

Original Article

Prevalence and Antibiotic Susceptibility of Pathogenic Bacteria Associated with Ocular Infections in Adult Patients

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Abstract

Ocular infection is a significant public health issue in many countries and is a leading cause of morbidity and blindness worldwide. The current study aimed to determine the prevalence of bacterial isolates, and their role in patients with external ocular infections, according to age, sex, and residence in rural and urban areas. In addition, investigate the antibiotic sensitivity and resistance patterns commonly used for treating patients suffering from external ocular infections in Babylon Governorate, Iraq. Two hundred patients with clinical suspicion of external ocular infections, ranging in age from 20 to 68 years old and from both sexes, participated in this study. The eye swabs were collected and cultured to distinguish between bacterial and viral isolates. About 105 isolates (52.5%) were found to be positive bacterial cultures and taken as a study sample, whereas 95 (47.5%) negative bacterial cultures were excluded. The results revealed that ocular infections most frequently occur in the age groups between 20-49 years, and most patients were males 65 (61.9%). The most common eye infection was conjunctivitis, which affected 45.7% of patients. This was followed by blepharitis, which affected 21.9% of patients; blepharoconjunctivitis, which affected 14.3% of patients; dacryocystitis, which affected 12.4% of patients; and keratitis, which affected 5.7% of patients. Also, the results indicated that the most common bacteria implicated in ocular infections are *Staphylococcus aureus* (37.1%), followed by Coagulase negative *Staphylococci* (CoNS) (26.7%), *Haemophilus influenzae* (21.9%), *Streptococcus pneumoniae* (6.7%), *Klebsiella pneumoniae* (3.8%), *Streptococcus pyogenes* (1.9%), *Pseudomonas aeruginosa* (1.9%). Furthermore, some antibiotics were tested for these pathogenic bacterial isolates to show their effects against these bacteria. It was found that most bacterial isolates were resistant to Ampicillin, Penicillin, and Tetracycline, whereas they were susceptible to Ciprofloxacin, Gentamycin and Chloramphenicol.

Keywords: Conjunctivitis, Blepharitis, Bacteria, Multidrug resistance

1. Introduction

Ocular infection is a significant public health issue in many countries and is a leading cause of morbidity and blindness worldwide (1). The most prevalent pathogens that cause external ocular infections are bacteria. Conjunctivitis, blepharitis, periorbital cellulitis, dacryocystitis, and orbital are the most prevalent

external ocular infections (2). Infections like these are linked to higher rates of morbidity and blindness worldwide (3).

Gram-positive commonly cause ocular infections, and Gram-negative bacteria such as *S. aureus*, *St. pneumoniae*, *Haemophilus influenzae*, *Pseudomonas aeruginosa*, *Bacillus*, Enterobacteriaceae, *Moraxellasp.*,

and *Neisseria gonorrhoeae* are among bacteria that cause ocular infections (4). Significant contributors to the development of ocular infections include "the virulence of the pathogen, inadequate personal cleanliness, poor living conditions, a compromised immune system, surgery, trauma, chronic nasolacrimal duct obstruction, and systemic disorders" (5). In hospitals, the rise of resistant bacterial strains to widely used antibiotics is a global problem (2). Furthermore, bacterial agents that cause ocular illnesses and their sensitivity to different antibiotics differ according to geographic location and from one hospital to another (6).

The current study aimed to determine the prevalence of bacterial isolates, and their role in patients with external ocular infections, according to age, sex, and residence in rural and urban areas. In addition, investigate the antibiotic sensitivity and resistance patterns commonly used for treating patients suffering from external ocular infections in Babylon Governorate, Iraq.

2. Materials and Methods

2.1. Study Populations and Specimen Collection

All 200 patients with clinical signs and symptoms of external ocular infections of age groups ranging from 20 to 68 years old and from both sexes were attending the Ophthalmic Unit of Hilla Teaching Hospital and other primary health centers from the interval beginning of September (2020) to the end of February (2021) in Babylon Governorate. The clinical features of ocular infection cases and other information were recorded, including patient age, sex, and residence. Also, the patients should not have antibiotics to avoid false negative results.

The eye swabs were obtained from patients and put into a sterilized swab tube that contained specific transport media and kept in an ice box—then transferred to the laboratory within two hours from collecting.

