

BACTERIAL CAUSES AND ANTIMICROBIAL SENSITIVITY PATTERN OF EXTERNAL OCULAR INFECTIONS IN SELECTED OPHTHALMOLOGY CLINICS IN SANA'A CITY

ABSTRACT

Background: Bacteria are one of the main factors causing external ocular infections; and the antibacterial resistance of the ocular bacteria is a global concern.

Objectives: The aim of this study, was to determine the bacterial profile and pattern of sensitivity to antibiotics for external ocular infections for patients who attended selected ophthalmology clinics in the city of Sana'a.

Methods: A cross-sectional study design was used from September 2016 to October 2017 where a total of 197 patients with external eye infections were included in the study which included conjunctivitis, keratitis, blepharitis and blepharo-conjunctivitis. Samples were collected and transferred to the National Center of Public Laboratories (NCPHL), in Sana'a. Possible bacterial pathogens have been isolated and identified using standard laboratory techniques, and microbial sensitivity testing has been carried out using a disc diffusion method.

Results: A total of 197 ocular samples were collected for microbiological evaluation, of which 146 (74.1%) had bacterial growth. Gram positive bacteria accounted for 52.1% and the predominant isolation was *S. aureus* (30.1%). Gram negative bacteria made up 47.9% and the predominant isolation was *Pseudomonas aeruginosa* (26.7%). The majority of Gram-positive bacteria were sensitive to ciprofloxacin (90% - 100%), vancomycin (86% - 100%) and Gram-negative isolates sensitive for amikacin (100%) and ciprofloxacin (63% - 100%).

Conclusion: These results indicated that Gram-positive bacteria were the most common bacteria isolated from external eye infections and were more susceptible to ciprofloxacin and vancomycin while Gram-negative isolates were more susceptible to amikacin and ciprofloxacin. The high rate of resistance for most antibiotics in Yemen, leaves ophthalmologists with very few options of drugs to treat eye infections. Large-scale ongoing studies in the future should also be conducted in order to monitor the antimicrobial resistance of the external ocular bacterial isolates.

Keywords: external ocular infections, bacterial causes, antimicrobial sensitivity, Sana'a, Yemen

INTRODUCTION

Pathogenic microorganisms cause ocular infections due to virulence and low host resistance in some circumstances such as personal hygiene, living conditions, socioeconomic status, low immunity status, and other related factors^{1,2}. The conjunctiva, the lid and the cornea are the frequently affected areas of the eye^{1,2}. Bacteria are one of the main causative agents that cause eye infections, which may lead to blindness. Thus, an immediate treatment is needed for a serious bacterial eye infection that threatens the cornea². For precise antibacterial treatment, isolation and identification of bacterial pathogens along with an antibiotic sensitivity spectrum is required³. Because there is a global problem with the appearance of bacterial resistance to topical antimicrobial agents that are influenced by pathogen properties and antibiotic prescribing practices including systemic antibiotic use and general health care guidelines^{4,5}.

This developing resistance increases the risk of treatment failure with potentially severe consequences^{6,7}. Bacterial etiology and sensitivity, as well as patterns of resistance, may vary according to geographical and regional location^{6,7,8}. Hence, recent information is vital for ophthalmologists for proper antimicrobial therapy^{1,4,6,7}. In Yemen, there was no previous study conducted on external ocular infections and patterns of sensitivity to antibiotics before this study, The study carried out by Al-Shamhi and others studied epidemiology and the diagnosis of corneal ulcers in the city of Sana'a, could be considered as part of this study as it only focused on corneal infection⁹. Due to the high rate of drug resistance to antibiotics in medicine in Yemen¹⁰, the ophthalmologists is left with a very few choices of drugs for the treatment of ocular infections. Hence, knowledge of the causative agents of this infection is essential to proper case management.

