Emerging Challenges: Methicillin and Vancomycin Resistance in *Staphylococcus aureus* from Urinary Tract Infections in Ewes of Diyala Governorate, Iraq

Bak AIH[2], Al-Ezzy AIA[1], Al-Zubaidi RMH[2]

[1]*Department of pathology, College of Veterinary Medicine, University of Diyala, Iraq*
[2]*Department of Medicine, College of Veterinary Medicine, University of Diyala, Iraq*
Corresponding author: alizziibrahim@gmail.com

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Abstrak

Bak AIH, Al-Ezzy AIA, Al-Zubaidi RMH. 2024. Tantangan: resistensi methicillin dan vancomycin terhadap *Staphylococcus aureus* akibat infeksi saluran kemih pada domba betina di Provinsi Diyala, Irak. JITV 29(4):201-207. DOI:http://dx.doi.org /10.14334/jitv.v29i4.3407.

S. aureus yang resistan terhadap methicillin merupakan penyebab infeksi saluran kemih (ISK) yang jarang terjadi. Penelitian saat ini bertujuan untuk mengisolasi dan mengidentifikasi S. aureus dari sampel urin domba betina yang mengalami ISK dan menentukan pola sensitivitas antimikroba untuk resistan terhadap methicillin-Vancomycin. Sebanyak 71 sampel urin dikumpulkan. S. aureus diisolasi menggunakan agar garam manitol dan dikonfirmasi oleh sistem VETEK2 dan PCR konvensional, menggunakan primer (Staur 4, 6) dan (mecA). S. aureus diisolasi dari (9,85%). Semua S. aureus yang diisolasi dari domba betina, (100%) memiliki resistensi terhadap Penisilin, sefalosporin dan resistensi methicillin dideteksi oleh uji skrining cefoxitin dan dikonfirmasi oleh deteksi gen MecA. Resistensi s. aureus terhadap antibiotik polipeptida dideteksi pada 100% untuk vancomycin dan (85,72%) untuk Teicoplanin. Resistensi S. aureus terhadap antibiotik makrolida dan Lincosamides terdeteksi pada (14,28%) untuk Azitromisin dan Klindamisin masing-masing. aureus yang diisolasi dari domba betina memiliki sensitivitas absolut terhadap aminoglikosida, kuinolon, makrolida (Eritromisin), oksazolidinon, tetrasiklin, antibiotik nitrofuran, Fusidane, An-samycins dan Sulfonamida. Studi ini menemukan prevalensi MRSA yang signifikan di antara galur S. aureus yang diisolasi dari wanita dengan ISK. Ini menunjukkan tingkat resistensi yang tinggi terhadap antibiotik beta-laktam, yang menimbulkan tantangan untuk pengobatan yang efektif. Studi ini juga mengidentifikasi resistensi vankomisin di antara isolat MRSA. Vankomisin sering dianggap sebagai pertahanan garis terakhir terhadap bakteri yang resistan, sehingga keberadaan S. aureus yang resistan terhadap vankomisin mengkhawatirkan dan membatasi pilihan pengobatan.

Kata Kunci: Domba Betina, *Staphylococcus aureus,* Infeksi Saluran Kemih

Abstract

Bak AIH, Al-Ezzy AIA, Al-Zubaidi RMH. 2024. Emerging challenges: Methicillin and vancomycin resistance in *Staphylococcus aureus* from urinary tract infections in ewes of Diyala Governorate, Iraq. JITV 29(4):201-207. DOI:http://dx.doi.org/ 10.14334/jitv.v29i4.3407.

Methicillin resistant s. aureus is a rare cause of urinary tract infections (UTIs). Current study aims to Isolates and identify *S. aureus* from urine samples of ewes with UTIs and determination of antimicrobial sensitivity pattern for methicillin-Vancomycin resistant. A total of 71 urine samples were collected. S. aureus was isolated using mannitol salt agar and confirmed by VETEK2 system and conventional PCR, using (Staur 4, 6) and (mecA) primers. *S. aureus* was isolated from (9.85%). All *S. aureus* isolated from ewes, (100%) have resistance for Penicillines, cephalosporins and methicillin resistance was detected by cefoxitin screen test and confirmed by detection of MecA gene. Resistance of s. aureus to polypeptides antibiotics was detected in 100% for vancomycin and (85.72%) for Teicoplanin. Resistance of S. aureus to macrolides and Lincosamides antibiotics was detected in (14.28%) for Azithromycin and Clindamycin respectively. *S. aureus* isolated from ewes have absolute sensitivity for aminoglycosides, quinolones, macrolides (Erythromycin), oxazolidinone, tetracyclines, nitrofuran antibiotic, Fusidane, Ansamycins and Sulfonamides. The study found a significant prevalence of MRSA among the isolated strains of S. aureus from ewes with UTIs. This indicates a high level of resistance to beta-lactam antibiotics, posing challenges for effective treatment. The study also identified vancomycin resistance among the MRSA isolates. Vancomycin is often considered a last-line defense against resistant bacteria, so the presence of vancomycin-resistant S. aureus is concerning and limits treatment options.

