Prevalence of Gram-Positive and Negative Bacteria Associated with External Ocular Infection in Wasit Province, Iraq

Mukal Assaad Hussein, Rana H. Raheema, Hassan Khalil Melek[1], Hayder Abdul-Amir M. Al-Hindy[2]

Departments of Medical Microbiology and [1]Surgery, College of Medicine/University of Wasit, Kut, [2]Department of Pharmacology and Toxicology, College of Pharmacy, University of Babylon, Babylon, Iraq

Abstract

Background: In developing countries, ocular infections are a significant public health concern, particularly bacterial infections, which are common. This research aimed to estimate how often Gram-positive and negative bacteria are present in individuals who have external ocular infections in Wasit province. **Materials and Methods:** One hundred twenty-one eye swabs were gathered from patients with various eye infections in the consulting clinics of AL-Zahra Hospital between October 1, 2021, and March 1, 2022. Various bacterial colonies with different morphological and biochemical characteristics were detected through growth testing. **Results:** Of all the external ocular samples collected, 56 (46.2%) had a positive culture for bacterial pathogens. Most of the isolated bacteria were Gram-positive, accounting for 73.2% (41 cases) of the total. Coagulase-negative Staphylococci (CoNS) were the most commonly identified Gram-positive bacteria, followed by *Staphylococcus epidermidis*, *Staphylococcus hominis*, *Staphylococcus haemolyticus*, *Staphylococcus aureus*, *Staphylococcus lentus*, *Staphylococcus hominis spp. Novobiosepticus*, *Staphylococcus pseudintermedius*, *Staphylococcus lugdunesis*, *Staphylococcus gallinarum*, *Staphylococcus vitulinus*, *Staphylococcus cohnii*, *Streptococcus pneumoniae*, *Kocuria rosea*, *Micrococcus luteus*, *Leuconostoc mesenteroides*, and, among which, Gram-negative bacteria accounted for 26.8% such as *Klebsiella pneumonia*, *Proteus mirabilis*, *Enterobacter aerogenes*, *Pantoea spp.*, *Yersinia enterocolitica*, *Citrobacter sedlakii*, *Pseudomonas aeruginosa*, *Pseudomonas oryzihabitans*, and *Sphingomonas paucimobilis*. DNA extraction and their purity and concentration were confirmed using Nanodrop. A monoplex pattern of polymerase chain reaction (PCR) was employed to detect Gram-positive specific 16S rRNA. **Conclusion:** A majority of the bacterial strains identified in patients with ocular infections, particularly Gram-positive bacteria, also concluded that the largest percentage of eye infections was for patients who lived in rural areas, this explains the reason for the isolation of some bacterial species that inhabit animals such as *S. lentus*, *S. gallinarum*, *S. pseudintermedius*, *S. vitulinus*, *S. cohnii*, and *L. mesenteroides*. In addition, most of the bacterial species found in patients with eye infections were resistant to multiple drugs, indicating the development of multidrug-resistant bacteria in the study population, also concluded that the most prevalent forms of external ocular infections were blepharitis, followed by conjunctiva.

Keywords: 16SrRNA, antibiotic susceptibility pattern, external ocular infection, Gram-positive and negative bacteria isolate

Introduction

Ocular infections are a principal cause of blindness and visual loss globally. Similarly, if treatment for an eye infection or inflammation is delayed, blindness might result.[1] The World Health Organization reports that approximately 285 million individuals worldwide have visual impairments. Ninety percent of them are from nations with low median incomes.[2] There is a wide range of morbidity associated with infections of the external ocular region, ranging from minor infections that clear up on their own to infections that jeopardize a patient's vision.[3] Bacteria can lead to a diverse range of eye diseases, including conjunctivitis, orbital cellulitis, keratitis, endophthalmitis, blepharitis, and dacryocystitis, among others.[4,5] Generally, bacterial infections affecting

Access this article online

Quick Response Code:

Website: https://journals.lww.com/mjby

DOI: 10.4103/MJBL.MJBL_558_23

Address for correspondence: Prof. Hayder Abdul-Amir M. Al-Hindy, Department of Pharmacology and Toxicology, College of Pharmacy, University of Babylon, Babylon, P-51001, Iraq. E-mail: makihayder68@gmail.com

Submission: 15-May-2023 **Accepted:** 06-Jul-2023 **Published:** 27-Sep-2023

How to cite this article: Hussein MA, Raheema RH, Melek HK, Al-Hindy HA-AM. Prevalence of Gram-positive and negative bacteria associated with external ocular infection in Wasit province, Iraq. Med J Babylon 2023;20:600-7.

the surface of the eye are the most common type of eye infection.[6]

Most cases of bacterial ophthalmic infections are typically treated by broad-spectrum antibiotics as a first-line approach, which often results in the development of antibiotic resistance. In addition, the widespread misuse of broad-spectrum antibiotics, for instance, to treat viral or bacterial infections or as a prophylactic measure, has contributed to the development of antibiotic resistance issues, which is a global issue.[7] The establishment of bacterial resistance to several antimicrobial drugs might raise the chance of medical failure. Multidrug resistance is becoming a severe problem that is becoming more widespread.[8] This project was presented to detect the incidence of common Gram-positive and negative bacteria from cases with an external eye infection in Al-Kut city, Wasit province, Iraq, and to evaluate the *in vitro* drug susceptibility patterns of these isolates and then molecular analyses.

