

Detection of *mecA*, *icaA*, *hla*, *sea* genes, and histological changes in mice for *Staphylococcus aureus* isolated from vaginosis in Iraqi women

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ARTICLE INFO

Received 30 March 2024
Revised 3 July 2024
Accepted 15 July 2024
Published 31 December 2024

Keywords :

Staphylococcus Aureus,
mecA ,*icaA* ,*hla* ,*Sea*

Citation: H. M. Abase, E. A. Hussain, J. Basrah Res. (Sci.) 50(2), 9 (2024).
[DOI:https://doi.org/10.56714/bjrs.50.2.2](https://doi.org/10.56714/bjrs.50.2.2)

ABSTRACT

Staphylococcus aureus causes critical infections in humans such as urinary infections, mastitis, pneumonia, meningitis, endocarditis, and osteomyelitis. It represents the main cause of nosocomial infection in surgical wounds. This study aimed to investigate the prevalence of *S.aureus* resistance and its virulence by using genetic markers. 150 samples were collected from vagina swabs for pregnant and nonpregnant women admitted to Ibn Al-Baladi and Al-Imamain Al-Kadhimain hospitals/Iraq. The study was performed from July to October 2023. The results showed that 13 isolates of *S.aureus* have *mecA* genes at (86.7%), 11 isolates have *icaA* gene (73.3%), and 8 isolates have the *sea* gene (53.3%), while *hla* gene was found in all 15 isolates (100%). *S.aureus* isolates showed histopathological changes in liver ,lung and vagina of mice .There was a prevalence of methicillin resistance *S. aureus* due to the presence resistance gene in most isolates. *S.aureus* isolates in this study showed their virulence through the occurrence of histopathological changes in liver ,lung and vagina .

1. Introduction

Staphylococcus aureus causes critical infections in humans, such as urinary infections, mastitis, pneumonia, meningitis, endocarditis, and osteomyelitis. It represents the main cause of nosocomial infection in surgical wounds [1,2,3]. *Staphylococcus* spp. belong to the family of Micrococcaceae. These are gram-positive, non-spore-forming, non-motile, and facultative anaerobic, or aerobic. There are more than twenty species that are known for the genus of *Staphylococcus*, spread in different habitats. *Staphylococcus* colonizes the surface of skin, dermal glands, and mucosal membranes in humans and animals [4,5]. Also, in one Iraqi study, it was isolated of *Staphylococcus aureus* from burn wounds with a high percentage of 25% compared with other bacteria [6]. Other Iraqi studies diagnosed *Staphylococcus aureus* infection in the urinary system of pregnant women [7]. A previous study detected *S.aureus* in the nasal cavity of workers in health care. The prevalence of MRSA was 94% among *S. aureus* [8]. Also, it isolated *S. aureus* in the nasal cavity from nurses who worked in two hospitals in Baghdad. Where the percentage of infection was 38.4% and 37.5% of *S. aureus* isolates [9]. The molecular diagnosis applications for pathogenic bacteria have improved the results of septic cases, particularly in synchronism with antibiotics management programs[10]. MRSA

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MRSA (methicillin-resistant *Staphylococcus aureus*) contagion still to be an important reason for illness and death in the world and forms a challenge to treatment efficiency [11].

