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# Characterization of Some Virulence Genes in Gram Positive Bacteria Isolated from External Eye Infections

**Conflict of interest:** nothing to declare.
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**Abstract**

**Introduction.** External ocular infections are common ophthalmic conditions caused mainly by Bacterial pathogens and may lead to serious complications if untreated.
**Purpose.** To identify some virulence factors of common Gram-positive bacteria isolated from patients with an external bacterial eye infection.
**Materials and methods.** A total of 209 eye swabs were collected from patients attending the Specialized Eye Center in Al-Haboubi General Hospital, Al-Nasiriyah, Iraq, between November 2025 to February 2026. Standard microbiological techniques including morphological characterization on culture media, biochemical tests, API 20 Staph system and VITEK compact system were used for bacterial identification. PCR technique was used for molecular detection of virulence genes (hlg, eap) in S. aureus, and S. epidermidis and (ply, lytA) genes in S. pneumoniae.
**Results.** The current results recorded that only (100; 47.85%) were positive for bacterial infection; even though (109; 52.15%) showed no bacterial growth. According to the distribution of bacterial species, the most frequently isolated bacteria were S. aureus (60.0%), followed by S. epidermidis (36.0%); at that point, one isolate identify as S. pneumoniae. The present data showed Conjunctivitis and Blepharitis were the most common diseases of external ocular infections, for isolation of Gram- positive bacteria. The PCR results recorded that 96%, and 72% of S. aureus isolated harbored eap and hlg genes; while approximately 89% % of S. epidermides iisolated had eap gene, and 57% give positive results of hlg gene. Also, a S. pneumoniae harbored ply gene, and no had lytA gene.
**Conclusion.** This study concluded that conjunctivitis and blepharitis were the most common external eye infections. Also, a S. aureus is the main pathogens of Gram-positive bacteria of eye infections.
**Keywords:** eye infections, S. aureus, S. epidermidis, S. pneumoniae, hlg, eap, ply, lytA

## INTRODUCTION

The eye is a vital sensory organ responsible for vision, through which most environmental information is obtained. It consists of specialized structures that work together to receive, focus, and transmit visual signals to the brain, making sight the dominant human sense [1]. The eye is composed of several functional structures including the cornea, aqueous humor, iris, pupil, lens, ciliary body, vitreous humor, and retina. These parts collectively ensure light refraction, image formation, and signal transmission to the brain for visual perception [2]. Common ocular infections include conjunctivitis, blepharitis, keratitis, and dacryocystitis, which range from mild inflammation to vision-threatening conditions [3, 4]. Conjunctivitis is inflammation of the conjunctiva, mainly caused by viral or bacterial infections, with Staphylococcus aureus and Streptococcus pneumoniae among the key pathogens [5]. While the blepharitis is a chronic inflammatory disorder of the eyelid margins associated with bacterial colonization, particularly Staphylococcus species. It leads to irritation and discomfort and is linked to biofilm formation and gland dysfunction [6]. Also, Keratitis is an inflammation of the cornea caused by infectious or non-infectious agents. It can lead to corneal damage, ulceration, and vision loss due to inflammatory cell infiltration and tissue destruction [4]. Dacryocystitis is an infection or inflammation of the lacrimal sac, usually due to nasolacrimal duct obstruction. The S. pneumoniae and Staphylococcus species were common causes of dacryocystitis [7]. S. aureus are the major causes of ocular infections due to their ability to produce toxins, form biofilms, and evade immune responses, leading to increased pathogenicity [8]. The gamma hemolysin (Hlg), and extracellular adherence protein (Eap) are key virulence factors of S. aureus; while pneumolysin (Ply) and autolysin (LytA) are major virulence determinants of S. pneumoniae. SpA, encoded by the spa gene, promotes immune evasion in addition to contributing to biofilm formation and inflammation [9]. Gamma hemolysin, encoded by hlgAB and hlgCB, is a pore-forming toxin that damages leukocytes and erythrocytes, enhancing immune evasion and tissue injury [10]. S. pneumoniae is a Gram-positive diplococcus responsible for respiratory and ocular infections such as conjunctivitis and keratitis. Its virulence is mainly due to its polysaccharide capsule and toxins such as pneumolysin [11]. In S. pneumoniae, pneumolysin, encoded by the ply gene, is a cholesterol-dependent cytolysin that forms pores in host cell membranes, leading to cell death and strong inflammatory responses [12]. Autolysin, encoded by lytA gene, mediates bacterial cell lysis and the release of pneumolysin and other pro-inflammatory components, thereby amplifying tissue damage and disease severity [13].

