

# In vitro biofilm formation and antimicrobial susceptibility patterns of bacteria isolated from suspected external ocular infected patients attending Jimma University Medical Center eye clinic, southwest Ethiopia

**Kuma Diriba**

Dilla University

**Tesfaye Kassa** (✉ [ktes36@gmail.com](mailto:ktes36@gmail.com))

Jimma University College of Public Health and Medical Sciences <https://orcid.org/0000-0001-5259-2245>

**Yared Alemu**

Jimma University

**Sisay Bekele**

Jimma University

---

## Research article

**Keywords:** External ocular infection, Bacterial pathogen, Biofilm formation, Multidrug resistance, Pseudomonas aeruginosa, Jimma

**Posted Date:** April 3rd, 2019

**DOI:** <https://doi.org/10.21203/rs.2.1780/v1>

**License:**  This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

---

## Abstract

Background: Ocular disease with its complications is a major public health problem which significantly impacts on quality of life in developing countries. An ocular infection due to microbial agents, can lead to reduced vision and blindness. This study was aimed to assess the antimicrobial susceptibility pattern and biofilm forming potential of bacteria isolated from suspected external ocular infected patients attending Jimma University Medical Center (JUMC). Method: A cross sectional facility based study was conducted on 319 suspect patients with external ocular infections from March 2017 to June 2017 at JUMC in Southwest Ethiopia. External ocular specimens were collected and standard operating procedures were followed to handle and culture throughout the study period. Antimicrobial susceptibility pattern of the isolates was determined by disk diffusion method according to CLSI 2015. Microtiter (96 wells) plate method was used to screen biofilm formation by measuring optical density at 570nm. Result: Out of 319 study participants with external ocular infection, the prevalence of bacterial pathogens was 46.1%. The predominant bacterial isolates were Coagulase negative staphylococcus (CoNS) (27.7%) followed by Staphylococcus aureus (19.7%). Among Gram negatives, Pseudomonas aeruginosa (6.8%) was the leading isolate. Increased antimicrobial resistance was observed for tetracycline (64%), erythromycin (66.7%) and penicillin (77.1%). Amoxicillin-clavulanic acid, ciprofloxacin and gentamicin were the most effective drugs for both Gram negative and Gram positive ranging from about 70 to 100% with the later two drugs for external ocular infections. Methicillin resistant S. aureus (MRSA) accounted for 13.8% of S. aureus isolates. Multidrug resistance (MDR) accounted for 68.7%. The overall biofilm formation rate of bacterial ocular pathogens was 66.1%; with P. aeruginosa (40%), CoNS (34.1%) and S. aureus (31%) formed strong biofilm phenotype. Conclusion: The prevalence of bacterial isolates among external ocular infection was high. Almost all bacterial isolates were resistant to atleast one or more drugs. MDR pathogens were observed increasingly among biofilm formers or vice versa. Therefore, antimicrobial susceptibility testing should be practiced to guide treatment of external ocular cases and to control the emergence of drug resistant bacteria.

## Introduction

The human eye, which is constantly exposed to the external environment, is a unique organ considered to be the window of our body. Ocular disease with its complications, which happen due to microorganisms, is a significant health problem worldwide; particularly it further impacts quality of life in least income countries. Ocular infection can damage the structure of the eye which can lead to reduced vision or even blindness if it is inappropriately diagnosed and treated. The most frequently affected parts of the eye are the conjunctiva, eyelid and cornea as a result of pathogenic micro-organisms (1). Conjunctivitis, blepharitis, and dacryocystitis are considered the most common manifestations of external ocular infections (2). The pathogenic micro-organisms in external ocular infections include bacteria, fungi, viruses and parasites (3).

Pathogenic bacteria are the major causative agents of ocular surface infections with possible loss of vision (4). Control of eye infections may involve the use of broad spectrum antimicrobial agents. Nevertheless the emerging and increasing antimicrobial resistance is a problem worldwide (5). In this regard, inappropriate and irrational use of antimicrobial medicines provide favorable conditions for resistant microbes to emerge, spread and persist (6). The development of bacterial biofilms is presently recognized as one of the most relevant drivers of persistent infections. It constitutes a serious challenge for clinical microbiologists and physicians being 100 to 1000 fold more resistant to antimicrobial agents than their counter parts in planktonic forms (7). Phenotypic and physiological changes in biofilm platform restrict penetration of antibiotics into biofilm forming bacteria. This expression of biofilm provides a higher resistance to antimicrobial treatments (8).

