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Identification of Antibiotic Resistance Genes in *Staphylococcus aureus* Isolated from Animals with Ocular Infection

KARAR ALI ABDULKHUDER* AND ABDUL KAREEM SALMAN ALYASSARI

College of Veterinary Medicine, Al-Qasim Green University, 51013 Babylon, Iraq.

Abstract | Antibiotic resistance poses a significant challenge in the management of *Staphylococcus aureus* infections, particularly those affecting the eyes. Understanding the antibiotic susceptibility profiles and genetic mechanisms underlying resistance is crucial for guiding effective treatment strategies. This study aimed to assess the antibiotic susceptibility patterns and molecular characteristics of *Staphylococcus aureus* strains isolated from eye infections. For this purpose, a total of 20 *Staphylococcus aureus* isolates were obtained from eye infection samples and then were subjected to antibiotic susceptibility testing using standard methods. Additionally, a subset of isolates demonstrating high resistance was selected for molecular analysis to detect the presence of antibiotic resistance genes, including *mecA*, *ermA*, and *ermB*. The antibiotic susceptibility testing revealed varying degrees of effectiveness among the antibiotics tested. Notably, *mecA*, *ermA*, and *ermB* genes were detected in all samples analyzed, indicating widespread methicillin and macrolide resistance. These findings underscore the complex nature of antibiotic resistance among *Staphylococcus aureus* strains causing eye infections, indicating the failure of antibiotic efficacies in these infections.

Keywords | *Staphylococcus aureus*, Eye infections, Antibiotic susceptibility

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***Correspondence** | Karar Ali Abdulkhuder, College of Veterinary Medicine, Al-Qasim Green University, 51013 Babylon, Iraq; Email: hamza14shukri72@gmail.com**Citation** | Abdulkhuder KA, Alyassari AKS (2024). Identification of antibiotic resistance genes in *Staphylococcus aureus* isolated from animals with ocular infection. J. Anim. Health Prod. 12(s1): 40-44.**DOI** | <http://dx.doi.org/10.17582/journal.jahp/2024/12.s1.40.44>**ISSN (Online)** | 2308-2801**Copyright:** 2024 by the authors. Licensee ResearchersLinks Ltd, England, UK.This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

INTRODUCTION

Ocular infections are a few of the illnesses which have unfold broadly across the world, and have significantly affected animals. It has been estimated that a range of different bacterial species are involved in ocular infection and are predominantly contributed by the Gram-positive bacteria (Durand, 2022). Due to rise in infection rates among small animals, there is interest in understanding the similarities and differences in infection patterns between humans and animals. Infections may be induced in animals, and information of the consequences of those accidents are critical in identify remedy and prevention pathways (Mellata, 2013). Bacteria are a main motive

of ocular infections worldwide related to a whole lot of factors, consisting of touch lenses, trauma, surgery, age, dry eye state, continual nasolacrimal duct obstruction, and former ocular infections. Bacteria are typically related to many styles of ocular infections, along with conjunctivitis, keratitis, endophthalmitis, blepharitis, orbital cellulitis, and dacryocystitis (Janik et al., 2020). Conjunctivitis is the maximum not unusualplace ocular infection, continual conjunctivitis may be a chance thing for different more or intraocular infections. Bacteria account for about 50-70% of infectious conjunctivitis. Bacterial conjunctivitis is maximum not unusual place in youngsters and the elderly, however, also can arise in neonates and adults (Azari and Arabi, 2020).

Blepharitis, an inflammation of the eye, can cause vision loss and spread to other parts of the eye. Keratitis, a highly contagious eye disease, is the leading cause of corneal blindness and can progress to endophthalmitis if not diagnosed early (Cabrera-Aguas et al., 2022). Endophthalmitis may be spontaneous, resulting from transmission of a virus during surgery or trauma, or endogenous, resulting from systemic spread of the virus. Both types can cause serious illness if not diagnosed and treated in a timely manner (Malmin et al., 2021). Dacryocystitis is a condition that occurs when the nasolacrimal duct becomes inflamed. In chronic cases, it is associated with infection, conjunctival inflammation, fluid retention, and chronic crying. This disease can damage eye tissue, such as the cornea, and lead to endophthalmitis after surgery (Taylor and Ashurst, 2023).