Material and Methods

One hundred twenty-one cases of eye infections were collected for a cross-sectional study from patients who were admitted to the Al-Zahra teaching hospital in the Waist province between October 1, 2021, and March 1, 2022. These patients were of both sexes, male and female. Collecting sampling swabs, sterile cotton-tipped swabs presaturated with hygienic physiological saline were aseptically used. These swabs were then placed in an Amies transport medium and transported to a clinical bacteriology laboratory. Identification of bacterial isolates was completed via biochemical and morphological tests, as well as Vitek2The Kirby-Bauer method for assessing antibiotic susceptibility was used on Muller Hinton agar,[9] and the results were interpreted following the standards.[10]

Using Nanodrop (Thermo Scientific, USA), the purity and concentration of the DNA that was isolated from 33 Gram-positive bacteria were verified. The samples' nucleic acid concentrations ranged from 50 to 360 mg/L, and their purity ranged from 1.8 to 2. Then, primers were created following the instructions provided by studies by [11,12] [Table 1].

Programmable thermal controller

The 1.5%–2% agarose gel wells were filled with the polymerase chain reaction (PCR) product and the DNA

Table 1: Primers' sequence of 16SrRNA genes

No.	Bacteria	Sequences (5′–3′)	Product size (bp)	References
1.	*Staphylococcus cohnii*	F: CCGTGGAGGGTCATTGGAAA R: TTGTCACCGGCAGTCAACTT	550	This study
2.	*Staphylococcus epidermidis*	F: GGAGGCAGCAGTAGGGAATC R: ACCTTCCTCCGGTTTGTCAC	839	
3.	*Staphylococcus haemolyticus*	F: TAACTTCGGGAAACCGGAGC R: TTTCCAATGACCCTCCACGG	501	
4.	*Staphylococcus lugdunensis*	F: GGAAGCAACAACGCAGAACA R: CATCTGGGGCTTGGAAACGA	533	
5.	*Staphylococcus hominis*	F: CTGGAACTGAGACACGGTCC R: TTTCCAATGACCCTCCACGG	327	
6.	*Streptococcus pneumoniae*	F: CCACACTGGGACTGAGACAC R: CCCCGGAAAGGGTCTAACAC	539	
7.	*Staphylococcus aureus*	F: GTGCCAGCAGCCGCGGTAA R: AGACCCGGGAACGTATTCAC	886	[13]
1.	*Enterobacter aerogence*	F: CTGGAACTGAGACACGGTCC R: CGGGGATTTCACATCCGACT	300	This study
2.	*Klebsiella pneumoniae*	F: TGCCTGATGGAGGGGGATAA R: TGCCTGATGGAGGGGGATAA	382	
3.	*Pseudomonas aeruginosa*	F: TCAACCTGGGAACTGCATCC R: CTTCTGGAGCAACCCACTCC	816	
4.	*Sphingomonas paucimobilis*	F: CCACACTGGGACTGAGACAC R: AGGCAGTTCTGGAGTTGAGC	301	
5.	*Proteus mirabilis*	F: CTGCCCGATAGAGGGGGATA R: GGAGTTAGCCGGTGCTTCTT	383	[14]
6.	*Yersinia enterocolitica*	F: GATGACCAGCCACACTGGAA R: GTTAAGCGCGGGGATTTCAC	322	This study
7.	*Pantoea spp.*	F: CCTGGACAAAGACTGACGCT R: CGCTTCTCTTTGTATGCGCC	523	[15]

