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Eye bacterial infections and antibiotic resistance patterns in patients attending Abdel Fadeel Almaz Hospital, Khartoum State, Sudan

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Abstract

Antimicrobial resistance is a global public health concern involving bacteria becoming resistant to antibiotics, making infections harder to treat. This study aimed to isolate and identify bacteria associated with eye infections in humans in Khartoum State. We collected 121 eye swabs from patients at Abdel Fadeel Almaz Hospital between February and May 2017. Of these samples, 85 (70.3%) yielded bacterial isolates. Identified Gram-positive bacteria included *Staphylococcus spp* and *Bacillus spp*, while Gram-negative bacteria included *Pseudomonas spp*. The predominant bacterium was *Staphylococcus aureus*. Antibiotic sensitivity tests showed that all isolates were sensitive to ciprofloxacin and azithromycin, indicating these drugs as effective treatments for eye infections. The study concluded that Gram-positive bacteria are the most common causative agents of eye infections in humans, and ciprofloxacin and azithromycin are effective treatment options.

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Introduction

Eye infections are a global health concern, affecting millions of people each year. These infections can be caused by a variety of pathogens, including bacteria, viruses, fungi, and parasites. The prevalence and types of eye infections can vary depending on geographic location, climate, socioeconomic conditions, and healthcare infrastructure (Modarres *et al.*, 1998). The common types of eye infections are : (1) conjunctivitis : an inflammation of the conjunctiva, often caused by bacteria (such as *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Haemophilus influenzae*). (2) Keratitis : An infection of the cornea, which can be bacterial (e.g., *Pseudomonas aeruginosa*, *Staphylococcus aureus*), viral (e.g., herpes simplex virus), fungal (e.g., Fusarium species), or parasitic (e.g., Acanthamoeba). (3) Endophthalmitis: a severe inflammation of the interior of the eye, typically caused by bacteria (e.g., *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Streptococcus species*) or fungi (e.g., *Candida species*). (4) Blepharitis: an inflammation of the eyelids, often associated with bacterial infections (e.g., *Staphylococcus aureus*) or skin conditions like seborrheic dermatitis (Modarres *et al.*, 1998).

The susceptibility of bacterial isolates from eye infections varies depending on the region and the specific bacterial strain. Generally, the following antibiotics are commonly tested and used: 1. Fluoroquinolones: Such as ciprofloxacin, moxifloxacin, and ofloxacin, are often effective against a broad range of bacteria, including *Pseudomonas aeruginosa*. 2. Aminoglycosides: Such as gentamicin and tobramycin, are effective against Gram-negative bacteria and some Gram-positive bacteria. 3. Macrolides: Such as erythromycin and azithromycin, are used primarily for Gram-positive bacteria and some Gram-negative bacteria. 4. Tetracyclines: Such as doxycycline, are used for atypical pathogens like Chlamydia trachomatis. 5. In the context of ocular infections, ciprofloxacin and azithromycin are commonly used antibiotics. Ciprofloxacin is a fluoroquinolone antibiotic that is effective against a broad range of Gram-negative and Gram-positive bacteria. It is often used in the treatment of bacterial conjunctivitis and keratitis.

Azithromycin, a macrolide antibiotic, is also widely used to treat ocular infections due to its broad spectrum of activity and good tissue penetration, especially in cases of bacterial conjunctivitis and trachoma. (Tzu *et al.*, 2021).

Cataracts remain the leading cause of blindness globally, with trachoma also being a significant cause (World Health Organization, 2017). Although cataracts are often age-related, they can also result from infections caused by microbiota (Forbes *et al.*, 2002).

Limited research has been conducted on eye infections, particularly in Sudan, emphasizing the need for further studies in this field.

This study aims to :

- identify potential bacterial isolates responsible for eye inflammation in adult patients attending an eye hospital in Khartoum State from February to May 2017.
- To isolate and identify pathogenic bacteria causing eye infections.
- To evaluate the antibiotic sensitivity of each bacterial isolate.

Materials and methods

Materials

The study was conducted at Abdel Fadeel Almaz Hospital in Khartoum State, Sudan, to isolate and identify pathogenic bacteria causing eye infections from February to May 2017.

Sample size

A total of 121 swab samples were collected from infected eyes of patients at Abdel Fadeel Almaz Hospital during the study period (Abdel Fadeel Almaz Hospital records, 2017).

Study design

This descriptive cross-sectional study was conducted from February to May 2017 , according to Forbes *et* *al.*, 2002).

Study area

The study took place at Abdel Fadeel Almaz Hospital in Khartoum State (Abdel Fadeel Almaz Hospital records, 2017).