In recent years, the growing concern about the spread of bacterial resistance to antibiotics has severely restricted the use of antibiotics in animal feeds (Arsène et al., 2022). Specifically, *S. aureus* poses an important problem in hospitals, nursing homes, veterinary health and other health care settings (Algammal et al., 2020). This study aimed to investigate of gram-positive bacteria that cause ocular infection by molecular and bacteriological methods.

MATERIALS AND METHODS

SAMPLE COLLECTION

A cross-sectional study was carried out in Al Qasim Green University -Iraq from July 2023 to December 2023. The study included collecting 200 samples from ocular infection in small animals and humans.

STUDY DESIGN

The current study included collecting 200 samples from ocular infection (Human: n=100 and animals n=100) and then culture on appropriate media. The bacteria were then diagnosed by phenotypic diagnosis and biochemical tests. Attempts to isolate *Staphylococcus aureus* were made from positive samples. The rest of the bacterial species were excluded. Confirmatory diagnosis of *Staphylococcus aureus* isolates was performed using the Vitek-2 system.

Antibiotic susceptibility testing was also performed with the same device. Thereafter, DNA was extracted and *mecA*, *ermAb* and *ermB* genes were detected using PCR technology, and finally statistical analysis was performed.

BACTERIA DIAGNOSIS

Bacterial diagnosis through phenotypic and biochemical characterization was performed according to methods of Abdulretha et al. (2016). The most frequently bacterial species that show in ocular infection were confirmed using Vitek-2 system. The Vitek-2 system from Biomerieux-France was used to diagnose bacteria with the following steps: Injection: prepared samples were injected into a multi-well card. These spaces contain inactive proteins that interact with enzymes or specific metabolites produced by the microbe. The vaccination program has been carefully designed for accurate and reliable results. Incubation: An incubation test card was included in the Vitek-2 system to provide a method of control. In this step, microbes in the sample transfer the substrate to the wells, causing a change in color or fluorescence. Detection and Identification: The Vitek-2 system uses optical scanning technology to accurately track test cards. Detection and analysis of the color or fluorescence of microorganisms is conducted at this stage. By comparing this method with a comprehensive database of known microbial characteristics, the system quickly and accurately identifies specific bacteria in a sample.

ANTIBIOTIC RESISTANT GENES DETECTION

Detection of different virulence genes including *mecA*, *ermA* and *ermB* (antibiotic resistance genes) was performed using PCR technique through following steps.

DNA EXTRACTION

The first step was to extract DNA from the bacterial sample using boiling methods as reported earlier (Mahuku, 2004).

PRIMER DESIGN

Specific primers targeting three genes were identified and were validated using Primer 3 software (Table 1). These primers bind to the target DNA and serve as starting points for DNA amplification.

Table 1: Primers were used in current study.

Gene	Target primer sequences	PCR condition	Size (bp)	Reference
<i>mecA</i>	5'-TCCAGATTACAACCTCACCAGG-3' 5'-CCACTTCATATCTTGTAACG-3'	32 cycles of 94°C for 30s, 53°C for 30s, and 72°C for 50s	162	Oliveira and Lencastre (2022)
<i>ermA</i>	5'-GTTCAAGAACAATCAATACA GAG-3' 5'-GGATCAGGAAAAGGACATTT TAC-3'	32 cycles of 94°C for 30s, 52°C for 30s, and 72°C for 60s	421	Lina et al. (1999)
<i>ermB</i>	5'-CCGTTTACGAAATTGGAACAGGTAAAGGGC-3' 5'-GAATCGAGAC TTGAGTGTGC-3'	32 cycles of 94°C for 30s,55°C for 30s, and 72°C for 60s	359	Lina et al. (1999)

BACTERIA DIAGNOSIS

The results showed that *Staph. aureus* appeared in 20 samples, which is about 10% of the total samples collected (Figure 1).

