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Molecular Study to Detect Some Virulence Factors in *S.aureus* Bacteria Isolated from Different Types of Eye Infections

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Abstract

Staphylococcus aureus is an opportunist that causes systemic infections and eye infections in the human being body. This organism increases its resistance to many categories of antibiotics and turns out to be more resistant. A total of one hundred fifty-four samples from different clinical cases had been collected from patients with conjunctivitis 63 (49.2 %). Blepharoconjunctivitis 29 (22.6 %), blepharitis 21 (16.4 %), dacryocystitis 8 (6.2 %). Moreover, 7 (5.4 %) with external eye infections attended Ibn-AL-Hythem Hospital and private clinics in Baghdad province during the period from 1st of September 2023 to 1st March 2024. The current study shows 63/154 (40.9 %) were *S. aureus*. These isolates were identified according to culture, microscopic examination, biochemical tests, and APIstaph system identification kits. The current study shows the highest percentage of *S. aureus* infections that was observed in conjunctivitis 23 (36 %) followed by 15 (24 %) with blepharoconjunctivitis. 8 (16 %) with blepharitis, 5 (13 %) with dacryocystitis, and 3 (5 %) with other external eye infections. The results showed 37/63 (58.73 %) of isolated *S. aureus* were VRSA strains. Regarding virulence factor the results showed Eta gene is present in all cases in percent 100 %, while other genes appear in different percentages: SeA (94.5 %), Seb (39.68 %), Sec (34.92 %), Sed (28.57 %), Tst (34.92 %), and Etb (17.46 %).

Keywords: Virulence Factors, S.aureus, and Eye Infections.

1. Introduction

Human eye, which is constantly exposed to the external environment, is a unique organ serving as the window of our body. Ocular disease with its complications, due to microorganisms, is a significant health problem worldwide particularly in the least-income countries [1]. Conjunctivitis, blepharitis, and dacryocystitis are considered the most

common manifestations of external eye infections [2]. These pathogenic microorganisms include bacteria, fungi, viruses, and parasites [3]. Drug-resistant *S. aureus* strains are seen in a few *S. aureus* strains [4] developed. The strains of *S. aureus* were resistant to antibiotics containing beta-lactams, such as penicillin's, amoxicillin, methicillin, ampicillin, cephalosporins, oxacillin, and others [5, 6].

Antibiotic resistance was acquired by aureus, resulting in a worldwide clone distribution of antimicrobial resistance expressions. Instead of MRSA strains, numerous bacterial diseases cause mortality in the public and clinics *S. aureus* infection, like MRSA strains, has been around for a long time [7]. Since the indiscriminate use of antibiotics is not a standard process, hospital facilities are not sanitary, and patients and health personnel are overburdened Infectious bacteria, such as *S. aureus*, are spread more easily [8]. Because of its specific role in eye infections, *S. aureus* is thought to be involved [9].

As a result, it makes sense to assess the state of microbial resistance to the most widely used antibiotics for treating *S.aureus*-caused eye infections. Recently, encapsulation of antimicrobial medicines in nanoparticle systems has emerged as a promising carrier approach for increasing therapeutic efficacy while decreasing unwanted side effects [10]. Antibiotic treatment through NPS offers numerous benefits, including controlled and uniform dispersion in the target area, higher solubility, longer release, improved patient compliance, fewer side effects, and improved cellular internalization.

2. Materials and Methods

From ophthalmic consultative clinic in hospital Ibn-AL-Hythem for eye infection in Baghdad, 213 clinical samples were collected from three parts of eyes the conjunctiva, the cornea, and the eyelids. From the 1st of September 2023 to the 1st of March 2024. Approval has been obtained from Baghdad health Directorate to conduct the study using a simple random sampling technique. Patients' genders were enrolled to obtain specimens from different clinical cases. Moreover, the sample size was calculated using the following formula [11].

$$Z_{1-\alpha/2}{}^2 P(1-P) / d^2$$

Where, the standard normal variate $Z_{1-\alpha/2}$ was 1.96 at 5 % type 1 error ($p = 0.05$), and 2.58 at 1 % type 1 error ($p=0.01$). As $p < 0.05$ is typically regarded as significant in research, 1.96 was utilized in the formula in which P was the population expected proportion based on previous studies [12]. Furthermore, d was the absolute inaccuracy or precision determined by the researchers. Briefly, the patient was requested to look up

while lowering the eyelid, and the sample was collected from one or both eyes based on the nature of the infection.