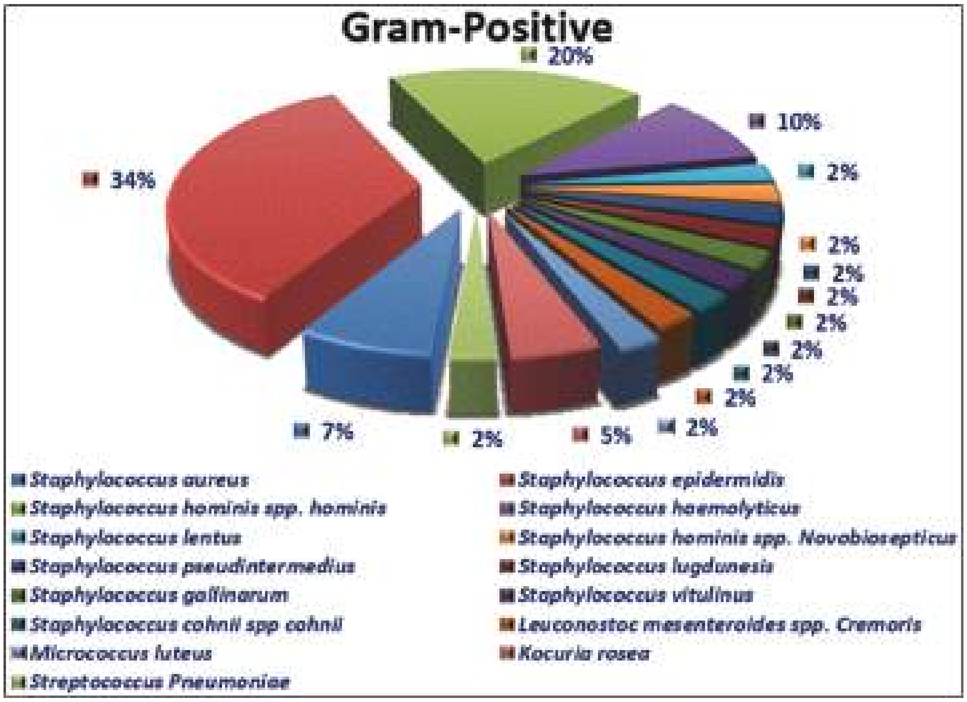

Figure 1: Gram-positive isolates bacteria from external ocular infection of the studied patients

ladder, and the gel electrophoresis was carried out at either 70 or 100 V. The gel was subsequently documented.

Statistical analysis

To conduct the statistical analyses, version 9.1 of the Statistical Analysis System was utilized. Also, the "Chi-square test" was carried out to analyze the differences in percentages. A *P* value of less than 0.05 is considered to be an acceptable significance threshold in statistics.[16]

Results

The current study included a total of 121 patients with external eye infections, and 56 of these patients (46.2%) had a positive culture. The majority of participants (23 [56.1%]) were males, from rural areas (60.9%), and 78.1% were earners. Among the various types of external ocular infection observed, blepharitis was the most prevalent (41.4%), followed by conjunctivitis (24.3%).

Results of Gram-negative showed that 15 (26.8%) isolates of the total belonged to Gram-negative bacteria (*Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterobacter aerogenes*, *Pantoea spp.*, *Yersinia*, *Citrobacter freundii*, *Pseudomonas aeruginosa*, *Pseudomonas oryzihabitans*, and *Sphingomonas paucimobilis*). Isolate was first determined by morphological and biochemical tests and Vitek2. The males and females were nearly equal. Most of the patients (60%) came from remote regions, and the majority (93.3%) were working. The most prevalent external ocular infection was blepharitis 10 cases (66.6%) and conjunctivitis 2 cases (13.3%), whereas the viral infection was 3 cases (20%).

It was found that 41 isolates (73.2%) belonged to Gram-positive bacteria [Figure 1]. All isolates were tested for resistance against antibiotics from different classes, and the results showed that all *Staphylococcus aureus* isolates were (100%) resistant to amoxicillin-clavulanic acid, ampicillin, penicillin, cefoxitin, and erythromycin, and resistant to chloramphenicol (66.7%). However, all isolates were sensitive (100%) to ciprofloxacin, amikacin, trimethoprim-sulphamethoxazole, and gentamicin.

Isolates of *Staphylococcus epidermidis* were resistant (100%) to penicillin followed by 92.9% to ampicillin then 50% to amoxicillin-clavulanic acid, whereas sensitive (100%) to amikacin followed by 92.9% to clindamycin, 78.6% and 71.4% to chloramphenicol and ciprofloxacin, respectively, and 57.1% for both cefoxitin and trimethoprim-sulphamethoxazole. *S. epidermidis* showed a high degree of resistance to penicillin (97%), cotrimoxazole (47%), gentamicin (26.5%), and a low degree of resistance toward ciprofloxacin (58.8%).

Staphylococcus hominis spp., Hominis, most of these isolates were resistant (87.5%) to penicillin and erythromycin followed by 62.5% to ampicillin, 50% to amoxicillin-clavulanic, cefoxitin, and trimethoprim-sulphamethoxazole, whereas all of these isolates were sensitive (100%) to amikacin followed by 87.5% to tetracycline and gentamicin, 75.0% to clindamycin, and 62.5% to ciprofloxacin. All isolates of *Staphylococcus haemolyticus* were resistant (100%) to ampicillin followed

