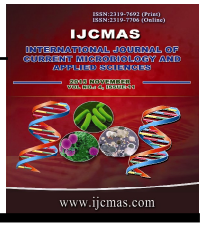
Int.J.Curr.Microbiol.App.Sci (2015) 4(11): 383-393

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 11 (2015) pp. 383-393**
http://www.ijcmas.com



Original Research Article

Potential Bacterial Pathogens of Red Eye infections and their Antibiotic Susceptibility Patterns in Taif, KSA

Ahmad F. Shahaby[1,2*], Abdulla A. Alharthi[1] and Adel E. El Tarras[1,2]

[1]Biotechnology and Genetic Engineering Unit, College of Medicine,
Taif University, Taif, Saudi Arabia
[2]Cairo University, College of Agriculture, Department of Microbiology, Genetics, Cairo, Egypt
**Corresponding author*

A B S T R A C T

Eye diseases are common worldwide, sometimes to an epidemic degree Ocular infections can cause damage to structures of the eye, which can lead to reduced vision and even blindness if left untreated. The present study investigated bacterial conjunctivitis. The objective of this study was to identify potential bacterial isolate of external ocular infections and their antimicrobial susceptibility patterns in patients attending university clinic. A total of 89 ocular specimens were collected among conjunctivitis cases. The participants were divided into 5 categories according to their occupation. The rate of culture-positivity was found to be significantly higher among study subjects of workers, children and students with conjunctivitis. Samples were processed for culture, isolation and identification by morphological, physiological, APi profiles and 16S-rRNA techniques. Out of 89 ocular specimens, 70 (78.7%) were positive. *S. aureus* 54 (19.6%) were the most predominant bacteria in mixed growth followed by coagulase- negative *Staphylococci* (CNS) 45 (16.4%) and *B. subtilis* 42 (15.3%). The rate of isolation was higher among the workers 61(22.2%) and children 59 (21.5%) than the other three populations. Isolates were identified as *S. aureus, S. epidermidis* (CNS), *S. pneumoniae, S. pyogenes, K. pneumoniae, B. subtilis, Micrococcus roseus, E. coli, P. aeruginosa* and *Enterobacter aerogenes.* Susceptibility testing was done according to Clinical and Laboratory Standards Institute (CLSI) guideline. *In vitro* ceftriaxone was effective against 74.9% of isolated pathogens. Gram positive isolates were more susceptible to erythromycin and ceftriaxone. Whereas Gram negative isolates were more susceptible to gentamycin and chloramaphenicol. These data reveal relatively erythromycin and/or gentamycin are effective against most isolated pathogens.

K e y w o r d s

External ocular infections, Conjunctivitis, Isolation, Identification, 16S-rRNA, Antibiotics, Susceptibility

Introduction

A red eye infection is the most common ocular disorder that primary care physicians encounter. The red eyes disease is present everywhere and there was an epidemic in one of Taif counties, Mahni County in 1998. The department of health in Kingdom of

Saudi Arabia organized many programs for protection from diseases. There are four different types of conjunctivitis: bacterial conjunctivitis, viral conjunctivitis, chlamydial conjunctivitis and allergic conjunctivitis (Leibowitz, 2005). In this communication we will consider the bacterial conjunctivitis. Bacteria are the most common pathogens and involved in infections of all the tissues of the eye. The most frequently affected part of the eye is conjunctiva, lid and cornea (Jorgensen *et al.,* 2015; Ubani, 2009) which are external part of the eye. A healthy conjunctiva is necessary for the maintenance of a healthy cornea and thus the visual acuity of the eye. Bacterial infections of the eye are usually localized but may frequently spread to adjacent tissue due to some predisposing factors such as during trauma, previous surgery, ocular surface disease, contact lens wear, ocular adnexal dysfunction and other exogenous factors, systemic diseases (Bharathi *et al.,* 2003) and immunosuppression may alter the defense mechanisms of the outer eye and permit bacteria to spread (Leibowitz, 2005; Seal and Uwe, 2007). Many opportunistic pathogenic agents are increasingly encountered in ocular infections due to widespread use of topical and systemic immunosuppressive agents, increasing numbers of patients with human immunodeficiency virus (HIV) infection and with organ transplants who are on immunosupressive therapy. These opportunistic pathogens also cause ocular infections due to increased use of contact lens. The dreaded infections endophthalmitis following cataract extraction and lens implantation often are caused by opportunistic pathogens (Leibowitz, 2005; Caldwell *et al.,* 1992).