## PURPOSE OF THE STUDY

To identify some virulence factors of common Gram-positive bacteria isolated from patients with an external bacterial eye infection.

## MATERIALS AND METHODS

### Bacterial Identification

A total of 209 eye swab samples were collected in a cross-sectional study from male and female patients with clinically diagnosed external ocular infections attending Al-Haboubi General Hospital between November 2025 to February 2026. Diagnosis was confirmed by ophthalmological examination using slit lamp microscopy. Sterile cotton-tipped swabs

pre-moistened with physiological saline were used, then immediately placed in Amies transport medium and sent to the microbiology laboratory. Samples were cultured on Blood agar, Mannitol salt agar, MacConkey agar, and Chrom agar, and incubated at 37 °C for 24 hours. Bacterial identification was based on colony morphology, hemolysis, Gram staining, and biochemical tests (catalase and coagulase), with further confirmation using the API Staph system and VITEK compact system for diagnosis S. pneumoniae.

**DNA Extraction and Molecular Detection**

Genomic DNA was extracted from bacterial isolates using Presto™ Mini gDNA Bacteria Kit (Geneaid, Taiwan) following the manufacturer's instructions. Polymerase chain reaction (PCR) was performed for the detection of virulence genes in bacterial isolates. The PCR reaction mixture consisted of 12 µL of master mix, 3 µL of DNA template, 1 µL of each forward primer and reverse primers, and nuclease-free water to a final volume of 20 µL. Specific primers targeting of hlg, and eap genes in S. aureus and S. epidermidis. Even though the ply and lytA genes in S. pneumoniae; the primer sequences and annealing temperature, product size of all genes was listed in Table 1. The PCR products were visualized using 2% agarose gel electrophoresis [14]. Statistical analysis was performed by using Statistical Package for the Social Sciences (SPSS) version 26, based in using Chi-square at p. value <0.05.

**Table 1**
**Sequences of primers used in gene amplification**

| Gene | Primer Sequences (5´- 3´) | Product size | Annealing Temperature, °C, time | Reference |
|---|---|---|---|---|
| hlg | F: GCCAATCCGTTATTAGAAAATGC<br>R: CCATAGACGTAGCAACGGAT | 937bp | 55°C, 60 sec | [15] |
| eap | F: TAC TAA CGA AGC ATC TGC C<br>R: TTA AAT CGA TAT CAC TAA TACCTC | 230bp | 50°C, 60 sec | [16] |
| lytA | F: CGGTTGGAATGCTGAGACCT<br>R: AGTACCAGTTGCCGTCTGTG | 461bp | 50°C, 30 sec | [17] |
| Ply | F: GGCGCCAAGTCTATCTCAAG<br>R: GCTGTAAAGCGACTGCCTTC | 238bp | 50°C, 60 sec | [18] |

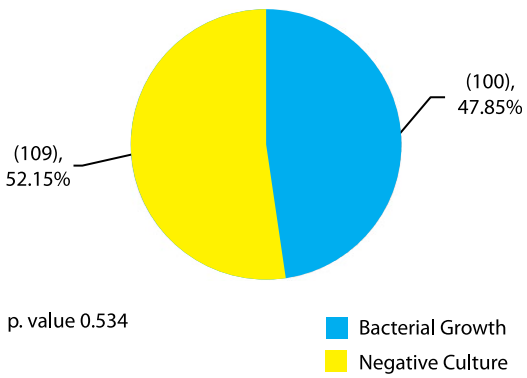



**Fig. 1. Prevalence of bacterial infection in patients with bacterial eye infection**

**Statistical Analysis**

The data of the present study was statistically analysed by using SPSS version 26, based on using Chi-square for independent at p. value <0.05.