A sterile cotton swab that had been premoistened with sterile physiological saline was used gently to collect eye discharge. An Eswab was rubbed softly over the lower conjunctival sac from medial to lateral side and back again [13]. Then, sample were cultured on several different media, such as blood agar, MacConkey agar, and mannitol salt agar the latter being a selective medium for *S. aureus*. Samples were incubated at 37 °C for 24 hours after which cultured bacteria was isolated and identified according to colony morphology, shape, size, colour, and pigment production.

Deoxyribonucleic acid (DNA) extractions were carried out using a commercial kit (Presto™ Mini gDNA Bacteria Kit, Geneaid, Thailand) to obtain DNA templates for use in PCR assays. The DNA of *P. aeruginosa* isolates were extracted as per the manufacturer's instructions. For cell harvesting pre-lyses, the bacterial strains were cultured on mannitol salt agar for 18 hours at 37 °C. Then, they were harvested by centrifugation for one minute at a speed of 14,000 rpm, with the supernatant being discarded.

Furthermore, a 20 µL of proteinase potassium (K) (solution and 180 µL of buffer Guanidinium thiocyanate (GT) were added to the pellet and mixed, with the sample tubes, inverted every three minutes for the duration of the incubation period. After mixing for 10 seconds with 200 µL of buffer Guanidine Brochloride (GB).

The cell lysate was incubated for 10 minutes at 70 °C, with sample tubes mixed by inversion every 3 minutes to induce lysis. The elution buffer was pre-heated 200 L/sample at 70 °C for DNA elution. For DNA binding, lysate samples were treated with 200 µL of 100 % ethanol and thoroughly mixed by shaking. The mixture was transferred to a spin column in a 2 mL collection tube and placed in a new 2 mL collection tube for the genome DNA (GD) column.

For DNA elution, the spin column was placed in a 1.5 microcentrifuge tube, and 100 µL of pre-heated elution buffer was added to the middle of the column matrix. After letting the mixture stand for 3 minutes to ensure that all the elution buffers had been absorbed. The spin column was centrifuged for 30 seconds at 14,000 rpm to elute the purified DNA. The extracted DNA was stored in the freezer at -20 °C till use. The concentration and purity of the DNA was measured by using an instrument (NanoDrop) and agarose gel electrophoresis.

During the process, 1 µL of the extracted DNA was added to the instrument

to detect DNA concentration and purity by analysing the optimal degree OD (260/280) ratio to verify the protein and DNA concentration.

For agarose gel electrophoresis, 1 × Tris-borate-EDT(TBE) buffer was placed in the electrophoresis tank, after which the agarose tray was immersed in the electrophoresis tank. It was ensured that the buffer was roughly several milliliters above the agarose surface. Each well was filled with 5 µL of the sample and 2 µL of dye, and the tank was then filled and closed. Electrophoresis was performed using 70 volt/cm of gel run swat electrophoresis. With the use of gel paper, the agarose was extracted from the tank and visualized. For the optimization of the primers used, 2.5 µL of the master mix was mixed with 5-6 µL of DNA.

Along with 1µL of the forward and reverse primers. Optimization was programmed for SeA, Seb, Sec, Sed, Tst, eta, mecA, and Etb genes and primer of gene grades were chosen, and the annealing temperature of PCR was set at 55 °C, 58 °C and 52 °C. Detection of SeA, Seb, Sec, Sed, Tst, eta, mecA, and Etb genes were carried out by mixing 12.5 mL master mix, 5-6 mL DNA, 1 mL each of forward and reverse primers, and nuclease-free deionized water to a final volume of 20 mL, as per the manufacturers' instructions.

PCR cycling program parameters used in the reaction for the detection of the genes of interest were noted (table 1). Data was analysed using SPSS 20. The chi-square test was used to analyse the data. P < 0.001 was considered statistically significant [13].