Bacterial sensitivity to many antimicrobial agents changes from location to location and in the same place from time to time^{6,8}. Consequently, the changing spectrum of microorganisms concerned in eye infections and the emergence of acquired microbial resistance determine the need for continuous monitoring to guide experimental treatment^{6,7,11}. The experimental choice of effective treatment has become more difficult as ocular pathogens are becoming increasingly resistant to commonly used antibiotics⁷. Regarding the study area in Yemen, there is a scarcity or lack of published data on the spectrum of etiological agents

responsible for external eye infections. Thus, the purpose of this study was to determine the spectrum of bacterial etiology for external ocular infections, and to assess the susceptibility of these bacterial ocular isolates to in vitro antibiotics commonly prescribed among patients with external eye infections in Yemen.

MATERIALS AND METHODS

This cross-sectional study included 197 eye samples for microbiological assessment of patients clinically diagnosed with external eye infections such as conjunctivitis, keratitis, blepharitis and blepharoconjunctivitis in selected ophthalmology clinics in Sana'a between September 2016 to October 2017. Patients diagnosed clinically with external eye infections, with informed consent taking, were included in the study. Excluding patients with trachoma, viral keratitis, peripheral ulcerative keratitis, allergic and viral conjunctivitis, and severe eye trauma, recent eye surgery, and patients who received antimicrobial therapy within two weeks before the requirement. All patients were examined on a slit lamp biomicroscope and the infectious diseases included in this study were clinically diagnosed by a group of ophthalmologists. After detailed eye examination using standard techniques¹², samples from the eyelid, conjunctiva, and cornea were collected by ophthalmologists. Immediately obtained eye samples were inoculated in blood agar, chocolate agar, selective media for MNYC [if the newborn patient and *N. gonorrhoea* were suspected], as well as the Loeffler serum slope of the *Moraxella* infection. Then the plates and tubes were incubated in appropriate conditions. Possible bacterial pathogens were isolated and identified using standard laboratory techniques, and microbial sensitivity testing was performed by a disc diffusion method¹³. The following antimicrobials were used with their respective concentration: Amikacin (AK, 30µg), ceftriaxone (CRO, 30µg), gentamicin (CN, 10µg) ciprofloxacin (CIP, 5µg), penicillin (P, 10U), tetracycline (TE, 30µg), erythromycin (E, 15µg), doxycycline (DO, 30µg), chloramphenicol (C, 30µg), trimethoprim-sulphamethoxazole (SXT, 1.25/23.75µg), and vancomycin (VA, 30µg).

RESULTS

A total of 197 external ocular infection patients (121 (61.4%) male and 76 (38.6%) female) were enrolled in this study. The most frequent age groups were ≤ 15 years (23.9%), and age group ≥ 46 years (24.4%); while young adult groups were less frequent (Table 1). Bacterial growth yielded on 146 (74.1%) while 51 (25.9%) were negative for bacterial culture (Table 2). The isolates in 146 patients with external ocular infections were Gram positive bacteria (52.1%), the predominant species of Gram positive was *S.aureus* (30.1%), while *beta hemolytic streptococci* counted 6.2%, *S.pneumoniae* was 6.2%, and CoNs was 8.2%. Gram negative isolates counted for 47.9%, the predominant Gram negative bacteria was *P.aeruginosa* (26.7%), while other species were less frequent e.g *E.Coli* (7.5%), *Moraxella* species (3.4%), *H.influnzae* (8.9%) and *Proteus* species was 1.4% (Table 2). Table 3 illustrate the susceptibility of Gram-positive bacteria. Most of Gram-positives showed resistance against penicillin up to 97%; but they were highly susceptible to ciprofloxacin (96%), vancomycin (92%), doxycycline (83%), tetracycline (59.2%), ceftriaxone (73.7%), erythromycin (92%). Table 4 illustrate the susceptibility of Gram-negative bacteria. The Gram-negative bacteria and showed high rate of susceptibility to amikacin (100%) and gentamicin (89.7%).

DISCUSSION

In wide-ranging, the eye isolates recognized in this study were similar to those of many other studies performed in different regions. Although the major bacteria known to cause external eye infections around the world is *S. aureus*^{1,2,8,14}. The most common isolates in this study was *S. aureus* (30.1%) followed by *P. aeruginosa* (26.7%). Similar studies performed in India^{1,8}, Nigeria^{2,15}, Gondar¹⁶, and Ethiopia^{14,17} also indicated that *S. aureus* is a predominant eye isolate. However, some other studies have reported that *S.aureus* is the first but has reported *E. coli*^{9,15}, *S. albus*², *S. pneumoniae*^{1,8} as the second common bacterial isolation not *P.aeruginosia* such as the current study.

