Diyala Journal of Medicine

ORIGINAL RESEARCH
Published: 30 October 2023
Doi: 10.26505/DJM.25016940912

Molecular prevalence of MecA and Blaz Genes with phenotypic analysis of Antibiotic Sensitivity Pattern for S.aureus Isolated From Dermal lesions of Sheep Breeders In Diyala Governorate – Iraq

Zainab Bressam Fajer(DVM)[1], Ali Ibrahim Ali Al-Ezzy (PhD)[2], Ahmed H. AL-Zuhairi (PhD)[3]

[1,2,3] College of Veterinary Medicine, University of Diyala , Diyala , Iraq

Abstract

Background: *S. aureus* is one of the dominant bacterial pathogens among dermal infections in human and animals, which have resistance to different antimicrobial drugs.

Objective: Isolation and identification of *S.aureus* from skin lesions among sheep breeders by traditional methods, Vitek 2 system and PCR using Staur 4, 6 specific pri-mers for validation, Detection of genes (methicillin resistant (mecA), Beta- lac-tamase gene (blaZ) by conventional PCR ,determine antibiotic sensitivity pat-tern by kirby bauer disc diffusion method.

Patients and Methods: A total of 44 swaps were collected from sheep breeders suffered from variety of infected skin lesions to detect methicillin sensitive and resistant s. aureus by employing traditional methods in addition to confirmatory techniques through fast rapid VETEK2 system , detection of genes (methicillin resistant (mecA), Be-ta-lactamase gene (blaZ) by conventional PCR , and determine antibiotic sensi-tivity pattern by kirby bauer disc diffusion method.

Results: *S.aureus* was isolated from 15/44,(34.09%) of skin lesion of sheep breeders . A total of 6/15 ,(40%) were methicillin resistant S.aureus (MRSA) ,which represent (13.63%) from total samples .Beta lactamase gene primers was detected in all S.aureus isolates. A 6/15,(40%) of S.aureus have resistance for members of antibiotics classes, penicillins, polypeptides, fluoroquinolones, macrolide which include the following : methicillin that was confirmed early by detection of me-cA gene , levofloxacin , ofloxacin , erythromycin and vancomycin. A 6/15,(40%) of S.aureus have multidrug resistant trait for Penicillins, Polypep-tides, Fluoroquinolones, Macrolide antibiotics. Non multidrug resistant S.aureus was reported for Penicillins (oxacillin,4/15, 26%) and Polypeptides antibiotics (vancomycin 8/15, 53.33%) of *S.aureus* . Absolute sensitivity was reported for gentamycin, tetracycline, rifampicin, imipenem and chloramphenicol.

OPEN ACCESS

Correspondence Address: Ali Ibrahim Ali Al-Ezzy
College of Veterinary Medicine, University of Diyala , Diyala , Iraq
Email: alizziibrahim@gmail.com

Website: https://djm.uodiyala.edu.iq/index.php/djm

Received: 12 September 2022
Accepted: 21 September 2022
Published: 30 October 2023

ORIGINAL RESEARCH
Published: 30 October 2023
Doi: 10.26505/DJM.25016940912

Conclusion: Methicillin sensitive *S.aureus* was more common compared with MRSA isolated from dermal infections of sheep breeders. Blaz gene was predominantly ex-pressed by *S.aureus* isolates followed by Mec A gene. *S.aureus* have resistance for penicillins, polypeptides, fluoroquinolones, macrolide which include the fol-lowing : methicillin, levofloxacin , ofloxacin , erythromycin and vancomycin . S.aureus have multidrug resistant trait for Penicillins, Polypeptides, Fluoroquin-olones, Macrolide antibiotics. S.aureus was absolutely sensitive to gentamycin, tetracycline, rifampicin, imipenem and chloramphenicol.

Keywords: Staphylococcus aureus, skin ,sheep breeders ,antibiotics, MecA, Beta lactamase

Introduction

Staphylococcus aureus is the most abundant skin-colonizing bacteria and the most important cause of nosocomial and community-associated skin infec-tions [1]. *S. aureus* is an opportunistic patho¬gen, and its virulence depends on extracellular proteins(enzymes and exo-toxins) , that contribute to causing a wide range of diseases in human [2]and animals [3]. The main cause for the suc-cessful distribution is the variability and resistance patterns for many antibiotic [4]. S.aureus has become resistant to penicillin due to the production of β-lactamases enzymes that hydrolyze β-lactams antibiotics such as penicillin, thereby rendering them biologically inert. Methicillin-resistant Staphylococcus aureus (MRSA) possesses reduced affin-ities for binding to β-lactam antibiotics by producing a specific penicillin-bind-ing protein, PBP2 (or PBP2a), resulting in β-lactam antibiotic resistance [5, 6]. The resistance acquired by Methicillin (oxacillin [OX])-resistant S. aureus is extended to most of the commonly used antimicrobial agents, including the ami-noglycosides, macrolides, chlorampheni-col, tetracycline, and fluoroquinolo¬nes[7,8].They are also reported to be resistant to all cephems, cephalosporins, and other β-lactams (such as amoxicillin-clavulanic acid, ticarcillin-clavulanic acid, ampicil-lin-sulbactam, carbapenems, and the piperacillin-tazobactam) regardless of the in vitro test results obtained with those agents [9].

This study aimed to Isolation and identi-fication of *S.aureus* from skin lesions among sheep breeders by traditional methods, Vitek 2 system and PCR us-ing Staur 4, 6 specific primers for vali-dation, Detection of genes (methicillin resistant (mecA), Beta-lactamase gene (blaZ) by conventional PCR ,determine antibiotic sensitivity pattern by kirby bauer disc diffusion method.

Patients and Methods

Samples collection

A total of 44 dermal swab samples col-lected from sheep breeders with in-fected skin . sample collection was ex-tended from 1st October 2021 to the end of February 2022. All collected samples were sent to microbiology la-boratory at the college of veterinary medicine –university of Diyala, Iraq in a cool box for initial isolation on manni-tol salt agar for 18-24 h. A golden yel-low colonies were selected for further investigation; (Gram staining, Nigrosin capsule staining, catalase test, coagu-lase test, DNase), identified S. aureus and (MRSA) through Vitek system 2 and PCR[10].