Table 1: The sequence and source of the gene primers used in the study.

Primer	Oligonucleotide sequence (5'-3')	Location within gene	Size of amplific d product (bp)	Multiplex PCR set
icaA	GSEAR-1	GGTTATCAATGTGOGGGTGG	349-368	936
	GSEAR-2	CGGCACTTTTTTCTCTTCGG	431-450	
Hla	GSEBR-1	GTATGGTGGTGTAACTGAGC	666-685	209
	GSEBR-2	CAAATAGTGACGAGTTAGG	810-829	
Hlb	GSECR-1	AGATGAAGTAGTTGATGTGTATGG	432-455	309
	GSECR-2	CACACTTTTAGAATC AACCG	863-882	
Pvl	GSEDR-1	CGAATAATAGGAGA AAATAAAAG	492-514	433
	GSEDR-2	ATTGGTATTTTTTTTCGTTC	750-769	
seb	GMECAR-1	ACTGCTATCOCACCCT CAAACC	1182-1201	404
	GMECAR-2	CTGGTGAAGTTGTAATCTGG	1325-1344	
sea	GETAR-1	GCAGGTGTTGATTTAG CATT	775-794	676
	GETAR-2	AGATGTCCCTATTTTTGCTG	848-867	
fnbB	GETBR-1	ACAAGCAAAAGAATA CAGCG	509-528	524
	GETBR-2	GTTTTTGGCTGCTTCTCTTG	715-734	
fnbA	GTSSTR-1	ACCCCTGTTCCCTTAT CATC	88-107	191
	GTSSTR-2	TTTTCAGTATTTGTAACGCC	394-113	

3. Statistics Analysis

Design of the study cross-sequential comparative study. Statistical research was carried out using the Statistical Kit of Social Science (SPSS) software V. 20 analysed descriptive statistics and the exact test of Chi-square. ($\chi 2$) or Fisher (typically used

where sample sizes are small) used to evaluate P value < 0.05 was considered statistically significant in the connection between the variables.

4. Results and Discussion

4.1 Prevalence of bacteria among various eye infections

Out of 213 ocular specimens processed for culture, bacteria were isolated from 122 (57.27 %). No mixed bacterial isolate per patient was found in this study. Among the bacterial isolates, 94 (77.04 %) of samples were Gram-positive groups and 28 (22.95 %) were Gram-negative groups. From the former groups, *S. aureus* was the most frequent isolate accounting for 49 (27.9 %), followed by CoNS and *S. pneumoniae* with 30 (19.7 %) and 8 (8.8 %) respectively.

From the latter groups, *P. aeruginosa* was the predominant isolate accounting for 9 (6.8 %), followed by 8 (6.1 %) *K. pneumoniae*. The spectrum of bacterial isolate varies with the age of patients. Most of the bacterial isolates were recovered from cases that were between one month and two years of age group (table 2). The result is comparable with a previous study conducted in Iraq [14]. However, the result is lower than the prevalence reported from elsewhere ranging between (74 %) and (88 %) [15].

This result of the study agrees with a study with *staphylococcus aureus*, *streptococcus pneumoniae*, *klebsiella pneumoniae*, *pseudomonas aeruginosa*, *enterobacter aerogenes*, *proteus mirabilis*, *citrobacter freundii*, and *streptococcus spp*. were identified as causative agents of eye infections [16].

Additionally, a study reported that 9 % of cases of conjunctivitis were caused by *S. pneumonia* [17]. The high prevalence of *S. aureus* infections can be attributed to their normal presence on the skin as part of the normal flora. However, infections become pathogenic under certain conditions, such as in immunocompromised patients with chronic diseases, and can cause infections in the conjunctiva, cornea, and eyelids.

These bacteria can also be transmitted by contaminated hands and have virulence factors such as enzymes and toxins for example protease and lipase. *S. epidermidis* is also part of the normal skin flora but can cause infections in immunocompromised hosts [18]. The presence of various bacterial species causing external ocular infections indicates variations in environmental conditions, personal hygiene practices, age, and the site of infection.