The predominance of *P.aeruginosia* in our study, which differs from previous studies, can be supported by finding similar studies conducted in Sudan¹⁸, Australia¹⁹, Malaysia²⁰, India²¹ and Thailand²². These results can be explained by the fact that as part of the eye's natural flora, *Pseudomonas* grow better in the eye than any known culture media and cause infection when mechanical shock to the corneal epithelium occurs, also, it produces external toxins A, which cause tissue necrosis leading to corneal ulceration^{2,12}. The present study showed fewer isolates of intestinal bacteria (*E.coli* = 7.5%; *Proteus* = 1.4%) when compared to a similar study performed in Nigeria¹⁵, and Gondar¹⁶ where variable *enterobactericiae* were more common isolates from external eye infections. This low number of intestinal bacteria in our study may be due to decreased in hand-faecal contamination and / or increased access to safe drinking water sources in the study area².

Laboratory-based resistance and sensitivity may not reflect the true clinical resistance and response to the antibiotic due to host factors and drug penetration⁸. On the other hand, these findings afford data that allows the doctor to make a rationale-based decision in choosing a primary regimen for ocular pathogens¹. Based on the results of the sensitivity test in the current study, most Gram-positive bacteria were susceptible to ciprofloxacin (96%) followed by vancomycin (92.1%). Vancomycin coverage against *S. aureus* and CoNS was 86.4% and 100%, respectively. This result corresponds to the study carried out in India⁸. In contrast, a study in Iran¹¹ reported low coverage of vancomycin against *S. aureus*.

Gentamicin covered against 71% of Gram-positive isolates and obtained high coverage against *S. aureus* (90.9%) This finding is consistent with studies conducted in Nigeria^{2,14}, Iran¹¹, and India^{8,21}. Though, this study showed low gentamicin coverage against CoNS (50%) compared to a study carried out in Nigeria^{2,14}, and India²¹.

Gentamicin coverage for *Pseudomonas aeruginosa* was 97.4%. *P. aeruginosa*, which makes up 26.7% of all isolated bacteria, was highly sensitive to amikacin (100%), ciprofloxacin (89.8%), ceftriaxone (76.9%), doxycycline (53.8%), and chloramphenicol (59%). These results were reported for ciprofloxacin from studies conducted in Saudi Arabia²⁴ and Nigeria^{14,25}. On the other hand, the study in India²¹ described low coverage of ciprofloxacin for *P. aeruginosa*.

The coverage of gentamicin against Gram-negative bacteria in this study was 89.7%. This can be compared to similar studies conducted in Nigeria² and Iran¹¹. However, the study carried out in India²⁶ indicated that gentamicin coverage for Gram-negative bacteria including *P. aeruginosa* was low.

Tetracycline coverage against Gram-positive bacteria was 59.2%. This result is comparable with the study carried out in Iran and Nigeria^{11,14}. Tetracycline coverage against Gram-positive bacteria was 59.2%. This result is comparable with the study carried out in Iran and Nigeria^{11,14}. The majority of Gram-negative bacteria (72%) appeared to be resistant to penicillin. However, coverage of penicillin against *S. pneumoniae* was high in this study (81.8%). This is comparable to studies done in Iran¹¹ and Nigeria². Amikacin has high coverage against *S. aureus* (95.51%) and CoNS (83.3%). This is consistent with studies done in Iran¹¹ and India^{8,21}. There is an increase in the resistance of studied antibiotics against isolated bacteria in the current study as in other studies elsewhere, the emergence of bacterial resistance due to pathogen properties and antibiotic prescribing practices including the widespread use of systemic antibiotics and health care guidelines^{5,6,27}. Other contributing factors may include an improper dose regimen, misuse of antibiotics for viral infections and other non-bacterial infections, and a long period of treatment rather than in the least globalization and migration⁶.