The spectrum of organisms causing conjunctivitis varies around the world. Microorganisms are known to cause external ocular infections such as conjunctivitis, keratitis, blepharitis, hordeolum, dacryocystitis, etc. which are responsible for increased incidence of morbidity and blindness worldwide (Modarrres *et al.,* 1998; Sharma, 2011). Infections of the conjunctiva can spread to the cornea and can cause a perforation, e.g., gonococcal infection. Bacterial conjunctivitis or limbal catarrh can spread over the cornea. Dryness will damage the surface of the cornea (Leibowitz, 2005; Wood, 1999). Infection is usually bilateral. It may start in one eye and later spread to the other. The spectrum of organisms causing conjunctivitis varies around the world. Clinical observation only are not diagnostic of the cause, so microbiological isolation and identification of bacterial pathogens along with antibiotic susceptibility pattern is essential (Finegold *et al.,* 1990). The clinical importance of external eye infections has been reported in some studies (Alene and Abebe, 2000; Tiliksew, 2002), by clinical presentation only, there are no much microbiological work with culture and drug sensitivity test which showed the magnitude of the problem.

Antibiotics can be purchased without prescription in less development countries, which leads to misuse of antibiotics. This may led to the emergence and spread of antimicrobial resistance (Caldwell *et al.* 1992, Anagaw *et al.* 2011; Tewelde *et al.* 2013). Also, poor hygienic and infection control practice in the area may play an important role in an increased prevalence of resistant bacteria in a community. Therefore, periodic controlling of etiology and re-evaluation of antibiotics is essential to make a rational choice of initial antimicrobial therapy.

The increasing use of PCR, over the last few years, rapid template purification, and automated DNA sequencing has

dramatically reduced the time necessary to yield a high-quality sequence. The use of 16S- rRNA gene sequencing to study the relatedness of prokaryotic species is well established and has led to increased availability of 16S- rRNA databases. The convergence of these technical and computational advances has also enhanced the application of 16S- rRNA gene sequence analysis to bacterial identification (Rantakokko-Jalava *et al.,* 2000). It was recently reported that subtle sequence differences in the 16S rRNA gene could be used for species identification (Sacchi *et al.,* 2002) and for subtyping and identifying hyper virulent bacterial clones (Nilsson *et al.,* 2003).

To our knowledge, no research has been conducted on prevalence, isolation and identification of potential bacterial pathogens and its distribution in the case of red eye infection as well as their antibiotic susceptibility pattern in this area. Therefore, the objective of this work was to isolate and identify potential bacterial isolates using morphological, physiological and 16S-rRNA techniques, in addition to, its distribution in the case of external ocular infection and their antibiotic susceptibility pattern.

Materials and Methods

Study area and design: The study was conducted on the campus of Taif University where samples were randomly sampled from volunteers from October, 2013 to December, 2014.

Sampling: A total of 89volunteer were randomly sampled from both in the halls as well as in the faculties aseptically swabbing the entire eye using dry sterile cotton swab in a standardized aseptic fashion with sterile cotton-tipped applicators in sterile caped plastic tube, CITOSWAB (Citotest labware manufacturing Co., LTD, China). Tubes were supplied with 3 ml of LB broth Miller media and incubated overnight at 35^{o}C. Samples were collected from 5 subjects: 21 faculty member, 20 personnel, 19 students, 14 workers and 15 children at the university clinic. Control samples were collected from healthy individuals and free of red eye diseases. Volunteers on antibiotics for the past 1 week were excluded. Sample from external parts of the eye (eyelid, conjunctiva, lacrimal sac and cornea) was collected using either swabbing or scraping as per the routine clinical management of the patients (Tabbara and Robert, 1995). Specimens were immediately delivered to the microbiology laboratory for culture and other bacteriological analysis.

Laboratory methods and procedures: Laboratory analysis was undertaken in the laboratories of Biotechnology and Genetic Engendering Research Center (BGERC) of the Taif University, Taif, KSA.