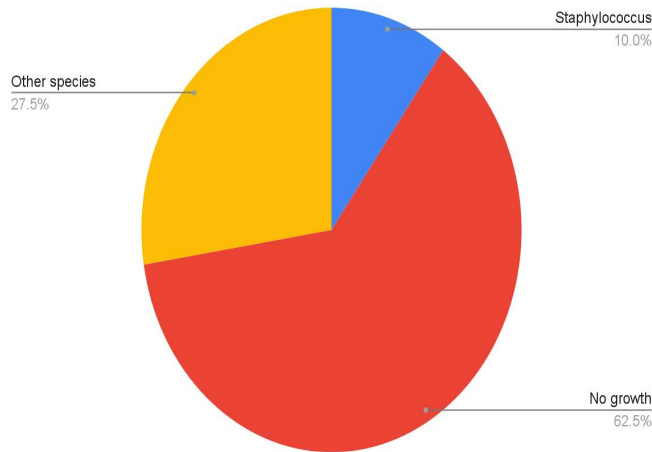


Figure 1: Distribution of bacterial samples.

ANTIBIOTIC SENSITIVITY TEST

The antibiotic susceptibility testing results reveal varying degrees of effectiveness among the antibiotics tested against *Staphylococcus aureus* strains isolated from eye infections (Figure 2). Cefoxitin screen demonstrated 50% resistance, indicating potential sensitivity of 50% to methicillin. Benzylpenicillin displayed a resistance rate of 65%, with 35% of samples showing intermediate susceptibility and no samples being sensitive. Oxacillin showed a resistance rate of 60%, with 40% of samples being sensitive and none showing intermediate susceptibility. Gentamicin and Tobramycin both exhibited excellent efficacy, with 100% of samples being sensitive, and only a small percentage showing resistance. Levofloxacin and Moxifloxacin demonstrated high sensitivity at 90%, with a minor proportion showing resistance. Inducible Clindamycin Resistance was observed in 55% of samples, with 45% showing sensitivity. Erythromycin and Clindamycin both showed sensitivity in 10 and 16 of samples, with resistance rates at 50% and 20%, respectively. Linezolid, Teicoplanin, Vancomycin, Tigecycline, Fusidic Acid, and Rifampicin displayed excellent sensitivity, with no samples showing resistance.

Tetracycline exhibited sensitivity in 60% of samples, with 20% showing resistance and intermediate susceptibility. Fosfomycin demonstrated sensitivity in 90% of samples, with 10% showing resistance. Nitrofurantoin showed sensitivity in 70% of samples, with a resistance rate of 30%. Mupirocin displayed sensitivity in 60% of samples, with 20% showing resistance and intermediate susceptibility.

Trimethoprim/Sulfamethoxazole exhibited sensitivity in 90% of samples, with 10% showing resistance.

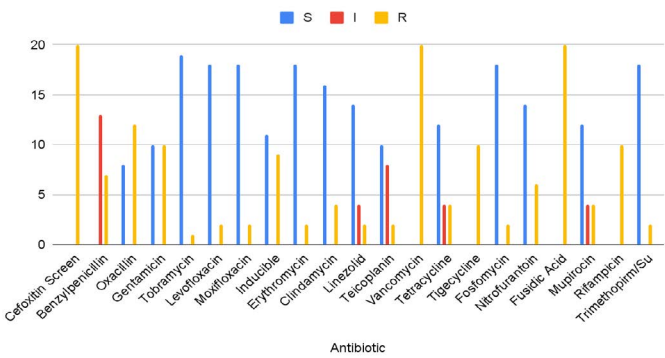


Figure 2: Antibiotic sensitivity test of 20 *Staphylococcus aureus* isolates.

ANTIBIOTIC RESISTANCE GENES DETECTION

In the current study, a total of 10 isolates were selected for molecular study, and they are the most resistant to antibiotics. The analysis of antibiotic resistance genes revealed consistent findings across all samples of *Staphylococcus aureus* isolated from eye infections as shown in Table 2. The *mecA* gene, associated with methicillin resistance, was detected in every sample, indicating a 100% positive rate. Similarly, the *ermA* and *ermB* genes, which confer resistance to macrolide antibiotics, were also present in all samples, resulting in a 100% positive rate for each gene.

Table 2: Prevalence of antibiotic resistance genes among 10 *Staphylococcus aureus* isolates.