ORIGINAL RESEARCH
Published: 30 October 2023
Doi: 10.26505/DJM.25016940912

Bacterial isolation

Each swab stick was immediately inoculated onto mannitol salt agar (Ox-oid Limited, England) plates and incu-bated at 37°C for 24 h. The organisms were isolated aseptically and character-ized using established microbiological methods, including colonial morpholo-gy, Gram stain characteristics, catalase, and coagulase tests [11]. Isolates that were Gram-positive cocci, catalase-positive, and coagulates human plasma were considered S. aureus. The main methods are coagulate and catalase test as mentioned above. Therefore, no need to mention the additional bio-chemical test because they are not so significant in S. aureus identification.

Molecular diagnosis for *S. aureus* and antimicrobial drug resistant genes :

PCR based detection was applied ac-cording to the instructions of references as illustrated in Table (1)

Antimicrobial Susceptibility Test:

All positive samples cultured on mannitol agar were submitted for anti-microbial susceptibility testing on Mueller- Hinton agar as stated by Clin-ical and Laboratory Standards Institute [12, 13]. Susceptibility was tested to antibiotics illustrated in Table (2). As stated by CLSI guidelines, *S.aureus* isolates were classified to (susceptible, intermediate, or resistant), as shown in Table (3).

Statistical Analysis

Calculation down by the Statistical Package of the Social Sciences for windows version 17 (SPSS, Armonk, NY: IBM Corp) [26, 27]. Pearson's chi-square and Pearson's correlation coeffi-cient was utilized for the correlation between the changeable of 2 test. P value of ≤ 0.05 and ≤ 0.01(2-tailed) were set to be statistically important [28,29]

Results

As shown in table (4) ,15/44, (34.09%) of samples from skin lesion of sheep breeders were positive for S.aureus on mannitol salt agar (MSA) , con-firmed by Vitek 2 system and conven-tional PCR by using S.aureus 23s RNA gene sequence specific primer (staur4 and staur6) as shown in Figure (1). On the other hand, a total of 6/15 ,(40%) were methicillin resistant S.aureus (MRSA) ,which represent (13.63%) from total samples according to methicillin resistance on Muller Hin-ton medium and results of conventional PCR by using *S.aureus* (MecA gene) as shown in Figure (2) .Beta lactamase gene primers was detected in all S.aureus isolates as shown in Table(5) and Figure (3).

As shown in Table (6) and Figure (4), 6/15,(40%) of S.aureus have resistance for members of antibiotics classes, penicillins, polypeptides, fluoroquin-olones, macrolide which include the following : methicillin that was con-firmed early by detection of mecA gene , levofloxacin , ofloxacin , eryth-romycin and vancomycin .

A shown in Table (7), 6/15,(40%) of S.aureus have multidrug resistant trait for Penicillins, Polypep-tides, Fluoroquinolones, Macrolide an-tibiotics. Non multidrug resistant S.aureus was reported for Penicillins (oxacillin,4/15, 26%) and Polypeptides antibiotics (vancomycin 8/15, 53.33%) of S.aureus . Absolute sensitivity was reported for gentamycin, tetracycline, rifampicin, imipenem and chloramphen-icol.

ORIGINAL RESEARCH
Published: 30 October 2023
Doi: 10.26505/DJM.25016940912

Table (1): Primers Used For PCR Based Detection Of *S.aureus* and Antimicrobial Drug Resistant Genes

Gene	Primers	Base pair	Sequence (5'- 3')	PCR Protocol			Reference
				Denaturation	Annealing	Extension	
***S.aureus* 23srRNA**	Staur4	1250bp	5'-ACGGA GTT AC A AAGGAC GAC-3'	94 oC/ 45 sec	64 oC / 60 sec	72 oC / 2min	[30]
	Staur6		5'-AGCTCAGCCT TAAC GAG TAC-3'				
Methicillin Resistant Gene A	*mec*A-F	162bp	5-TCCAGATTACAACTTCAC CAGG-3	94 oC / 45sec	50 oC / 30sec	72 oC / 30sec	[30]
	*mec*A-R		3-CCACTTCATATCTTGTAACG-5				
Beta lactamase gene	Blaz -F	517 bp	5'-AAGAGATTTGCCTAT GC TTC-3'	94oC /4min	94oC /60 secon	94oC /60 secor	[30]
	Blaz-R		5'-GCTTGACCACTTTTAT C A GC-3'				

Table (2): Antibiotic Discs Utilized Throughout The Study[31]

Antibiotic	Abbreviation	Weight
Methicillin	MET	5 µg
Oxacillin	OX	5 µg
Levofloxacin	LE	5 µg
Ofloxacin	OF	5 µg
Erythromycin	E	15 µg
Gentamicin	GEN	10 µg
Tetracycline	TE	30 µg
Rifampicin	RIF	5 µg
Imipenem	IPM	10 µg
Vancomycin	VA	30 µg
Ciprofloxacin	CIP	5 µg
Chloramphenicol	C	30 µg

Table (3): Criteria for Antibiotic Sensitivity of *S.aureus* [31]

Antimicrobial category	Antibiotic	Sensitive more than (mm) ≥	Intermediate (mm)	Resistant (less than) (mm)
Penicillins	Methicillin (5µg)	22	17-22	17
	Oxacillin(5µg)	13	11-12	10
Fluoroquinolones	Levofloxacin (5µg)	19	16-18	15
	Ofloxacin (5µg)	18	15-17	14
	Ciprofloxacin (5µg)	21	16-20	15
Macrolides	Erythromycin(15µg)	21	18-20	18
Aminoglycosides	Gentamycin(10µg)	15	13-14	12
Tetracyclines	Tetracycline(30µg)	19	15-18	14
Ansamycins	Rifampicin(5µg)	20	17-19	16
Carbapenems	Imipenem (10µg)	16	14-15	13
Polypeptides	Vancomycin (30µg)	21		17
phenicols	Chloramphenicol (30µg	18	13-17	12

ORIGINAL RESEARCH
Published: 30 October 2023
Doi: 10.26505/DJM.25016940912

Table (4): Isolation Rate of *S. aureus* & MRSA From skin of Sheep Breeders

Source of skin swabs	Total no. of samples	No.(%) Of *S. aureus* Isolates	No.(%) Of MRSA from positive samples	No.(%) Of MRSA from total samples
Sheep breeders	44	15(34.09%)	6/15,(40%)	6/44,(13.63%)

