Bacterial isolates and their antimicrobial susceptibility pattern among patients with ocular infections at the University of Gondar Hospital, Northwest Ethiopia: A 10-years retrospective study

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Research note

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Abstract

Abstract Objective: The aim of this study was to identify potential bacterial isolates and their antimicrobial susceptibility pattern among patients who visited eye clinic of Gondar university hospital. Results: From year of January 2009 to January 2019 a total of 319 eye discharge specimens were submitted for microbiological analysis, of which 133(41.7%) were culture positive. A total of 42(31.6%) Gram negatives and 91(68.4%) Gram-positive bacteria were isolated. Among isolated Gram-negative bacterial species, Klebsiella spp was the most predominant, 42.9% followed by E. coli 26.2%. Among isolated Gram-positive pathogenic bacterial species, S. aureus was the most prevalent, 59.5% followed by S. pyogenes , 8% and S. pneumoniae , 5.5%. Most of the bacterial isolates were resistant to cotrimoxazole, 81.6%, amoxicillin, 78.7%, tetracycline,76.2%, and ampicillin 75.9%. About 47.4 % of bacterial isolates showed multi drug resistance to three or more classes of antimicrobials. Antimicrobials like: ampicillin, amoxicillin, ceftriaxone, ciprofloxacin, and norfloxacin exhibited year to year increment of resistance ($p<0.0001$).

Introduction

The eye is one of the sense organs which is very important throughout our life. Ocular infection is a challenge for all age groups and can cause damage to structures of the eye, which can lead to loss of vision and even blindness if left untreated [1]. Dust, high temperature, microorganisms, and other factors can lead to various eye diseases which can lead to blindness [2]. However, bacteria are the major contributor to ocular infections throughout the world [3].

Ocular disease and its complications are a significant health problem worldwide that impacts greatly on quality of life. World Health Organization (WHO) estimates that about 285 million peoples are visually impaired worldwide and 90% of these individuals live in low-resource countries [4].

In the case of sub-Saharan Africa, an estimated 26 million individuals live with visual impairment, of whom 5.9 million individuals are classified blind [5]. The costs of conjunctivitis are substantial. Bacterial conjunctivitis, which comprises about 50 percent of all cases of conjunctivitis, costs an estimated $377 million to $857 million annually in the U.S [6].

Internationally, there is a growing concern over antimicrobial resistance (AMR) which is currently estimated to account for more than 700,000 deaths per year worldwide [7]. In this study, we will review the microbiology and antimicrobial susceptibility pattern of ocular bacterial infection in Gondar University Comprehensive Referral Hospital over the past 10 years.

Materials And Methods

Study Area, Study Design and Period

The study was conducted at University of Gondar Hospital, North Gondar, based on the 2005 census, the total population of Gondar is 194,773 (97,625 males and 97,148 females) [8]. Hospital based cross-sectional retrospective study was conducted at the University of Gondar Hospital among patients with ocular infection from the year January,2009 to January,2019.

Data collection

A standard data extraction sheet was prepared by using different literature and the data were collected from the registration book.

Laboratory methods

The eye discharge specimens were collected aseptically and streaked on blood agar, MacConkey agar and chocolate agar (Oxoid, UK). The inoculated plates were incubated at 37^0C for 24-48hrs. The growth on the plates were identified by standard microbiological techniques. AST was done by the modified Kirby-Bauer disk diffusion method on Mueller Hinton agar (Oxoid, UK). For fastidious isolates, Mueller Hinton agar supplemented with 5% sheep blood was used. Diameters of a zone of inhibition around the disk were measured with the ruler and interpreted according to the Clinical Laboratory Standard Institute guidelines. Bacteria resisting for more than two drugs of the different classes are considered as “multi-drug resistant” [9].

Data Analysis and Interpretation

Data was entered to SPSS version 20 statistical packages for analysis. Descriptive statistics were done to calculate frequencies of categorical variables. Chi-square test was done to see trend of drug resistance among bacterial isolates. Results were presented in the form of tables, texts, and graphs. P value<0.05 were considered as statistically significant.