Inoculation: The cotton swab end was soaked in 10ml LB broth and incubated aerobically over-night at 37°C and then sampled swabs were streaked over sheep blood agar, mannitol salt agar, eosin methylene blue agar (EMB), *Streptococcus* selective agar media and MacConkey agar plates, for characterization of aerobic bacteria; no anaerobic / fungal cultures were taken. Plates were incubated aerobically at 37°C for 48 h.

Quantification of bacteria: Serial dilutions from the resulting growth from the LB medium were pour-plated on plate count agar (PCA) and incubated for 24hrs at 37^{o}C under aerobic condition. The number of estimated Colony Forming Units (CFU) for each sample was then counted using the Quebec colony counter (Reichert, USA). Serial dilutions from the resulting growth from the nutrient broth medium were pour-plated on count agar (PCA) and incubated

for 24 hrs at 37^{o}C under aerobic condition. The number of estimated Colony Forming Units (CFU) for each sample was then counted. Samples were inoculated in mannitol salt agar (MSA), sheep blood agar (BA), and plate count agar (PCA) media for counting total bacteria. Duplicate plates for each media were made for each dilution. All pure isolated colonies were sub-cultured onto sheep blood agar plates (for growth of heterotrophic bacteria) and MacConkey agar plates (for coliforms) for 24 hrs at 37^{o}C for colony isolation and morphological identification (Koch, 1984).

Isolation of microorganisms: All pure isolated colonies were sub-cultured onto blood agar plates (for growth of heterotrophic bacteria) and MacConkey agar plates (for coliforms) for 24hrs at 37^{o}C for colony isolation and morphological identification.

Identification of microorganisms

Identification and characterization of isolates based on the morphological, physiological, serological, the internal resistance for antibiotics method and the biochemical characteristics presented in Bergey's Manual of Systematic Bacteriology (Holt *et al.* 1994) and the APi Kits profiling (API 2009). Also, 16S-rRNA sequencing techniques were adopted to characterize and identify the selected isolates at molecular level.

Molecular genetics analysis

DNA extraction: The cell pellets form all isolates were used to extract genomic DNA using (Jena Bioscience, Germany) extraction kit following the manufacturer's instructions.

PCR amplification of 16S-rRNA gene: Primer sequences used to amplify the 16S-rRNA gene fragment were: U1 [5CCA GCA GCC GCG GTA ATA CG3] and U2 [5ATC GG(C/T) TAC CTT GTT ACG ACT TC3] according to Kumara *et al.* (2006). The PCR master mix contained10 *P*mol of each primer and 12.5 µl of 2xSuperHot PCR Master Mix (Bioron, Ludwigshafen, Germany) mixed with 50 to 100 ng of DNA template. Sterile d.H2O was added to a final volume of 25 µl. Thermal cycler (Uno II, Biometra, Germany) program was 94°C for 4 min., 94°C for 1 min., 55°C for 1 min., 72°C for 1.5 min, the number of cycles was 35 cycle and the post PCR reaction time was 72°C for 5 min.

Analysis of the PCR products: After the amplification, the PCR reaction products were electrophoresed with 100 bp ladder marker (Fermentas, Germany) on 10 x 14 cm 1.5% -agarose gel (Bioshop; Canada) for 30 min using Tris-borate- EDTA Buffer. The gels were stained with 0.5ug/ml of ethidium bromide (Bioshop; Canada), visualized under the UV light and documented using a GeneSnap 4.00- Gene Genius Bio Imaging System (Syngene; Frederick, Maryland, USA).

Sequencing of 16S-rRNA gene: The 990bp PCR-products of each isolate were purified from excess primers and nucleotides by the use of AxyPrep PCR Clean-up kit (AXYGEN Biosciences, Union City, California, USA) and directly sequenced using the same primers as described for the amplification process. The products were sequenced using the Big Dye Terminator Cycle Sequencing Ready Reaction Kit (ABI Applied Biosystems, Foster City, California, USA) on a 3130XL Genetic Analyzer (Applied Biosystems). The bacterial 16S-rDNA sequences obtained were then aligned with known 16S rDNA sequences in Genbank using the basic local alignment search tool (BLAST) at the National Center for Biotechnology Information, and percent

homology scores were generated to identify bacteria.