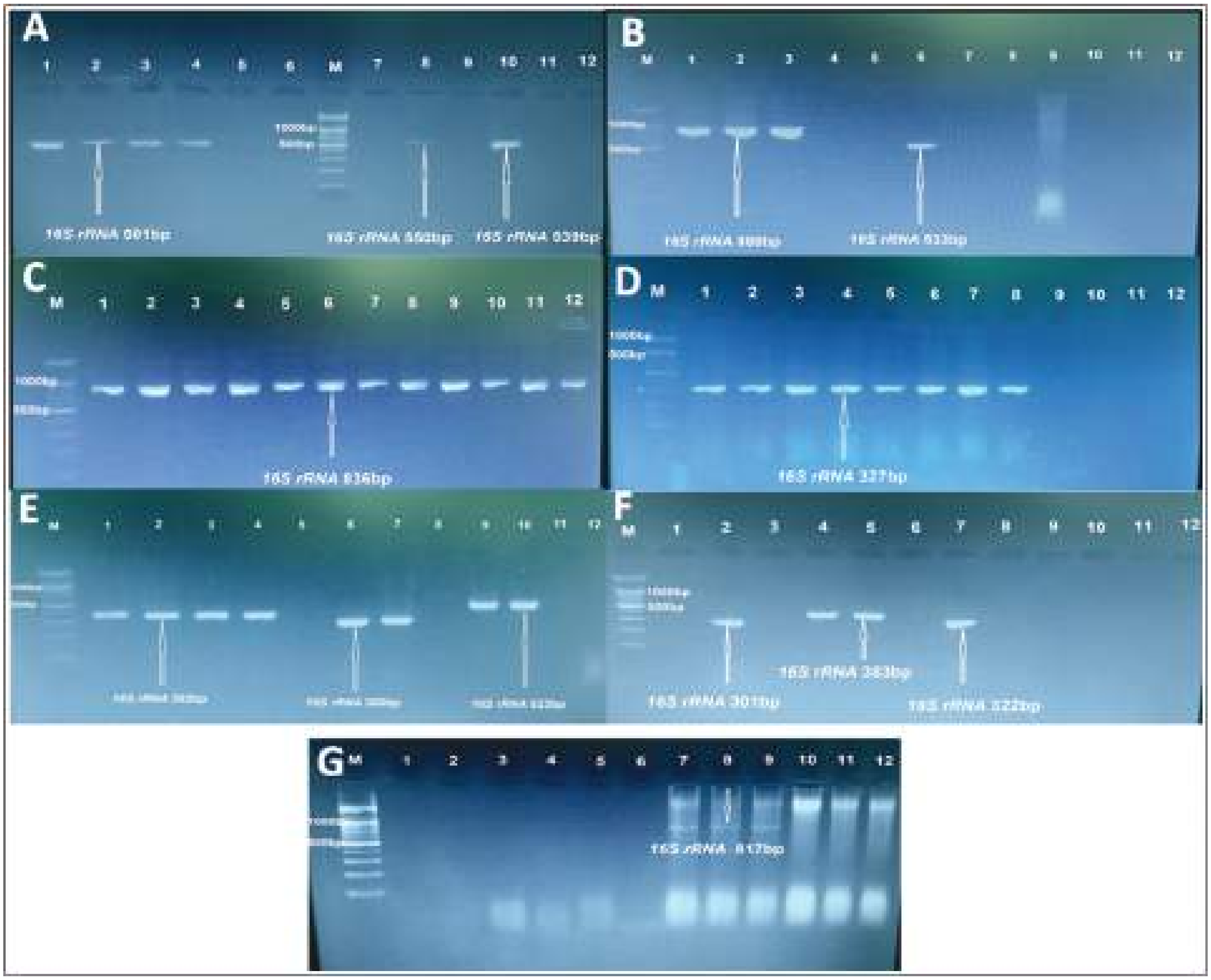

Figure 2: Using traditional PCR, samples were run by gel electrophoresis, ethidium bromide dye staining, UV transilluminator visualization, and agarose 1.8%, 100 V/cm for 35 min. DNA ladder, lane, 2000 bp was used as the reference marker (M), in lanes 1 through 12: (A) *Staphylococcus haemolyticus, Staphylococcus cohnii*, and *Streptococcus pneumoniae* 16S rRNA gene,16S rRNA 539 bp, 550 bp, and 501 bp. (B) The 16S rRNA gene from *Staphylococcus lugdunesis* and *Staphylococcus aureus*. 16S rRNA 886 bp and 16S rRNA 533 bp. (C) *Staphylococcus epidermidis* 836 bp of 16S rRNA. (D) *Staphylococcus hominis* 16S rRNA gene, 327 bp of 16S rRNA. (E) *Klebsiella pneumoniae, Enterobacter aerogenes*, and *Pantoea*: 16S rRNA genes with sizes 382 bp, 300 bp, and 523 bp were observed. (F) *Sphingomonas paucimobilis, Proteus mirabilis*, and *Yersinia pestis*: 16S rRNA gene with sizes of 301 bp, 383 bp, and 322 bp. (G) *Pseudomonas aeruginosa*:16S rRNA, 817 bp. PCR = polymerase chain reaction.

by 75.0% to all of the following antibiotics (cefoxitin, ciprofloxacin, trimethoprim-sulphamethoxazole, and gentamicin). However, their sensitivity was relatively lower (50%) to amoxicillin-clavulanic acid and tetracycline.

Staphylococcus pseudintermedius isolate was resistant (100%) to cefoxitin and penicillin, whereas sensitive to all of the following antibiotics: ampicillin, clindamycin, amoxicillin-clavulanic acid, erythromycin, ciprofloxacin, chloramphenicol, amikacin, tetracycline, trimethoprim-sulphamethoxazole, and gentamicin.

Results of antibiotic susceptibility of Gram-negative bacteria are as follows: *E. aerogenes* were resistant (100%) to ceftriaxone, ampicillin, clindamycin, penicillin, and erythromycin, whereas sensitive (100%) to tobramycin, amoxicillin-clavulanic acid, cefoxitin, ciprofloxacin, chloramphenicol, tetracycline, amikacin, trimethoprim-sulphamethoxazole, and gentamycin.

The resistance to ceftriaxone was 100% across all *K. pneumoniae* isolates, clindamycin, penicillin, and erythromycin, respectively, followed by 75% to ampicillin, whereas sensitive (100%) to tobramycin, amoxicillin-clavulanic acid, cefoxitin, ciprofloxacin, chloramphenicol, tetracycline, amikacin, and gentamycin, respectively.