Collection and transportation of samples

Eye samples were collected using the method described by Forbes *et al.* (2002). Purulent material from the lower conjunctival sac and inner canthus of the infected eye was aseptically collected with sterile swabs to avoid skin contamination.

Samples were immediately transported to the Tropical Medicine Research Institute Laboratory for bacteriological examination.

Tests for gram-positive bacteria

(Forbes *et al.*, 2002) **:** (Tables 1 – 2)

Catalase test

2 ml of 3% hydrogen peroxide solution was placed in a test tube. A small amount of the tested organism's colony from nutrient agar was added to the hydrogen peroxide using a wooden stick. Gas bubble production indicated a positive result.

Coagulase test

This test identifies bacteria producing the coagulase enzyme.

Slide method

A single colony from a 24-hour nutrient agar plate was emulsified in diluted plasma on a microscopic slide. Clumping within 5 seconds indicated a positive result.

Tests for gram-negative bacteria

Urease test

A urea agar medium slope was inoculated with the tested organism and incubated at 37°C for 24 hours.

A color change to pink indicated a positive reaction, while no change indicated a negative reaction.

Citrate utilization

Simmon's citrate agar medium was inoculated with the tested organism and incubated at 37°C for 24 hours. A blue color indicated positive citrate utilization, while no color change indicated a negative result.

Kligler's iron agar test (KIA)

The tested organism was inoculated into Kligler ion agar medium and incubated at 37°C for 24 hours. Yellow butt-red or pink slant indicated glucose fermentation only; yellow slant and yellow butt indicated fermentation of lactose and possibly glucose. Red-pink slant and butt indicated no fermentation. Cracks and bubbles in the medium indicated gas production from glucose fermentation, and blackening along the stab line or throughout the medium indicated hydrogen sulfide production.

Indole production

Peptone water medium was inoculated with the tested organism and incubated at 37°C for 24 hours. After incubation, 2 drops of Kovac's reagent were added and shaken well. A red ring formation at the surface of the media indicated a positive reaction.

Oxidase test

A solution of oxidase reagent was prepared by dissolving 1g of reagent in 100 ml of distilled water. The solution was added to isolated bacteria on nutrient agar for five minutes, and the excess reagent was discharged. After 15 minutes, a color change to purple indicated a positive result, while no color change indicated a negative result.

Sensitivity tests

Antibiotic sensitivity tests were performed using the diffusion susceptibility test (Kirby-Bauer method, Cowan and Steel, 1974). Five similar colonies from a 24-hour nutrient agar pure culture were selected using a sterile wire loop, inoculated in 5 ml of nutrient broth medium, and incubated at 37°C for 8 hours until light visible turbidity appeared, comparable to the 0.5 McFarland barium sulfate turbidity standard. Using a sterile cotton swab, the colony suspension was spread on Muller Hinton agar medium, left to dry at room temperature for 15 minutes, and antibiotic discs were placed on the medium surface with sterile forceps.

The plates were incubated at 37°C for 18-24 hours, and the zone of growth inhibition around the discs was measured with a ruler. Four antibiotic discs—fusidic acid, azithromycin, ciprofloxacin, and optochin were used for the sensitivity test (Table 3).

Results

This study was conducted at Abdel Fadeel Almaz Hospital in Khartoum State, Sudan, and included 121 patients with eye infections. The study population comprised both males and females, aged between 20 and 80 years. The highest frequency of eye infections was observed in the age group 61-70 years (27 patients, 22.3%). Females were more affected than males, with 92 (76%) female patients and 29 (24%) male patients (Fig. 1).

Table 1. Biochemical Test for *Staphylococcus aureus* and *Staphylococcus intermedius* Isolated from Infected Eyes.

Characters	*S. aureus*	*S. intermidius*
Catalase test	+	+
Coagulase test.	+	+
Vp test	+	_
Gram reaction	+	+

+ : Positive reaction.

- : Negative reaction.

Table 2. Biochemical Test for *Pseudomonas aeruginosa*, *Bacillus lentus*, *Bacillus spp*, and *Staphylococcus epidermidis* Isolated from Infected Eyes.

Characters	*Pseudomonas aeruginosa*	*Bacilus lentus*	*S.epidermidis*
Gram reaction	-	+	+
Catalase test			+
Co-agulase test.			-
Oxidase test	+	+	
KIA test	+		
Peptone water test	-	-	
Citrate test	+	-	
Urease test	-	+	

+ : Positive reaction.

- : Negative reaction.