Antibiotic resistance genes	mecA	ermA	ermB
Positive %	100	100	100
Negative %	0	0	0

The presence of high antibiotic resistance in our study could be attributed to several factors. Firstly, the resistance or sensitivity of bacteria to antibiotics can vary depending on the geographic location, prevalence of antibiotic usage, and bacterial strain (Urban-Chmiel et al., 2022). Additionally, the emergence of antibiotic-resistant lines because of misuse or overuse of antibiotics in healthcare and agriculture performs a large role. Moreover, the genetic make-up of micro-organism can have an impact on their susceptibility to antibiotics, as a few lines can additionally own particular resistance mechanisms, which include efflux pumps or enzymatic degradation of antibiotics (Zhang and Heng, 2022).

Previous research has proven each similarity and variations with the present-day study's findings. For instance, research carried out in special areas may record versions in antibiotic susceptibility styles because of variations in bacterial traces

customary in the one's regions or versions in antibiotic utilization practices (Gonçalves et al., 2023; Pervaiz et al., 2024). Additionally, research that specialize in unique affected person populations, which include hospitalized sufferers as opposed to outpatients, can also additionally showcase differing susceptibility styles because of various stages of antibiotic exposure (Asbell et al., 2020).

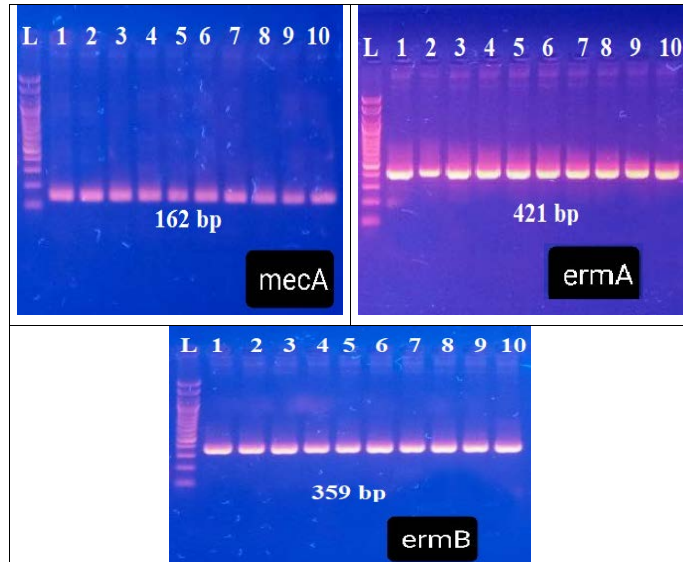


Figure 3: Gel electrophoresis of *mecA*, *ermA* and *ermB* genes that are detected in *Staph. aureus*.

In the present study, a subset of 10 isolates, decided based on excessive resistance to antibiotics, underwent molecular analysis. The results, exact in Table 2, tested constant findings throughout all samples of *Staphylococcus aureus* remoted from eye infections. Specifically, the presence of antibiotic resistance genes turned into observed. The *mecA* gene, usually related to methicillin resistance, turned into detected in each sample, indicating a 100% fantastic fee. Likewise, the *ermA* and *ermB* genes, chargeable for conferring resistance to macrolide antibiotics, have been additionally found in all samples, ensuing in a 100% fantastic fee for every gene. These findings align with preceding research which have diagnosed *mecA*, *ermA*, and *ermB* genes as key determinants of antibiotic resistance in *Staphylococcus aureus*.

CONCLUSIONS AND RECOMMENDATIONS

The findings of this study highlight the complicated panorama of antibiotic susceptibility and resistance amongst *Staphylococcus aureus* lines remoted from eye infections. Despite the various ranges of effectiveness determined most of the antibiotics tested, numerous key traits emerged. The detection of *mecA*, *ermA*, and *ermB* genes in all samples underscores the pervasive nature of methicillin and macrolide resistance genes in those isolates.

Identification of Antibiotic Resistance Genes in *Staphylococcus aureus* Isolated from Animals with Ocular Infection is a one health topic and can be useful for future studies of veterinarian, ophtamatolists and microbiologists.

AUTHOR'S CONTRIBUTION

Both of authors had similar roles and participation in all process of study.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Ethical refulation of present study was approved and monitored by Al Qasim Green University Ethical Comitte.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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