Yersinia isolate was resistant (100%) to ampicillin, clindamycin, cefoxitin, and penicillin, whereas sensitive (100%) to ceftazidime, ceftriaxone, tobramycin, amoxicillin-clavulanic acid, erythromycin, ciprofloxacin,

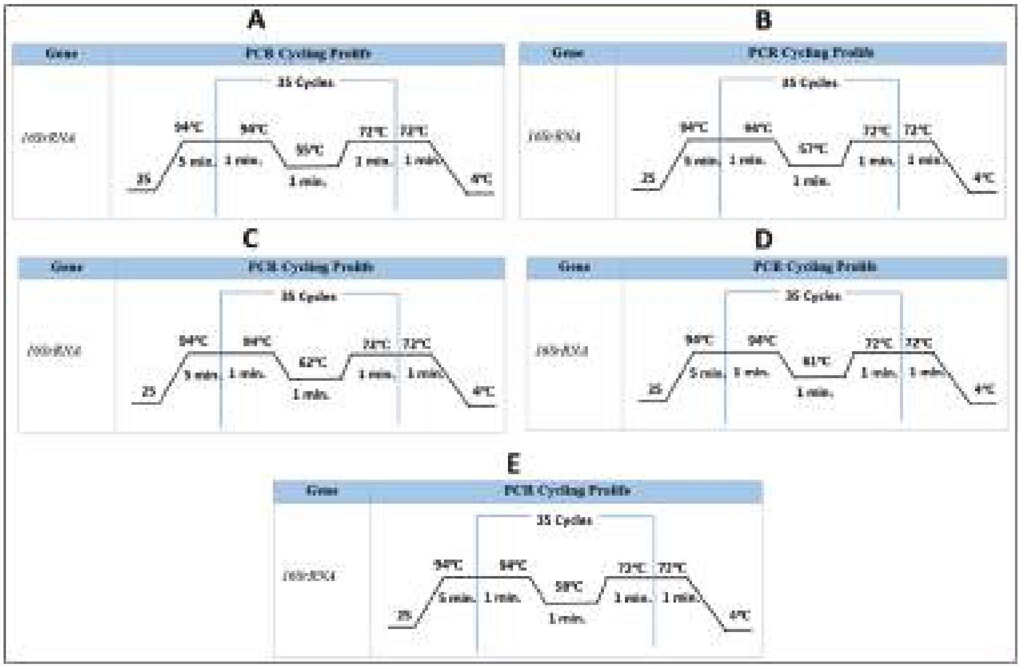

Figure 3: Thermal cycling program for 16S rRNA genes of: (A) *Staphylococcus homins*, (B) *Staphylococcus lugdunesis*, *Streptococcus pneumoniae*, and *Staphylococcus epidermidis*, (C) *Staphylococcus cohnii*, (D) *Staphylococcus haemolyticus*, and (E) *Staphylococcus aureus*

chloramphenicol, amikacin, tetracycline, trimethoprim-sulphamethoxazole, and gentamicin.

Both Pantoea isolates resisted (100%) penicillin and erythromycin, whereas sensitive (100%) to ceftazidime, ceftriaxone, ampicillin, tobramycin, cefoxitin, amoxicillin-clavulanic acid, amikacin, chloramphenicol, gentamicin, and tetracycline, respectively.

P. oryzihabitans isolate was resistant (100%) to clindamycin, amoxicillin-clavulanic acid, penicillin, trimethoprim-sulphamethoxazole, and intermediate (100%) to ceftriaxone and ampicillin, whereas sensitive (100%) to ceftazidime, tobramycin, cefoxitin, erythromycin, ciprofloxacin, chloramphenicol, amikacin, tetracycline, and gentamicin.

P. aeruginosa isolate was resistant (100%) to ceftriaxone, ampicillin, clindamycin, amoxicillin-clavulanic acid, cefoxitin, penicillin, and trimethoprim-sulphamethoxazole, respectively, and intermediate (100%) to erythromycin and tetracycline, whereas sensitive (100%) to ceftazidime, tobramycin, ciprofloxacin, chloramphenicol, amikacin, and gentamycin.

Sphangomonas paucimbilis isolate was resistant (100%) to amoxicillin-clavulanic acid, and intermediate (100%) to clindamycin, erythromycin, ciprofloxacin, and tetracycline, whereas sensitive (100%) to ceftazidime, ceftriaxone, tobramycin, ampicillin, cefoxitin, penicillin, chloramphenicol, amikacin, trimethoprim-sulphamethoxazole, and gentamicin, respectively.

C. freundii isolate was resistant (100%) to ceftriaxone, ampicillin, clindamycin, amoxicillin-clavulanic acid, penicillin, and erythromycin, and intermediate (100%) to cefoxitin, whereas sensitive (100%) to ceftazidime, tobramycin, ciprofloxacin, chloramphenicol, amikacin, tetracycline, trimethoprim-sulphamethoxazole, and gentamicin, respectively.

P. mirabilis isolates were resistant (100%) to ampicillin, clindamycin, penicillin, erythromycin, chloramphenicol, and trimethoprim-sulphamethoxazole, whereas sensitive (100%) to tobramycin and amoxicillin-clavulanic acid.