Key Words: Ewes, *Staphylococcus aureus*, Urinary Tract Infection

INTRODUCTION

The urinary system is one of the most important systems in an animal's body. It removes harmful waste from the body and regulates the components of body fluids, as well as controlling hormone production which promotes bone marrow to red blood cell synthesis (Pugh et al. 2020) The colonization and infection of one or more urinary system sections is referred to as urinary tract infection (UTIs) (Mohammed et al. 2020). Bacteria infiltrate the gastrointestinal system and colonize the external genitalia, as well as invade the bladder and urethra and impede urine flow UTI also causes vascular damage in the urinary bladder, reducing kidney competence and interfering with metabolic end product excretion (El-Deeb & Elmoslemany 2016).

S. aureus is opportunistic and its virulence depends on enzymes and exotoxins, that contribute to causing a wide range of diseases in human (Cheung et al. 2021)and animals (Dai et al. 2019) . Its variable sensitivity and resistance for numerous antibiotics represent the major reason behind the cosmopolitan distribution (Rasmi et al. 2022). Production of β-lactamases was the main trait for *S. aureus* that cause continues failure in treatment with beta lactam antibiotics as penicillin. Methicillin-resistant *S. aureus* (MRSA) possesses reduced affinities for binding to β-lactam antibiotics by producing a specific penicillin-binding protein, PBP2 , resulting in β-lactam antibiotic resistance (Lade & Kim 2021; Bush & Bradford 2020). Methicillin resistance is extended to different antimicrobial agents, including “the aminoglycosides, macrolides, chloramphenicol, tetracycline, and fluoroquinolones (Cabrera et al. 2020). While (Bitrus et al. 2015) was reported resistant to all cephems, cephalosporins, and other β-lactams such as (amoxicillin-clavulanic acid, ticarcillin-clavulanic acid, ampicillin-sulbactam, carbapenems, and the piperacillin-tazobactam). This study aimed to isolation and characterization of *S.aureus* from urine samples of ewes with UTIs by classical bacteriological methods, Vitek 2 system and conventional PCR using Staur 4, 6 specific primers, Determine antimicrobial sensitivity pattern for methicillin-Vancomycin resistant s.aureus in ewes with urinary tract infections.

MATERIALS AND METHODS

Collection of urine specimens

Urine specimens were collected from 71 ewes with UTIs from 1st October 2022 to the end of May 2023. Samples were delivered to “the postgraduate lab.at the college of veterinary medicine –university of Diyala, Iraq” in a cool box.

Processing of specimens

Samples were cultured on MSA for 18-24 h. A golden yellow colonies were selected for full identification of MRSA through Vitek system 2 and PCR according to (Fajer et al. 2023).

Molecular detection of *S. aureus* specific and Methicillin resistance genes

Conventional PCR was applied to confirm the diagnosis and detection of methicillin resistance gene according to Table 1.

Antimicrobial Susceptibility Test (AMS)

AMS test was achieved by Vitek 2 system as stated by Clinical and Laboratory Standards Institute (Humphries et al. 2018 ; Aljboori et al. 2022; Fajer et al. 2023).

Ethical consideration

This study conducted according to the approval of an ethical review committee of pathology department, college of veterinary medicine, Diyala University, Iraq (Al-Khalidi et al. 2020; Hameed et al 2020; Hassan et al. 2020).

Table 1: Molecular markers used in conventional PCR for Detection of *S. aureus* and MRSA from urine specimens of ewes

Gene	Primers	Base pairs	Sequence (5’- 3’)	PCR Protocol			Reference
				Den.	Ann.	Ext.	
S.aureus 23srRNA	Staur4	1250bp	5’-ACGGA GTT AC A AAGGAC GAC-3’	94°C/ 45 sec	64°C/ 60 sec	72°C/ 2min	(Sheela 2017)
	Staur6		5’-AGCTCAGCCT TAAC GAG TAC-3’				
Methicillin Resistant Gene A	mecA-F	162bp	5-TCCAGATTACAACTTCAC CAGG-3	94°C/ 45sec	50°C/ 30sec	72°C/ 30sec	
	mecA-R		3-CCACTTCATATCTTGTAACG-5				

PCR= Polymerase Chain Reaction, MRSA= Methicillin-resistant *Staphylococcus aureus*; Den= denaturation; Ann= annealing, Ext= extention

Statistical analysis

All Calculations by using the Statistical Package of the Social Sciences for windows version 17 (SPSS, Armonk, NY: IBM Corp) (Hameed et al. 2024; Hameed & Al-Ezzy 2024).