Table (5): Conventional PCR based Detection of Staur4,6, mecA and BlaZ genes of *S.aureus* isolated From skin of Sheep breeders

Source	Staur 4,6	Mec A	Blaz
Sheep breeders	15/44 (34.09%)	6/15 (40%)	5/5 (100%)

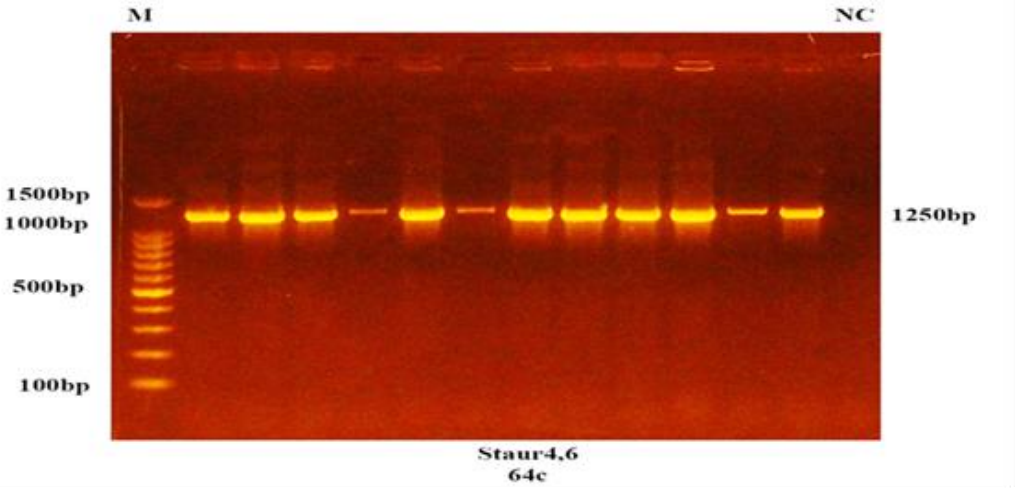

Figure (1): Amplification for staur primers 4&6 (1250bp) by conventional polymerase chain reaction for S. aureus recovered from skin lesions of sheep breeder.NC: Negative control.

ORIGINAL RESEARCH
Published: 30 October 2023
Doi: 10.26505/DJM.25016940912

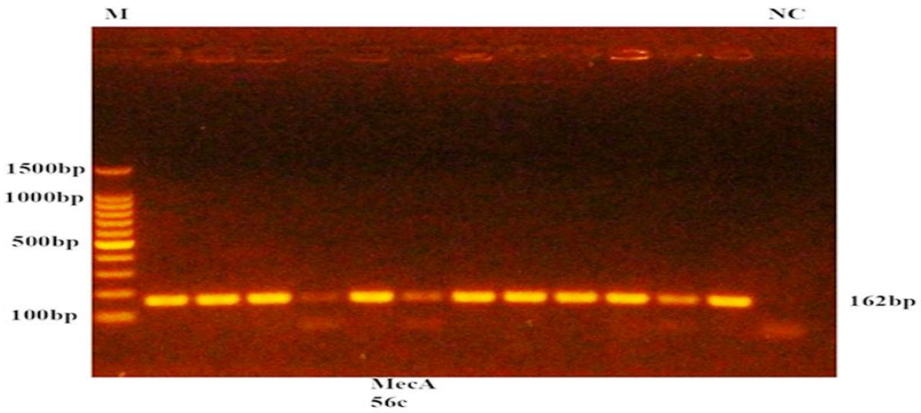

Figure (2): Amplification MecA (162bp) by conventional polymerase chain reaction for S. aureus recovered from skin **lesions**

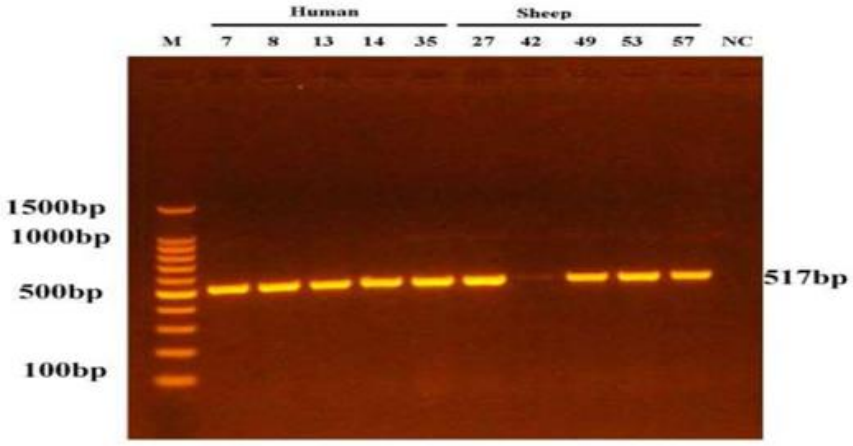

Figure (3): amplification *blaZ*(517 bp) By conventional polymers chain reaction for *S. aureus* recovered from skin lesion of Sheep breeders

ORIGINAL RESEARCH
Published: 30 October 2023
Doi: 10.26505/DJM.25016940912

Table (6): Antibiotic Sensitivity Pattern For *S.aureus* Isolated From Sheep breeders

