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Phenotypic, Biochemical Identification And Antibiotic Sensitivity Pattern Of Staphylococcus Species From Urinary Tract Infections Among Female Sheep Breeders And Ewes With Special Emphasis To Methicillin And Vancomycin Resistant *Staphylococcus aureus*

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Abstract :

Aims: Isolation, phenotypic identification and antibiotic sensitivity pattern of staphylococcus species from urinary tract infections in female sheep breeders and ewes with special emphasis to methicillin and vancomycin resistant S. aureus

Methods: A total of 120 urine samples were collected from female sheep breeders (49) and ewes (71) with signs of UTI were cultured on mannitol salt agar and subjected to full biochemical identification by Vitek 2 system. Methicillin resistance was detected by Cefoxitin Screen test and MecA gene was detected by PCR.

Results:

S. aureus was isolated from 3(4.83%), in female sheep breeders and 7(11.29%) among ewe. S. aureus shown (100%) resistance for Penicillines and Cephalosporins, Methicillin which confirmed early by detection of MecA gene. All MRSA was resistant to Polypeptides, (100%) for Vancomycin and 2/3, (66%) for Teicoplanin in female sheep breeders. In ewes, All MRSA was resistant to polypeptides antibiotics, (100%) for vancomycin and 6/7, (85.72%) for Teicoplanin. Resistance of S. aureus to macrolides antibiotics was detected in 1/7, (14.28%) for Azithromycin. Resistance of S. aureus to Lincosamides antibiotics, Clindamycin was detected in 1/7, (14.28%)

Conclusion: Although, S. aureus constitutes a minimal in the form of frequency as a cause of UTIs in human and ewes, Methicillin and Vancomycin Resistant S. aureus should be considered seriously and potentially to get rid the possible sequels

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Introduction

The primary job of urinary system is for eliminating harmful waste from the body in human and animals and to the control of the body's fluid [1]. urinary tract infection (UTI) can be defined as the urothelium's inflammatory reaction to bacterial invasion and the second most prevalent kind of infection in the body[2]. The bacteria that cause UTIs originate in the gastrointestinal system and colonize the external genitalia, invading the bladder and urethra to obstruct the flow of urine [3].UTIs harm the vascular system of the bladder, which lowers kidney function and disrupts the excretion of metabolic end products[4]. Methicillin-Resistant S. aureus (MRSA) was identified as the primary cause of hospital-associated infections and as one of the most nosocomial bacteria globally [5]. S. aureus is related to animal mucous membranes and skin [6] . When Comparing sheep to other ruminant species, infections of the urinary tract have not been as common and most frequent outcome in sheep is inflammation of the kidneys[7]. This ultimately causes urinary tract infections, which result in significant economic losses, and lowers animal productivity, both quantitatively and qualitatively.

Current study aims to isolation, phenotypic identification and antibiotic sensitivity pattern of staphylococcus species. from urinary tract infections in female sheep breeders and ewes with special emphasis to methicillin and vancomycin resistant *S.aureus*

Materials and Methods

Ethical Agreement:

This cross-sectional study was approved by scientific committee of medicine department, college of veterinary medicine, university of Diyala, Iraq

Area of the study:

Current study was conducted in Khanaqin district of Diyala governorate which is located at the northeast of the capital Baghdad [8-10]and Kalar district of Sulaymaniyah governorate from the first of October 2022 to March 2023.

Samples:

One hundred and twenty urine samples were collected from patients with clinical signs of urinary tract infection, ewes (71 samples) and their breeders (49 samples),

Laboratory investigations:

In order to prevent contamination, the urine specimens were first cultured instantly on mannitol salt agar and incubated for 24 hours at 37 °C.[11, 12] .The morphological characteristics (colony size, shape, color, hemolysis, translucency, edge, elevation, and texture) on culture media and biochemical tests, antibiotics susceptibility test were used to identify *S.aureus*. Gram stain was applied to the isolates in order to observe how they responded to the stain and how they were arranged[12, 13].

Confirmatory Diagnosis of S. aureus:

Testing for antibiotic susceptibility and bacterial identification is done by the fully automated VITEK® 2 System. Based on the manufacturer's instructions, which called for planting urine samples—isolated from sheep and humans—on Mannitol Salt Agar (MSA) and incubating them for 24 hours at 37 °C, the diagnosis was made. Once bacterial colonies started to form on the medium, a pure colony was transferred into a sterile inoculating loop and combined with physiological normal saline in a manufacturer-approved tube [6, 11, 12, 14]

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Statistical analysis:

Statistical analysis depends on frequency analysis [15-17].Calculation down by the Statistical Package of the Social Sciences for windows version 17 (SPSS, Armonk, NY: IBM Corp) [18-20].