## RESULTS

**Bacterial Isolation and Identification**

A total of 209 eye samples were collected from patients with various ocular infections, nearly 100 samples (47.85%) were positive for bacterial infection; while, 109 samples (52.15%) showed no bacterial growth, as exposed in Figure 1. The difference was statistically non-significant at (p>0.05).

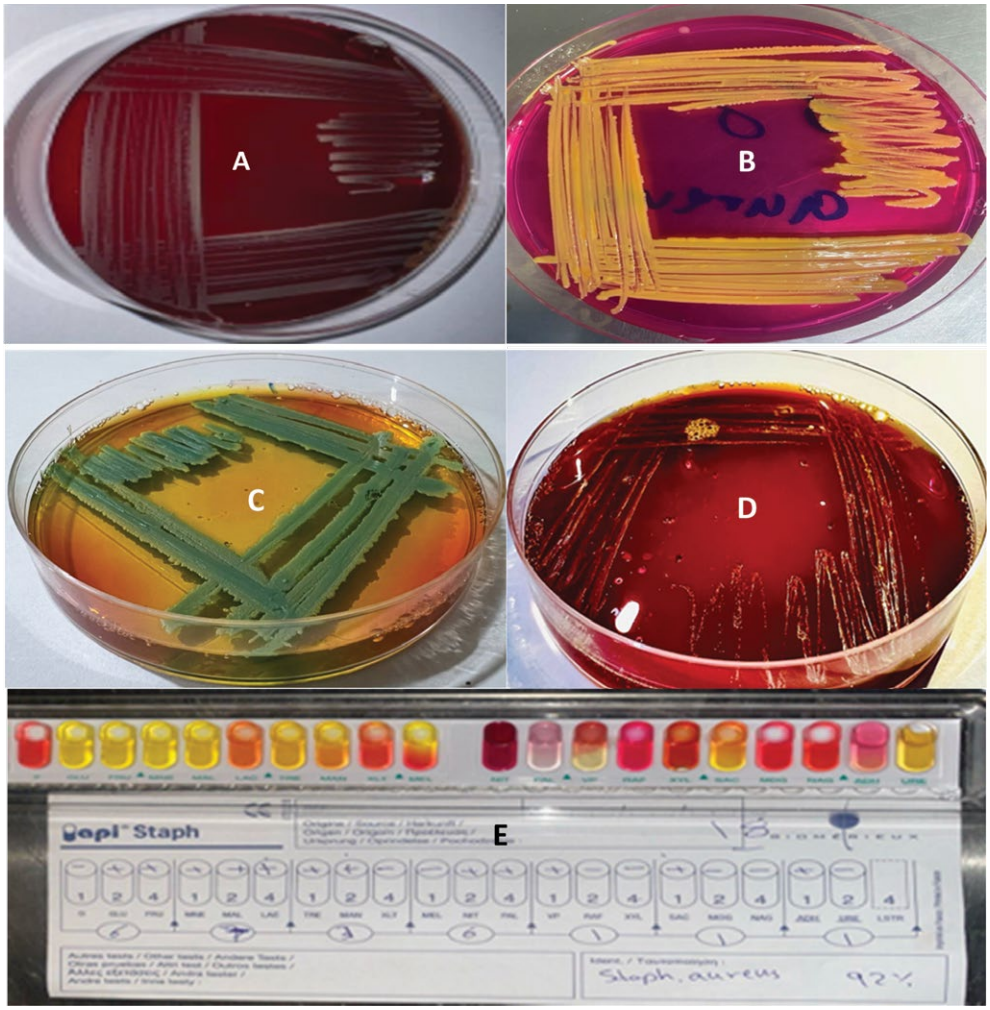



**Fig. 2. The appearance of S. aureus on A – blood agar, B – MSA, C – chrom agar, D – for confirming identification of S. aureus isolates used the API Staph System (BioMerieux/France), E – the appearance of S. pneumoniae on blood agar**