Antibiotic	Minimum inhibition zone Diameter (mm)	Maximum inhibition zone Diameter (mm)	Mean± SE Inhibition zone Diameter (mm)	Sensitive No.(%)	Intermediate No.(%)	Resistant No.(%)	Total No. of Isolates
Methicillin	**9**	**22**	**16.87± 1.352**	**0(0%)**	**9(60%)**	**6(40%)**	**15(100%)**
Oxacillin	**9**	**23**	**17.20 ± 1.192**	**11(73.33%)**	**0(0%)**	**4(26.67%)**	
Levofloxacin	**12**	**25**	**18.00 ± 1.231**	**9(60%)**	**0(0%)**	**6(40%)**	
Ofloxacin	**11**	**25**	**17.67 ± 1.326**	**9(60%)**	**0(0%)**	**6(40%)**	
Ciprofloxacin	**20**	**29**	**24.40 ± 0.735**	**14(93.33%)**	**1(6.67%)**	**0(0%)**	
Erythromycin	**11**	**24**	**18.53 ± 1.305**	**9(60%)**	**0(0%)**	**6(40%)**	
Gentamycin	**22**	**28**	**25.33 ±0 .475**	**15(100%)**	**0(0%)**	**0(0%)**	
Tetracycline	**19**	**26**	**21.93 ± 0.492**	**15(100%)**	**0(0%)**	**0(0%)**	
Rifampicin	**24**	**33**	**28.53 ± 0.616**	**15(100%)**	**0(0%)**	**0(0%)**	
Imipenem	**30**	**33**	**31.13 ± 0.256**	**15(100%)**	**0(0%)**	**0(0%)**	
Vancomycin	**17**	**22**	**18.40 ±0 .375**	**1(6.67%)**	**0(0%)**	**14(93.33%)**	
Chloramphenicol	**20**	**25**	**23.00 ±0 .447**	**15(100%)**	**0(0%)**	**0(0%)**	

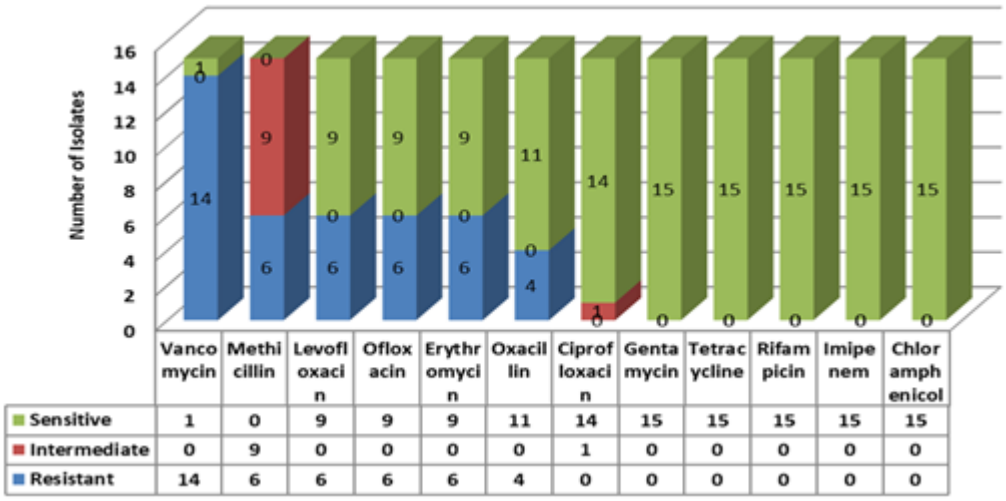

Figure (4): Antimicrobial susceptibility of *S.aureus* Isolated From sheep

ORIGINAL RESEARCH
Published: 30 October 2023
Doi: 10.26505/DJM.25016940912

Table (7): Drug resistance among S.aureus isolated from sheep Breeders

Classification of *S.aureus* according to antimicrobial drugs resistant	**Resistance for Antimicrobial class**	**No.(%)**
Non Multidrug resistant isolates	Penicillins Polypeptides antibiotics	4/15, (26.67%) 8/15,(53.33%)
Multidrug resistant isolates	Penicillins, Polypeptides, Fluoroquinolones, Macrolide	6/15, (40 %)
Pan Drug resistant isolates	None	(0%)
Total		15,(100%)

Discussion

S.aureus one of the dominant pathogenic bacteria among skin infec-tions, the aim of this study isolation and identification of S.aureus and MRSA in skin lesions of sheep breeders in addition to molecular detection through PCR assay for genes (staur4,6, MecA, blaz). A 44 skin lesion samples collected from sheep breeders cultured on Blood agar isolated and mannitol salt agar (MSA) which is a medium en-couraging growth of certain bacteria while inhibiting the growth of others, short time distinguishes. Contains a high concentration of salt7% + Manni-tol sugar1% + Agar (solidifying agent) + Enzymatic digest of casein + Enzy-matic digest of animal tissue + Beef extract + Phenol red indicator) which inhibit most bacteria that makes MSA selective against most gram negative and selective for some gram positive bacteria that tolerate high salt concen-trations. It is also a differential medium for mannitol-fermenting staphylococci [32] containing carbohydrate mannitol and the indicator phenol red, a pH in-dicator for detecting acid produced by mannitol-fermenting staphylococci. S. aureus produces yellow colonies, by acidic byproduct formation that causes the phenol red in the agar to turn yel-low. It is used for the selective isolation of staphylococcus species [33], verified by Gram stain, Nigrosin stain, biochemical tests (Catalase, Coagulase, DNase), confirming technique by Vi-tek2 system then evaluation of antibi-otic sensitivity, 10 pure positive sam-ples (five for each) referred to conven-tional PCR, same result in this study finds on a Vitek® 2 system of bioMé-rieux Inc. automated machine confirm matching 96%, 94% at different incu-bation periods according to the manu-facturers procedures [34].

In current study S.aureus 23srRNA gene sequence specific pri-mers (staur4, 6,) for S.aureus was de-tected in all S.aureus isolates by con-ventional PCR which come in line with that reported by [30].

This study reported S. aureus from sheep breeders skin infections ,15/44,(34.09%) and among those infected with MRSA ,6/15 ,(40%) in Diyala Iraq. This study was lower than that reported by [35]in which S.aureus was recovered from 40% ,MRSA from 17% of human samples. On the other hand, [35] postulated that" The presence of MRSA obtained among the veterinary staff and the students possesses great dissemination of MRSA in the commu-nity due to the environmental setting of rearing a small number of animals in the household very to humans.

ORIGINAL RESEARCH
Published: 30 October 2023
Doi: 10.26505/DJM.25016940912

Current results come closely to that reported by [36] stated that "a 15% MRSA nasal colonization rate among preclinical students in Nepal". In addi-tion, [37] reported that MRSA was isolated from 18% among veterinary staff in a small animal referral hospital in the UK . However, current results come in contrary with that reported by [38] ,stated that MRSA was isolated from 7% of veterinary staff and 6.5% reported among veterinary personnel [39] also disagree with [40] ,who stated that MRSA was Isolated from 3.4% among contact people handling animals in households, most of which work in the veterinary profession .on the other hand current study disagree with [41],stated that MRSA was isolated from 1.1% of sheep farmers in South-ern Italy.