2.2. Cultivation and Identification of Bacteria

MacConkey's agar, Blood agar, and Chocolate agar (heated blood agar) were inoculated with the laboratory's collected clinical sample of eye swabs,

then incubated at 37°C for 24 hours. A single colony of pure bacterial isolates were identified using morphological characteristics such as the following assays: Gram's stain and biochemical tests, including "Oxidase, Catalase, Coagulase, Optochin and Bacitracin tests, Lysine decarboxylation, Indole production, Motility, Urease, Carbohydrate fermentation, Citrate utilization, Gas production, and H₂S production,". In addition, satellitism test was done to identify *Haemophilus influenzae* isolates (7).

2.3. Antibiotic Susceptibility Test

It was done for all identified bacterial isolates on Muller-Hinton agar using the disc diffusion method suggested by the Clinical and Laboratory Standard Institute (CLSI) 2014 guideline (8). The inoculum was prepared by adding growth from (3–5) isolated colonies of the test organism to 5 ml of nutrient broth. The culture was incubated at 37°C for 2 hours to produce a moderate turbidity bacterial suspension. An inoculum was obtained from the standardized culture using a sterile swab. The inoculum was then streaked onto the surface of the Muller-Hinton agar plate, and antibiotic discs were equally dispersed throughout the agar's surface using sterile forceps. After that, the plates were incubated at 37°C for 24 hours, and the zone inhibition diameters were determined.

3. Results

In the present study, 200 good clinical samples of eye swabs were collected from patients (113 male and 87 female) suffering from external ocular infections, ranging in age from 20 to 68 years old. Of them, 105 isolates (52.5%) were found to be positive bacterial cultures, and 95 isolates (47.5%) had negative bacterial cultures (Table 1).

Table 1. Distribution of eye swabs based on the bacterial culture results

Bacterial culture results	No. of eye swabs (%)	Gender	
		Male	Female
Positive bacterial culture	105 (52.5)	65	40
Negative bacterial culture	95 (47.5)	48	47
Total	200 (100%)	113	87

The negative bacterial isolates 95 were excluded, whereas all 105 positive bacterial isolates from patients (65 male and 40 female) suffering from ocular infections were taken as a study sample (Table 2).

Table 2. Distribution of adult patients with ocular infections based on their age groups and gender

Age groups in years	No. of patients (%)	Gender	
		Male	Female
20 – 29	25 (23.8%)	15	10
30 – 39	30 (28.6%)	19	11
40 – 49	33 (31.4%)	21	12
50 – 59	10 (9.5%)	6	4
≥ 60	7 (6.7%)	4	3
Total	105(100%)	65 (61.9%)	40 (38.1%)

The results in table 2 reveal that ocular infections occur in patients of all age groups, but it most frequently occurs in the age groups between 20-49 years.

Also, it is clear that ocular infections occur in males and females, and most patients were males 65 (61.9%). Male predominance was observed in patients aged 20-49 years, while there was no difference in other age groups.

Furthermore, the high prevalence of ocular infections in a rural areas (74.3%) than in urban areas (25.7%) (Figure 1).

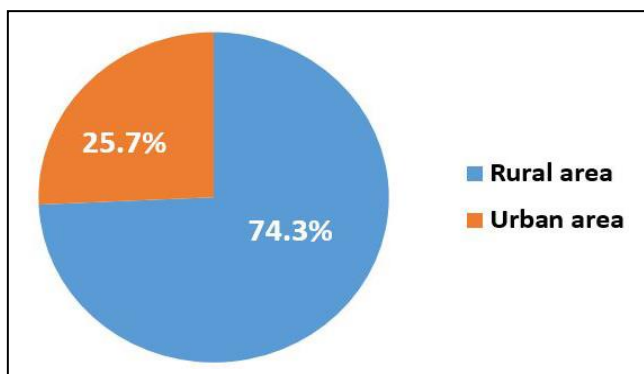


Figure 1. The prevalence of ocular diseases in rural and urban areas

Among the 105 positive bacterial isolates obtained from individuals with ocular infections, conjunctivitis was found to be the most prevalent eye

infection, accounting for 45.7%. This was followed by blepharitis (21.9%), blepharoconjunctivitis (14.3%), dacryocystitis (12.4%), and keratitis (5.7%) (Table 3).