**Prevalence of Bacterial Species in Eye Infection Patients**

Regarding the distribution of bacterial species, statistically a significant difference was observed at ($p<0.01$). The most frequently isolated organism was S. aureus (60.0%), followed by S. epidermidis. (36.0%), and 1 isolate (1%) identified as S. pneumoniae, as listed in Table 2. To identify S. aureus, the bacterial isolates were streaked onto MSA medium that used for the isolation, purification, and identification of Staphylococcus spp., and Chrom agar that used to accurate identification of Staphylococcus species. A S. aureus isolates were mannitol fermenter and appeared as yellow colonies on this medium; while colonies appeared as small, and green colonies on chrom agar, as shown in Figure 2.

**Table 2**
**Prevalence of bacterial species in eye infection patients**

| Bacterial species | No. | Percentage % |
|---|---|---|
| S. aureus | 60 | 60.0 |
| S. epidermidis | 36 | 36.0 |
| S. pneumoniae | 1 | 1.00 |
| G-ve Bacteria | 3 | 3.00 |
| Total | 100 | 100 |
| **CalX²=96.2, TabX²=7.81, DF=3, p. value <0.01** | | |

**Table 3**
**Result of Viteks system tests for S. pneumoniae**

| No. | Test | Result | NO | Test | Result | NO | Test | Result |
|---|---|---|---|---|---|---|---|---|
| 1 | ADH1 | – | 13 | dGAL | + | 25 | NAG | + |
| 2 | ADH2s | – | 14 | dMAN | + | 26 | NC6.5 | – |
| 3 | AGLU | + | 15 | dMAL | + | 27 | NOVO | + |
| 4 | AGAL | + | 16 | dRAF | – | 28 | OPTO | + |
| 5 | AMY | – | 17 | dRAB | – | 20 | PHOS | + |
| 6 | APPA | + | 18 | TyrA | – | 30 | PIPLC | – |
| 7 | ASPA | – | 19 | dSOR | – | 31 | ProA | – |
| 8 | BACL | – | 20 | dTRE | + | 32 | PUL | – |
| 9 | BGAL | + | 21 | dXYL | – | 33 | PyrA | + |
| 10 | BGAR | + | 22 | ILATK | + | 34 | SAC | + |
| 11 | BGUR | – | 23 | LAC | + | 35 | SAL | + |
| 12 | CDEX | – | 24 | LeuA | – | 36 | URE | – |

**Table 4**
**Prevalence of Gram-positive bacteria in eye infection patients**

| | S. aureus | | S. epidermidis | | S. pneumonia | |
|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % |
| Blepharitis | 24 | 40.00 | 11 | 30.56 | 0 | 0.00 |
| Conjunctivitis | 26 | 43.33 | 21 | 58.33 | 1 | 100 |
| Hordeolum | 2 | 3.33 | 3 | 8.33 | 0 | 0.00 |
| Dacryocystitis | 8 | 13.33 | 1 | 2.78 | 0 | 0.00 |
| Total | 60 | 61.86 | 36 | 37.11 | 1 | 1.03 |
| **CalX²=89.5, TabX2=12.59, DF=6, p. value <0.01** | | | | | | |