The low occurrence of Gram-negative enteric bacteria in this study may be attributed to effective personal hygiene

since the primary mode of transmission for enteric pathogens is through fecal-oral contamination of the eye. Furthermore, previous studies have shown that wearing contact lenses is the main cause of Gram-negative bacterial ocular infections [19]. Some of the patients in the current study had a history of contact lens wearing 9 (16 %).

Table 2: Prevalence of bacteria among various eye infections.

Isolate	Age in years				
	0-2	3-16	17-45	< 45	Total
Gram-positive bacteria					
S. Aureus	19	9	11	10	49
**CoNS*	11	7	8	4	30
S. pneumoniae	5	2	1	0	8
S. pyogenes	2	0	1	1	4
S. agalactiae	1	1	0	1	3
Gram negative bacteria					
P. aeruginosa	4	2	2	1	9
K. pneumoniae	2	0	0	6	8
P. mirabilis	2	1	1	0	4
Enterobacter spp.	3	1	0	0	4
E. coli	1	0	1	1	3
Total	50	23	25	24	122

4.2 Prevalence of *S. aureus* among various eye infections

Table 3 shows the prevalence of *S. aureus* among various eye infections. In the current study, the highest percentage of *S. aureus* infections was found in conjunctivitis 23 (36 %), this bacterium can be considered one of the major agents of community-acquired *S. aureus* infection in eye disease, followed by blepharoconjunctivitis and the frequency of *S. aureus* was 15 (24 %), then blepharitis 8 (16 %), dacryocystitis, 5 (13 %), While the lower incidence was 3 (5 %) in external eye infections.

Table 3: Prevalence of S. aureus among various Eye infections.

Eye infections type	Number	%
Conjunctivitis	21	36
Blepharoconjunctivitis	12	24
Blepharitis	8	16
Dacryocystitis	5	13
External eye infections	3	5
Total	49	100
X2		
P value		

* Highly significant difference (P<0.01)

The present study's findings revealed that *S. aureus* isolated from eye infections, which may be caused by ophthalmic disease, can operate as a reservoir for opportunistic microorganisms. If antibiotics are used to treat ophthalmic disease or other infections, can caues an increase in *staphylococcus spp*. in the eye.

S. aureus strains can cause antibiotic resistance is widespread and can ophthalmic development because of antibiotic therapy. The fact that *S. aureus* is more prevalent in the eye might result in a more severe illness. The current percentages of isolated *S. aureus* are consistent with those reported who found that conjunctivitis was 36 (33.8 %), followed by blepharitis and external eye infections 19 (26.8 %) and 12(16.9 %),

respectively [20]. Also, according to the findings of a prevalence of *S. aureus* in the eye blepharoconjunctivitis of (21 %) and conjunctivitis of (11 %) in 110 patients attending a hospital with a variety of eye illnesses.

Because The case for *S. aureus* in the pathogenesis of eye disease is challenging due to the diversity of the normal flora and the healthy carriage of *S. aureus* in particular patient groups. However, given the high rates of *S. aureus* recovery in patients with eye mucosal symptoms such as pain, burning, erythema, and swelling, physicians should consider the potential of this pathogen playing a role in eye mucosal illness [21].

Prevalence of *S. aureus* because the transient contamination of the patient's hand may be the source of infection of external eye structures. Proper sanitary and hygienic measures including frequent hand and face washing with antimicrobial or no antimicrobial soap can minimize facial area colonizing bacteria which in turn reduces transient organisms [22].

5. Detection of Virulence Factors to *staphylococcus aureus*

5.1 Conventional PCR Screening for icaA gene

Twenty isolates of *S. aureus* were positive for (icaA) gene 20/49 (40.81 %), PCR product of this gene was 930 bp as shown in (figure1). The M Lane is the standard DNA sized from 1500-100 bp. Lanes 1, is a negative sample for the icaA gene. Lanes 2-7 represent positive samples for the 930 bp. icaA gene. Electrophoresis was performed on 100V for 55 minutes on (1.2 %) agarose