The *mecA* gene detection considers the key mechanism of methicillin impedance in staphylococcus species, enabling the evolution of fast molecular screening to recognize between MRSA colonies and methicillin-sensitive *S. aureus* (MSSA) via PCR. Whereas, MRSA detection directly in clinical samples represents a challenge, where the *mecA* gene may exist in the methicillin resistance strain of coagulase-negative staphylococcus, which is found in different types of clinical samples [12]. In the last decades, *S. aureus* has developed resistance to many drugs worldwide due to the common antibiotics used. The development of *S. aureus* resistance is referred to by many mechanisms, involving the generation of enzymes that suppress antimicrobial factors, the anti-microbial efflux pump activated, the reduction of permeability in the cell wall of bacteria to antibiotics, and the modulation of target position to the antibiotic [13]. The appearance of virulent and resistant drug strains of *S. aureus*, especially MRSA, causes a critical challenge to the therapy of staphylococcus infections. The methicillin resistance strains are more resistant to many antibiotics (beta-lactams, macrolides, aminoglycosides,) and therefore difficult to treat infection. The penicillin resistance mechanism is beta-lactamase generation to suppress penicillin via the destroy ring of beta-lactam. Another mechanism related to the existence of penicillin-binding protein 2a encoded via *mecA* gene, that found on SCCmec (staphylococcus cassette chromosome mec) [14]. *S. aureus* pathogenicity is associated with different virulence genes, such as *S.aureus* enterotoxins (SEs), and hemolysins (*hla* and *hlb*), which contribute to the adhesion, colonization, and invasion tissue , consequently inducing pathogenicity [15]. SEA (Staphylococcal enterotoxin A) is one of the most commonly involved enterotoxins in outbreaks of food poisoning [16]. The *icaA* (intercellular adhesion gene A) gene responsible for PIA (polysaccharide intercellular adhesin) poly-N-succinyl β -1-6 glucosamine formation has a distinct role in the synthesis of biofilm and cell adhesion. *Staphylococcus aureus* produces several layers of biofilm formed within a slime or glycocalyx layer with various protein expressions [17]. One study indicated the occurrence of histological changes in the lungs of mice infected with *S.aureus* bacteria. The lungs showed pneumonia with pus, moderate to severe, and acute multifocal necrosis with prominent perivascular edema, multifocal hemorrhage, and accumulation of neutrophil and macrophage cells [18].

This study aimed to investigate the prevalence of virulence genes of *S.aureus* and the detection of histological alteration in mice .

2.Materials and Methods

2.1. Ethical consent

It was obtained oral consent from women to collect samples, and it also obtained approval from the ethics committee at Baghdad University/ College of Education for Pure Science (Ibn Al-Haitham) to conduct this study and from the ethics committee in the Ministry of Health for a sample collection from hospitals (Ref: 1256 / Date 3 March 2023). Moreover, consent was obtained from the Ministry of Health to obtain animals from the National Center for Pharmaceutical Control and Research/ Ministry of Health.

2.2. Study design and Sample collection

Total 150 samples from pregnant and nonpregnant women using vagina swabs. The study was performed from July to October 2023 at Ibn Al-Baladi and Al-Imamain Al-Kadhimain hospitals in Iraq. Inclusion criteria included the ages (15-72 years), symptoms of infection, history of contraception use, and treatment received. There were excluded nonpregnant women and women who suffered from bleeding vaginas. The swab inserted half space between the introitus and the cervix to prevent contamination by uterus mucus. After that the swab is pressed gently on the vagina walls, swirled to collect the sample, and carefully removed to avoid touching another part of the body [19].

2.3. Identification of bacteria

The swabs were cultured on a blood agar plate, and a mannitol agar plate, then incubated for 24 hours at 37⁰ C to grow bacteria. The bacteria isolates were identified using the Vitek2 compact system (ID GP card:21342; bioMérieux/France) according to manufacturer instructions. These isolates were re-cultured and stored at 4° C to be used in other analyses.

2.4. Detection of genes by PCR

Genomic DNA in bacteria was extracted using ABIOPure Total DNA/USA. Quantus Fluorometer (Promega /USA) was used to detect DNA concentration. It detected the presence of genes in *S. aureus* using specific primers for these genes (Macrogen company /Korea) as in Table (1). PCR (Polymerase chain reaction) technique was applied to 15 isolates of *S. aureus*, the PCR reaction contents (20 µl) included 10µl GoTaq Green master mix PCR (2X) (Promega, USA), 1 µl for each forward and reverse primer (10 picomoles/µl) (Macrogen/ Korea), 3µl of DNA template, 5µl of free nuclease water. PCR program is shown in Table 2 for genes. Also, electrophoresis was applied using agarose gel (2%) to reveal PCR amplicon using staining with ethidium bromide stain.