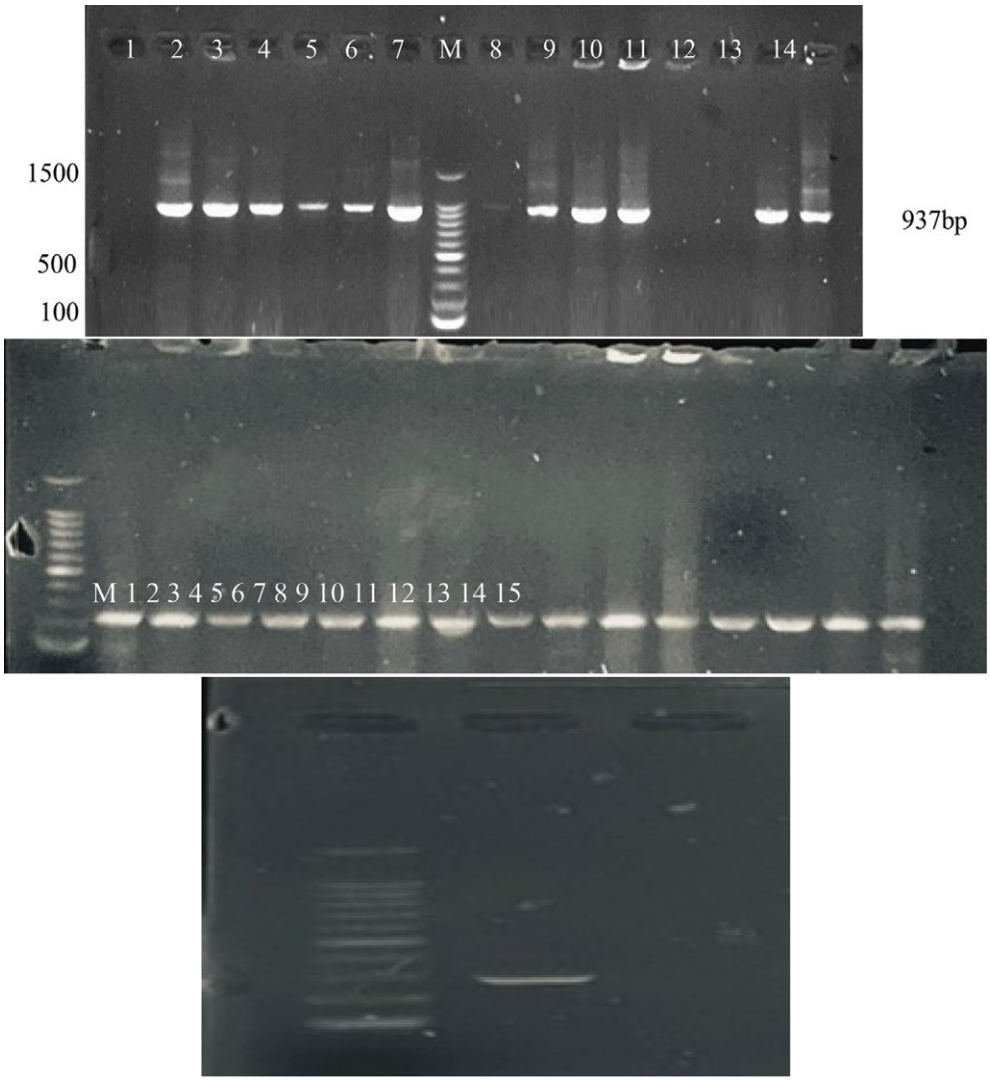



**Fig. 3. A – Agarose gel electrophoresis of hlg gene, where M: marker; 2–11: positive results; 1, 12, 13: negative results. B – Agarose gel electrophoresis of eap gene, where M: marker; 1–15: positive results. C – Agarose gel electrophoresis of ply gene, where M: marker; 1: positive results; 2: negative control**

**Table 5**
**Distribution of genes in Gram positive bacteria isolates.**

| S. aureus | | | S. epidermidis | | | S. pneumoniae | |
|---|---|---|---|---|---|---|---|
| **Gene** | **No.** | **%** | **No.** | **%** | **Gene** | **No.** | **%** |
| hlg | 43 | 72% | 21 | 57% | lytA | 0 | 0% |
| eap | 58 | 96% | 32 | 89% | Ply | 1 | 100% |

The S. pneumoniae isolate was cultured on blood agar, and it incubated at 37 ºC for 24 h, as in Figure 4. Also, for accurate identification of this Bacteria used of Viteks compact system, the results of 36 biochemical tests to identification S. pneumoniae, as in Table 3.