Molecular analysis

This study has revealed that *S. haemolyticus*, a species of Coagulase-negative Staphylococci (CoNS), is a significant cause of infected corneal ulcers, despite it being a part of the normal flora and found everywhere. The advancements in identification and diagnosis have helped identify CoNS as a major cause of such ulcers. The study has found four (100%) cases of *S. haemolyticus*, one (100%) case of *Staphylococcus cohnii*, and one (100%) case of *Streptococcus pneumoniae* through diagnostic tests for *16S rRNA* bacteria. Figure 2 shows the PCR results after they were separated using agarose gel electrophoresis.

The study found that all three samples of bacteria identified as *S. aureus* tested positive for the *16S rRNA* diagnostic test. In addition, one sample of bacteria identified as *Staphylococcus lugdunensis* also tested positive for the same test.

Based on the 16S rRNA diagnostic test used in this investigation [Figure 3], all 14 samples tested positive for the presence of *S. epidermidis.* This information was visualized in Figure 2 (C) using an agarose gel electrophoresis technique.

According to the results of this study, all eight of the bacterial samples tested were identified as *S. hominis* through the use of a 16S rRNA diagnostic test. Figure 2 (D) displays the visualization of PCR products through an agarose gel.

In the case of Gram-negative, the study's findings revealed that the 16S rRNA bacterial diagnostic test successfully identified all four *K. pneumoniae* strains (100%), both *E. aerogenes* strains (100%), and both Pantoea strains (100%). Figure 2 (E) shows the outcomes from the visualization of the PCR products on an agarose gel.

Based on the study findings, the diagnostic test for 16S rRNA bacteria indicated that *S. paucimobilis* (one strain), *P. mirabilis* (two strains), and *Yersinia pestis* (one strain) showed 100% detection accuracy. The results were illustrated in Figure 2 (F) by showing the agarose gel of PCR products.

The study found that the diagnostic test for detecting *P. aeruginosa* using 16S rRNA was 100% accurate in identifying one instance of this bacteria. The results were illustrated in Figure 2 (G) by showing the agarose gel of the PCR products.

Discussion

Hameed[17] reported the highest rate of cases being conjunctivitis, blepharoconjunctivitis followed by blepharitis, dacryocystitis, and other external eye infections. Also, the findings of the study by Zimam *et al*.[18] showed the highest rate was also for conjunctivitis followed by blepharitis and dacryocystitis.

The presence of different bacterial species causing external ocular infections suggests that variations in environmental conditions, personal hygiene practices, age, and the site of infection may be contributing factors. The findings of this study are consistent with a previous study conducted by Hameed,[17] which also reported a high resistance rate of *S. aureus* to cephalexin and penicillin, at 92.5% and 91.6%, respectively. Similarly, the same study reported a high sensitivity rate of these bacteria to ciprofloxacin at 7.4%. This study's findings were in line with those of studies by Raheema[19] and Raheema and Abed.[20] Isolates of *S. epidermidis* were resistant (100%) to penicillin followed by 92.9% to ampicillin then 50% to amoxicillin-clavulanic acid; these outcomes are in line with the results of a study by Pate *et al*.,[21] where 100% of resistance was observed for cefoxitin, and ciprofloxacin and gentamicin were determined to be the two antibiotics that were most efficient against *S. pseudintermedius*. Also, findings were consistent with those of the study by Raheema *et al*.,[22] which also reported that *S. pseudintermedius* isolates were sensitive to ampicillin, ciprofloxacin, gentamicin, erythromycin, tetracycline, and amikacin.

Methicillin-susceptible *S. aureus* (MSSA) acquired in the hospital setting causes keratitis less frequently but leads to a higher incidence of conjunctivitis compared with MSSA, which is acquired in the community.[23] Intraocular infection caused by *Staphylococcus lugdunensis* is not common but can cause severe vision impairment. This kind of infection has been linked to trauma, intravitreal injections of antivascular endothelial growth factor medications, dexamethasone implants, and cataract surgery. Therefore, *S. lugdunensis* would be considered an infrequent but grave cause of postoperative endophthalmitis, principally in diabetic people.[23,24]

However, *K. Pneumoniae* was resistant to ampicillin, amoxicillin, and tetracycline, which is in agreement with Jassim *et al*.s[25] findings.

Both Pantoea isolates resistance results were similar to the result of the studies by Gajdács[26] and Muslem *et al*.,[27] in which all of the isolates were amikacin-susceptible. In addition, the majority were responsive to gentamicin, ceftriaxone, and chloramphenicol.