In Yemen, it is a common practice that antibiotics can be purchased without a prescription, leading to misuse of antibiotics. This may contribute to the emergence and spread of antimicrobial resistance^{16,17}. Other factors may include substandard quality or substandard antimicrobial drugs, increased use of a specific antimicrobial agent, poor sanitation, contaminated food and fecal contamination from humans or animals^{2,5,17}. As a result of patterns of bacterial sensitivity to many antimicrobial agents, they may vary from place to place and in the same place from time to time^{7,8,23}.

CONCLUSION

These results indicated that Gram-positive bacteria were the most common bacteria isolated from external eye infections and were more susceptible to ciprofloxacin and vancomycin while Gram-negative isolates were more susceptible to amikacin and ciprofloxacin. The high rate of resistance for most antibiotics in Yemen, leaves ophthalmologists with very few options of drugs to treat eye infections.

LIMITATION

Mono and gati floxacin, fusidic acid, tobramycin, neomycin that are used as eye drops were not included in the tested antibiotics because they were not available in our laboratory during the study. We usually only test antibiotics used for systemic infections. Extensive future studies should also be conducted in order to monitor antimicrobial resistance including topical antibiotics such as mono, and gati fluoxacin, fusidic acid, tobramycin, and neomycin. External fungal eye infections should also be studied.

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CONFLICT OF INTEREST

"No conflict of interest associated with this work".

AUTHOR'S CONTRIBUTION

This research work is part of the National Center for Public Health Laboratories (NCPHL) and MSc project. The first author did clinical work and the second and third authors did the lab. The corresponding author (HAA) supervised laboratory works, reviewing and editing the research.

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Table 1: The age and gender distribution of patients with external ocular infection in selected hospitals and eye clinics in Sana'a city, Yemen

characters	Male (n= 197)	
	No.	%
Sex		
Male	121	61.4
female	76	38.6
Age group		
≤ 15 years	47	23.9
16 – 25 years	31	15.7
26 – 35 years	42	21.3
36 – 45 years	29	14.7
≥ 46 years	48	24.4
Total	197	
Mean age	29.4 years	
S D	9.5 years	
Min	1 month	
Max	80 years	

Table 2: Distribution of bacterial isolates of external ocular infection in selected hospitals and eye clinics in Sana'a city, Yemen

Bacterial isolates	Positive for bacterial growth N=146	
	No	%
Gram positive bacteria	76	52.1
<i>S.aureus</i>	44	30.1
<i>Beta-haemolytic streptococcus</i>	9	6.2
<i>S. pneumonia</i>	11	7.5
<i>CoNs</i>	12	8.2
Gram negative bacteria	70	47.9
<i>H.influenzae</i>	13	8.9
<i>Pseudomonas aeruginosa</i>	39	26.7
<i>Moraxella lacunata</i>	5	3.4
<i>E.coli</i>	11	7.5
<i>Proteus spp</i>	2	1.4
Total n=197	146	74.1

Mixed infection cases were excluded.

Table 3: Antibiotic susceptibility test of Gram positive isolates from external ocular infections in selected hospitals and eye clinics in Sana'a city, Yemen