Table1.Primers sequences of genes

Gene	5`-3` sequence	Annealing temp(C ⁰)	Product size(bp)	References
<i>mecA</i> -F	AAAAAA GGT GGT ATC GATTGG C	55	533	[20]
<i>mecA</i> -R	AGT TCT GCA GTA CCG GAT TTG C			
<i>sea</i> -F	TTG GAA ACG GTT AAA ACG AA	55	120	[21]
<i>sea</i> -R	GAA CCT TCC CAT CAA AAA CA			
<i>icaA</i> -F	GAT TAT GTA A TG TGC TTG GA	50	770	[21]
<i>icaA</i> -R	ACT ACT GCT GCG TTA ATA AT			
<i>hla</i> -F	CTG ATT ACT ATC CAA GAA ATT CGA	58	209	[21]
	TTG			
<i>hla</i> -R	CTT TCC AGC CTA CTT TTT TAT CAG T			

Genes	Initial Denaturation	Denaturation	Annealing	Extension	Final Extension	Hold
<i>mecA</i>	95°C(5min) 1cycle	95°C30(sec) 30cycle	55°C(30sec) 30cycle	72°C(30sec) 30 cycle	72°C(7min) 1 cycle	10 ⁰ C(10min) 1cycle
<i>sea</i>	95°C(5 min) 1 cycle	95°C(30sec) 30cycle	55°C(30sec) 30cycle	72°C(30sec) 30cycle	72°C(7min) 1cycle	10 ⁰ C(10min) 1cycle
<i>icaA</i>	95°C(5 min) 1 cycle	95°C(30sec) 30cycle	50°C(30sec) 30cycle	72°C(30sec) 30cycle	72°C(7min) 1cycle	10 ⁰ C(10min) 1cycle
<i>hla</i>	94°C(5 min) 1 cycle	95°C(30sec) 30cycle	58°C(30sec) 30cycle	72°C(30sec) 30cycle	72°C(7min) 1cycle	10 ⁰ C(10min) 1cycle

Table 2. PCR programs for genes

2.5. Sequencing of genes

It was conducted using sequence PCR amplicons for studied genes of *S.aureus* isolates by Macrogen DNA sequencing company /Korea. Nucleotide sequences were aligned with global isolates by NCBI's Basic Local Alignment Search Tool Bio Edit program to identify NCBI (<http://www.ncbi.nlm.nih.gov>).

2.6. Evaluation of ability virulence for *S.aureus* in mice

It was used in Swiss albino mice Balb/c (8 weeks) with (51-55) grams. The animals were divided into 4 Groups and one control group, the first group was injected with the bacterial suspension in an intraperitoneal membrane with 0.1 ml of *S.aureus* ($\sim 1 \times 10^4$ CFU cell/ml). The second group was injected with 0.1 ml of *S.aureus* ($\sim 1 \times 10^8$ CFU cell/ml). The third group was injected orally with 0.1 ml of *S.aureus* ($\sim 1 \times 10^4$ CFU cell/ml). The fourth group was injected orally with 0.1 ml of *S.aureus* ($\sim 1 \times 10^8$ CFU cell/ml). It monitored the mortality of mice through 48 hours and 168 hours. Mice were sacrificed and dissection to obtain liver, lung, and vagina for mice. These organs are fixed in 10% formalin and then embedded in paraffin, sectioned, and stained by haematoxylin-eosin stain for histological examination [22].

3.Results

3.1. Identification of bacteria

The results of the Vitek2 compact system showed a probability of 92-99% for *S.aureus* isolates.

3.2. Genes detection

The results showed that 13 isolates of *S.aureus* have *mecA* genes at (86.7%) ,11 isolates have *icaA* gene (73.3%), and 8 isolates have *sea* gene (53.3%),while *hla* gene is found in all 15 isolates (100%) as shown in Figures (1,2,3,4).