Table 3. Frequency of ocular infections types among patients in relation to the identified clinical features

Ocular infections types	No. of cases	Percentage
Conjunctivitis	48	45.7%
Blepharitis	23	21.9%
Blepharoconjunctivitis	15	14.3%
Dacryocystitis	13	12.4%
Keratitis	6	5.7%
Total	105	100%

Out of 105 bacterial isolates, 17 (35.4%) were confirmed to be *Haemophilus influenzae*, followed by *Staphylococcus aureus* 16 (33.3%), Coagulase negative *Staphylococci* (CoNS) 11 (22.9%), *Streptococcus pneumoniae* 2 (4.2%), *Klebsiella pneumoniae* 1 (2.1%), and *Streptococcus pyogenes* 1 (2.1%). (2.1 percent). From the cases of blepharitis and blepharoconjunctivitis, *Staphylococcus aureus* was the most common bacteria, accounting for 8 (34.8%) and 8 (53.3%), respectively. In addition, Coagulase-negative *Staphylococci* (CoNS) 8 (61.5 %) were identified as the most prevalent bacteria associated with dacryocystitis, followed by *Staphylococcus aureus* 4 (30.8%). Furthermore, the most common bacteria associated with dacryocystitis were Coagulase-negative *Staphylococci* (CoNS) (n=8, 61.5 %), followed by *Staphylococcus aureus* (n=4, 30.8 %). While *Staphylococcus aureus* was the most common bacteria isolated from keratitis cases, accounting for 3 (50%), it was followed by *Pseudomonas aeruginosa* (n=2, 33.3 %) and 1 (16.7%) *Streptococcus pneumoniae* (Table 4).

The results tabulated in table 4 revealed that *Staphylococcus aureus* is the most prevalent bacteria leading to external ocular infections being responsible for (37.1%) of infections, followed by Coagulase negative *Staphylococci* (CoNS) (26.7%), *Haemophilus influenzae* (21.9%), *Streptococcus*

pneumoniae (6.7%), *Klebsiella pneumoniae* (3.8%), *Streptococcus pyogenes* (1.9%), and *Pseudomonas aeruginosa* (1.9%) (Figure 2).

Moreover, certain antibiotics were tested for pathogenic bacteria isolated from patients with ocular infections to show their effects against these bacteria (Table 5).

Table 4. Frequency of pathogenic bacteria in patients with various clinical features of ocular infections types

Pathogenic bacteria	Ocular infections types					Total (%)
	Conjunctivitis	Blepharitis	Blepharoconjunctivitis	Dacryocystitis	Keratitis	
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	
<i>S. aureus</i>	16 (33.3)	8 (34.8)	8 (53.3)	4 (30.8)	3 (50)	39 (37.1)
CoNS	11 (22.9)	6 (26.1)	3 (20)	8 (61.5)	0	28 (26.7)
<i>H. influenzae</i>	17 (35.4)	4 (17.4)	2 (13.3)	0	0	23 (21.9)
<i>S. pneumoniae</i>	2 (4.2)	2 (8.7)	1 (6.7)	1 (7.7)	1 (16.7)	7 (6.7)
<i>K. pneumoniae</i>	1 (2.1)	2 (8.7)	1 (6.7)	0	0	4 (3.8)
<i>St. pyogenes</i>	1 (2.1)	1 (4.3)	0	0	0	2 (1.9)
<i>P. aeruginosa</i>	0	0	0	0	2 (33.3)	2 (1.9)
Total	48 (45.7)	23 (21.9)	15 (14.3)	13 (12.4)	6 (5.7)	105 (100)

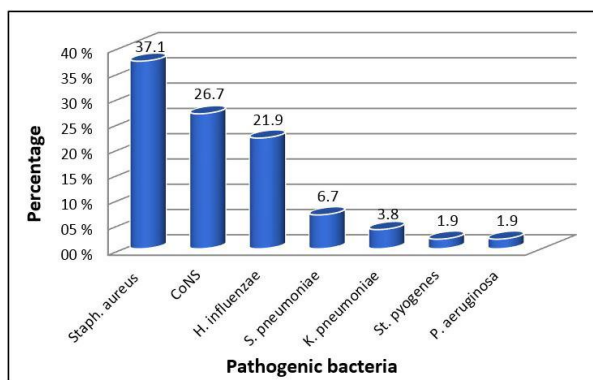


Figure 2. Frequency of pathogenic bacteria among patients with external ocular infections

Table 5. Antibiotic susceptibility of pathogenic bacteria isolated from adult patients with external ocular Infections