Aerobic incubation of the eye swab samples revealed different bacterial species. Out of the 121 samples, 36 showed no growth while 85 exhibited bacterial growth. The isolated bacteria included *Staphylococcus aureus* (62 isolates, 72.9%), *Staphylococcus intermedius* (15 isolates, 17.6%), *Staphylococcus epidermidis* (4 isolates, 4.7%), Bacillus *spp* (2 isolates, 2.3%), *Bacillus lentus* (1 isolate, 1.7%), and *Pseudomonas aeruginosa* (1 isolate, 1.7%) (Fig. 2).

Table 3. Standard zone of inhibition to different Antibiotics.

Antibiotic disk	Disk potency	Zone of inhibition (diameter in mm)		
		Resistant	Intermediate	Sensitive
Azithromycin(AZM)	15mcg	13 or less	14-17	18 or more
Ciprofloxacin(CIP)	5mcg	15 or less	16-20	21 or more
Fusidic acid(FA)	10mcg	24 or less	-	24 or more
Optochin (OP)	10mcg	14 or less	-	14 or more

The prevalence of eye infections was higher in urban residents (87 cases, 71.9%) compared to rural residents (34 cases, 28.1%) (Fig. 3).

Housewives had the highest frequency of eye infections (63 cases, 52.1%), while teachers had the lowest frequency (2 cases, 1.7%) (Fig. 4).

Table 4. Comparison of sensitivity of bacterial *spp* to antibiotics used.

Isolated bacteria	OP. N (%)	CIP. N (%)	FA. N (%)	AZM. N(%)
B.lentus	0	1(100)	1(100)	1(100)
Bacillusspp	0	2(100)	0 (0)	1(50.0)
Pseudomonasspp	0	1(100)	1(100)	1(100)
S.aureus	0	45(72.6)	34(54.8)	26(41.9)
S.epidermidis	0	2(50.0)	2(50.0)	2(50.0)
S.intermidus	0	9(60.0)	6(40.0)	7(46.7)
P-value	0.996	0.837	0.406	0.672

CIP = ciprofloxacin, AZM = azithiromycin, OP = Optochin, FU = Fusidic acid.

The highest percentage of eye infections was among illiterate individuals (56 cases, 46.3%), while the lowest percentage was among those with secondary school education (12 cases, 9.9%) (Fig. 5).

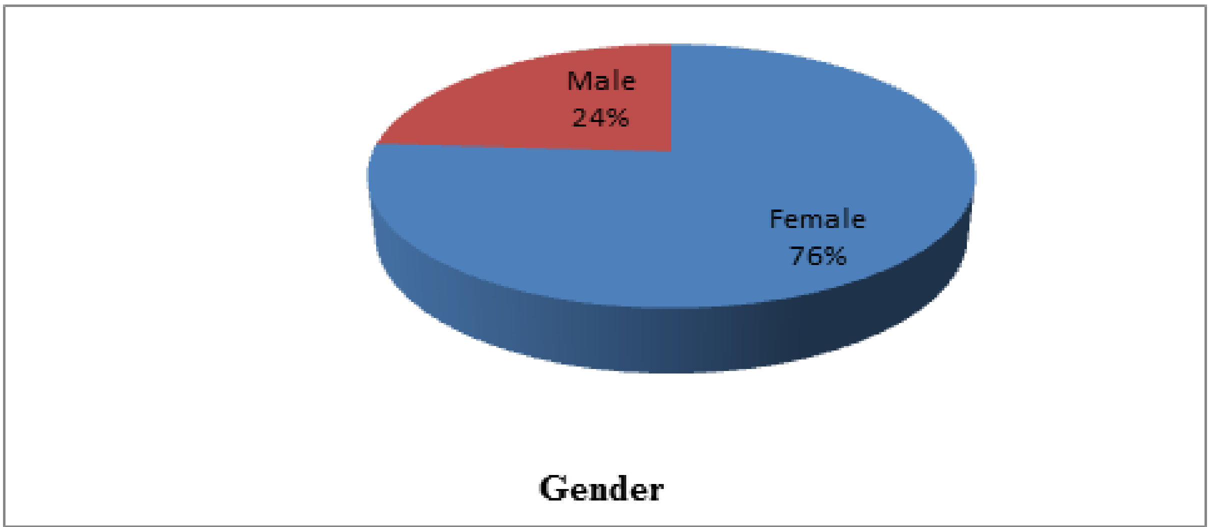

Fig. 1. Distribution of the eye infections according to the gender.