Ethical Consideration

Ethical clearance was obtained from Research and Ethics Committee of the School of Biomedical and Laboratory Science, Collage of Medicine and Health Science.

Results

Socio-demographic characteristics

Within the last ten years (January 2009-January 2019), a total of three hundred nineteen (319) ocular infection suspected patients visited University of Gondar Comprehensive specialized referral hospital. Among this, a total of 133(41.7% patients were culture positive. About 81(60.3%) patients were males and 52(39.7%) were females. However, the age of most patients was in the age group of 1-5 years [Table 1].

Prevalence of bacterial isolates from ocular specimens

In this study, the overall bacterial isolation rate was 133(41.7%). Both Gram-negative and Gram-positive bacteria were isolated from the positive cases. A total of 42(31.6%) Gram negatives and 91(68.4%) Gram-positive bacteria were isolated. Among isolated Gram-negative bacterial species, *Klebsiella* spp was the most predominant, 42.9% followed by *E. coli* 26.2%. Among isolated Gram-positive bacterial species, *S. aureus* was the most prevalent, 59.5% followed by CONS 18.7% [Table 2].

Antimicrobial susceptibility pattern of bacterial isolates

Overall, most of the bacterial isolates showed high level of resistance to cotrimoxazole 62/76 (81.6%), amoxicillin 37/47(78.7%), tetracycline48/63 (76.2%) and ampicillin 44/58 (75.9%) [Table 3].

Antimicrobial susceptibility pattern of Gram-negative bacterial isolates

Among recovered Gram-negative bacterial isolates, *K. pneumoniae* and *E. coli* showed high level of resistance. Ampicillin, amoxicillin, cotrimoxazole, gentamycin, chloramphenicol, norfloxacin and ceftriaxone were among tested antimicrobials resisted by *K. pneumoniae* with the resistance rate of 100%, 100%,84.6%,73.3%, 66.7%, 66.7% and 60% respectively. *E. coli* also showed significant level of resistance to ampicillin (77.8%), cotrimoxazole (77.8%), tetracycline (72.7%) and amoxicillin (71.4%) [Table S1].

Antimicrobial susceptibility pattern of Gram-positive bacterial isolates

In this study, *S. aureus* showed great extent of resistance to ampicillin (100%), penicillin G (80.8%), tetracycline (77.8%), oxacillin (66.7%) and amoxicillin (75%) [Table S2].

Multi drug resistance property of isolates

The overall multidrug resistance rate of bacterial isolates was 47.4% [Table S3].

Antimicrobial resistance trend in the study setting

In this study, increased trend of resistance to many tested antimicrobials was observed in the past 10 years. Increased trend of resistance to ampicillin was observed over a period of 10 years (January 2009-January 2019) ($p < 0.0001$). In 2009, 80%, 45.5%, 25 %, 12.5 % and 36.8% of the isolates were resistant to ampicillin, amoxicillin, ceftriaxone, ciprofloxacin, and norfloxacin, respectively. However, by 2019, after 10 years, the resistance rates were 100%, 100%, 60%, 51.8% and 56% to ampicillin, amoxicillin, ceftriaxone, ciprofloxacin and norfloxacin, respectively [Table S4].

Discussion

Ocular infection is a major public health problem especially in developing countries like Ethiopia. The external ocular infections are responsible for increased incidence of morbidity and blindness worldwide, their morbidity vary from self-limiting light infection to sight threatening infection [10,11]. In this study, the overall, prevalence of isolated bacteria was 133/319(41.7%). This finding is in agreement with the previous studies carried out in Hawassa [12], in which, about 48.8% of the study participants were culture positive. However, our result was significantly lower than findings reported in Sudan [13], Tigray [14] and Jimma [10], about 63.7%, 66.7% and 74.7% positivity rate respectively. This difference may result from difference in geography of the study setting and types of study design. However, compared to study findings in China [15], 20.43% and India [16], 34.5%, this finding is higher. This difference may be resulted from difference in self-hygiene, socioeconomic status of the study population and disease prevention practices.