Antibiotic susceptibility test: Antibiotic susceptibility was determined by the agar diffusion technique on Mueller-Hinton agar according to the direction of the Clinical and Laboratory Standards Institute (CLSI) (Bauer *et al.,* 1966; CLSI, 2007) using 8 antibiotic discs (Biotec Lab. UK) corresponding to the drugs most commonly used in the treatment of human and animal infections caused by bacteria; ampicillin (10 µg), tetracycline (10 µg), gentamicin (10 µg), chloramaphenicol (25 µg), refamycine(30 µg), penicillin (10 iu), ciprofloxacin (5 µg), ceftriaxone (30 µg) and erythromycin (5 µg). In this test, antibiotic disks are placed on a Petri dish that contains the bacterial being tested. In general, if the bacteria can grow close to the disk, this indicates that the bacteria are resistant to that particular antibiotic. *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC 25922), were used as reference strains for culture and sensitivity testing.

This research work was approved by the Department of Higher Studies and Scientific Research, Taif University and the University Medical Centre. All the data obtained were kept confidential by using only code numbers and locking the data. Participation of the study subjects was purely voluntary.

Statistical analysis: Analysis was performed using the SPSS 10 software. Data from this study was analyzed descriptively using one-way ANOVA test, and means were compared using Duncan's multiple range tests at 5% significance level.

Results and Discussion

A total of 89participants diagnosed as ocular infection (Red eye) gave specimens for microbiological evaluation during the study period. Of these, 21 (23.6%) were faculty members, 20 (22.5%) were personal, 19 (21.3%) were students, 14 (15.7%) were workers, and 15 (16.9%) were children. Most of the study participants were adult and from inside the campus and in educational status except the children (Table 1). Of the total processed external ocular specimens 14(66.7%) faculty members, 15(75%) personal, 15(78.9%) students, 12(85.7%) workers and 14(93.1%) children gave good growth in LB media were found positive (Table 1). The highest growth rate of cultures was recorded in children (93.1%).

Red eye infection is a major public health problem in world. In this study, the overall prevalence of bacterial external ocular infections was 78.7%, where prevalence was higher than previous result (47.4%) recorded by Nigatu (2004) and 54.2% by Anagaw *et al.* (2011). Also, it is higher than result from other study (60.8%) by Dagnachew *et al.* (2014), 74.7% by Tewelde *et al.* (2013) and other countries such as India (58.8%) (Bharathi *et al.,* 2010). The varying rate of isolation from one category to another might be due to varying distribution of bacterial etiology with geographic variation, study period, variation with the study population and infection prevention practice in diverse settings.

All 89 participants sampled were positive with varied numbers of bacteria (Table 2). The range of numbers were $4x10^4$–$3.6x10^9$ CFU/mL for total bacterial flora, 0–$4.1x10^6$ for *Streptococcus* spp., the highest was obtained from red eye children, $3.3x10^2$–$6.3x10^8$ for *Staphylococcus* spp., $2.1x10^3$–$3.8x10^8$ for heterotrophic bacteria (hemolysin), 0–$6.3x10^8$for faucal coliform, and 0–$3.3x10^8$for *E. coli* (Table 2). *Staphylococcus* recorded the highest number

of bacteria being $6.3x10^8$ CFU/ mL for workers.

In this study, highest numbers of bacterial pathogens were obtained in workers and children. This may be due to study period, variation with the study population and infection prevention practice in diverse settings. The high number of Gram positive cocci *Staphylococcus* bacteria may be due to contamination of the eye from skin normal flora, study period, health of cornea and geographic location.

Identification, morphological, and biochemical characterization

Strains were local isolates isolated by enrichment technique and deposited in our microbial bank at Taif University, Saudi Arabia in our laboratory. The isolates were identified on the basis of their cultural and biochemical characteristics according to Bergey's Manual of Determinative Bacteriology (9^{th} edition) (Holt *et al.*, 1994) and Api kit profiles (ApiBioMerieuxsa, 2009). Phenotypic examination of the recovered microorganisms revealed that they belong to the genera of *Staphylococcus*, *Streptococcus, Klebsiella, Enterobacter, Escherichia, Micrococcus, Bacillus* and *Pseudomonas* (Table 3). All selected strains showed optimal growth at 30^oC. These findings were in agreement with a study by Sandford (1999).