RESULTS AND DISCUSSION

As shown in Table 2, S. aureus was isolated from 7/71, (9.85%) urine sample of ewes. All S. aureus isolates have resistant to methicillin by cefoxitin screening test in Vitek 2 system. All S. aureus have resistant for vancomycin. As shown in Table 3 and Figure 1 and Figure 2, all *S. aureus* isolates have Staur 4,6 gene and MecA A gene. Table 3 revealed that all *S. aureus* isolated have resistance for Penicillines and Cephalosporins. All *S. aureus* isolates have methicillin resistance that detected by Cefoxitin Screen test and confirmed early by detection of MecA gene. All *S. aureus* isolates have resistance to vancomycin polypeptides antibiotic and (85.72%) for Teicoplanin. Resistance of *S. aureus* to macrolides, mainly Azithromycin was detected in (14.28%). Resistance to Lincosamides antibiotics, Clindamycin was detected in 1/7, (14.28%) .*S. aureus* isolated from ewes have absolute sensitivity for the following classes Aminoglycosides, Quinolones, Macrolides (Erythromycin), oxazolidinone, Tetracyclines, nitrofuran antibiotic, Fusidane, Ansamycins and Sulfonamides.

S. aureus one of the rare pathogenic bacteria among urinary tract infection. In the current study, ***S.** aureus* was isolated from 7/71, (9.85%) urine samples of ewes. All *S. aureus* isolates have resistance for Penicillines, cephalosporins and methicillin which confirmed by detection of MecA gene. A total of 77 urine samples collected from ewes cultured on blood agar and mannitol salt agar which is a medium encouraging growth of certain bacteria while inhibiting the growth of other. As stated by Fajer et al. 2023 that as selective and differential medium, MSA contains a NaCL,7% and mannitol sugar1% inhibit most bacteria that makes MSA selective against most gram negative and selective for some gram-positive bacteria that tolerate high salt concentrations. As stated by Abubaker & Alythi (2021) that MSA contain a pH indicator, phenol red for detection of acid produced by mannitol-fermenting staphylococci which turn the *S. aureus* colonies in the medium appear yellowish in color. As stated by Anderson et al. (2013) that in addition to MSA which was used for the selective isolation of presumptive pathogenic Staphylococcus species, Gram stain, Catalase and Coagulase tests, Vitek2 system were used for confirmation. Current study come in line with that reported by Alzolibani et al. (2012) in which seven positive samples referred to conventional PCR and VITEK® 2 System confirm matching 99% . In the current study, the rate of *S. aureus* UTIs in ewes was 7/71, (9.85%). All isolates were MRSA which higher than that reported In north-western Greece by Papadopoulos et al. (2018) who found that 57.8% of specimens were *S. aureus* positive and only 3% MRSA positive. While in China (Zhou et al. 2017).

reported that *S. aureus* was isolated from 43.24% of specimens while in Italy, Giacinti et al. (2017) reported that *S. aureus* was isolated from 53.5% among dairy sheep farms and MRSA rates 7%. While in Norway Mørk et al. (2012) recoded *S. aureus* was (32.6%) in sheep.

In current study *S.aureus* 23srRNA gene sequence specific primers (staur4, 6,) for *S. aureus* was detected in all *S.aureus* isolates by Conventional PCR which come in line with that reported by Sheela & Krupanidhi (2017). All *S. aureus* have methicillin resistant gene, (*mecA*) by conventional PCR which is higher than that reported by Galia et al. (2019)

in Italy, they stated that MecA was detected among 58/65(89%) of *S. aureus* isolates. Acquisition of *mec*A renders β-lactams useless against MRSA and alternative therapies. In Italy, Mascaro et al. (2019) reported that in a dairy sheep farms, *S. aureus* isolate might act as a mec C-MRSA reservoir thus, it is recommended that laboratories must search for the *mecC* gene in all the *mecA*-negative isolate . Less percentage done by Omidi et al. (2020) of 146 *S. aureus* isolates, 24 (16.4%) carried *mec*A genes and identified as MRSA strains. In a study by Lakhundi & Zhang (2018) that counted mecA and its new homologues (mecB, mecC, and mecD) on thirteen types in more than ten Allele.

