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RESEARCH ARTICLE

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

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Abstract

Objectives: The aim of the current study was to reveal 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 infection of external eye were included in the study which included conjunctivitis, keratitis, blepharitis and Blepharoconjunctivitis. 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 regular laboratory techniques, and microbial sensitivity testing has been carried out using a disc diffusion method.
Results: A total of 197 ocular samples were obtained for microbiological evaluation, of these 146 (74.1%) have bacterial growth. Bacteria of Gram positive accounted for 52.1% and the prevalent 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 revealed that Gram-positive bacteria were the generally common bacteria isolated from infections of external eye and were more susceptible to vancomycin and ciprofloxacin while Gram-negative isolates were more susceptible to ciprofloxacin and amikacin. 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: antimicrobial sensitivity, bacterial causes, external ocular infections, Sana'a, Yemen.

INTRODUCTION

Pathogenic microorganisms cause ocular infections as a result of virulence and low host resistance in some circumstances such as living conditions, personal hygiene, 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 iimmediate treatment of a serious bacterial eye infection that threatens the cornea is needed[2]. For precise antibacterial treatment, isolation and identification of bacterial pathogens along with an antibiotic sensitivity spectrum is required[3]. Because there is a global problem with the appearance of bacterial resistance to topical antimicrobial agents that are effected by pathogen properties and antibiotic prescribing practices including systemic antibiotic use and general health care guidelines[4,5]. This growing 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[6-8]. 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 infection[9]. Due to the high rate of drug resistance to antibiotics in medicine in Yemen[10], the ophthalmologists is left with a very few options 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 complicated as ocular pathogens are becoming increasingly resistant to commonly used antibiotics[7]. 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 regularly prescribed among patients with external eye infections in Yemen.

MATERIALS AND METHODS

This cross-sectional study included 197 eye specimens for microbiological assessment of patients clinically diagnosed with external eye infections such as keratitis, conjunctivitis, blepharitis and blepharo-conjunctivitis 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 viral conjunctivitis, trachoma, viral keratitis, peripheral ulcerative keratitis, allergic and severe eye trauma, current 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 techniques[12], 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. gonorrhea* were suspected], as well as the Loeffler serum slope of the *Moraxella* infection (all culture media from Difco Laboratories USA). 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 method[13]. The following antimicrobials were used with their respective concentration (Difco Laboratories, USA): Ceftriaxone (CRO, 30 μg), amikacin (AK, 30 μg), gentamicin (CN, 10 μg) ciprofloxacin (CIP, 5 μg), penicillin (P, 10U), tetracycline (TE, 30 μg), erythromycin (E, 15 μg), doxycycline (DO, 30 μg), chloramphenicol (C, 30 μg), trimethoprim-sulphamethoxazole (SXT, 1.25/23.75 μg), and vancomycin (VA, 30 μg).

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 shows the sensitivity 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 illustrates the susceptibility of Gram-negative bacteria. The Gram-negative bacteria and showed high rate of susceptibility to amikacin (100%) and gentamicin (89.7%).

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**
Staphylococcus aureus	44	30.1
Beta-hemolytic streptococcus	9	6.2
Streptococcus pneumonia	11	7.5
Coagulase negative staphylococci	12	8.2
Gram negative bacteria	**70**	**47.9**
Haemophilusinfluenzae	13	8.9
Pseudomonas aeruginosa	39	26.7
Moraxella lacunata	5	3.4
Escherichia coli	11	7.5
Proteus species	2	1.4
Total n=197	146	74.1

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	*S. aureus* N=44		*S. pneumoniae* N=11		CoNs N=12		*Beta hemolytic streptococci* 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)
Erthromycin (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)

DISCUSSION

In wide-ranging, the eye isolates recognized in the current study were comparable to those of many other studies performed in different regions. Although the major bacteria recognized to cause external eye infections around the world is *S. aureus*[1,2,8,14]. The most common isolates in the current study was *S. aureus* (30.1%) followed by *P. aeruginosa* (26.7%). Similar studies performed in India[1,8], Nigeria[2,15], Gondar[16], and Ethiopia[14,17] also indicated that *S. aureus* is a predominant eye isolate. On the other hand, 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. aeruginosa* such as the current study. The predominance of *P. aeruginosa* in our study, which differs from previous studies, can be supported by finding similar studies conducted in Sudan[18], Australia[19], Malaysia[20], India[21] and Thailand[22]. 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 recognized 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 Nigeria[15], and Gondar[16] where variable E*nterobacteriaceae* 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 area[2]. Laboratory-based resistance and sensitivity may not reflect the true clinical resistance and response to the antibiotic due to drug penetration and host factors[8]. 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 pathogens[1]. 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 India[8]. In contrast, a study in Iran[11] 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%)

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	*H. influenzae* N=13		*Pseudomonas aeruginosa* N=39		*E.coli* N=11		*Moraxella lacunata*, 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)
Erthromycin (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=resista

This finding is similar with studies conducted in Nigeria[2,14], Iran[11], 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 India[21]. Gentamicin coverage for *P. 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 Arabia[24] and Nigeria[14,25]. On the other hand, the study in India[21] described low coverage of ciprofloxacin for *P. aeruginosa*. The gentamicin coverage against Gram-negative bacteria in this study was 89.7%. This can be compared to similar studies conducted in Nigeria[2] and Iran[11]. In spite of this, the study carried out in India[26] 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. In spite of this, coverage of penicillin against *S. pneumoniae* was high in the current (81.8%). This is comparable to studies done in Iran[11] and Nigeria[2]. Amikacin has high coverage against *S. aureus* (95.51%) and CoNS (83.3%). This is regular with studies done in Iran[11] 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 preparations including the extensive use of systemic antibiotics and health care guidelines[5,6,27]. Other causal factors may comprise an improper dose regimen, mistreatment of antibiotics for viral infections and other non-bacterial infections, and a long period of treatment rather than in the least globalization and migration[6]. In Yemen, it is a widespread practice that antibiotics can be acquired 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, contaminated food, poor sanitation, and fecal contamination from animals or humans[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].

CONCLUSIONS

These results revealed that Gram-positive bacteria were the most common bacteria isolated from external eye infections and were more susceptible to vancomycin and ciprofloxacin while Gram-negative isolates were more susceptible to ciprofloxacin and amikacin. The high rate of resistance for most antibiotics in Yemen, leaves ophthalmologists with very few options of drugs to treat eye infections. Mono and gatifloxacin, 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 gatifloxacin, fusidic acid, tobramycin, and neomycin. On the other hand external fungal eye infections should also be studied.

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AUTHOR'S CONTRIBUTION

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Alshamahi EYA: writing original draft, methodology, investigation. **Al-Shami HU:** formal analysis, data curation, conceptualization. **Al-Shamahy HA:** review, supervision. **Musawa YA:** methodology, formal analysis. All authors read and approved the final manuscript for publication.

DATA AVAILABILITY

The datasets generated during this study are available from the corresponding author upon reasonable request.

CONFLICT OF INTEREST

None to declare.

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