Different scholars reported that both Gram-negative and Gram-positive bacterial species involved in ocular infection [13,15,17,18] and this finding is in line with our findings. Among isolated Gram-negative bacterial species, *Klebsiella* spp was the most predominant bacterial isolate recovered from eye discharge. Similarly, this bacterial species was isolated from eye discharge specimens at University of Gondar in 2016 [19]. In contrast to our finding, the study done in Sudan [13], Hawassa [12], Tigray [14] and Jimma [10] reported that *P. aeruginosa* was the most isolated bacteria. Geographical location and acquisition of hospital acquired infection in the study participants may be the possible reason for this difference. *E. coli* was another Gram-negative bacterial isolate recovered in this study. Similarly, this bacterium was also reported in different studies [15, 16,19].

In this study, the dominant bacterial isolates from eye discharge specimens were Gram positive cocci. This finding is in agreement with other several previous studies done in many parts of Ethiopia [10,12,14], and countries like China [15], India [17] and Sudan [13]. In the present study, *S. aureus* was the predominant Gram-positive bacterial isolate concordant with previous study findings reported in Hawassa [12], Tigray [14], Jimma [10] and India [17].

On the contrary, other study findings from Saudi Arabia [18] and Uganda [20] stated that CoNS was the most prevalent Gram-positive bacterial species. This difference may result from the immune status of patients and specimen collection procedures.

In this study, Gram-positive bacterial species like *S. pyogenes* and *S. pneumoniae* were also significantly isolated. This finding is supported by findings reported in Sudan [13] and Hawassa [12]. However, bacterial species like *N. gonorrheae,* H*. influenzae* and *Pseudomonas* were not isolated in this study unlike to other studies [12,13, 17].

The antimicrobial susceptibility patterns of both Gram-negative and Gram-positive bacterial isolates was also determined. Overall, most isolated bacterial species showed significant level of resistance to tested antimicrobial agents. Ampicillin (75.9%), tetracycline (76.2%), amoxicillin (78.7%) and cotrimoxazole (81.6%), were among antimicrobials resisted by isolated bacteria. This finding is supported by different studies [10, 12,13,14,17,19].

Specifically, antimicrobial resistance by Gram-negative bacterial species become a big threat for the world, especially for developing countries [21]. Ampicillin, amoxicillin, cotrimoxazole, gentamycin, chloramphenicol, norfloxacin and ceftriaxone were among tested antimicrobials resisted by *K.*

pneumoniae, one of the isolated Gram-negative bacteria with the resistance rate of 100%, 100%,84.6%,73.3%, 66.7%, 66.7% and 60% respectively. In case of ampicillin, this finding is comparatively in agreement with results reported in India [16] in which, *K. pneumoniae* was 100% resistant to ampicillin. In a reverse, unlike to our findings, results reported in Gondar [22] showed that complete sensitivity was observed to gentamycin (100%), chloramphenicol (100%), ciprofloxacin (100%) and cotrimoxazole (100%). In the study setting, an irrational use of antimicrobials without prescription, improper dosage regimen, misuse of antimicrobials for viral and other non-bacterial infections and extended duration of therapy could result in increased antimicrobial resistance.

E. coli also showed a significant level of resistance to ampicillin (77.8%), cotrimoxazole (77.8%), tetracycline (72.7%) and amoxicillin (71.4%). Similarly, a high level of resistance was observed in the study done by Yared A *et al*., 2016 [22], in which, *E. coli* showed 100% resistance to amoxicillin and tetracycline. In the study setting, however, some tested antibiotics like nalidixic acid (100%), erythromycin (100%) and norfloxacin (75%) are still effective antimicrobial agents against *E. coli.* This finding was consistent with study done in southern parts of Ethiopia [12], gram negative isolate was more susceptible to ciprofloxacin and gentamicin.