The differences in the MRSA in-fection rate among humans may likely be due to "the difference in contact hours between humans and animals, the timing and site of sample collections, previous antibiotic therapy, geograph-ical location, and the protocols applied to search for carriage (sites tested and enrichment protocols such as culture method) and antibiotic usage restriction law in the study area". Furthermore, the observation in the present study could probably suggest "the possible occur-rence of community-acquired MRSA and livestock-associated MRSA in the study area due to the lack of strict laws guiding against the unnecessary use of antibiotics in the study area".

In a study by[42] ,they counted mecA and its new homologues (mecB, mecC, and mecD) on 13 types in more than ten Allele. Resistance bestowed by the mecA gene product is demonstrat-ed via a reduced rate of β-lactam-mediated enzyme acylation and decreased affinity for β-lactams compared to that of native PBPs. The crystal structure of the mecA gene product (i.e., PBP2a) provided the structural basis for this resistance. PBP2a is an elongated protein with a transpeptidase domain, a transmembrane domain, and a non-penicillin-binding domain, which possesses an allosteric site [43]. Com-pared to the active sites of native PBPs, the active site of PBP2a is less accessi-ble to β-lactams, as it is located in a narrow extended cleft. Hence, it does not affect the synthesis of peptidoglycan, given the antibiotic strength reached in vivo [42].

Molecular detection, beta-Lactamase gene in *S. aureus* isolate of sheep breeders by conventional PCR, gene (blaZ) detected among 5 pure isolate from breeders ,(5/5) 100% of S. aureus. In a study by [44] ,stated 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 ex-hibit resistance to antibiotics in other classes produced on plasmid encoded as class A β-lactamase (penicillinase)[45]. hence, its hydrolytic activity against oxacillin, cephems, and carbapenems. Additionally, site-directed mutagenesis within amino acid sequences showed that an alanine at position 112 of BlaZ plays an important role in the hydrolysis of oxacillin [45]. They are two mechanisms for resistance of beta-lactam antibiotics. One is pro-duction of beta-lactamases; enzymes hydrolytically destroy beta-lactams. Other is expression of penicillin-binding protein (PBP 2a), which is

ORIGINAL RESEARCH
Published: 30 October 2023
Doi: 10.26505/DJM.25016940912

not susceptible to inhibition by beta-lactam antibiotics. S. aureus either beta-lactamase or PBP 2a-directed resistance (or both)[42].

This study detect antibiotic sensi-tivity pattern for S. aureus isolated from sheep breeders in which (34.09%) was confirmed as methicillin sensitive S.aureus , and A total of 6/15 ,(40%) were methicillin resistant S.aureus (MRSA) ,which represent (13.63%) from total samples confirmed by detection of (mecA) gene .Beta lac-tamase gene primers was detected in all S.aureus isolates. A 6/15,(40%) of S.aureus have resistance for members of antibiotics classes, penicillins, poly-peptides, fluoroquinolones, macrolide which include the following : methicil-lin that was confirmed early by detec-tion of mecA gene , levofloxacin , of-loxacin , erythromycin and vancomy-cin . A 6/15,(40%) of S.aureus have multidrug resistant trait for Penicillins, Polypeptides, Fluoroquinolones, Mac-rolide antibiotics. Non multidrug re-sistant S.aureus was reported for Peni-cillins (oxacillin,4/15, 26%) and Poly-peptides antibiotics (vancomycin 8/15, 53.33%) of *S.aureus*.

Absolute sensi-tivity was reported for gentamycin, tet-racycline, rifampicin, imipenem and chloramphenicol.While [46]recorded that MRSA was determined by PCR and resistance to cefoxitin. Although [47] stated that antimicrobial resistance of MRSA detected by penicillin 93.4%, ampicillin 88.9%, and cloxacillin 83.3%, whereas .In Palestine [48] claimed that MRSA isolates identified by cefoxitin disc diffusion and all were vancomycin sensitive and Gentamicin.

Numerous studies confirm that MRSA always exhibits resistance to multiple antimicrobial agents, including; penicil-lin, methicillin, oxacillin, cefoxitin, amoxicillin-clavulanic acid, amoxicillin-sulbactam, quinolones, macrolides, cephalosporins, tetracycline, and chlo-ramphenicol [49]. Multi drug resistant trait MRSA always not affected by the first line of antibiotic treatment in most cases, many studies illustrated MRSA resistance to the new generations of antibiotics such as vancomycin, linezol-id and daptomycin [50]. The release of β-lactamase enzyme by S. aureus is the main cause of penicillin and penicillin derivatives resistance, while the mecA gene (encodes for Penicillin-binding protein production) is responsible for methicillin resistance. The mecA gene is found on the MRSA chromosome (SCCmec); seven types of SCCmec were identified up to date. There are seven types of SCCmec (I–VII). The production of MRSA penicillin-binding protein is considered the most im-portant cause of penicillin and methicil-lin resistance [51].

Conclusions

Methicillin sensitive S.aureus was more common compared with MRSA isolated from dermal infections of sheep breed-ers. Blaz gene was predominantly ex-pressed by S.aureus isolates followed by Mec A gene. S.aureus have resistance for penicillins, polypeptides, fluoro-quinolones, macrolide which include the following : methicillin, levofloxacin , ofloxacin , erythromycin and vanco-mycin . S.aureus have multidrug re-sistant trait for Penicillins, Polypeptides, Fluoroquinolones, Macrolide antibiot-ics.

Recommendations

The findings of this study indicate a high Further investigations for molecular mecha-

ORIGINAL RESEARCH
Published: 30 October 2023
Doi: 10.26505/DJM.25016940912

nisms of drug resistance by S.aureus to dimin-ish its spread throughout the local community *S.aureus* was absolutely sensitive to gentamycin, tetracycline, rifampicin, imipenem and chloramphenicol.

Source of funding: The current study was funded by our charges with no any other funding sources elsewhere.

Ethical clearance: Ethical approval was obtained from the College of Medicine / University of Diyala ethical committee for this study.

Conflict of interest: Nil

References

[1]Turner NA, Sharma-Kuinkel BK, Maskarinec SA, Eichenberger EM, Shah PP, Carugati M, et al. Methicillin-resistant Staphylococcus aureus: an overview of basic and clinical research. Nature Reviews Microbiology. 2019;17(4):203-18.