Genetic identification and molecular characterization

Sequencing of 16S- rRNA gene as a PCR based technique was used to identify the selected bacterial isolates. According to the alignment at the National Center for Biotechnology Information (NCBI), the sequences of studied isolates in (Table 3) were identified as *S. pneumonia, S. pyogenes, K. pneumonia, S. aureus, S. epidermidis* (CNS), *B. subtilis, Micrococcus roseus, E. coli, P. aeruginosa, Enterobacter aerogenes* (DeSantis *et al.*, 2006).

Bacterial isolate and features

Of the total processed external ocular specimens, 70 (78.7%) were found culture positive (Table 1). The majority of population participated in this study had mixed infection except faculty members, personals and students were free of *Micrococcus* spp, *Enterobacter* spp and *P. aeruginosa*, respectively (Table 3). This makes the total number of bacterial isolates reached 275 strains. *S. aureus* 54 (19.6%) were the most predominant bacteria in mixed growth followed by coagulase negative-*Staphylococci* 45(16.4%) and *B. subtilis* 42(15.3%). The rate of isolation was higher among the workers 61(22.2%) and children 59(21.5%) than the other three populations. The overall isolation rate of *S. pneumoniae*, *S. pyogenes* and *K. pneumoniae* ranged from 20–22(7.3–8%). The rate of culture-positivity was found to be significantly higher among study subjects of workers, children and students with conjunctivitis.

No *Micrococcus roseus* from faculty members or students populations were recovered. *P. aeruginosa* was recovered from all populations except children and *Enterobacter* spp was recovered from all participants except faculty members (Table 3).The overall predominant isolated pathogen was *S. aureus* (54; 19.6%) followed by coagulase negative-*Staphylococci* (CNS) (45; 16.4%), *S. pneumoniae* (22; 8%) and *Klebsiella pneumoniae* (21; 7.6%).

The rate of isolation was higher among

workers (22.2%) and children (21.5%) followed by students (20.4%). The prevalence of ocular infection was not significantly associated with study subjects. However, statistically significant association was observed in the age (Dagnachew *et al.*, 2014; Modarrres *et al.*, 1998). The reason for increased susceptibility to infection in workers and children may be that they are at a greater risk after their maternal immunity has disappeared and before their own immunity system had matured (Jorgensen *et al.*, 2015; Ubani, 2009), while in workers it may be due to dry eye and weaning immunity. Moreover, similar to previous study in Iran prevalence of ocular infection has no significant association with age or sex (Anagaw *et al.*, 2011; Modarrres *et al.*, 1998).

Antibiotic resistance profile of bacterial isolate

In this study, antimicrobial susceptibility of isolated bacteria is: chloramphenicol (79.3%), ceftriaxone (74.9%), ciprofloxacin (73.5%), gentamycin (67.6%), erythromycin (65.1%), rifamycine (62.5%), tetracycline (62.2%), penicillin (57.1%), and ampicillin (52%) (Table 4).

Table.1 Categories and samples collected from Taif University campus. Distribution of investigated subjects and prevalence of bacterial growth patterns

Serial	Subjects	Total samples	growth	Growth rate (%)
1	Faculty member	21	14	66.7
2	Personal	20	15	75.0
3	Student	19	15	78.9
3	Workers	14	12	85.7
5	Children	15	14	93.1
	Total	89	70	78.7

Table.2 Quantification of bacterial growth found on external ocular infections

Subjects No. Bacteria	Faculty members	Personal	Students	workers	children
Total bacterial count (Plat count)	$1.3x10^5$ - $5.3x10^6$	$4X10^4$ - $4X10^8$	$1.4X10^5$ – $3X10^7$	$1.1x10^5$ – $1.2X10^8$	$5.1X10^5$ – $3.6X10^9$
Streptococcus spp	$0\text{-}1.7X10^5$	$0 – 4.1X10^6$	$0.5X10^2$ - $1X10^4$	$1x10^2$ – $1X10^4$	$1.3X10^2$ – $0.5X10^3$
Staphylococcus spp	$3.3x10^2$- $6.2X10^4$	$2.1X10^5$ - $1.3X10^7$	$3.5X10^4$ – $2.8 X 10^8$	$1.1x10^3$ – $6.3X10^8$	$4.2X10^5$ – $4X10^7$
Blood agar (Heterotrophic bacteria)	$3.3x10^3$- $4.4X10^6$	$2.4X10^5$– $2.1X10^8$	$2.1X10^3$ – $6.4X10^7$	$2.3x10^4$ – $3.7X10^8$	$5.9X10^6$ – $1.8X10^8$
MacConky (Faecal coliform)	$6.3x10^3$- $7.2X10^6$	$3.2X10^3$ – $4.2X10^8$	$1.2X10^3$ – $4.4X10^5$	$0 - 6.3X10^8$	$1.5X10^4$ – $1X10^7$
E. coli(EMB)	$0\text{-}3.3X10^8$	$1.3X10^3$ – $3.3X10^4$	$2.1X10^2$ – $3.3X 10^5$	$0- 1.3X 10^3$	$0 –1.4X10^4$