In this study, antimicrobials susceptibility pattern of Gram-positive bacterial species was also determined. *S. aureus*, one of the most predominantly isolated Gram-positive bacteria, had great extent of resistance to ampicillin (100%), penicillin G (80.8%), tetracycline (77.8%) and amoxicillin (75%). Different literatures supported our findings. For instance, a prospective study done in India [16] showed that *S. aureus* was resistant to penicillin (100%), erythromycin (100%), tetracycline (87.5%) and amoxicillin (62.5%). However, rate of resistance in our study by *S. aureus* was higher than findings reported in Uganda [20], about 31% and 55.2% of S. aureus resisted against erythromycin and tetracycline, respectively. Similar study done in Sudan [13] also showed that about 100% and 72% of *S. aureus* was resistant to penicillin and tetracycline, which is almost comparable to the present study.

The overall prevalence of multi-drug resistance (MDR) to three or more classes of commonly prescribed antimicrobials was 47.4%. this is lower than results reported in Hawassa [12], 69.9% and Gondar [19], 87%. But, MDR rate in the present study is higher than finding reported in Tigray [14], only 34.8% MDR rate was observed.

Investigation on antimicrobial resistance trend is indispensable for empirical treatment of infections, implementing resistance control measures, and preventing the spread of antimicrobial-resistant microorganisms. In the present study, the antimicrobial resistances of bacteria isolated from eye discharge was analyzed. In this study, increased trend of resistance to many tested antimicrobials was observed in the past 10 years. Increased trend of resistance to ampicillin was observed over a period of 10 years (2009-2018) ($p < 0.0001$). In 2009, 80%, 45.5%, 25 %, 12.5 % and 36.8% of the isolates were resistant to ampicillin, amoxicillin, ceftriaxone, ciprofloxacin, and norfloxacin, respectively. However, by 2018, after 10 years, the resistance rates were 100%, 100%, 60%, 51.8% and 56% to ampicillin, amoxicillin, ceftriaxone, ciprofloxacin and norfloxacin, respectively [Table S4]. However, this finding was not further compared with others because of insufficient data.

Limitation of the study

- Antibiotic disc sensitivity test may vary with hospital settings, where infection rate depends on environment and locality, type of infection, its control practices and antibiotic use. Thus, this finding is only applicable to the study setting.
- Being a retrospective study may also a little bit compromise this finding.

List Of Abbreviations

AMR: Antimicrobial resistance, AST: Antimicrobial susceptibility test, MDR: Multidrug resistance, SD: Standard deviation

Declarations

Ethics approval and consent to participate

An ethical clearance letter was obtained from the Departmental Research and Ethics Review Committee of school of biomedical laboratory science. The reference number of the ethical letter was "Ref no-SBMLS/2123/11". This ethical letter was obtained from Mr. Mekonnen Girma (mekonnen2302@cmail.com), Markos Negash (markosnegash@yahoo.com) and Bamilaku Enawgaw (bamlak21@gmail.com). Verbal permission to extract data from registration book was obtained from the Medical Microbiology laboratory head. Confidentiality was maintained at all levels of the study.

Consent for publication

All authors read the manuscript and have provided their consent to publish.

Availability of data and material

Data and supporting materials associated with this study will be shared upon request

Competing interests

The authors declare that they have no competing interest.

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This study was not funded.

Authors' contribution

DS did conceptualization, analyzing the data, methodology designing, , writing original draft and review the final manuscript.

AT did conceptualization, analyzing the data, methodology designing, , writing original draft and review the final manuscript.

ZY did conceptualization, analyzing the data, methodology designing, writing original draft and review the final manuscript.

MM did conceptualization, analyzing the data, methodology designing, , writing original draft and review the final manuscript.

GB did conceptualization, methodology designing, and review the final manuscript.