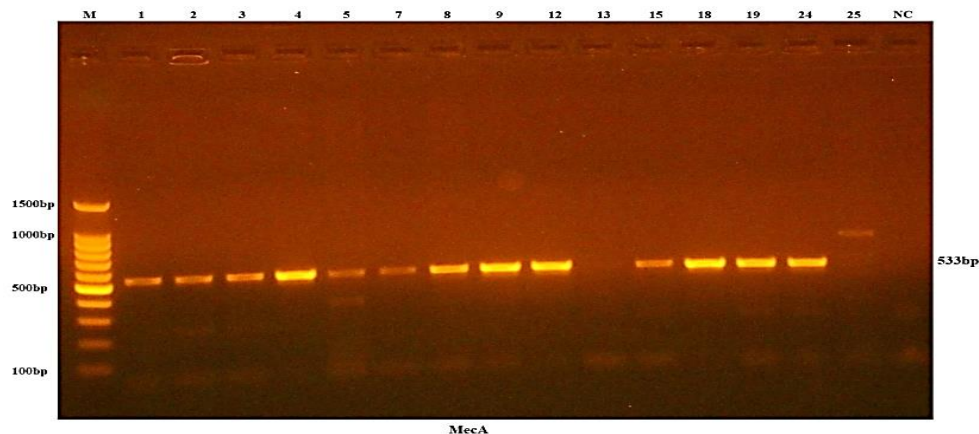


Fig.1.The amplification PCR product of *mecA* gene (533bp) for *S.aureus* isolates was electrophoresed on a 2% agarose gel. M: 100bp ladder marker.

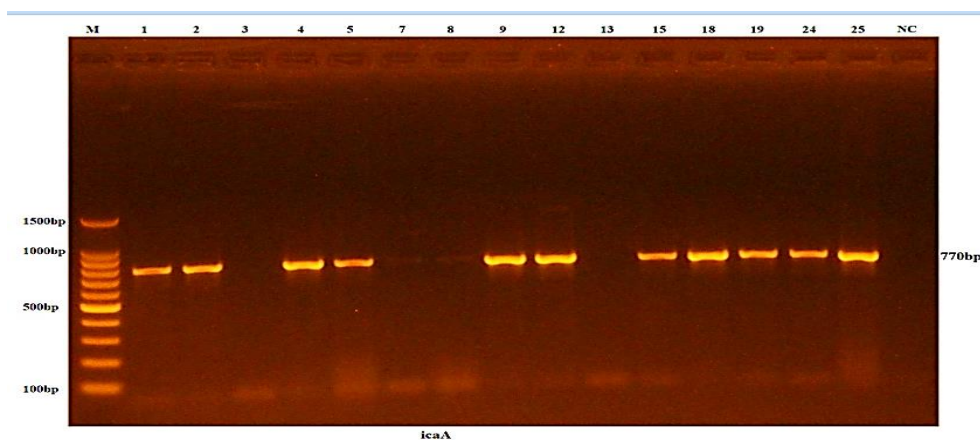


Fig.2. The amplification PCR product of *icaA* gene (770bp) for *S.aureus* isolates was electrophoresed on a 2% agarose gel. M: 100bp ladder marker

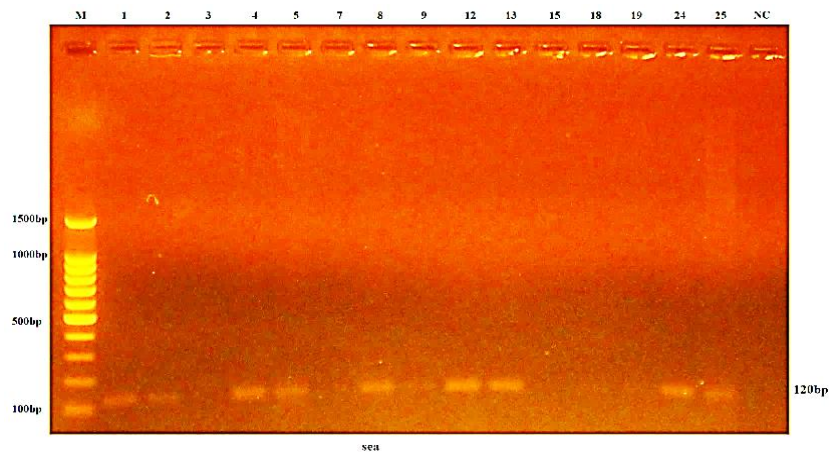


Fig.3. The amplification PCR product of *sea* gene (120bp) for *S.aureus* isolates was electrophoresed on a 2% agarose gel. M: 100bp ladder marker.