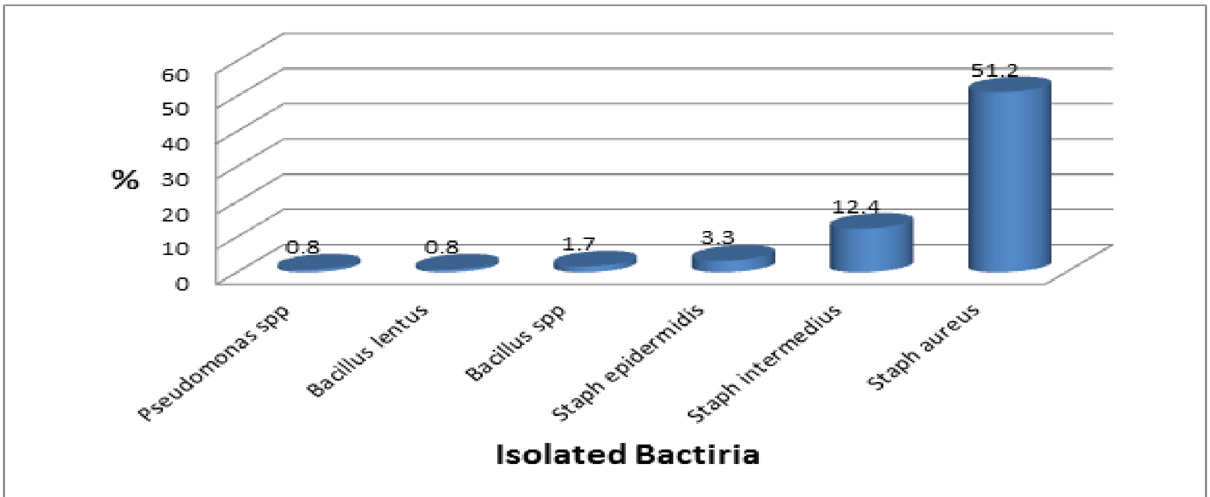

Fig. 2. Percentage of bacteria isolated from infected eyes of human.

Non-diabetic patients exhibited the highest percentage of eye infections (99 cases, 81.8%), whereas diabetic patients had a lower percentage (22 cases, 18.2%) (Fig. 6). The highest distribution of eye infections was in patients aged 61-70 years (27 cases, 22.3%), while the lowest distribution was in patients over 70 years (14 cases, 11.6%) (Fig. 7).

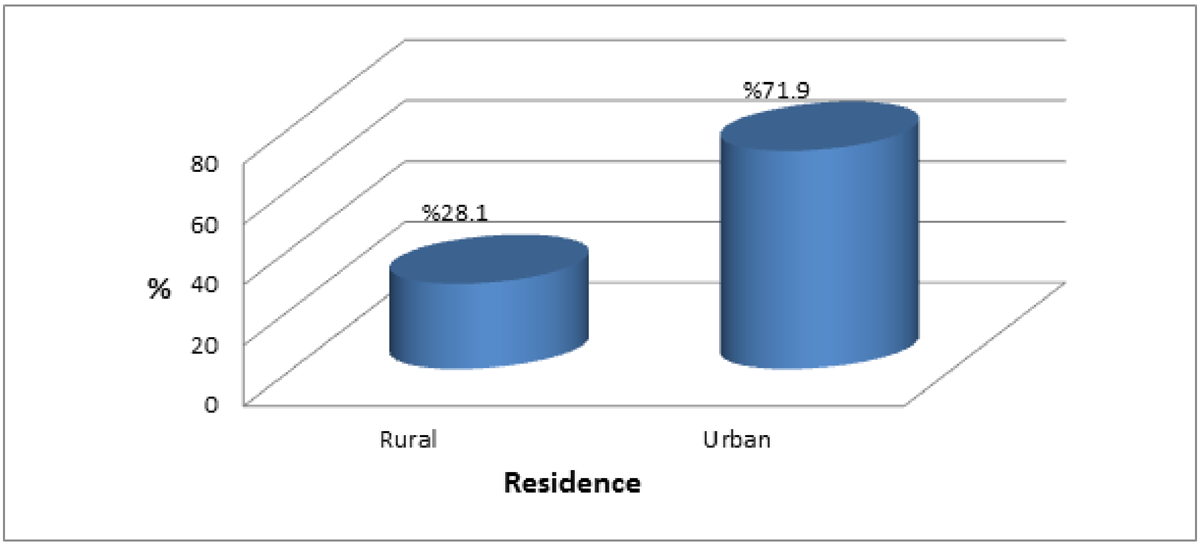

Fig. 3. Prevalence of the eye infections according to the resident.