SE did conceptualization, analyzing the data, methodology designing, and review the final manuscript.

TB did conceptualization, analyzing the data, methodology designing, , writing original draft and review the final manuscript.

All authors have read and approved the manuscript.

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Tables

Variables	**frequency**	**Percentage**
Sex		
Male	81	60.9
Female	52	39.1
Age group in years		
<5	100	75.2
5-9	5	3.8
10-19	7	5.3
20-39	18	13.5
40-49	3	2.3
>=50	1	0.7
Total	133	100

Table 1: Socio-demographic characteristics of culture positive patients with ocular bacterial infection at Gondar University specialized referral hospital, Gondar, Northwest, Ethiopia.

Bacterial isolates	**Frequency**	**Percentage**
Gram-negative bacterial isolates		
Klebsiella species	18	42.9%
E. coli	11	26.2%
NLFGNR	5	11.9%
Citrobacter species	3	7.1%
Other	3	7.1%
proteus species	2	4.8%
Total	**42**	**100%**
Gram-positive bacterial isolates		
S. aureus	45	49.5%
CONS	17	18.7%
0ther *streptococcus* species	16	17.6%
S. pyogenes	8	8.8%
S. pneumoniae	5	5.5%
Total	**91**	**(100%)**

Table 2: Number and percentage of bacteria isolates from eye discharge specimens at University of Gondar specialized and referral hospital (January 2009 -January 2019), Gondar, Northwest, Ethiopia.

Antimicro. agent	No. of bacteria tested	Sensitive N (%)	Resistant N (%)
Ampicillin	58	14(24.1%)	44(75.9%)
Amoxicillin	47	10(21.3%)	37(78.7%)
Ceftriaxone	81	53(65.4%)	24(29.6%)
Chloramphenicol	81	43(53.1%)	38(46.9%)
Ciprofloxacin	80	63(78.8%)	17(21.3%)
Clindamycin	25	17(68%)	8(32%)
Erythromycin	80	42(52.5%)	38(47.5%)
Gentamycin	81	39(48.1%)	43(53.1%)
Nalidixic Acid	8	4(50%)	4(50%)
Norfloxacin	49	37(75.5%)	12(24.5%)
Cotrimoxazole	76	17(22.4%)	62(81.6%)
Tetracycline	63	15(23.8%)	48(76.2%)

Table 3: Overall antimicrobial susceptibility profiles of bacterial isolates from eye discharge at University of Gondar referral hospital (January 2009 – January 2019), Gondar, Northwest, Ethiopia

Additional Files

Table S1. Antimicrobial susceptibility patterns of Gram-negative bacterial isolates from eye discharge at University of Gondar referral hospital laboratory (2009-2018), Gondar, Northwest, Ethiopia. This table shows the antimicrobial susceptibility pattern of some gram-negative bacterial species isolated from eye discharge. This table should be put just after Table 3 (see above the "Discussion").

Table S2. Antimicrobial susceptibility patterns of Gram-positive bacterial isolates from eye discharge at University of Gondar referral hospital (2009-2018), Gondar, Northwest, Ethiopia. This table shows the antimicrobial susceptibility pattern of some gram-positive bacterial species isolated from eye discharge. This table should be put just after Table S1 (see above the "Discussion").

Table S3. Multidrug Resistance Patterns of Bacteria Isolates from eye discharge at University of Gondar referral hospital laboratory (2009-2018), Gondar, Northwest, Ethiopia. This table shows the Multidrug resistance pattern of bacterial isolates from eye discharge. This table should be put just after Table S2 (see above the "Discussion").

Table S4. Chi-square test for trend applied to bacterial resistance rates to various antimicrobials at University of Gondar comprehensive specialized hospital laboratory (2009 - 2018), Gondar, Northwest, Ethiopia. This table shows the trend of antimicrobial resistance by recovered bacterial isolates from eye discharge. This table should be put just after Table S3 (see above the "Discussion").

Supplementary Files

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