P. aeruginosa isolate was resistant to different antibiotics and sensitive to ceftazidime, tobramycin, ciprofloxacin, chloramphenicol, amikacin, and gentamycin; this result disagreed with the study by Abdul-Husseein *et al*.[28] The study's findings of *P. mirabilis* isolates revealed high resistance to ampicillin. A comparable finding was verified by studies done by Aljeeliz *et al*.,[29] Raheema *et al*., [30] and Albadri.[31]

This study's results agree with other studies by Acharya *et al*.,[32] which also noted the prevalence of *S. paucimobilis* as a pathogen that may trigger ophthalmic infections such as endophthalmitis, panophthalmitis, and keratitis after surgery for cataracts. *P. mirabilis* has been identified in bacterial conjunctivitis less frequently, and this finding was consistent with another study.[33] The study found that the diagnostic test for detecting pathogenic bacteria using 16S rRNA was 100% accurate in identifying bacteria. Our result is consistent with recent studies,[30,33] and the effectiveness of personal cleanliness may be responsible for the study's low incidence of Gram-negative colonic bacteria. This is due to fecal-oral contamination of the eyes being the main method of transmission for enteric infections. Educating patients in general, and especially patients living outside of the city and animal breeders, on the importance of seeking urgent treatment from an ophthalmologist if a symptom indicates an eye infection, and not using any ointment or antibiotic without first consulting an ophthalmologist. A similar finding was reported by Ibrahim *et al*.,[34] Mohammed *et al*.,[35] and Al-khafaji *et al*.[36] on the use of antibiotics that they should be kept under the supervision and advice of doctors and should be given in appropriate doses for an appropriate period.[37]

Conclusions

The largest percentage of eye infections was for patients who lived in rural areas, this explains the reason for the isolation of some bacterial species that inhabit animals such as *Staphylococcus lentus*, *Staphylococcus gallinarum*, *S. pseudintermedius*, *Staphylococcus vitulinus*, *S. cohnii*, and *Leuconostoc mesenteroides*. Blepharitis was the most typical kind of external eye infection, followed by conjunctivitis. CoNS were found to be the primary bacterium in eye infections. Gram-positive bacteria were the most frequent causal agents of ocular infections, and amikacin is the preferred medication for Gram-positive bacteria. In addition, tobramycin is the preferable treatment option for Gram-negative bacterial infections in individuals with ocular infections, and Gram-negative bacteria were the less frequent causal agents of ocular infections.

Authors' contributions

Each author contributed equally to the creation of this manuscript.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Wang N, Yang Q, Tan Y, Lin L, Huang Q, Wu K. Bacterial spectrum and antibiotic resistance patterns of ocular infection: Differences between external and intraocular diseases. J Ophthalmol 2015;2015:1-7.
2. Aklilu A, Bitew A, Dessie W, Hailu E, Asamene N, Mamuye Y. Prevalence and drug susceptibility pattern of bacterial pathogens from ocular infection in St. Paul's Hospital Millennium Medical College, Ethiopia. J Bacteriol Mycol 2018;5:1085.
3. World Health Organization. Available from: www.who.int/news-room/fact-sheets/detail/blindness-and-visual-impairment. 2022. Fact sheets/Detail/Blindness and vision impairment.
4. Bertino JS, Jr. Impact of antibiotic resistance in the management of ocular infections: The role of current and future antibiotics. Clin Ophthalmol 2009;3:507-21.
5. Gopinath B, Rochtchina E, Flood V, Mitchell P. Association of elevated homocysteine level and vitamin B12 deficiency with anemia in older adults. Arch Intern Med 2009;169:901-2.
6. Tesfaye T, Beyene G, Gelaw Y, Bekele S, Saravanan M. Bacterial profile and antimicrobial susceptibility pattern of external ocular infections in Jimma University specialized hospital, Southwest Ethiopia. Am J Infect Dis Microbiol 2013;1:13-20.
7. Yeshambel B, Feleke M, Mengistu E. Ocular bacterial infections and antibiotic resistance patterns in patients attending Gondar Teaching Hospital, Northwest Ethiopia. BMC Res Notes 2018;11:597.
8. Amsalu A, Abebe T, Mihret A, Delelegne D, Endale T. Potential bacterial pathogens of external ocular infections and their antibiotic susceptibility pattern at Hawassa University Teaching and Referral Hospital, Southern Ethiopia. Afr J Microbiol Res 2015;9:1012-9.
9. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single diffusion method. Am J Clin Pathol 1966;45:493-6.
10. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing, M100. 31st ed. Wayne, PA: Clinical and Laboratory Standards Institute; 2020.
11. Nasser DD, Raheema HR, Ahmed IA. Phylogenetic and molecular characterization of β-lactamases among uropathogenic Escherichia coli from pediatric patients in Wasit province. Biochem Cell Arch 2022;22.
12. Dahwash SM, Raheema RH, Albahadili MA, Maslat AH. Distribution of phylogenetics and virulence genes of uropathogenic Escherichia coli among urinary tract infection in pregnant women. Biochem Cell Arch 2021;21:449-56.
13. Poulsen AB, Skov R, Pallesen LV. Detection of methicillin resistance in coagulase-negative staphylococci and staphylococci directly from simulated blood cultures using the EVIGENE MRSA Detection Kit. J Antimicrob Chemother 2003;51:419-21.
14. Acharya M, Farooqui JH, Spencer H, Arora V, Mathur U. Sphingomonas paucimobilis keratitis post cataract surgery: A first case report from India. Nepal J Ophthalmol 2019;11:82-5.
15. Albaaj AAA, Al-Ramahy SK. Molecular study of bacteria Pantoea spp. in Diwaniyah city, Iraq. J Phys Conf Ser 2019;1294:062060.
16. SAS. ETS 9.1 User - SAS Institute Inc. Users Guide. Cary, NC: SAS Institute Inc.; 2004.
17. Hameed FA. Bacteriological study of eye infection in Baghdad city. Prof. (Dr) RK Sharma 2020;20:183.
18. Zimam A, Wondemagegn M, Fantahun B. Common bacterial causes of external ocular infections, associated risk factors, and antibiotic resistance among patients at ophthalmology unit of Felege Hiwot Referral Hospital, Northwest Ethiopia: A cross-sectional study. J Ophthalmic Inflamm Infect 2021;11:1-10.
19. Raheema RH. Isolation of methicillin-resistant Staphylococcus pseudintermedius strains from human otitis cases in Wasit province, Iraq. Syst Rev Pharm 2021;12:372-7.
20. Raheema RH, Abed KA. Molecular identification of virulence genes Staphylococcus aureus isolated from different clinical isolates. Indian J Public Health Res Dev 2019;10.
21. Pate M, Zdovc I, Avberšek J, Ocepek M, Pengov A, Podpečan O. Coagulase-negative staphylococci from non-mastitic bovine mammary gland: Characterization of Staphylococcus chromogens and Staphylococcus haemolyticus by antibiotic susceptibility testing and pulsed-field gel electrophoresis. J Dairy Res 2012;79:129-34.
22. Raheema RH, Melek HK, Jassim AT. Characterization of antibiotic resistance of Staphylococcus aureus isolated from patients with diabetic foot ulcers in Wasit province. J Sci Res Arch 2021;03:201-8.
23. Al-Charrakh AH, Obayes MH. First record of isolation and characterization of methicillin resistant *Staphylococcus lugdunensis* from clinical samples in Iraq. Biomed Res Int 2014;2014:736259.
24. Chen KJ, Chen YP, Wang NK, Sun MH, Sun CC, Wu WC, *et al*. Retinal vasculitis and posterior pole preretinal exudates in exogenous bacterial endophthalmitis: Management and visual outcomes. Ocul Immunol Inflamm 2022;30:491-6.
25. Jassim AT, Raheema RH, Melek HK. Characterization of multidrug-resistant bacteria isolated from patients with diabetic foot ulcers in Wasit province. Biochem Cell Arch 2022;22:000-000.
26. Gajdács M. Epidemiology and antibiotic resistance trends of Pantoea species in a tertiary-care teaching hospital: A 12-year retrospective study. Dev Health Sci 2019;2:72-5.
27. Muslem RE, Raheema RH, Yasir QD. Molecular detection of plasmid-mediated AmpC in Gram-negative bacteria isolated from intensive care unit patients in Wasit province, Iraq. Egyp J Hosp Med 2022;89:6488-95.