In a study by Joseph et al. (2018) they stated that most strains of *S. aureus* possess ability to produce beta-lactamases, an enzyme that can open beta-lactam rings in cephalosporin and penicillin. Some acquire resistance genes from the environments and/or from other bacteria and thus may exhibit resistance to antibiotics in other classes produced on plasmid encoded as class A β-lactamase (Nomura et al. 2020). Resistance for beta-lactam antibiotics usually by production of beta-lactamases; or by expression of penicillin-binding protein (PBP 2a), which is not susceptible to inhibition by beta-lactam antibiotics. *S. aureus* have beta-lactamase or PBP 2a-directed resistance (or both) (Lakhundi & Zhang 2018).

In the current study, *S. aureus* 100% resistant to methicillin that confirmed by detection of (mecA) gene which records resistant for Oxacillin. All s.aureus have resistance to vancomycin polypeptides antibiotics and 6/7, (85.72%) have resistance for Teicoplanin. Resistance of *S. aureus* to macrolides antibiotics, mainly Azithromycin and Lincosamides antibiotics, mainly Clindamycin was detected in 1/7, (14.28%). *S. aureus* isolated from ewes have absolute sensitivity for Aminoglycosides, Quinolones, Macrolides (Erythromycin), oxazolidinone, Tetracyclines, nitrofuran antibiotic, Fusidane, Ansamycins and Sulfonamides.

Table 2: Isolation rate of *S. aureus*, MRSA and VRSA from urine of ewes

Samples source	Sample number	A (%)	B (%)	C (%)
Ewes	71	7(9.85%)	7/7,(100%)	7/7 ,(100%)

A= Number of S. aureus isolates based on MSA growth, Vitek 2 system, and PCR; B= Number of MRSA from total positive samples based on Cefoxitin Screen test, conventional PCR using Mec A gene primers, C= number of VRSA from total samples based on Vitek 2 system; PCR= Polymerase Chain Reaction, MRSA= Methicillin-resistant *Staphylococcus aureus*

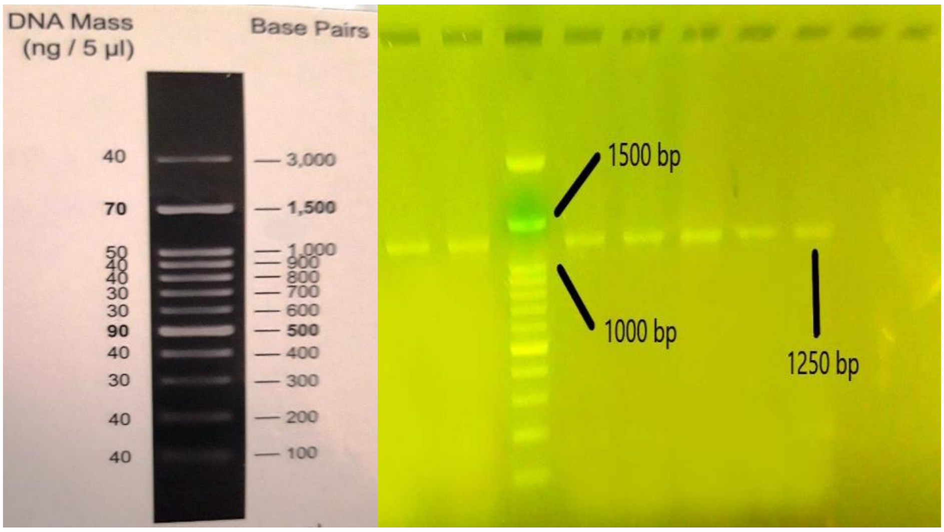

Figure 1. Amplification for staur primers 4&6 (1250bp) by conventional PCR for *S. aureus* isolated from urine of ewes