Bacterial isolates	No. of isolates	Antibiotic susceptibility pattern	AMP	C	CIP	CN	P	TE
<i>S. aureus</i>	39	S%	2 (5.1)	35 (89.7)	37 (94.9)	39 (100)	1 (2.6)	10 (25.6)
		R%	37 (94.9)	4 (10.3)	2 (5.1)	0	38 (97.4)	29 (74.4)
CoNS	28	S%	3 (10.7)	23 (82.1)	25 (89.3)	26 (92.9)	2 (7.1)	8 (28.6)
		R%	25 (89.3)	5 (17.9)	3 (10.7)	2 (7.1)	26 (92.9)	20 (71.4)
<i>H. influenzae</i>	23	S%	14 (60.9)	18 (78.3)	22 (95.7)	19 (82.6)	1 (4.3)	4 (17.4)
		R%	9 (39.1)	5 (21.7)	1 (4.4)	4 (17.4)	22 (95.7)	19 (82.6)
<i>S. pneumoniae</i>	7	S%	4 (57.1)	5 (71.4)	6 (85.7)	5 (71.4)	4 (57.1)	5 (71.4)
		R%	3 (42.9)	2 (28.6)	1 (14.3)	2 (28.6)	3 (42.9)	2 (28.6)
<i>K. pneumoniae</i>	4	S%	0	2 (50)	4 (100)	4 (100)	0	1 (25)
		R%	4 (100)	2 (50)	0	0	4 (100)	3 (75)
<i>S. pyogenes</i>	2	S%	1 (50)	2 (100)	2 (100)	2 (100)	1 (50)	1 (50)
		R%	1 (50)	0	0	0	1 (50)	1 (50)
<i>P. aeruginosa</i>	2	S%	1 (50)	1 (50)	2 (100)	2 (100)	0	1 (50)
		R%	1 (50)	1 (50)	0	0	2 (100)	1 (50)

S: Susceptible; R: Resistance; AMP: Ampicillin (10 µg); C: Chloramphenicol (30 µg); CIP: ciprofloxacin (5 µg); CN: Gentamicin (10 µg); P: Penicillin (10 µg); TE: Tetracycline (30 µg)

4. Discussion

Ocular bacterial infections are a significant public health problem in developing countries and are strongly linked to antibiotic resistance (9). Exogenous bacterial pathogens are the most common cause of ocular infections. They can, however, penetrate the eye and cause infection in some conditions.

In the current study, the total prevalence of bacterial isolates associated with external ocular infection was (52.5%). Our result agrees with the previous studies reported in Iran and Gondar at a prevalence rate of about (52.4% and 54.2%) respectively (10, 11). However, this study's results are lower than those of other previous studies reported in Babylon, Jimma, and India at a prevalence rate of about (92.1% and 74.7%) respectively (12, 13). On the other hand, the results of this study are higher than those of a study carried out in Bangalore (34.5%) (14).

This study showed that ocular infections most frequently occur in ages (20-49). This result corresponded to the result conducted in Yemen, which found that human eye infection can affect people of all age groups, and the infection is more common in the age groups (21-30) (15). The results also showed that ocular infection had affected both sexes, but most patients were males 65 (61.9%) because of their outside activities. These results corresponded to those reported in Ethiopia (16), which indicated that external ocular infections were more common in male than female patients.

On the other hand, the high prevalence of ocular infections in a rural areas (74.3%) than in urban areas (25.7%). The difference in distribution is probably due to poor hygiene and the fact that many rural communities use river waters for swimming, making them a source of eye infection. External ocular infections were more common in farmers and individuals without work (15).

Additionally, the results revealed that conjunctivitis is the most common external ocular infection, accounting for 45.7%, followed by blepharitis (21.9%) and

blepharoconjunctivitis (14.3%), dacryocystitis (12.4%), and keratitis (5.7%). These findings are consistent with those reported in Ethiopia (1), which stated that conjunctivitis is the most prevalent external ocular infection (40.5%), followed by blepharitis (22.3%), blepharoconjunctivitis (12.6%), dacryocystitis (10.2%) while keratitis the least common (4.2%). In contrast, the findings of this study disagree with the findings of another study carried out in India (17), which found that dacryocystitis is the most predominant external ocular infection than other infections.

Furthermore, the results of our study showed that the most prevalent bacteria leading to external ocular infections is *Staphylococcus aureus* being responsible for (37.1%) of infections, followed by Coagulase negative *Staphylococci* (CoNS) (26.7%), *Haemophilus influenzae* (21.9%), *Streptococcus pneumoniae* (6.7%), *Klebsiella pneumoniae* (3.8%), *Streptococcus pyogenes* (1.9%), and *Pseudomonas aeruginosa* (1.9%). These results agreed with the other previous studies (18), which stated that Gram-positive cocci were more common as the leading cause of bacterial ocular infections. On the other hand, previous studies reported in Jimma (19) and India (20) revealed that *Staphylococcus aureus* was the most predominant pathogen identified from ocular infections.