The present study was noted the prevalence of Gram-positive bacteria across a common external ocular infection. The prevalence of the predominant pathogen (S. aureus), with the highest percentage in conjunctivitis (43.33%) of all S. aureus cases; then in blepharitis (40.00%); followed by in Dacryocystitis (13.33%). S. epidermidis was the second most common pathogen, showing a high percentage in conjunctivitis (58.33%), then in Blepharitis (30.56%). S. pneumoniae was rarely isolated bacteria; in addition, only conjunctivitis patients have infection with S. pneumonia (100%), as in Table 4.

**Molecular Study**

The results of PCR technique showed that 72%, 96% of S. aureus isolates harbored hlg and eap genes; while the percentage of both genes in S. epidermidis was 57% and 89%, respectively. According to current results, the bands that had shown in Figure 3A documented the size of hlg gene that was nearly 937 bp. Although, Figure 3B revealing the size of eap gene was closely 230 bp. A S. pneumoniae isolate has ply gene; while this isolated no harbored lytA gene, as listed in Table 5. The molecular size of Ply gene recorded that in Figure 3C, that was nearly 238 bp.

## ■ DISCUSSION

The present study revealed that conjunctivitis and blepharitis were the most common type of eye infections. This finding is consistent with previous studies that identified conjunctivitis as the predominant ocular infection due to its high transmissibility and association with environmental exposure and poor hygiene practices [3, 19]. However, these results disagreed with other studies like [20] reported that the blepharitis was more prevalent ocular infections in certain populations, particularly among contact lens users and hospitalized patients.

In this study, the bacterial Growth were detected in 47.85% of samples collected from ocular eye infections, whereas a 52.15% showed no bacterial infection. The current finding differed from the results of [21] indicated that a considerable proportion of ocular infections may be non-bacterial in origin. In contrast, other studies have reported a significantly higher bacterial isolation rates exceeding (60–70%) of collected samples, and who suggested that regional and methodological variations in detection [22].

The predominance of S. aureus was observed in (60%) total isolates; this study agrees with several studies that identified Gram-positive bacteria as the leading causative agents of ocular infections [19, 20, 23]. However, the recent results differed from other studies that reported a higher prevalence of coagulase-negative staphylococci such as S. epidermidis or even Gram-negative organisms like Pseudomonas aeruginosa, particularly in hospital-acquired infections [24].

The results of molecular study showed that the hlg gene was detected in 72% and 57% of S. aureus and S. epidermidis isolates, respectively. Even though the eap gene was detected in 96%, and 89% of both S. aureus and S. epidermidis isolates. The high percentage of presence of both virulence genes is related with enhance bacterial pathogenicity. These findings are consistent with [25], who reported a high prevalence of the hlg gene in clinical isolates of S. aureus, highlighting its importance in bacterial



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virulence. Similarly, [26] emphasized that hemolysin genes are key virulence determinants in S. aureus due to their role in tissue damage and infection progression.

About 96% and 89% of S. aureus and S. epidermidis harbored eap gene, these results agreed with [27], who reported that a widespread distribution of this gene among clinical isolates and confirmed its crucial role in bacterial adhesion, colonization, and biofilm formation. In contrast [28], reported a lower prevalence of this gene, which may be attributed to differences in sample sources, geographical distribution, or molecular detection methods.

The current study showed the S. pneumoniae isolate had ply gene only, and no had lytA gene. This study is unlike with study performed by [29] who reported that the lytA gene is considered one of the most specific and conserved molecular markers for the identification of S. pneumoniae and is usually detected at a high prevalence among clinical isolates. Therefore, the absence of the gene (0%) in the present study is considered an unusual finding and may be attributed to genetic variations of this isolate. In contrast, the detection of the ply gene in 100% of this isolate agreed with the findings of [30, 31] confirmed the widespread distribution of this gene among S. pneumoniae isolates due to its important role as a major virulence factor. Similarly [32], indicated that the ply gene is strongly associated with pneumococcal pathogenicity and is commonly detected at a high frequency in clinical isolates.

## ■ CONCLUSION

This study concluded that conjunctivitis and blepharitis were the most common external eye infections. Also, a S. aureus is the main pathogens of Gram-positive bacteria that recovered from eye infections.

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