Table.3 Prevalence and composition of bacterial isolates (%) amongvisitors attending the University clinic at Taif University campus

Subjects / Isolates	Faculty members	Personal	Students	workers	children	Total
S. pneumoniae	5(22.7)	5(22.7)	4(18.2)	2(9.1)	6(27.3)	22(8)
S. pyogenes	2(10)	5(25)	3(15)	6(30)	4(20)	20(7.3)
K. pneumoniae	4(19)	5(23.8)	3(14.3)	4(19)	5(23.8)	21(7.6)
S. aureus	12(22.2)	9(16.7)	10(18.5)	10(18.5)	13(24.1)	54(19.6)
CNS*	10(18.5)	7(15.6)	11(24.4)	9(20)	8(17.8)	45(16.4)
B. subtilis	6(14.3)	5(11.9)	10(23.8)	8(19)	13(31)	42(15.3)
Micrococcus spp.	-	2(20)	-	5(50)	3(30)	10(3.6)
E. coli	3(13.6)	3(13.6	5(22.7)	7(31.8)	4(18.2)	22(8)
P. aeruginosa	4(17.4)	7(30.4)	6(26.1)	6(26.1)	-	23(8.4)
Enterobacter spp.	-	5(31.3)	4(25)	4(25)	3(18.8)	16(5.8)
Total	46(16.7)	53(19.3)	56(20.4)	61(22.2)	59(21.5)	275

*CNS= Coagulase- negative *Staphylococci*

Table.4 Antibiotics sensitivity pattern of bacterial isolates at Taif University Campus

Antibiotic* Isolates	Number of strains sensitive to antibiotics (%)									
	No.	Tet	Ref	Amp	Cef	Chl	Gen	Cip	Ery	Pen
G^+ bacteria										
S. pneumoniae	22	15 68.2**	16 72.7	17 77.3	22 100	20 90.9	7 31.8	20 90.9	21 95.5	15 68.2
S. pyogenes	20	14 70	15 75	19 95	20 100	18 90	10 50	18 90	18 90	20 100
S. aureus	54	20 37	19 35.2	18 33.3	40 74.1	40 74.1	39 72.2	40 74.1	35 64.8	10 18.5
CNS	45	25 55.6	26 57.8	31 68.9	35 77.7	34 75.6	25 55.6	30 66.7	40 88.9	45 100
B. subtilis	42	22 52.4	25 59.5	29 69.1	36 85.7	33 78.6	26 61.9	28 66.7	39 92.9	40 95.2
Micrococcus roseus	10	8 80	5 50	6 60	7 70	5 50	5 50	7 70	8 80	9 90
Total G^+	193	104 53.9	106 54.9	120 62.2	160 82.9	150 77.7	112 58	143 74.1	161 83.4	139 72
G^- bacteria										
E. coli	22	20 90.9	20 90.9	12 54.5	14 63.6	20 90.9	20 90.9	17 77.3	0	0
P. aeruginosa	23	14	13	0	3	15	23	23	nd	nd

		60.9	56.5		13	65.2	100	100		
Enterobacter aerogenes	16	14 87.5	15 93.8	8 50	10 62.5	14 87.5	13 81.3	nd	nd	nd
K. pneumoniae	21	19 90.5	18 85.7	3 14.3	19 90.5	19 90.5	18 85.7	19 90.5	18 85.7	18 85.7
Total G^-	82	67 81.7	66 80.5	23 28.1	46 56.1	68 82.9	74 90.2	59 72	18 22	18 22
Overall total	275	171 62.2	172 62.5	143 52	206 74.9	218 79.3	186 67.6	202 73.5	179 65.1	157 57.1

