8.69%). Absolute sensitivity was reported for ciprofloxacin, gentamycin, tetracycline, rifampicin, Imipenem and chloramphenicol among sheep isolates.

Non Multidrug resistant *S. aureus* isolated from sheep have resistant for fluoroquinolones (17.39%), Penicillins (13.04%), polypeptide antibiotics, (8.69%). Multidrug resistant *S. aureus* isolated from sheep have resistance for Penicillins, Fluoroquinolones, Macrolide, (17.39%).

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List of Abbreviations

Abbreviate	TERM
Aap	**Accretion-attendant protein**
ABL	**Antimicrobial blue light**
Blaz- gene	**beta lactamase gene**
BHI	**Braine Heart Infusion**
BP	**Base pair**
CDC	**Centers for Disease Control**
Clf	**Clamping factor**
CLSI	**Clinical and Laboratory Standard Institute**
DNA	**De-oxy ribose Nucleic Acid**
DNase	**DNA hydrolytic enzyme**
ECM	**Extra Cellular Matrix**
EE	**Endogenous Endophthalmitis**
EPS	**Essential Parts *S. aureus***
ETs	**Extra foliative Toxins**
FAO	**Food Agriculture Organization**
FnbpA, B	**Fibronectin-binding proteins A & B**

Hib	**Beta hemolysin gene**
***ica*A**	**Inter cellular adhesion A gene**
***ica*A**	**Inter cellular adhesion D gene**
ICTV	**International Council Taxonomic Virology**
***mec*A**	**Mobile Element Complex type A**
MIC	**Minimum inhibitory concentrations**
MDR	**Multi drug resistance**
MRSA	**Methicillin Resistant *Staphylococcus aureus***
MSSA	**Methicillin Sensitive *Staphylococcus aureus***
HA-	**Hospital Acquired -Methicillin Resistant *S. aureus***
CA-	**Community Acquired-Methicillin Resistant *S. aureus***
LA-	**Livestock Associated-Methicillin Resistant *Staphylococcus aureus***
OIE	**Office International des Epizootics (France)** **(WOAH) World organization for animal health (English)**
PBP	**Penicillin-binding protein**
PDI	**Photo Dynamic Inactivation therapy**
PBS	**Phosphate Buffered Saline**

PCR	**Polymerase Chain Reaction**
PVL	**Panton-Valentine Leukocidin**
SCC *mec*	**Staphylococcal chromosome cassette *mec***
SEs	**Cito-Skeleton endotoxins**
SSSS	**Staphylococcal Scalded Skin Syndrome**
SEM	**Scanning Electron Microscope**
TSST-1	**Toxic Shock Syndrome Toxin-1**
TDR	**totally drug-resistant**
TEM	**Transmission Electron Microscope**
UK	**United Kingdom**
UTI	**Urinary Tract Infection**
UV	**Ultra Violet**
WHO	**World Health Organization**

CHAPTER ONE

INTRODUCION

1.1 Introduction

The *Staphylococcus aureus* capable on improving and obtaining its own strategies and provide resistance to different antimicrobial drugs, which is further line among the extraordinary collection belong to this infectious bacteria, thus development of resistance via different bacterial strains due to mismanagement and/or severe multiple usage of treatments antibiotics (Woodford and Livermore 2009).

Since 1880 when Scottish surgeon Sir Alexander Ogston, obtained a groups of bacteria in pus of surgical wound abscess that infected with, microscopically examined, described and named it Staphylococcus due to its appearance, later in 1884, Rosenbach differentiated it through colony golden pigmentation (aureus) as a genus type (Taylor and Unakal 2021).

Among the 30s of last century, Sir Alexander Fleming starts penicillin era to fight germs and bacteria, supposed extracted from mold, which contained active antibacterial agent, as a beta lactam ring which is powerful inhibitor of cell-wall biosynthesis Kranjec *et al.*, (2021) that prevents multiplications and colonization, thus considered to be antibiotics not bactericidal agents.

Historically the earliest records, in 3000 BC, in China uses of neglected soya beans to treat infected wounds, also 1600BC, in Greek using mold worn out cheese, nearly period in Egypt, offered treating with “rotten barley bread”. Healing usage of molds sustained without much attention of how the molds might be applying their effect (Page, 2012), that core of beta-lactam ring, starts to vanish by breaking down under the effects of Beta lactamase enzyme produced from new developing *Staphylococcus aureus* strains named MRSA noticed around 1960 as life threating extended phenomena, worldwide increasing yearly which causing changes in the roles of battle against pathogenic microbes (Pandey and Cascella 2019).

Emergence methicillin resistant *Staphylococcus aureus* MRSA have been reported in livestock were three different types three types depending on its transmissions through human population termed; Hospitals acquired, Community acquired and livestock associated (HA-MRSA), (CA-MRSA) and (LA-MRSA) respectively (Duraisamy *et al*., 2020), those infections have gained importance due to limitation of treatment possibilities against highly zoonotic MRSA (Chuang and Huang 2015), cross-species infections between humans and animals were documented for certain strains of MRSA (Iddah and Pete 2021).

MRSA was registered in numerous locations of the planet as probable causes life threating, septicemia forming, bone and cardiac disorders in mankind, while wildlife might playing potential role to infect accompanier human particularly, emergence increasing occurrences of LA-MRSA in hospitals and aggressive infections in humans (Cheung *et al*., 2021).

Authors reported another seriously virulent factors, although it conceder to be a microbiota harmless as a part of skin flora or nasopharyngeal inhabitation, related to skin diseases mostly due to its toxins, enzymes, external antigens and cell wall dipped in surrounded matrix called biofilm act as dangerous substance according to its adhering ability among different surfaces vital and non-vital for preserving and protecting through its metabolic activities, nutrition, defensing and structural dwelling allows to growth and division then colonizing, containing hemolysing, catalaseing, coagulaseing and clumping factors (Mistretta *et al*., 2019).

Researchers recently were trying to offer a variety of options for alternative treatments as possible solutions by new recommended methods by using short length photo waves (Reynoso *et al*., 2019), Nanoparticles techniques, (Malaekeh-Nikouei *et al*., 2020), bacteria-phages killing viruses (Ovchinnikov *et al*., 2020), combinations of antibiotics (Newstead *et al*., 2020) to remove or prevent bacterial adhesion, avoid formation of biofilm and use alternative plant-

based treatments extracts (Mala *et al.*, 2021), to solve the emergence of MRSA and MDR issues (Kranjec *et al.*, 2021).

1.2 Objective Aims of the Study

The current study designed to fulfill the following Objective aims;

[1] Isolation and identification of *Staphylococcus aureus* from sheep breeders and sheep by conventional laboratory method &automated Vitek 2 system.

[2] Identification of *Staphylococcus aureus* specific 23s RNA gene sequence (staur4 and staur6) by conventional PCR.

[3] Detection of methicillin resistant gene (*mecA*), Beta- lactamase gene (*blaZ*), Beta-hemolysin genes (*hib*) biofilm producing genes Inter Cellular Adhesion (*ica*A& D), by conventional PCR

[4] Study of antimicrobial susceptibility pattern for *Staphylococcus aureus*.

[5] Evaluation of relationship between *Staphylococcus aureus* infection and possible risk factors in human and sheep.