Patients who did not use antibiotics had a higher percentage of eye infections (94 cases, 78%) compared to those who used antibiotics (27 cases, 22%) (Fig. 8).

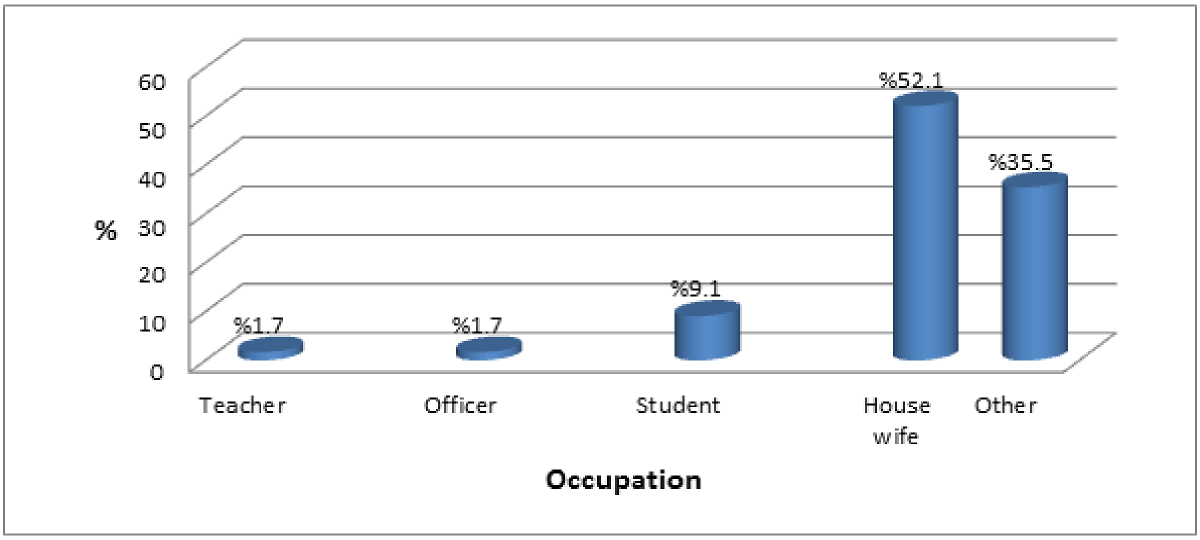

Fig. 4. Frequency of the eye infections according to the occupations.

Antibiotic sensitivity test

All isolates were resistant to optochin. *B. lentus* was sensitive to fusidic acid, ciprofloxacin, and azithromycin. *Bacillus spp* were sensitive to ciprofloxacin (2/2, 100%). *Pseudomonas aeruginosa* was sensitive to ciprofloxacin (1/1, 100%). *Staphylococcus aureus* was sensitive to ciprofloxacin (45/62, 72.6%), fusidic acid (34/62, 54.8%), and azithromycin (26/62, 41.9%). *Staphylococcus epidermidis* was sensitive to ciprofloxacin (2/4, 50%), fusidic acid (2/4, 50%), and azithromycin (2/4, 50%). *Staphylococcus intermedius* was sensitive to ciprofloxacin (9/15, 60%), fusidic acid (6/15, 40%), and azithromycin (7/15, 46.7%) (Table 4).