Results:

Population under Investigation

As shown in Table (1), the current study includes (49) females sheep breeders 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 female sheep breeders, versus 22 out of 49 (44.89%) give negative urine culture. A total of 35/71, (49.29%) of urine specimens gives positive urine culture among ewes, versus 36 out of 71 (50.70%) give negative urine culture.

Table 1: Population under investigation in current study

Source Of Urine Samples	Number of Individuals with positive urine culture	Number of Individuals with negative urine culture	Total number
Female Sheep breeders	27(55.10%)	22(44.89%)	49(100 %)
Ewes	35(49.29%)	36(50.70%)	71(100 %)

Spatial Distribution of Population under Investigation in Current Study

As shown in table (2), Current study was achieved in villages of Khanaqin district of Diyala governorate and of Kalar district of Sulaymaniyah governorate, as shown in figure (1). Barekah village was the main source of urine specimens19/120 (15.83%), followed by Tazade village 18/120, (15%). Minimum numbers of urine specimens were obtained from Gezhakan village, 5/120, (4.16%).

Table 2: Spatial Distribution of Population under Investigation in Current Study

Name of Village under investigation	Female Sheep breeders	Ewes	Total
Barika	5(4.16%)	14(11.66%)	19(15.83%)
Tazade	8(6.66%)	10(8.33%)	18(15%)
Ban Zamen	5(4.16%)	9(7.5%)	14(11.66%)
Berlot	6(5%)	6(5%)	12(10%)
Sayed Khalil	7(5.83%)	4(3.33%)	11(9.16%)
Top Askar	2(1.66%)	8(6.66%)	10(8.33%)
Haosh Kuro	5(4.16%)	5(4.16%)	10(8.33%)
Chala Rash	4(3.33%)	4(3.33%)	8(6.66%)
Qoratu	4(3.33%)	3 (2.5%)	7(5.83%)
Majeed Salar	2(1.66%)	4(3.33%)	6(5%)
Gezhakan	1(0.83%)	4(3.33%)	5(4.16%)
Total	49(40.83%)	71(59.16%)	120(100%)

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Bacterial Isolates from Female Sheep Breeders and Ewes with Clinical Manifestation of Urinary Tract Infections

As shown in table (3), a total number of 62 bacterial isolates that were diagnosed primarily as *Staphylococcus 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, primer (Staur4,Staur6)as shown in figure (1), *S.aureus* was reported in 3/62,(4.83%)among female sheep breeders with clinical manifestation of UTI versus 7/62 ,(11.29%) among ewes with clinical manifestation of Urinary tract infections . *S. epidermidis* were reported in 10/62,(16.12%) among female sheep breeders with clinical manifestation of UTI versus 10/62,(16.12%) among ewes with clinical manifestation of Urinary tract infections.

S.equorum and Staphylococcus haemolyticus were detected in 8 out of 62, (12.90%). *Xylosus*, *S. warneri,* S. gallinarum. *S.arlettae*, Enterococcus faecalis were recorded in 1out of 62 bacterial isolates (1.61%) among female sheep breeders with clinical manifestation of UTI. *S. warneri, S.cohnii* ssp. Urealyticus. Alloiococcus otitis, Aerococcus viridans were reported in 1 out of 62 bacterial isolates, (1.61%) among ewes with clinical manifestation of UTI.

Table (3): Bacterial Isolates from Female Sheep Breeders and Ewes with Clinical Manifestation of Urinary Tract Infections

Bacterial isolates from infected individuals	Female sheep breeders with clinical manifestation of UTI	Ewes with clinical manifestation of UTI	Total
Staphylococcus epidermidis	10(16.12%)	10(16.12%)	20(32.25%)
Staphylococcus aureus	3(4.83%)	7(11.29%)	10(16.12%)
Staphylococcus equorum	1(1.61%)	7(11.29%)	8(12.90%)
Staphylococcus haemolyticus	6(9.67%)	2(3.22%)	8(12.90%)
Staphylococcus lentus	2(3.22%)	2(3.22%)	4(6.45%)
Unknown	1(1.61%)	1(1.61%)	2(3.22%)
Staphylococcus xylosus	0(0%)	2(3.22%)	2(3.22%)
Staphylococcus warneri	1(1.61%)	1(1.61%)	2(3.22%)
Staphylococcus gallinarum	1(1.61%)	0(0%)	1(1.61%)
staphylococcus cohnii ssp. urealyticus	0(0%)	1(1.61%)	1(1.61%)
Staphylococcus arlettae	1(1.61%)	0(0%)	1(1.61%)
Enterococcus faecalis	1(1.61%)	0(0%)	1(1.61%)
Alloiococcus otitis	0(0%)	1(1.61%)	1(1.61%)
Aerococcus viridans	0(0%)	1(1.61%)	1(1.61%)
Total	27(43.55%)	35(56.45%)	62(100%)