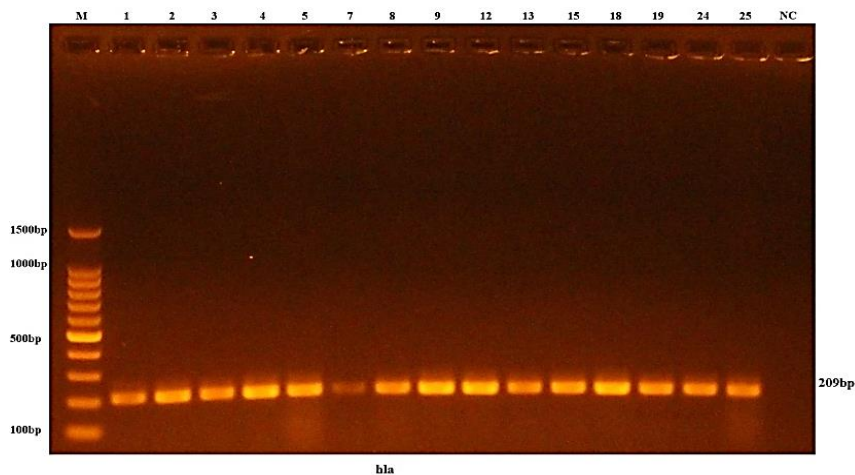


Fig.4. The amplification PCR product of *hla* gene (209bp) for *S.aureus* isolates was electrophoresed on a 2% agarose gel. M: 100bp ladder marker.

3.3. Sequencing of genes

The results of the *mecA* gene in the current study had a similarity of 99% with the reference strain in GenBank (CP133660.1). The *icaA* gene in the current study had a similarity of 99.79% with the reference strain in GenBank (AP028993.1). The *sea* gene had a similarity of 98% with the reference strain in GenBank (LC020109.1). The results sequence of *hla* gene had a similarity of 99.4% with reference stain in the gene bank (EF543163.1).

3.4. Histological examination

The results of histological tissue show the lung in control with normal bronchus, alveolar sac and pulmonary artery (Figure 5). Figure (6) shows the lung of mice infected with *S.aureus* with destructive bronchus, alveolar sac peribronchial lymphatic aggregation with pulmonary artery. Figure (7) shows the hepatic lobule (control) with a normal central vein, normal arrangement of hepatic cords and normal portal triad. Figure (8) shows the hepatic lobule (mice infected with *S.aureus*) with congestion of portal vein, disarrangement of hepatic cords, zonal cellular swelling with granular degeneration and necrosis of hepatocytes. Figure (9) shows the vagina (Control) with normal mucosal folds lined by pseudo-stratified columnar cells, normal submucosal lymphoid aggregates, and smooth muscle of tunica muscularis. Figure (10) shows the vagina (mice infected

with *S.aureus*) with mild hyperplasia (infiltration cells) of stratified epithelium with mild inflammation (vaginitis).

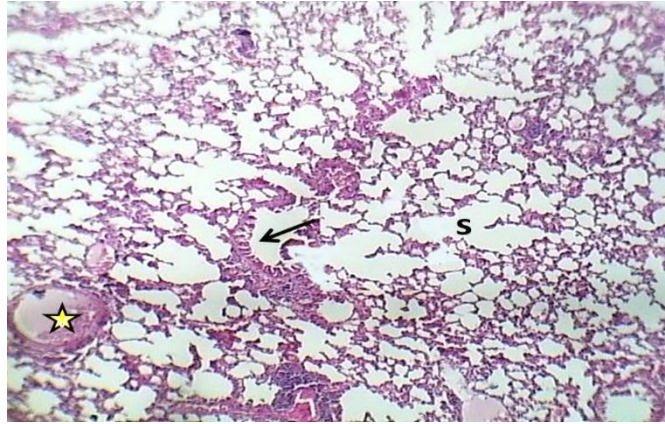


Fig.5. Section of lung (control) shows normal bronchus (Arrow), alveolar sac (S) & pulmonary artery (Asterisk). H&E stain.400x