28. Abdul-Husseein HK, Al-Akkam KA, Abdul-Husseein MK, Al-Hindy HA. Liver function abnormalities in COVID-19 patients and their association with age and sex: A cross-sectional study. Arch Razi Inst 2023;78:453-8.
29. Aljeeliz ZA, Raheema RH, Abood SF. Relationship between antibiotic resistance, biofilm-formation and virulence factors among multidrug-resistant Proteus mirabilis isolated from UTI patients in Wasit province, Iraq. HIV Nurs 2022;22:2082-8.
30. Raheema RH, Mahood SA, Karium. The effect of partial purified plantaricin against urinary tract infection (UTI) induced by escherichia coli and Proteus mirabilis in experimental rat model. Eur J Pharm Med Res 2016;3:623-30.
31. Albadri ATJ, Raheema RH, Melek HK. Characterization and molecular study to detect multidrug resistance bacteria isolated from patients with diabetic foot ulcers in Wasit province, Thesis.2021.
32. Acharya M, Farooqui JH, Spencer H, Arora V, Mathur U. Sphingomonas paucimobilis keratitis post cataract surgery: A first case report from India. Nepal J Ophthalmol 2019;11:82-5.
33. Haile Z, Mengist HM, Dilnessa T. Bacterial isolates, their antimicrobial susceptibility pattern, and associated factors of external ocular infections among patients attending eye clinic at Debre Markos Comprehensive Specialized Hospital, Northwest Ethiopia. PLoS One 2022;17:e0277230.
34. Ibrahim SA, Mohamed1 DA, Suleman SK. Microbial causes of urinary tract infection and its sensitivity to antibiotics at Heevi Pediatric Teaching Hospital/Duhok city. Med J Babylon 2020;17.
35. Mohammed HA, Sahi NM, Ahmed RT, Al-Rubaye AF. Antimicrobial activity of some nanoparticles synthesized by laser ablation technique against some bacteria isolated from oral cavity. Med J Babylon 2022;19:601-8.
36. Al-khafaji SFH, Al-mahdi ZKA, Wisam W. Microbial distribution and secretory IgA level among crossbite patients at an early stage of comprehensive orthodontic treatment. Med J Babylon 2023;20:160-7.
37. Al Sa'ady AT, Abdulrasol ZA, Obaid AF, Alhindy HAM, Al-Mumin AS. Prevalence of adverse effects from COVID-19 vaccine among Iraqi adults: A retrospective cross-sectional study. J Emerg Med Trauma Acute Care 2022;3:1-9.