Antibiotic Sensitivity Pattern for S. aureus Isolated from Female sheep Breeders and Ewes

As shown in table (4), Penicillins and cephalosporins are the antibiotic classes to which 3/3 (100%) of S. aureus isolated from female sheep breeders are resistant, as Table 2 illustrates. The cefoxitin screen test revealed

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methicillin resistance, indicating that 3/3 (100%) of S. aureus have resistance. The early discovery of the MecA gene further supported this finding. S. aureus was shown to be resistant to 2/3, 66% of teicoplanin and 3/3,100% of vancomycin when it came to polypeptide antibiotics.

Table 5 illustrates that 7/7 (100%) of the S. aureus isolates from ewes are resistant to cephalosporins and penicillins, two types of medicines. The cefoxitin screen test revealed methicillin resistance, indicating that 7/7 (100%) of S. aureus have resistance, which was verified early by finding the MecA gene.

7/7,100% of S. aureus samples showed resistance to vancomycin, and 6/7, 85.72% to teicoplanin, among other polypeptide antibiotics. 1/7 cases (14.28%) of *S.aureus* resistance to macrolide antibiotics was found to be azithromycin-resistant. Clindamycin, a Lincosamides antibiotic, was shown to be resistant to S. aureus in 1/7 (14.28%) cases.

1250bp

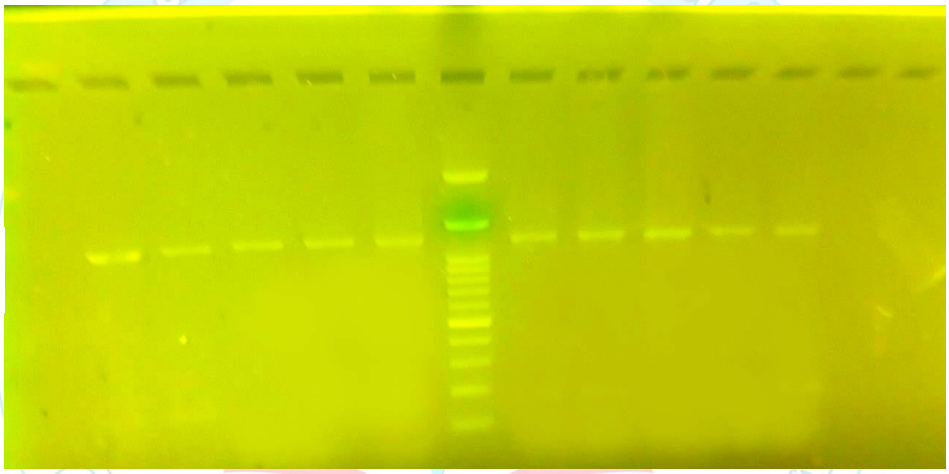

Figure (1) :Amplification for staur primers 4&6 (1250bp) by conventional PCR for *S. aureus* recovered from Urine sample of sheep breeder.

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Table (4): Antibiotic Sensitivity Pattern for S. aureus Isolated from Female sheep Breeders

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	3/3,(100%)	Macrolides	Azithromycin		S	3/3,(100%)
	Amoxicillin Clavulanic acid		R	3/3,(100%)		Erythromycin	<= 0.25	S	3/3,(100%)
	Oxacillin	>=4	R	3/3,(100%)	Lincosamides	Clindamycin	<= 0.25	S	3/3,(100%)
Cephalosporins	Cefoxitin Screen	Positive	Methicillin resistance	3/3,(100%)	oxazolidinone	Linezolid	2	S	3/3,(100%)
	Cefalexin		R	3/3,(100%)	Polypeptides	Teicoplanin	<=0.5	S	1/3, (33.33%)
								R	2/3, (66.67%)
	Cefazolin		R	3/3,(100%)		Vancomycin	*=32	R	3/3, (100%)
	Cefapime		R	3/3,(100%)					
Aminoglycosides	Gentamicin	<=0.5	S	3/3,(100%)	Tetracycline	Doxycycline		S	3/3,(100%)
	Tobramycin	<= 1	S	3/3,(100%)		Tetracycline	<=1	S	3/3,(100%)
Quinolones Fluoroquinolones	Ciprofloxacin		S	3/3,(100%)		Tigecycline	<= 0.12	S	3/3,(100%)
	Gatifloxacin		S	3/3,(100%)	nitrofuran antibiotic	Nitrofurantoin	<— 16	S	3/3,(100%)
	Levofloxacin	<= 0.12	S	3/3,(100%)	Fusidane	Fusidic Acid	°= 0.5	S	3/3,(100%)
	Moxifloxacin	<=0.25	S	3/3,(100%)	Ansamycins	Rifampicin	<= 0.5	S	3/3,(100%)
	Norfloxacin		S	3/3,(100%)	Sulfonamides	Trimethoprim/ Sulfamethoxazole	<= 10	s	3/3,(100%)