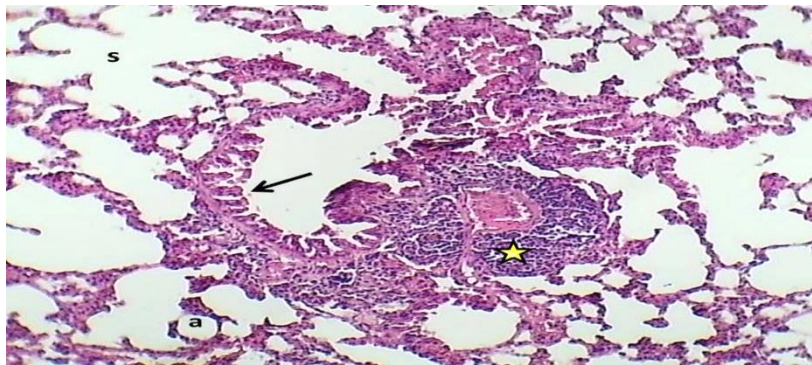


Fig.6. Section of the lung (mice infected with *S.aureus*) shows destructive bronchus (Arrow), alveolar sac (S) peribronchial lymphatic aggregation with pulmonary artery (Asterisk), alveolus (a). H&E stain.100x

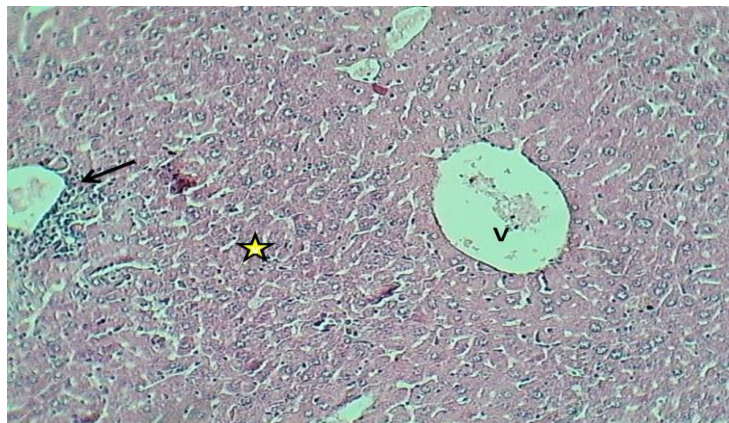


Fig.7. Section of hepatic lobule (control) shows normal central vein (V), normal arrangement of hepatic cords (Asterisk), normal portal triad (Arrow).H&E stain.100x

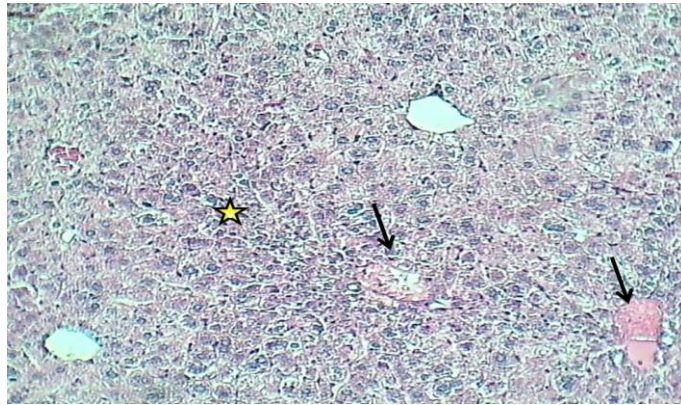


Fig.8. Section of the hepatic lobule (mice infected with *S.aureus*) shows congestion of portal vein (arrows), disarrangement of hepatic cords (Asterisk), zonal cellular swelling with granular degeneration and necrosis of hepatocytes (Asterisk).H&E stain 100x