[2]Cheung GY, Bae JS, Otto M. Pathogenicity and virulence of Staphylococcus aureus. Virulence. 2021;12(1):547-69.

[3] Dai J, Wu S, Huang J, Wu Q, Zhang F, Zhang J, et al. Prevalence and characterization of Staphylococcus aureus isolated from pasteurized milk in China. Frontiers in microbiology. 2019;10:641.

[4] Rasmi AH, Ahmed EF, Darwish AMA, Gad GFM. Virulence genes distributed among Staphylococcus aureus causing wound infections and their correlation to antibiotic resistance. BMC Infectious Diseases. 2022;22(1):1-12.

[5] Lade H, Kim J-S. Bacterial targets of antibiotics in methicillin-resistant Staphylococcus aureus. Antibiotics. 2021;10(4):398.

[6] Bush K, Bradford PA. Epidemiology of β-lactamase-producing pathogens. Clinical microbiology reviews. 2020;33(2):e00047-19.

[7] Kavya UR, Laxmi S, Ramkumar V. Effect of intravenous dexmedetomidine administered as bolus or as bolus-plus-infusion on subarachnoid anesthesia with hyperbaric bupivacaine. Journal of Anaesthesiology, Clinical Pharmacology. 2018;34(1):46.

[8] Cabrera R, Fernández-Barat L, Motos A, López-Aladid R, Vázquez N, Panigada M, et al. Molecular characterization of methicillin-resistant Staphylococcus aureus clinical strains from the endotracheal tubes of patients with nosocomial pneumonia. Antimicrobial Resistance & Infection Control. 2020;9(1):1-10.

[9] Bitrus AA, Zakaria Z, Bejo SK, Othman S. Persistence of Antibacterial Resistance and Virulence Gene Profile of Methicillin Resistant Staphylococcus Aureus (MRSA) Isolated From Humans and Animals. Pakistan Veterinary Journal. 2016;36(1).

[10] Fajer ZB, Al-Ezzy AIA, Al-Zuhairi AH. Evaluation of risk factors for dermal infection with Staphylococcus aureus and MRSA among Sheep In Diyala Governorate, Iraq. Diyala Journal for Veterinary Sciences. 2022;1(5):8-37.

[11] Becker K, Heilmann C, Peters G. Coagulase-negative staphylococci. Clinical microbiology reviews. 2014;27(4):870-926.

[12] Humphries RM, Ambler J, Mitchell SL, Castanheira M, Dingle T, Hindler JA, et al. CLSI methods development and standardization working group best practices for evaluation of antimicrobial susceptibility

ORIGINAL RESEARCH
Published: 30 October 2023
Doi: 10.26505/DJM.25016940912

tests. Journal of clinical microbiology. 2018;56(4):e01934-17.

[13]Wayne P. Clinical and laboratory standards institute. Performance standards for antimicrobial susceptibility testing. 2011.

[14] Al-Ezzy AIA. Isolation Of Malassezia Furfur And Evaluation Of Ivermectin And Calvatia Craniiformis As A Novel Antifungal Agents For Pityriasis Versicolor With Special Refer To Risk Factors …. IJCPR. 2017;8(4):311-9.

[15] Humadi A, AL-Ezzy A, Mohammed A. Role Of Acrylonitrile Toxicity In Lung of Albino Male Rats. Diyala Journal for Veterinary sciences. 2021;1(2):93-9.

[16] Hameed M, AL-Ezzy A, Jalil W, Al-Khalidi A. Physiological Protective Effects of Ascorbic acid Versus d-l-α-tocopheryl acetate -Sodium Selenite Combination in Mice under experimental Sodium Nitrate …. biochemical and cellular archives. 2020;20(1).

[17] Al-Khalidi MAAH, AL-Ezzy A. Effect Of Drinking Water Quality On physiological Blood Parameters And Performance Of Laying Hens In Diyala province-Iraq. Biochemical and Cellular Archives. 2020;20(1):2649-54.

[18] Al-Khalidi A, Hameed M, Al-Ezzy A. Effects Of Saccharomyces cerevisiae As Probiotic On Blood Indices ,Humoral Immunity and Performance Of Isa Brown Laying Hens In Diyala Province-Iraq. Biochemical and Cellular Archives. 2020;20(1).

[19] Al-Khalidi A, Al-Ezzy A, Hameed M. Correlation Between Aspergillosis And Renal Function Profile Analysis In Broilers Of Diyala Province -Iraq. Diyala Journal of Agricultural Sciences. 2018;10:177-93.

[20] Akram Ahmed Hassan EJK, Al-Ezzy, Ali Ibrahim Ali, MS Hameed. Correlation Between Aspergillosis And Liver Function Profile Analysis In Broiler. Research Journal of Pharmaceutical, Biological and Chemical Sciences 8 (5 …. 2017;8(5):432-42.

[21]Al-Ezzy A. Evaluation of the Performance of Melia Azedarach for skin wound healing in donkeys: clinical and histopathological study. AJPCT. 2015;3:1-9.

[22] Al-Ezzy A. Heamatological Changes Associated with Gastrointestinal Parasites Infection in Domestic Animals attended to Outpatient Clinic of Faculty of Veterinary Medicine of Diyala …. International journal of innovation and applied studies. 2014;9(3):1266-.

[23] Al-Ezzy A. Clinical, Epidemiological And Laboratory Investigations Of Mange infestation In Sheep In Khalis City-Diyala Province In Iraq. Biotechnology International. 2014;8(1):1-10.

[24] Awad AK, Al-Ezzy AIA, Jameel GH. Phenotypic Identification and Molecular Characterization of Malassezia spp. isolated from Pityriasis versicolor patients with special emphasis to risk factors in Diyala province, Iraq. Open access Macedonian journal of medical sciences. 2019;7(5):707.

[25] AL-Ezzy AIA. In Situ Nick End Labeling as a Molecular Immunopathological Indicator for the Severity of DNA Fragmentationand Gastroduodenal Tissue Damage among H. Pylori Cag APositive Patients. Indian Journal of Science and Technology. 2016;9(2).