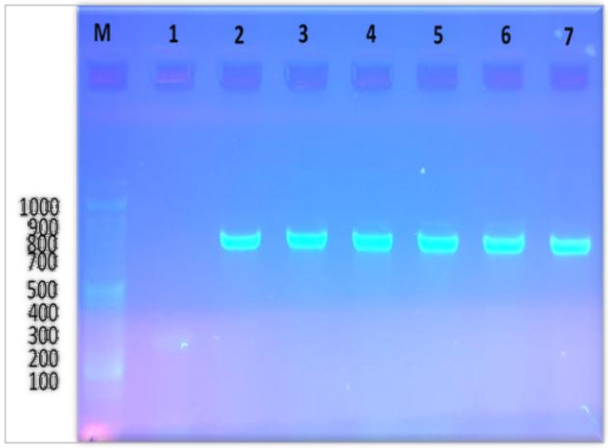

Figure 1: PCR product gel electrophoresis of icaA gene.

5.2 Conventional PCR Screening for FnbA gene

Twenty-eight isolates of *S. aureus* were positive for (FnbA) gene 28/49 (57.14 %), PCR product of this gene was 191 bp.

5.3 Conventional PCR Screening for FnbB gene

Eight isolates of *S. aureus* were positive for (FnbB) gene 8/49 (16.32 %), PCR product of this gene was 524 bp.

5.4 Conventional PCR Screening for HLA gene

Fifteen isolates belong *S.aureus* were positive for hla gene 15/49 (30.16 %), PCR product of this gene was 209 bp, as shown in (figure 2). The M Lane is standard DNA sized from 1500 -100 bp. 1-9 lanes represent positive samples for 209 bp. hla gene, Electrophoresis was performed on 100V for 55 minutes on (1.5 %) agarose.

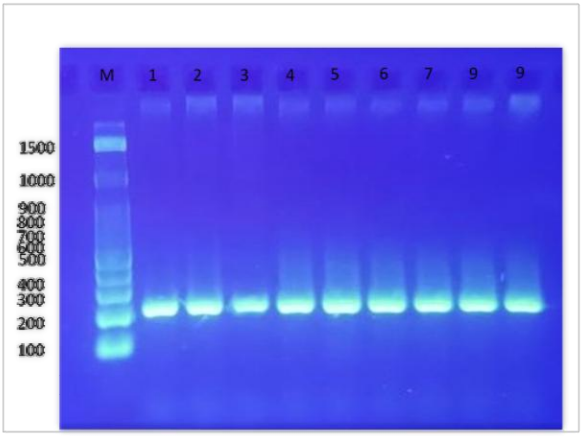

Figure 2: PCR product gel electrophoresis of HLA gene.

5.5 Conventional PCR Screening for hlb gene

Twenty-nine isolates belong *S.aureus* were positive for hlb gene 29/49 (59.18 %). PCR product of this gene was 433 bp. as shown in (figure 3). The M Lane is standard DNA sized from 1500-100 bp. Lanes 1, 2, 3, 4, 6, 7, and 9 were positive samples. Lanes 5 and 8 represent positive samples for the 433 bp PVL gene. Electrophoresis was performed on 100V for 55 minutes on (1.5 %) agarose.

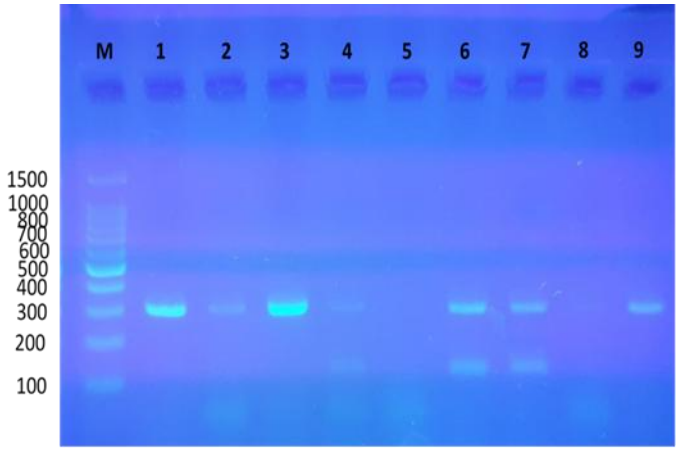

Figure 3: PCR product gel electrophoresis of hlb gene.