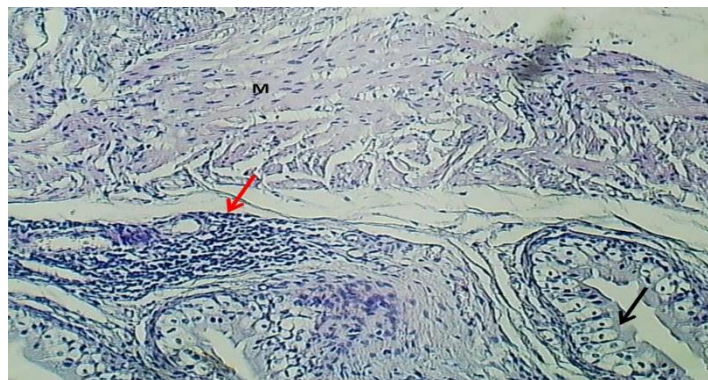


Fig.9.Section of vagina (Control) shows normal mucosal folds lined by pseudo stratified columnar cells (black arrows), normal submucosal lymphoid aggregates (red arrow) & smooth muscle of tunica muscularis (m). H&E stain.100x

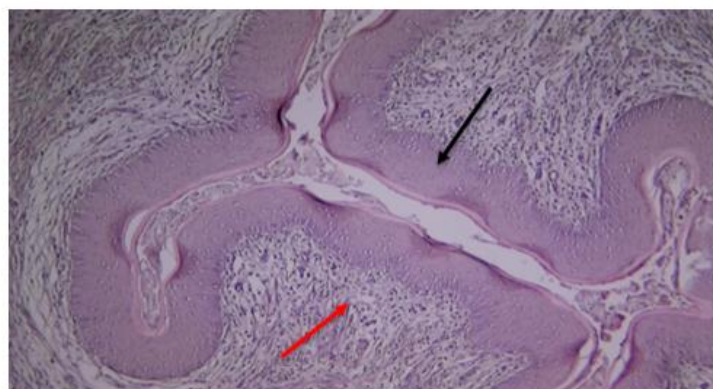


Fig.10. Section of the vagina (mice infected with *S.aureus*) shows mild hyperplasia (Black arrow) (infiltration of cells) of stratified epithelium with mild inflammation (vaginitis) (Red arrow) H & E stain 100x

4. Discussion

Virulence factors like enzymes, adhesin, toxins, and cell surface proteins that are produced by *S. aureus* have an important role in causing different diseases [21]. Methicillin-resistant *S.aureus* strains

represent a common reason for the establishment of nosocomial infection and are an important health issue. *S. aureus* is considered a bloodstream infection in most advanced countries [23].

The outcomes coincide with another study conducted that indicated histological alteration with *S. aureus* infection. Chen et al. [24] showed in their study that there were histological changes in the liver with focal necrosis of hepatic cells in mice infected with *S. aureus*. Another study indicated there were histological alterations in the liver with focal necrosis in hepatic cells. Moreover, there were changes in lung tissue showing thickening in the alveolar wall [25]. Also, AL-Zaidi et al. [26] indicated vaginal microbiota cause vaginosis that involves *Staphylococcus* spp, *Enterococcus* spp., *Acinetobacter* spp., *Escherichia coli*, group B *Streptococcus*, and *Klebsiella* spp.

One Iraqi study, that diagnosed *Staphylococcus aureus* in hospitals by PCR using 16SrRNA found that 100% were diagnosed as *S. aureus* [27]. Moreover, in one study, *mecA* gene was detected in 60% of *S. aureus* isolates that were isolated from Iraqi patients with furunculosis [28].

Abdulmanea et al. [14] showed in their study there is a relation between *S. aureus*, MRSA, and the presence of *icaA*, *hla* genes. Further, the appearance of high resistance in the isolates that carried these genes. Baz et al. [29] recorded in their study that 32.6% were positive for the *sea* gene in *S. aureus* that was resistant to ampicillin and amoxicillin.