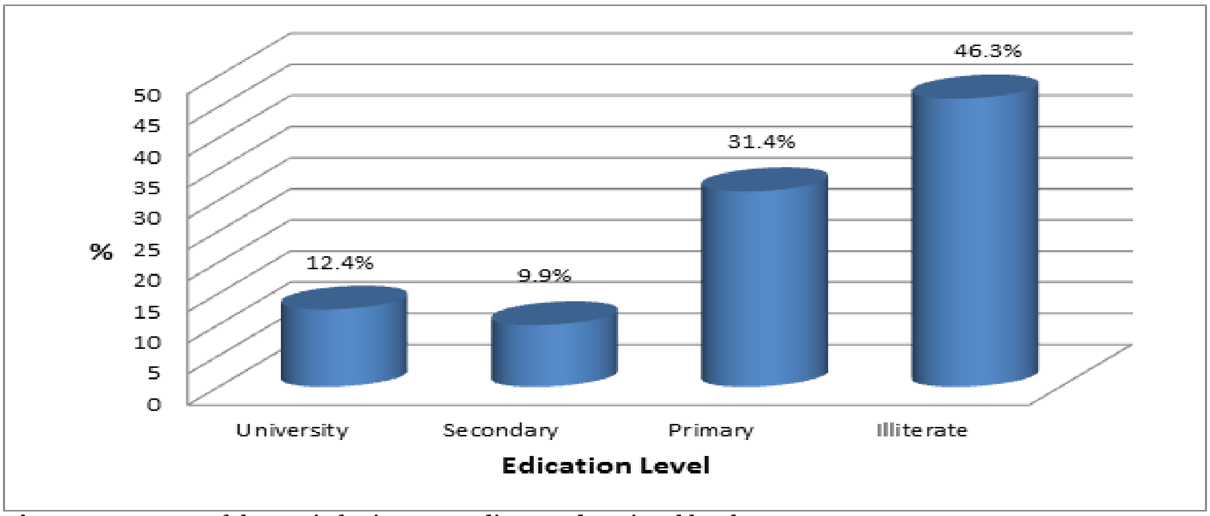

Fig. 5. Percentage of the eye infections according to educational levels.

The bacteria were classified as sensitive or resistant based on the diameter of the zone of inhibition for each antibiotic. *Staphylococcus aureus* was resistant to optochin, but sensitive to azithromycin (zone of inhibition: 2.5 cm), ciprofloxacin (zone of inhibition: 2 cm), and fusidic acid (zone of inhibition: 1.2 cm).

Isolation and identification bacteria associated with eye infections

Eye infections are frequently reported worldwide, with bacteria being the most common causative agents (Modarres *et al.*, 1998). Antimicrobial susceptibility testing provides critical data on which antibiotics are effective against specific bacterial isolates. Resistance patterns can vary widely based on geographic location, healthcare practices, and antibiotic usage.

This study aimed to isolate and identify bacteria associated with eye infections in humans. Eighty-five bacterial species were isolated, 84 of which were Gram-positive and one was Gram-negative.

The most prevalent bacterium was Staphylococcus aureus, which aligns with previous studies conducted in Sudan (Nabila, 1985; Fowzia, 2002; Salma, 2003; Iman, 2008) and in other regions (Jackson and Eykyn, 2003).

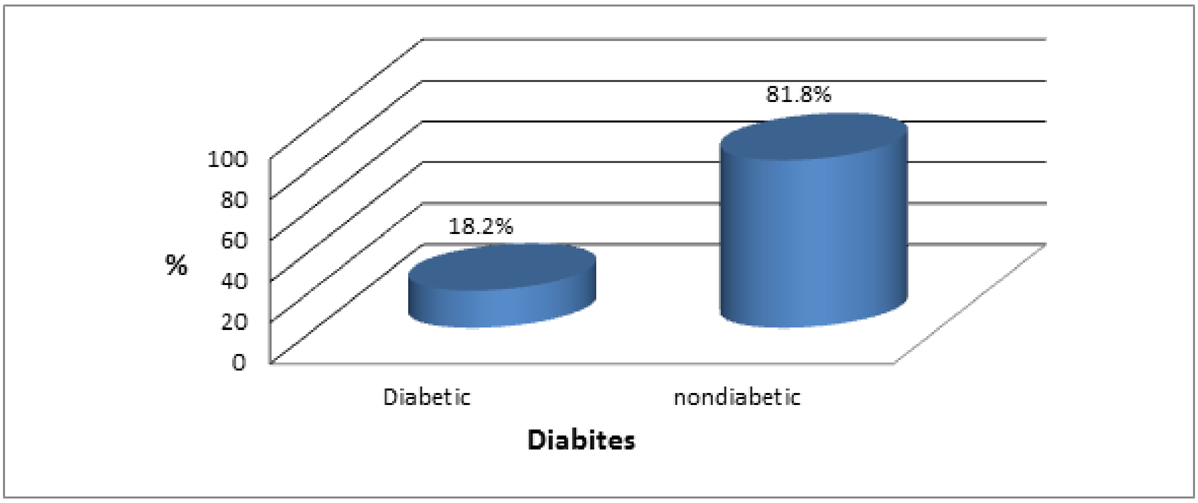

Fig. 6. Distribution of the eye infections according to diabetic status.

Staphylococcus epidermidis was also isolated, consistent with findings by NanWang *et al.* (2015) and Benz & Scott (2004). Only one *Pseudomonas aeruginosa* was isolated, in agreement with studies by Tilahun Aweke *et al.* (2014) and NanWang *et al.* (2015). Bacillus lentus was isolated, which concurs with multiple studies (Greding *et al.*, 1993; Fatimah *et al.*, 1994; Seham *et al.*, 1995; Moore *et al.*, 1995; Kawther, 1997; Iman, 1997; Fowzia, 2002; Salma, 2003), but disagrees with Iman (2008). The majority of bacterial isolates were from patients aged 61-70 years, consistent with a study by Deborah Dean *et al.* (2008). Female patients were more frequently infected than male patients, aligning with Salah *et al.* (2016), but conflicting with studies by Deborah Dean *et al* 2008.