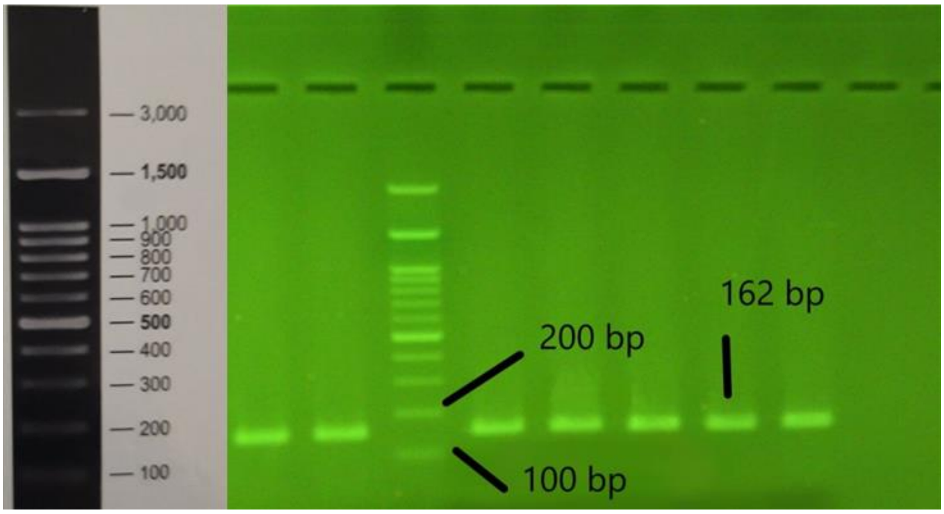

Figure 2. Amplification for methicillin Resistant gene (MecA) primer (1620bp) by Conventional PCR for S. aureus isolated from urine of ewes

In a study of Algammal et al. (2020) they stated that MRSA was determined by PCR and resistance to cefoxitin. Although Sallam et al. (2015) stated that antimicrobial resistance of MRSA detected by penicillin 93.4%, ampicillin 88.9%, and cloxacillin 83.3%, whereas .In Palestine (Azmi et al. 2019) claimed that MRSA isolates identified by cefoxitin disc diffusion and all were vancomycin sensitive and Gentamicin. In Italy, Giacinti et al. (2017) reported that 60.58% of MRSA were susceptible to all the antimicrobials drugs, and 39.42% were resistant to at least one antimicrobial drugs. In particular, 22.12% were resistant to tetracycline, 15.38% to sulfonomides, 13.46% to trimethoprim and sulfa methoxazole, and 8.65% to ampicillin, however only one isolate was resistant to both Fluoroquinolones and aminoglycosides, *S. aureus* isolates displaying resistance to oxacillin, cefoxitin, or both. Resistant to all the β-lactams tested and to erythromycin, streptomycin, kanamycin, and tetracycline.

Table 3. Sensitivity Pattern of S. aureus Isolated from Ewes with UTIs based on Vitek 2 system

Class of antimicrobial agents	Antimicrobial	MIC	Interpretation	No. (%) of s.aureus isolates	Class of antimicrobial agents	Antimicrobial	MIC	Interpretation	No. (%) of s.aureus isolates
Penicillines	Benzylpenicillin	>= 0.5	R	7/7,(100%)	Macrolides	Azithromycin		R	1/7, (14.28%)
	Amoxicillin Clavulanic acid		R	7/7,(100%)		Erythromycin	<= 0.25	S	7/7, (100%)
	Oxacillin	>=4	R	7/7,(100%)	Lincosamides	Clindamycin	<= 0.25	S R	6/7, (85.72%) 1/7, (14.28%)
Cephalosporins	Cefoxitin Screen	POS	Methicillin resistance	7/7,(100%)	oxazolidinone	Linezolid	2	S	7/7,(100%)
	Cefalexin		R	7/7,(100%)	Polypeptides	Teicoplanin	<=0.5	S R	1/7, (14.28%) 6/7, (85.72%)
	Cefazolin		R	7/7,(100%)		Vancomycin	*=32	R	7/7, (100%)
	Cefapime		R	7/7,(100%)					
Aminoglycosides	Gentamicin	<=0.5	S	7/7,(100%)	Tetracycline	Doxycycline		S	7/7,(100%)
	Tobramycin	<= 1	S	7/7,(100%)		Tetracycline	<=l	S	7/7,(100%)
Quinolones Fluoroquinolones	Ciprofloxacin		S	7/7,(100%)		Tigecycline	<= 0.12	S	7/7,(100%)
	Gatifloxacin		S	7/7,(100%)	nitrofuran antibiotic	Nitrofurantoin	<— 16	S	7/7,(100%)
	Levofloxacin	<= 0.12	S	7/7,(100%)	Fusidane	Fusidic Acid	<= 0.5	S	7/7,(100%)
	Moxifloxacin	<=0.25	S	7/7,(100%)	Ansamycins	Rifampicin	<= 0.5	S	7/7,(100%)
	Norfloxacin		S	7/7,(100%)	Sulfonamides	Trimethoprim/ Sulfamethoxazole	<= 10	s	7/7,(100%)

CONCLUSION

The study found a significant prevalence of MRSA among the isolated strains of S. aureus from ewes with UTIs. This indicates a high level of resistance to beta-lactam antibiotics, posing challenges for effective treatment. The study also identified vancomycin resistance among the MRSA isolates. Vancomycin is often considered a last-line defense against resistant bacteria, so the presence of vancomycin-resistant S. aureus is concerning and limits treatment options

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