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Table (5): Antibiotic Sensitivity Pattern for S. aureus Isolated from Ewes

Class of antimicrobial agents	Antimicrobial	MIC	Interpretation	No. (%) of *S.aureus* isolates	ss 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	6/7, (85.72%)
								R	1/7, (14.28%)
Cephalosporins	Cefoxitin Screen	POS	Methicillin	7/7,(100%)	oxazolidinone	Linezolid	2	S	7/7,(100%)
	Cefalexin		R	7/7,(100%)	Polypeptides	Teicoplanin	<=0.5	S	1/7, (14.28%)
								R	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	<=1	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%)

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Discussion:

Results showed that in sheep *S.aureus* represent (11.29%) this rate disagree with [21], who found in study in Baghdad city that *S.aureus* represent (5.6%),with [22], who found in study in Iran that *S.aureus* represent (3.4%),[23],who found in study in Nigeria that *S.aureus* represent (90%), with [7] who found in study in Egypt that *S.aureus* represent (25%), with [24]who found in study in Algeria that *S.aureus* represent (30.4%), with [25] who found in study in Wasit city that *S.aureus* represent (20%).

In sheep breeders Specimens results of microbial positive growth *S.aureus* represent (4.83%), and this rate disagree with finding of [26] who found in study in Kufa city that *S.aureus* represent (8.5%), with[27] who found in study in Countries in Africa and Asia that *S.aureus* represent(8.3%), this rate disagree with[28] who found in study in Libya that *S.aureus* represent (0.5%), with [29]who found in study in Iran that *S.aureus* represent (74.7%), with [30]who found in study in India that *S.aureus* represent (68.18%). The improper use of antibiotics and the development of bacterial resistance in the microorganisms that cause urinary tract infections have been extensively documented in the scientific literature [31].Results showed the resistance of *S.aureus* in sheep (100%) to Penicillines, Cephalosporins, Cefoxitin, polypeptides antibiotics and vancomycin, while for Teicoplanin was (85.72%), and for macrolides antibiotics, Azithromycin, Lincosamides antibiotics, Clindamycin was (14.28%).When comparing these results with the study he conducted [32] , in Egypt we notice that the results are consistent with resistance to Penicillines (96%), Lincosamides and Clindamycin (28%) and not consistent with the resistance results to Cefoxitin (56 %), vancomycin (16 %). in other study done by [33], in Portugal the results are not consistent with resistance to cefoxitin (0.0 %),vancomycin(0.0 %), ciprofloxacin (16.7%,), Teicoplanin (0.0 %). in other study done by [34], in Spain the results are not consistent with resistance to Cephalosporins, Cefoxitin ((21.05%), Vancomycin (0.0 %), Teicoplanin (0.0 %), but results are consistent with resistance to Penicillines (89.47%), Lincosamides and Clindamycin (15.79%). While in female sheep breeders' resistance of S. aureus shown (100%) resistance for Penicillines, Cephalosporins, Cefoxitin, Vancomycin and, and (66%) for Teicoplanin, when comparing these results with study done by [35] in Kirkuk city agreed with resistance to Penicillines (92%), and disagreed with resistance to Cefoxitin (48%), Teicoplanin (12%), while the study he conducted [36]in Nigeria show incompatibility in resistance to Vancomycin (0.0 %). In study done by [37] in Babylon City Show compatibility in resistance to Penicillines (100%) and incompatibility in resistance to Vancomycin (60%), the result also show compatibility with study done by [38] in Saudi Arabia in resistance to Vancomycin(100%) and incompatibility in resistance to Penicillines (0.0 %).

In conclusion: although, *S. aureus* constitutes a minimal in the form of frequency as a cause of UTIs in human and ewes , Methicillin and Vancomycin Resistant S. aureus should be considered seriously and potentially to get rid the possible sequels.

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