[26]AL-Ezzy AIA, Kadhim AT. Comprehensive Evaluation For The Life Style And Zoonotic Risk Factors Associated With Cryptosporidium Parvum Infection In

ORIGINAL RESEARCH
Published: 30 October 2023
Doi: 10.26505/DJM.25016940912

Children Under Five Years. Diyala Journal For Veterinary Sciences. 2021;1(2):77-92.
[27] AL-Ezzy AIA. Chromotrope Gram Hot And Giemsa Staining Techniques As Alternatives For Ziehl–Neelsen Hot Stains For Detection Of C. Parvum Infection In Children And Calves. Diyala Journal for Veterinary Sciences. 2021;1(3):100-11.
[28] Al-Ezzy AIA, Kadhim AT. Evaluation For sociodemographic Risk Factors associated with Cryptosporidium Parvum Infection In Children under Five years. Diyala Journal For Veterinary Sciences. 2021;1(2):100-13.
[29] Jameel GH, Al-Ezzy AIA. Evaluation of Antifungal Activity of Calvatia craniiformis and Ivermectin as Novel Alternative Therapies for Aspergillus niger Associated Acute Otitis Media with Special Refer to Socio Demographic Factors Among Rural Children of Diyala Province-Iraq. International Journal of Pharmaceutical and Clinical Research. 2017;9(8):581-9.
[30] Sheela GM. Study of pathogenic factors of Staphylococcus aureus from clinical cases of livestock and poultry. 2017.
[31] HiMedia. Antimicrobial Susceptibility Systems. India: HiMedia 2020.
[32] Abubaker NS, Alythi AG. The Presence Of Mec A Gene In Methicillin–Resistant Staphylococcus Aureus Strains (Mrsa) Isolated From Surfaces Of Plants In Al–Beida Hospital Garden. European Journal Of Pharmaceutical And Medical Research. 2021;8(3):5-9.
[33] Anderson C, Johnson T, Case C, Cappuccino J, Sherman N. Great adventures in the microbiology laboratory. Pearson California, USA; 2013.
[34] Alzolibani AA, Al Robaee AA, Al Shobaili HA, Bilal JA, Ahmad MI, Saif GB. Documentation of vancomycin-resistant Staphylococcus aureus (VRSA) among children with atopic dermatitis in the Qassim region, Saudi Arabia. Acta Dermatovenerol Alp Pannonica Adriat. 2012;21(3):51-3.
[35] Jauro S, Hamman MM, Malgwi KD, Musa JA, Ngoshe YB, Gulani IA, et al. Antimicrobial resistance pattern of methicillin-resistant Staphylococcus aureus isolated from sheep and humans in Veterinary Hospital Maiduguri, Nigeria. Veterinary World. 2022;15(4).
[36] Ansari S, Gautam R, Shrestha S, Ansari SR, Subedi SN, Chhetri MR. Risk factors assessment for nasal colonization of Staphylococcus aureus and its methicillin resistant strains among pre-clinical medical students of Nepal. BMC research notes. 2016;9(1):1-8.
[37] Loeffler A, Boag AK, Sung J, Lindsay JA, Guardabassi L, Dalsgaard A, et al. Prevalence of methicillin-resistant Staphylococcus aureus among staff and pets in a small animal referral hospital in the UK. Journal of Antimicrobial Chemotherapy. 2005;56(4):692-7.
[38] Espadale E, Pinchbeck G, Williams NJ, Timofte D, McIntyre KM, Schmidt VM. Are the hands of veterinary staff a reservoir for antimicrobial-resistant bacteria? A randomized study to evaluate two hand hygiene rubs in a veterinary hospital. Microbial Drug Resistance. 2018;24(10):1607-16.
[39] Mai-Siyama I, Okon K, Adamu N, Askira U, Isyaka T, Adamu S, et al. Methicillin-resistant Staphylococcus aureus (MRSA) colonization rate among ruminant

ORIGINAL RESEARCH
Published: 30 October 2023
Doi: 10.26505/DJM.25016940912

animals slaughtered for human consumption and contact persons in Maiduguri, Nigeria. African Journal of Microbiology Research. 2014;8(27):2643-9.

[40] Hanselman BA, Kruth SA, Rousseau J, Weese JS. Coagulase positive staphylococcal colonization of humans and their household pets. The Canadian Veterinary Journal. 2009;50(9):954.

[41] Mascaro V, Squillace L, Nobile CG, Papadopoli R, Bosch T, Schouls LM, et al. Prevalence of methicillin-resistant Staphylococcus aureus (MRSA) carriage and pattern of antibiotic resistance among sheep farmers from Southern Italy. Infection and Drug Resistance. 2019;12:2561.

[42] Lakhundi S, Zhang K. Methicillin-resistant Staphylococcus aureus: molecular characterization, evolution, and epidemiology. Clinical microbiology reviews. 2018;31(4):e00020-18.

[43] Peacock SJ, Paterson GK. Mechanisms of methicillin resistance in Staphylococcus aureus. Annu Rev Biochem. 2015;84(1):577-601.

[44] Joseph W, Oti B, Tsaku A, Ajegena S, Ajegena B. Molecular Detection of Beta-Lactam Resistance Genes in Staphylococcus Aureus Isolated From Women in Nasarawa State, Nigeria. International Journal of Healthcare and Medical Sciences. 2018;4(5):60-5.

[45] Nomura R, Nakaminami H, Takasao K, Muramatsu S, Kato Y, Wajima T, et al. A class A β-lactamase produced by borderline oxacillin-resistant Staphylococcus aureus hydrolyses oxacillin. Journal of Global Antimicrobial Resistance. 2020;22:244-7.

[46] Algammal AM, Hetta HF, Elkelish A, Alkhalifah DHH, Hozzein WN, Batiha GE-S, et al. Methicillin-Resistant Staphylococcus aureus (MRSA): one health perspective approach to the bacterium epidemiology, virulence factors, antibiotic-resistance, and zoonotic impact. Infection and Drug Resistance. 2020;13:3255.

[47] Sallam KI, Abd-Elghany SM, Elhadidy M, Tamura T. Molecular characterization and antimicrobial resistance profile of methicillin-resistant Staphylococcus aureus in retail chicken. Journal of Food Protection. 2015;78(10):1879-84.