5.6 Conventional PCR Screening for PVL gene

Nine isolates of *S.aureus* were positive for pvl gene 9/49 (18.36 %), PCR product of this gene was 433 bp, as shown in (figure 4). The M Lane is standard DNA sized from 1500-100 bp. Lanes 1, 4, 5, 6, and 7 were negative samples. Lanes 2, 3, 5, and 8 represent positive samples for the 433 bp PVL gene. Electrophoresis was performed on 100V for 55 minutes on (1.5 %) agarose.

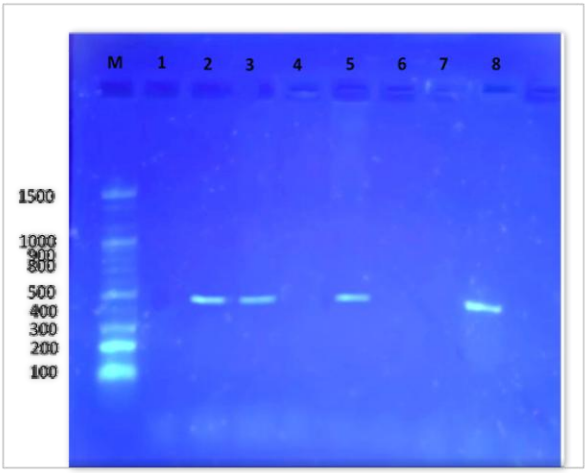

Figure 4: PCR product gel electrophoresis of PVL (panto valentine) gene.

5.7 Conventional PCR Screening for sea gene

Seven isolates of *S. aureus* were positive for (sea) gene 7/49 (14.28 %). PCR product of this gene was 676 bp.

5.8 Conventional PCR Screening for Seb gene

Nine isolates of S. aureus were positive for (seb) gene 9/49 (18.36 %). PCR product of this gene was 404 bp. Genes encoding for fnbA and fnbB were encountered in (57.14 %) and (16.32 %) respectively. In this study fnbA was the most common that concordance with studies from Chain and Palestine [23-24]. However, in both these reports, the prevalence of fnbA was higher than this study with (78.2 %) and (62.5 %), respectively. The occurrence of fnbB was (29 %) in both reports.

Polysaccharide intercellular adhesin, which is required for biofilm development, is encoded for and controlled by the intercellular adhesion (icaA) operon. Among the ica genes, icaA is crucial for *staphylococcus aureus* for the development of biofilms. The *N*-acetylglucosaminyltransferase enzyme, which generates *N*-acetylglucosamine oligomers from UDP-*N*-acetylglucosamine, is encoded by the icaA gene. In the current study, icaA was detected in 20/49 (40.81 %) isolates. The detection of the icaA gene was lower when compared to a study from North India, wherein (52.3 %) harboured the icaA gene.31 studies from Brazil, China, and Iran reported (100 %), (89.9 %) and (60.3 %) of icaA, respectively. Hence, icaA may not be the sole gene associated with biofilm formation [25-26].

HLA and hlb genes that encode for cytolytic toxins such as alpha-hemolysin and beta-hemolysin in *staphylococcus aureus* were the most common virulence genes in this study. Majority of isolates 15/49 (30.16 %), in the present investigation carried at least one of the hemolysin encoding gene, hla and/or hlb. Twenty-nine isolates did not harbor any of the hemolysins.

Hlb was more common than hla with their occurrence being (59.18 %) and (30 %). In Iran also hlb was the most common hemolysin observed, while in China hla was the most predominant [27]. *Staph aureus* could contain several enterotoxins (SEs) that could cause poisoning symptoms when taken [28]. *Staph aureus* enterotoxin also may be implicated as a virulence factor in some cases of toxic shock-like syndromes. In a local study, the Sea, Seb rate was (86.78 %), and (52.2 %) [29]. In general, the Sea gene was the most common compared to the Seb,

Sec, and Sed genes, and this corresponds to what was stated in the study results.

6. Conclusion

Data from the study revealed that different bacterial *spp.* were the causative agents of bacterial eye infection, but the most common bacterial was the *staphylococcus aureus*.

7. References

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