



جمهورية العراق
وزارة التعليم العالي
و البحث العلمي
جامعة ديالى
كلية الطب البيطري



التعريف السريري , البكتيري والجزئي لبكتيريا المكورات العنقودية الذهبية للاصابات المسالك البولية في النعاج و المربيين في ديالى و السليمانية - العراق

رسالة مقدمة إلى مجلس كلية الطب البيطري – جامعة ديالى
من متطلبات نيل درجة الماجستير علوم في الطب البيطري
(الامراض المشتركة)

من قبل
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إشراف

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Summary

In this study a total of 120 urine samples were collected from shepherds (49) and ewes (71) who suffered from urinary tract infections from the first of October 2022 to March 2023 in Sulaymaniyah province (Kalar district and surrounding villages) and Diyala province (Khanaqin district, villages of Qoratu district). Using Mannitol salt agar (as selective media) and employing traditional laboratory methods in addition to confirmatory techniques by the Vitek 2 system and conventional PCR, using specific primers (Staur 4, 6), and MRSA gene primers (*mecA*). Conventional PCR was used for the detection of Staphylokinase (*sak*), Lipase (*geh*), Intracellular Adhesion (*IcaA*), and protease (*SSPA*) genes.

Results:

The study includes (49) shepherds and (71) ewes suffering from clinical signs of urinary tract infections. A total of 27/49 (55.10%) of urine specimens give positive urine culture among shepherds, versus 35/71 (49.29%) among ewes. A total of 62 bacterial isolates were diagnosed primarily as *S. aureus* by traditional culture on mannitol salt agar and standard biochemical tests, but with further investigation with Vitek 2 system and conventional PCR using specific gene *S.aureus* 23srRNA primers (Staur 4, Staur 6). *S. aureus* was reported in 3/62 (4.83%), among shepherds, versus 7/62 (11.29%) among ewes with clinical manifestations of urinary tract infections.

S. equorum and *S. haemolyticus* detected in 8 out of 62 (12.90%). *S. xylosus*, *S. warneri*, *S. gallinarum*, *S. arlettae*, *Enterococcus faecalis* detected in 1 out of 62 bacterial isolates (1.61%) among shepherds. *S. warneri*, *S. cohnii ssp*, *Urealyticus*, *Alloiococcus otitis*, *Aerococcus viridans* detected in 1 out of 62 bacterial isolates (1.61%) among ewes with clinical manifestations of UTI.

S. aureus was reported mainly among shepherds in the age group (3–12 years). “A significant positive correlation was reported between age and infection with *S. aureus*. While in ewes *S.aureus* was reported mainly among

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antibiotics, fusidanes, ansamycins, and sulfonamides. A total of 1/3, 33% of *S. aureus* was sensitive to the polypeptide class, teicoplanin.

All *S. aureus* isolated from Ewes 7/7 (100%) have resistance to penicillins, cephalosporins, and methicillin. Resistance was detected by a cefoxitin screen test and confirmed early by detection of the MecA gene by conventional PCR. Resistance of *S. aureus* to polypeptide antibiotics was detected in 7/7, 100% for vancomycin and 6/7 (85.72%) for teicoplanin. Resistance of *S. aureus* to macrolide antibiotics was detected in 1/7 (14.28%) for azithromycin. Resistance of *S. aureus* to Lincosamide antibiotics, Clindamycin, was detected in 1/7 (14.28%). *S. aureus* isolated from ewes has an absolute sensitivity of 7/7 (100%) for the following classes: aminoglycosides, quinolones, macrolides (erythromycin), oxyazolidinone, tetracyclines, nitrofurantoin antibiotics, Fusidane, Ansamycins, and sulfonamides.

Chapter One

Introduction

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1.1 Introduction

One of the most vital System in animal's and human body is the urinary system, which performs functions including removing harmful waste from the body, controlling the components of body fluids, and regulating the hormone production that encourages the development of Red blood cells in the bone marrow (Lugo-Amador *et al.*, 2004).

When one or more urinary tract organs are invaded and infected, it's known as Urinary tract infections (UTI). UTIs are caused by bacteria that originate from the gastrointestinal tract, colonize the external genitalia, invade the bladder and urethra, and obstruct the flow of urine (Hydronephrosis), these bacteria can also originated from external sources, such as unhygienic practices, the normal flora of the genital areas, skin, and anus (Nicolle, 2008. Mohammed *et al.*, 2020).

As stated in the search of Zenad and Aljemaly (2007); the ureter transports the urine from the kidneys to the urinary bladder. The valve that stops urine from returning from the bladder to the ureter (posterior urethral valves) has been discovered to protect the urinary system against bacterial invasion. If invasion occurs, opportunistic bacteria created by the normal flora may become present. This particular infection spread from the kidneys to the ureter. Pyelonephritis is an inflammation of the whole kidney. When a bovine illness initially appears, it sometimes affects sheep (Mohammed *et al.*, 2020).

Gender, immune system issues, structural anomalies of the urinary tract, sexual activity, mixing medicines and antiseptics with the natural flora of the genital area, urinary catheters, and instrumentation are some of the risk factors for urinary tract infections (UTIs) (Nicolle, 2008. Foxman, 2003). Due to the urethra's close closeness to the anus, females are more likely than males to

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experience urinary tract infections (UTIs). In fact, every woman will experience at least one UTI in her lifetime (Nicolle, 2008).

Upon tissue examination, *Staphylococcus aureus* is a pyogenic pathogen with a typical appearance that is found nearly exclusively in extracellular spaces. This pathogen can cause a variety of life-threatening systemic diseases, including infective endocarditis, as well as localized infections like soft-tissue abscesses. It can also cause tissue destruction and host immune response defense by excreting enzymes, proteases, and exotoxins, which it uses to colonize several host tissue surfaces (Lowy, 2000).

Methicillin-resistant *Staphylococcus aureus* (MRSA) has been described in animals since 1972 (Devriese, Van Damme *et al.*, 1972). As a historically significant emerging zoonotic disease, MRSA is significant for both veterinary and public health. Serious diseases can be contracted by humans and animals from *Staphylococcus aureus*, which can withstand harsh environmental factors like sunshine and desiccation (Atoum *et al.*, 2003; Weese and van Duijkeren 2010). When compared to other species of ruminants, sheep have less frequent urinary tract infections; the most prevalent bacteria affected are *Corynebacterium pseudotuberculosis*, *Corynebacterium renale*, *Escherichia coli*, *Klebsiella pneumoniae*, *Actinomyces pyogenes*, *Staphylococcus aureus*, and *Proteus* (Petrovski, 2015).

1.2 Aims and objectives of the study

1. Isolation and identification of *S. aureus* from urine samples of shepherd and ewes by conventional methods and automated Vitek 2 system

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2. Evaluation of relationship between *S. aureus* infection and possible risk factors in human and sheep.
3. Molecular identification of *S. aureus* by detection of “*S. aureus* 23s RNA gene sequence specific primer (staur4 and staur6)” by conventional PCR.
4. Detection of methicillin resistant gene (*mecA*) by conventional PCR.
5. Identification of *S. aureus* biofilm producing gene (Inter Cellular Adhesion A ‘*icaA*’) by conventional PCR.
6. Identification of *S. aureus* virulence factors: Lipase (*geh*) gene, protease (*sspA*) gene, leukocidin (*lukS-PV*) genes, and staphylokinase (*sak*) gene by conventional PCR.
7. Examine the *S. aureus* antimicrobial susceptibility pattern by using Automated Vitek 2 system.