Antibiotic	<i>S.aureus</i> N=44		<i>S.pneumoniae</i> N=11		CoNs N=12		<i>Beta haemolytic streptococci</i> N=9		Total N=76	
	S No(%)	R No(%)	S No(%)	R No(%)	S No(%)	R No(%)	S No(%)	R No(%)	S No(%)	R No(%)
Amikacin (30 µg)	42(95.5)	2(4.5)	2(18.2)	9(81.8)	10(83.3)	2(16.7)	3(33.3)	6(66.7)	57(75)	19(25)
Penicillin (30 µg)	1(2.3)	43(97.7)	9(81.8)	2(18.2)	5(41.7)	7(58.3)	6(66.7)	3(33.3)	21(27.6)	55(72.4)
Vancomycin (30 µg)	38(86.4)	6(23.6)	11(100)	0(0.0)	12(100)	0(0.0)	9(100)	0(0.0)	70(92.1)	6(7.9)
Erythromycin (15 µg)	26(59)	18(41)	9(81.8)	2(18.2)	8(66.7)	4(33.3)	7(77.8)	2(22.2)	50(65.9)	26(34.1)
Trimethoprim-sulphamethoxazole (1.25/23.75 µg)	32(72.7)	12(27.3)	5(45.5)	6(54.5)	8(66.7)	4(33.3)	7(77.8)	2(22.2)	52(68.4)	24(31.6)
Chloroamphenicol (30 µg)	23(52.3)	21(47.7)	11(100)	0(0.0)	7(58.3)	5(41.7)	8(88.9)	1(11.1)	49(64.5)	27(35.5)
Gentamycin (10 µg)	40(90.9)	4(9.1)	3(27.3)	8(72.7)	6(50)	6(50)	5(55.6)	4(44.4)	54(71)	22(29)
Tetracycline (30 µg)	30(68.2)	14(31.8)	3(27.3)	8(72.7)	9(75)	3(25)	3(33.3)	6(66.7)	45(59.2)	31(40.8)
Doxycycline (30 µg)	34(77.3)	10(22.7)	10(90.9)	1(9.1)	11(91.7)	1(8.3)	8(88.9)	1(11.1)	63(83)	13(17)
Ceftriaxone (30 µg)	30(68.2)	14(31.8)	9(81.8)	2(18.2)	9(75)	3(25)	8(88.9)	1(11.1)	56(73.7)	20(26.3)
Ciprofloxacin(5 µg)	42(95.5)	2(4.5)	10(90.9)	1(9.1)	12(100)	0(0.0)	9(100)	0(0.0)	73(96)	3(4)

Table 4: Antibiotic susceptibility test of Gram negative isolates from external ocular infections in selected hospitals and eye clinics in Sana'a city, Yemen

Antibiotics	<i>H. influenzae</i> N=13		<i>Pseudomonas aeruginosa</i> N=39		<i>E.coli</i> N=11		<i>Moraxella lacunata</i> N=5		Total N=68	
	S No(%)	R No(%)	S No(%)	R No(%)	S No(%)	R No(%)	S No(%)	R No(%)	S No(%)	R No(%)
Amikacin (30 µg)	13(100)	0(0.0)	39(100)	0(0.0)	11(100)	0(0.0)	5(100)	0(0.0)	68(100)	0(0.0)
Erythromycin (15 µg)	9(69.2)	4(30.8)	NT	NT	NT	NT	NT	NT	9(69.2)	4(30.8)
Trimethoprim-sulphamethoxazole (1.25/23.75 µg)	2(15.4)	11(84.6)	8(20.5)	31(79.5)	2(18.2)	9(81.8)	1(20)	4(80)	13(19.1)	55(80.9)
Chloroamphenicol (30 µg)	9(69.2)	4(30.8)	23(59)	16(41)	4(36.4)	7(63.6)	5(100)	0(0.0)	41(60.3)	27(39.7)
Gentamicin (10 µg)	13(100)	0(0.0)	38(97.4)	1(2.6)	6(54.5)	5(45.5)	4(80)	1(20)	61(89.7)	7(10.3)
Tetracycline (30 µg)	10(76.9)	3(23.1)	10(25.6)	29(74.4)	8(72.7)	3(27.3)	4(80)	1(20)	32(47)	36(53)
Doxycycline (30 µg)	11(84.6)	2(15.4)	21(53.8)	18(46.2)	7(63.6)	4(36.4)	4(80)	1(20)	43(63.2)	25(36.8)
Ceftriaxone (30 µg)	12(92.3)	1(7.7)	30(76.9)	9(23.1)	5(45.5)	6(54.5)	4(80)	1(20)	51(75)	17(25)
Ciprofloxacin (5 µg)	11(84.6)	2(15.4)	35(89.8)	4(10.2)	7(63.6)	4(36.4)	5(100)	0(0.0)	58(85.3)	10(14.7)

NT= Not tested, S=sensitive, R=resistant