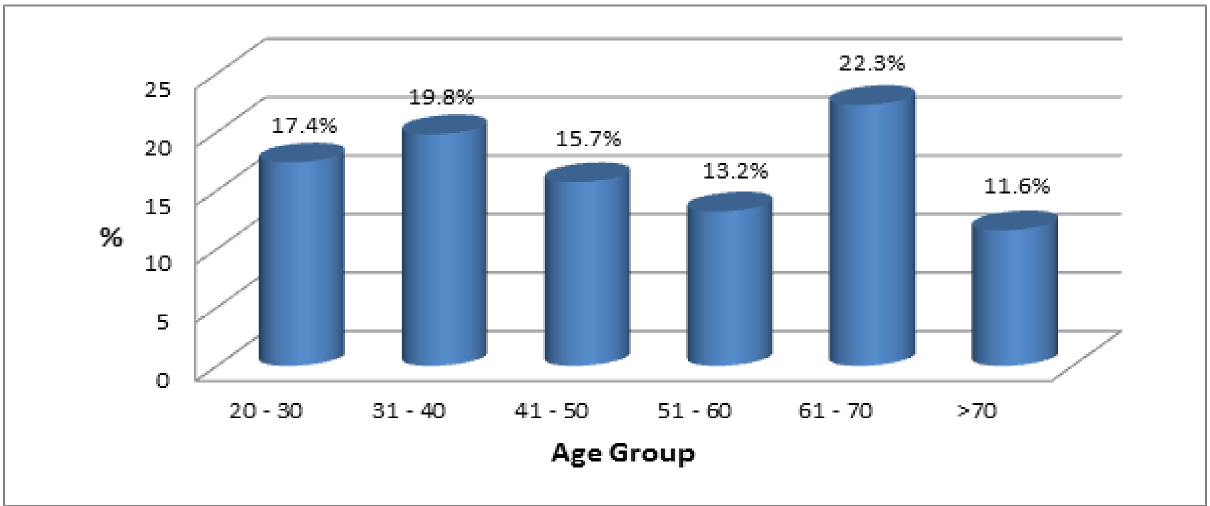

Fig. 7. Distribution of the eye infections according to the different ages.

The female patients (92(76%)) were more infected than male patients(29 (24%))this result agrees with Salah *et al.*,(2016),but disagrees with some previous studies reported a high prevalence of the disease in male patients than female patients (Deborah Dean *et al.*, 2008). This can be explained by the fact that gender differences may be due to the Hygiene and behavior from area to another area. The patients who were came from the urban areas (87(71.9%)) was more infected than patients which who came from the rural area (34(28.1%) which is agreement with study that reported by Mar Justel *et al.*,(2015).

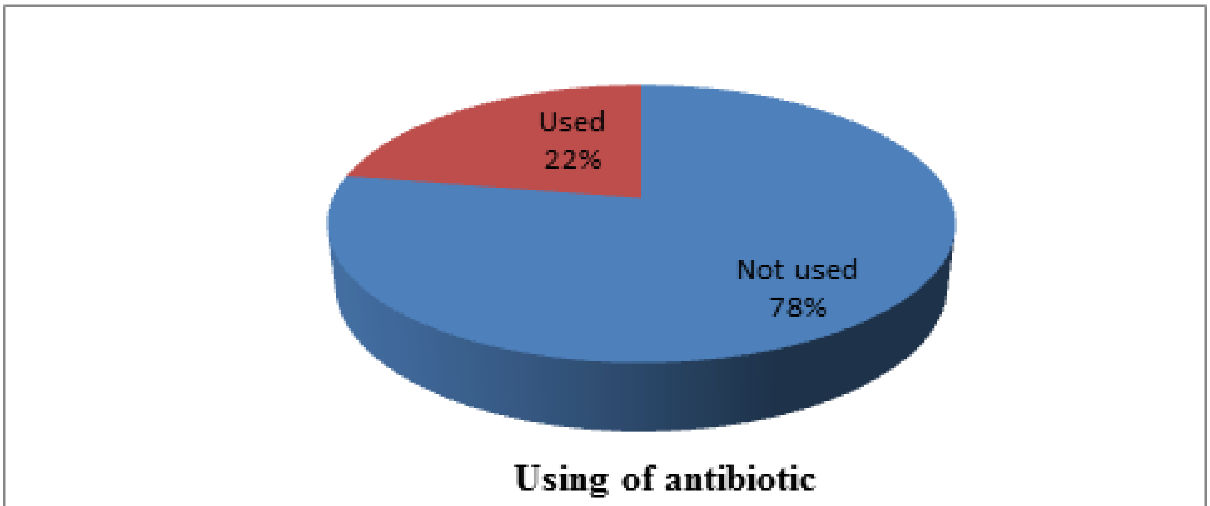

Fig. 8. Percentage of the infections according to patients using antibiotic and patients not using antibiotic.