*Tetracycline (Tet), Rifamycine (Ref), Ampicillin (Amp), Ceftriaxone (Cef), Chloramaphenicol (Chl), Gentamycin (Gen), Ciprofloxacin (Cip), Erythromycin (Ery), Penicillin (Pen), Not done (nd). CNS= Coagulase-negative *Staphylococci* (*S. epidermidis*). ** = % sensitive strains

Rapid use of antibiotics for severe ocular infections is routine in ophthalmic practice resulting in increased drug resistance. In this study, among the commonly used topical antibiotics 20.7% of all strains were chloramphenicol resistant; 22.4% of all strains and 69.2% of *S. pneumoniae* were resistant to gentamicin. Moreover, 37.8% of all strains were tetracycline resistant. While, tetracycline was susceptible in 52.4–91% of all strains except *S. aureus* (37%), rifamycine 50–93.8% except *S. aureus* (35.2%), ampicilin 50–95% except *K. pneumoniae* (14.3%), ceftriaxone 62.5–100% except *P. aeruginosa* (13%), chloramaphenicol 65.2–90.9%except *Micrococcus roseus* (50%), gentamycin 50 - 100% except *S. pneumonia* (31.8%), ciprofloxacin 74.7–100% except CNS and *B. subtilis* (66.7%), and penicillin 85.7–100% except *S. aureus* (18.5%). This is in agreement with the study conducted by Caldwell *et al.* (1992), Joseph, (2009), Anagaw *et al.* (2011) and Tewelde *et al.* (2013). The reason for increased resistance for some antibiotics may be earlier exposure of the isolates to these drugs. Moreover, these drugs are very common due to low cost and often purchased without prescription in different areas (Jorgensen *et al.,* 2015).

In conclusion, *Staphylococcus aureus* was the overall predominant isolated pathogen followed by CNS. High rate of culture-positivity was observed among study subjects with children (93.1%) followed by workers (85.7%). Gram negative isolates were more susceptible to gentamycin and chloramaphenicol, whereas Gram positive isolates were more susceptible to erythromycin and ceftriaxone. Relatively, Erythromycin and/or gentamycin are effective against most isolated pathogens.

Reference

Anagaw, B., Fantahun, B., Berhanu, A., Yeshambel, B., Andargachew, M. 2011. Bacteriology of ocular infections and their antibiotic susceptibility pattern in Gondar University Hospital, North West, Ethiopia. *Ethiop. Med. J.,* 49(2): 117–23.

Api, 2009. BioMerieux sa 69280 MarcyI' Etoile, France. http://www. biomerieux.com.

Bauer, A.W., Kirby, W.M.M., Sherris, J.C., Turck, M. 1966. Antibiotic susceptibility testing by standard single disc method. *Am. J. Clin. Pathol.,* 45: 493–496.

Bharathi, M.J., Ramakrishnan, R., Shivakumar, C., Meenakshi, R., Lionalraj, D. 2010. Etiology and antibacterial susceptibility pattern of community- acquired bacterial

ocular infections in a tertiary eye care hospital in south India. *Indian J. Ophthalmol.,* 58: 497–507.

Caldwell, D.R., VerinHartwick-Young, R., Meyer, S.M., Drake, M.W. 1992. Efficacy and safety of lodoxamide 0.1% vs. cromolyn sodium 4% in patients with vernal keratoconjunctivitis. *Am. J. Ophthalmol.,* 113: 632–37.

Clinical and Laboratory Standards Institute (CLSI), 2007. Performance standards for antimicrobial susceptibility testing; seventeenth information supplement. Wayne Pennsylvania: Clinical and Laboratory Standards Institute, CLSI document M100-S17.

Dagnachew, M., Yitayih, W., Feleke, M., Tesfaye, N., Getachew, F. 2014. Types and drug susceptibility patterns of bacterial isolates from eye discharge samples at Gondar University Hospital, Northwest Ethiopia. *BMC Res. Notes,* 7: 292.

DeSantis, T.Z. Jr., Hugenholtz, P., Keller, K., Brodie, E.L., Larsen, N. 2006. NAST: A multiple sequence alignment server for comparative analysis of 16S -rRNA genes. *Nucleic Acids Res.,* 34: 394–399.

Holt, John, G., Krieg, Noel, R., Sneath, Peter, H.A., Staley, James, T., Willims, Stanley, T. (Eds.), 1994. Bergey's manual of determinative bacteriology, 9th edn. Williams and Wilkins, Baltimore.

Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S., Warnock, D.W. 2015. Manual of clinical microbiology, 11th edn, V(1), ASM Press, Washington, DC. USA.

Joseph, S.B. 2009. Impact of antibiotic resistance in the management of ocular infections: the role of current and future antibiotics. *Clin. Ophthalmol.,* 3: 507–521.

Koch, A.L. 1984. Growth measurement. In: Gerhardt, P., Murray, R.G.E., Costllow, R.N., Nester, E.W., Wood, W.A. (Eds.), Manual of methods for general bacteriology. American Soci.forMicrobiol. Washington, DC, USA. Pp. 179–208.

Kumara, M.B., Leona, V., Materanoa, A., Ilzinsa, O., Galindo-Castroa, I., Fuenmayora, S. 2006. Polycyclic aromatic hydrocarbon degradation by biosurfactant-producing *Pseudomonas* sp. IR1. Verlag der ZeitschriftfürNaturforschung, Tübingen, Pp. 203–012.

Leibowitz, H.M. 1991. Antibacterial effectiveness of ciprofloxacin 0.3% ophthalmic solution in the treatment of bacterial conjunctivitis. *Am. J. Ophthalmol.,* 112(Suppl): 29S–33S.

Leibowitz, H.M. 2005. Primary care, the red eye. *N. Eng. J. Med.,* 343(5): 345–351.

Leibowitz, H.M., Pratt, M.V., Flagstad, I.J., Berrospi, A.R., Kundsin, R. 1976. Humanconjunctivitis, II treatment. *Arch. Ophthalmol.,* 94: 1752–6.

Miller, I.M., Wittreich, J., Vogel, R., Cook, T.J. 1992. The safety and efficacy of topical norfloxacin compared with placebo in the treatment of acute, bacterial conjunctivitis. *Eur. J. Ophthalmol.,* 2: 58–66.

Modarrres, S.H., Lasheii, A., Nassiri, O.N. 1998. Bacterial etiologic agents of ocular infection in children in the Islamic Republic of Iran. *East Med. Health. J.,* 4(1): 44–49.

Nigatu, A. 2004. Pattern of microbial agents of external ocular infections in Federal Police Hospital and Minilik II Memorial Hospital. MSc thesis, AAU.

Nilsson, W.B., Paranjype, R.N., DePaola,

A., Strom, M.S. 2003. Sequence polymorphism of the 16 S rRNA gene of Vibrio vulnificus is a possible indicator of strain virulence. *J. Clin. Microbiol.,* 41: 442–446.

Rantakokko-Jalava, K., Nikkari, S., Jalava, J., Eerola, E., Skurnik, M., Meurman, O., Ruuskanen, O., Alanen, A., Kotilainen, E., Toivanen, P., Kotilainen, P. 2000. Direct amplification of rRNA genes in diagnosis of bacterial infections. *J. Clin. Microbiol.,* 38: 32.

Sacchi, C.T., Whitney, A.M., Mayer, L.W., Morey, R., Steigerwalt, A., Boras, A., Weyant, R.S., Popovic, T. 2002. Sequencing of 16S rRNA gene: a rapid tool for identification of Bacillus anthracis. *Emerg. Infect. Dis.,* 8: 1117.

Sandford, S.J. 1990. Eye diseases in hot climates, 2nd edn, Butterworths.

Tabbara, F.RAA. 1995. Infections of the eye, 2nd edn, The eye institute, Riyadh, Saudi Arabia. Pp. 3, 98, 556.

Tewelde, T., Getnet, B., Yeshigeta, G., Sisay, B., Muthupandian, S. 2013. Bacterial profile and antimicrobial susceptibility pattern of external ocular infections in Jimma University Specialized Hospital, Southwest Ethiopia. *Am. J. Infect. Dis. Microbiol.,* 1(1): 13–20.

Ubani, U.A. 2009. Bacteriology of external ocular infections in Aba, South Eastern Nigeria. *Clin. Exp. Optometry,* 92(6): 482–489

Wood, M. 1999. Conjunctivitis: Diagnosis and management. FRCS, PCEA Kikuyu Eye Unit, PO Box 45, Kikuyu, Kenya. *Community Eye Health,* 12(30): 19–20.