[48] Azmi K, Qrei W, Abdeen Z. Screening of genes encoding adhesion factors and biofilm production in methicillin resistant strains of Staphylococcus aureus isolated from Palestinian patients. BMC genomics. 2019;20(1):1-12.

[49] Weese JS, van Duijkeren E. Methicillin-resistant Staphylococcus aureus and Staphylococcus pseudintermedius in veterinary medicine. Veterinary microbiology. 2010;140(3-4):418-29.

[50] Traczewski MM, Katz BD, Steenbergen JN, Brown SD. Inhibitory and bactericidal activities of daptomycin, vancomycin, and teicoplanin against methicillin-resistant Staphylococcus aureus isolates collected from 1985 to 2007. Antimicrobial Agents and Chemotherapy. 2009;53(5):1735-8.

[51] Pournaras S, J Sabat A, Grundmann H, Hendrix R, Tsakris A, W Friedrich A. Driving forces of mechanisms regulating oxacillin-resistance phenotypes of MRSA: truly oxacillin-susceptible mecA-positive Staphylococcus aureus clinical isolates also exist. Current Pharmaceutical Design. 2015;21(16):2048-53.

Diyala Journal of Medicine

ORIGINAL RESEARCH
Published: 30 October 2023
Doi: 10.26505/DJM.25016940912

الانتشار الجزيئي لجينات MecA و Blaz مع تحليل النمط الظاهري لحساسية المضادات الحيوية للمكورات العنقودية الذهبية المعزولة من الآفات الجلدية لمربي الأغنام في محافظة ديالى – العراق

زينب بريسم فجر [1], علي ابراهيم علي العزي [2] , احمد حنش الزهيري [3]

الملخص

خلفية الدراسة: المكورات العنقودية الذهبية هي واحدة من مسببات الأمراض البكتيرية المهيمنة بين عدوى الدير-مال في الإنسان والحيوان ، والتي لديها مقاومة للأدوية المختلفة المضادة للكروبي.

اهداف الدراسة: لعزل وتحديد البكتيريا العنقودية الذهبية من الآفات الجلدية بين مربي الأغنام بالطرق التقليدية ، نظام الفايتك السريع و بواسطة تفاعل البوليميراز المتسلسل التقليدي باستخدام Staur 4, 6 ، بادئات محددة للتحقق من الصحة ، الكشف عن الجينات (مقاومة الميثيسيلين (mecA) ، جين بيتا لاكتاماز (blaZ) بواسطة تفاعل البوليميراز المتسلسل التقليدي ، تحديد نمط المضادات الحيوية بطريقة انتشار قرص كيربي باور.

المرضى والطرائق: تم جمع ما مجموعه 44 مقايضة من مربي الأغنام الذين عانوا من آفات جلدية مصابة مختلفة للكشف عن حساسية ومقاومة الميثيسيلين. المذهبة من خلال استخدام الطرق التقليدية بالإضافة إلى التقنيات المؤكدة من خلال نظام الفايتك السريع ، والكشف عن الجينات (مقاومة الميثيسيلين (mecA) ، وجين بيتا لاكتاماز (blaZ) بواسطة بواسطة تفاعل البوليميراز المتسلسل التقليدي ، وتحديد نمط الحساسية المضادة للحيوية عن طريق طريقة انتشار قرص كيربي باور.

النتائج: تم عزل المكورات العنقودية الذهبية من 44/15 ، (34.09٪) من الآفات الجلدية لمربي الأغنام. اجمالى 15/6 (40٪) من بكتريا المكورات العنقودية الذهبية المقاومة للميثيسيلين تمثل (13.63٪) من مجموع العينات. (6/15 A، (40٪) من بكتريا المكورة العنقودية الذهبيه لديها مقاومة لأعضاء فئات المضادات الحيوية ، البنسلين ، عديد الببتيدات ، الفلوروكينولونات ، الماكروليد والتي تشمل ما يلي: الميثيسيلين الذي تم تأكيده مبكرًا عن طريق الكشف عن جين ميك أ ، الليفوفلوكساسين ، أوفلوكساسين ، الاريثروميسين والفانكومايسين. 15/6 (40٪) من بكتريا المكورة العنقودية الذهبيه لها سمات مقاومة للأدوية المتعددة للبنسلين ، بولييبب-تيدس ، الفلوروكينولونات ، المضادات الحيوية ماكرولايد. تم الإبلاغ عن بكتريا المكورة العنقودية الذهبيه غير المقاومة للبنسلين (أوكساسيللين ، 15/4 ، 26٪) والمضادات الحيوية متعددة الببتيدات (فانكومايسين 15/8 ، 53.33٪) من بكتريا المكورة العنقودية الذهبيه. تم الإبلاغ عن حساسية مطلقة للجنتاميسين والتتراسيكلين والريفامبيسين والإيميبينيم والكلورامفينيكول.

الاستنتاجات: المكورات العنقودية الذهبية الحساسة للميثيسيلين كانت أكثر شيوعًا مقارنة بالمكورات العنقودية الذهبية المقاومة للميثيسيلين المعزولة من العدوى الجلدية لمربي الأغنام. تم التعبير عن جين Blaz بشكل مسبق بواسطة عزلات المكورات العنقودية الذهبية تليها جين Mec A. المكورات العنقودية الذهبية لديها مقاومة للبنسلين ، عديد الببتيدات ، الفلوروكينولونات ، الماكروليد والتي تشمل ما يلي: ميثيسيلين ، ليفوفلوكساسين ، أوفلوكساسين ، إريثروميسين وفانكومايسين. المكورات العنقودية الذهبية لها سمات مقاومة للأدوية المتعددة للبنسلين ، بولي بيب-تيدس ، الفلوروكينولونات ، ماكرولايد. المكورات العنقودية الذهبية كانت حساسة تمامًا للجنتاميسين والتتراسيكلين والريفامبيسين والإيميبينيم والكلورامفينيكول.

الكلمات المفتاحية: المكورات العنقودية الذهبية ، الجلد ، مربي الأغنام ، المضادات الحيوية ، ميك أ ، بيتا لاكتاماز

البريد الالكتروني: alizziibrahim@gmail.com

تاريخ استلام البحث: 12 أيلول 2022

تاريخ قبول البحث: 21 أيلول 2022

[3,2,1] كلية الطب البيطري - جامعة ديالى - ديالى – العراق