In this study the highest frequency of infection according to educational level was illiterates (56(46.3%)) that may be due to low of education and poor hygiene system. In the present study non diabetic patients (99(81.8%)) were more infected than diabetic patients this might be due to good

Hygiene, periodic follow-up and personal attention of diabetic patients, this result was disagree with study that reported by Karim sab and Razak,(2013).

Antibiotic resistance patterns

Susceptibility to infection was found to increase in elderly, because they are at a greater risk due to their low immunity. In this study ciprofloxacin and azithromycin antibiotics were drugs of choice against ocular pathogens (Benz *et al.*, 2004).

The mechanisms of Antimicrobial Resistance may be one or more of the following: 1. Genetic Mutation: Bacteria can mutate their DNA, acquiring resistance to antibiotics they were once susceptible to. 2. Horizontal Gene Transfer: Bacteria can exchange genetic material (plasmids, transposons) containing resistance genes with other bacteria, even across different species. 3. Drug Inactivation or Modification: Bacteria produce enzymes that inactivate antibiotics (e.g., beta-lactamases break down beta-lactam antibiotics). 4. Reduced Permeability: Bacteria alter their cell walls or membranes to prevent antibiotics from entering the cell. 5. Efflux Pumps: Bacteria pump antibiotics out of their cells, reducing intracellular concentrations below effective levels.

The Common Antibiotic Resistance Mechanisms may be one or more of the following: (1) Beta-lactam Resistance that common in Gram-positive (e.g., *Staphylococcus aureus*) and Gram-negative bacteria (e.g., *Escherichia coli, Klebsiella pneumoniae*) due to beta lactamase production. (2) Fluoroquinolone Resistance: Often due to mutations in DNA gyrase and topoisomerase IV genes, reducing drug binding. (3) Aminoglycoside Resistance: Mediated by enzyme modification (e.g., acetyltransferases, phosphotransferases) or reduced uptake. (4) Macrolide Resistance: Often due to efflux pumps or modification of the ribosomal target site. (5) Tetracycline Resistance: Mediated by efflux pumps or ribosomal protection proteins. The factors may contributing to antimicrobial resistance may be one or more of the following: (1) Overuse and Misuse of Antibiotics: Inappropriate prescribing, inadequate dosing, and use in agriculture contribute to selective pressure. (2) Poor Infection Control: Spread of resistant bacteria in healthcare settings due to inadequate hand hygiene and environmental contamination. (3) Lack of new antibiotics: Few new antibiotics in development reduce treatment options for resistant infections. (4) Global Travel and Trade: Spread of resistant bacteria across regions due to international travel and trade. (5) Environmental Factors: Antibiotics in wastewater, agriculture runoff, and healthcare settings can contribute to resistance development.

Consequences of antimicrobial resistance may be one of the following (1) Treatment Failures: Infections become harder to treat, leading to prolonged illness and increased mortality. (2) Increased Healthcare Costs: Longer hospital stays and use of more expensive antibiotics contribute to economic burden. (3) Public Health Threat: Resistant bacteria can spread within communities and globally, limiting treatment options (CDC, 2023; WHO, 2022).

Conclusion

In conclution, this study found that Gram-positive bacteria were the most common causative agents of eye infections in human. *Staphylococcus aureus* was found to be the predominant bacteria in eye infections. All isolates were resistant to optochin. *B. lentus* was sensitive to fusidic acid, ciprofloxacin, and azithromycin. *Bacillus spp* were sensitive to ciprofloxacin. *Pseudomonas aeruginosa* was sensitive to ciprofloxacin. *Staphylococcus aureus* was sensitive to ciprofloxacin, fusidic acid, and azithromycin. *Staphylococcus epidermidis* was sensitive to ciprofloxacin, fusidic acid, and azithromycin.

Staphylococcus intermedius was sensitive to ciprofloxacin, fusidic acid, and azithromycin. Ciprofloxacin and azithromycin are drugs of choice for treatment of eye infections. These in vitro antibiotic resistance data may assist clinicians in selecting appropriate antibiotic for treat of eye infections.

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