The present study's data showed that the followed bacteria associated with ocular infections is Coagulase-negative *Staphylococci* (CoNS). This result is supported by the previous study obtained by Musa, Nazeerullah (21). In the current study, *H. influenzae* was the most common isolate (21.9%) among Gram-negative bacteria associated with ocular infections. This result contrasts with Getahun, Gelaw (22), who found that *K. Pneumoniae* was the most common Gram-negative bacteria leading to associate ocular infections. However, another study in Saudi Arabia showed that *P.aeruginosa* was the most prevalent bacteria associated with ocular infections (23). Also, in the current study, the predominant bacteria isolated from dacryocystitis cases were *Staphylococci* (CoNS). This

result corresponds to the result reported in Iran (24). In Iraq, Al-Buhilal, Rhumaid (25) showed that the non-typeable *H.influenzae* was a significant bacterium in eye specimens of patients suffering from conjunctivitis.

Also, the in vitro effects of some antibiotics for the bacterial isolates of the present study were investigated. It was shown that the antibiotic sensitivity in bacterial ocular infections was varied, with increased resistance to most antibiotics. The ciprofloxacin was highly effective against all bacteria isolates from adult patients with ocular infections, followed by chloramphenicol and gentamicin. These results parallel previous studies conducted in different locations (26, 27).

According to table 5, it was found that all isolates of *S. aureus* were highly resistant to ampicillin (94.9%) and penicillin (97.4%). Additionally, an antibiotic susceptibility pattern of *S. pneumoniae* and *S. pyogenes* isolates showed that (85.7% and 100%) sensitivity pattern to ciprofloxacin, (57.1% and 50%) sensitivity pattern to ampicillin, and penicillin. Other studies in Ethiopia reported the same pattern as our finding (16). *S. aureus* isolates appeared to have the highest resistance rate to penicillin and ampicillin, and this might be related to the synthesis of beta-lactamase enzymes and changes in the penicillin-binding proteins (28). All isolates of CoNS in the current study were resistant to penicillin, ampicillin, and tetracycline at a rate of (92.9%, 89.3%, and 71.4%), respectively. Besides, the results also showed that *H. influenzae* and *P. aeruginosa* isolates were highly sensitive to ciprofloxacin (95.7% and 100%), gentamycin (82.6% and 100%), and to a lesser degree to chloramphenicol (78.3% and 50%). Previous studies carried out in southwest Ethiopia showed similar results (29).

Moreover, *K. pneumonia* isolates were susceptible (100%) to ciprofloxacin and Gentamycin but showed highly resistant (100%) to ampicillin and penicillin, and some isolates showed resistance to a lesser degree to tetracycline (75%). Similar results were reported in Northwest Ethiopia (29).

Antimicrobial resistance may be raised probably due to ineffective dosing regimens, self-mediations, inappropriate use of antibiotics without a prescription, prolonged therapy duration, and lack of personal hygiene. However, regular face washing can help prevent bacteria-caused external eye infections; thus, health education is essential.

The bacterial infection is higher than other infections. External ocular infections most frequently occur in the age groups between (20-49) years and infect both males and females, but male predominance was observed in this age group. The predominant bacteria implicated in external ocular infections are *Staphylococcus aureus*, coagulase-negative Staphylococci (CoNS), *Haemophilus influenza*, *Streptococcus pneumoniae*, *Klebsiella pneumonia*, *Streptococcus pyogenes*, and *Pseudomonas aeruginosa*. Most bacterial isolates were showed resistance to Ampicillin, Penicillin, and Tetracycline, whereas susceptible to Ciprofloxacin, Gentamycin, and Chloramphenicol.

Authors' Contribution

All the authors contributed significantly to the study design, the study sample collection, laboratory testing, data analysis and interpretation, the creation of a draft, manuscript revisions, as well as final approval of the version that has been published.

Ethics

The study protocol was approved by the medical ethics board of the Middle Technical University, Baghdad, Iraq. The study included only adults and written informed consents were provided by all the subjects participated in the study.

Conflict of Interest

The authors declare that they have no conflict of interest.

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