This variation in resistance could be demonstrated due to the contact degree between the *S. aureus* strain and antibiotics. Many hypotheses demonstrated the increase in antibiotic impedance not just the excessive utilization of antibiotics in medicinal care but also the deficiency of organization in the marketing and use of antibiotics. This is increased due to self-therapy with high doses. The smuggled traffic of drugs is considered a factor in the appearance of resistant antibiotics [30].

6. Conclusion

The current study found that a large percentage of the isolated *Staphylococcus aureus* were resistant to methicillin and that these isolates showed histopathological effects on the liver, lung, and vagina of mice.

Recommendations

Study other virulence genes in vaginosis and associated with antibiotic resistance.

Acknowledgment

Thankful to all the participants and the staff of Ibn Al-Baladi and Al-Imamain Al-Kadhimain hospitals/ Iraq. We did not obtain the financial source of the study.

Novelty Statement

The current study represents the survey to monitor these virulence genes in vaginosis.

Conflict interest

The authors declare that they have no known competing financial interests .

Source support

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Authors' contributions

Hawraa Munther Abase: Conceptualization; Data curation; Investigation; Methodology; Project administration; Resources; Software; Writing - original draft; Writing - review and editing.

Enas Abdalhadi Hussain: Supervision; Validation; Writing - review and editing.

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كشف عن جينات *mecA* و *icaA* و *hla* و *sea* والتغيرات النسجية لبكتيريا *Staphylococcus aureus* المعزولة من التهاب المهبل لدى النساء العراقيات

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معلومات البحث	المخلص
الاستلام 30 آذار 2024	<p>تسبب المكورات العنقودية الذهبية <i>S.aureus</i> حالات عدوى خطيرة لدى البشر مثل التهابات المسالك البولية، والتهاب الضرع، والالتهاب الرئوي، والتهاب السحايا، والتهاب الشغاف، والتهاب العظم والنقي. وهو يمثل السبب الرئيسي لعدوى المستشفيات في جروح العمليات الجراحية. هدفت هذه الدراسة إلى معرفة مدى انتشار مقاومة بكتريا المكورة العنقودية الذهبية وضرورتها باستخدام الجينات المسؤولة عن الضراوة. تم جمع 150 عينة من المسحات المهبلية من نساء حوامل وغير حوامل في مستشفى ابن البلدي والإمام الكاظم/العراق. أجريت الدراسة في الفترة من تموز إلى تشرين الأول 2023. أظهرت النتائج أن 13 عزلة من بكتريا <i>S.aureus</i> تمتلك جين <i>mecA</i> بنسبة (86.7%)، و 11 عزلة تمتلك جين <i>icaA</i> (73.3%)، و 8 عزلات تمتلك جين <i>sea</i> (53.3%). بينما وجد جين <i>hla</i> في جميع العزلات الـ 15 بنسبة (100%). أظهرت عزلات بكتريا المكورة العنقودية الذهبية تغيرات نسجية مرضية في الكبد والرئة والمهبل في الفئران. وكانت هناك انتشار للمكورات العنقودية الذهبية المقاومة للميثيسيلين بسبب وجود جين المقاومة في معظم العزلات. أظهرت عزلات بكتريا المكورة العنقودية الذهبية في هذه الدراسة ضرورتها من خلال حدوث تغيرات نسجية مرضية في الكبد والرئة والمهبل.</p>
المراجعة 3 تموز 2024	
القبول 15 تموز 2024	
النشر 31 كانون الأول 2024	
الكلمات المفتاحية	
المكورات العنقودية الذهبية، <i>mecA</i> ، <i>hla</i> ، <i>icaA</i>	
Citation: H. M. Abase, E. A. Hussain, J. Basrah Res. (Sci.) 50(2), 9 (2024). DOI:https://doi.org/10.56714/bjrs.50.2.2	

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ISSN: 1817-2695 (Print); 2411-524X (Online)
Online at: <https://jou.jobrs.edu.iq>