In most parts of Ethiopia, getting antibiotics without prescription order is easy, and this can lead to underuse, overuse or misuse of antibiotics (9). These conditions contribute to the emergence and spread of antimicrobial resistant strains. Moreover, poor sanitary and infection control practice in the anatomic area (face) may play a serious part in an increased prevalence of resistant bacteria. This rising antimicrobial resistance increases the risk of treatment failure with potentially serious consequences (10,11).

Even though a study on ocular infection was conducted in 2012 in Jimma area (4), the bacterial profile and antimicrobial susceptibility pattern can vary over time and place as indicated in different studies (3,12). Therefore, the changing spectrum of microorganisms involved in external ocular infections and the emergence of acquired microbial resistance to antibiotics needs continuous surveillance to guide empirical therapy. As a result of this, updated knowledge of bacterial aetiologic agents in ocular infections and their antibiogram are crucial. On the other hand, bacterial biofilm development was not addressed in bacterial isolates from external ocular infections in Jimma area. Hence, the present study was intended to update profile of bacteria present in external ocular infection. Moreover, this study aimed to assess the antimicrobial susceptibility pattern along with biofilm forming potential of the isolates at Jimma University Medical Center (JUMC) eye clinic, Southwest Ethiopia.

## Materials And Methods

### Study Design and Population

A cross-sectional health facility based study was conducted on a total of 319 patients at JUMC, Department of Ophthalmology in Ethiopia from March to June 2017. All patients with external ocular infection that fulfill the eligibility criteria during the study period were recruited prospectively by ophthalmic nurse and confirmed based on clinical examination by ophthalmologists. Patients examined and diagnosed with slit lamp biomicroscope and had an external ocular infection with red eye, discharge, mucoid or mucopurulent secretion, thickening of the conjunctiva, in one or both eyes, and agreed to participate were included in this study. Patients on antibiotics within the last 5 days prior to sample collection date were excluded from the study since bacteria is less frequently detected in culture based tests collected after antibiotic use (13).

### Data Collection Procedures and Process

### Socio Demographic and Clinical Characteristics

Socio-demographic data (age, sex, monthly income, educational level, occupation and address), clinical data (history of repeated infections, duration of stay in hospital, use of contact lenses, surgery, previous antibacterial therapy, systemic diseases and use of traditional medicine) and others like source of light and use of fire wood at home were collected from each study participants by using structured questionnaire. Ophthalmologists diagnosed patients with clinical picture of external ocular infections.

## Specimen Collection, Handling and Transport

All consecutive patients examined clinically with the slit-lamp bio-microscope were set apart for suspected bacterial infection. Specimens from external ocular structures were collected from consented patients. Briefly, patients were requested to look up while lowering the eye lid down, and samples were collected from one or both eyes based on the nature of the infection. The discharge from the surface of the eye was collected gently using sterile cotton swab that had been pre-moistened with sterile physiological saline. The swab was rubbed over the lower conjunctival sac from medial to lateral side and back again. Purulent material was collected in the cases of dacryocystitis by everted puncta then applying pressure over the lacrimal sac area from the infected eye (12,14). In the cases of ulcerative blepharitis, lashes deposit, tear film foaming content, and corneal punctate erosions were swabbed. From each patient, two swabs were collected: one for Gram staining immediately after collection and the other one for bacterial culture. The collected swab were inserted into Amies transport media, placed in a cold box and transported to Jimma University Medical Microbiology Laboratory. Standard operating procedures were followed to handle specimens collected from patients throughout the study period.

## Isolation and Identification of Bacterial Pathogens

All specimens were streaked onto MacConkey agar, Mannitol salt agar, Blood agar and Chocolate agar plates (all media used from Oxoid, Hampshire, UK). The plates were incubated aerobically at 37°C for 24 to 48 hours. The Chocolate agar was kept at 37°C for 24 to 48 hours in a 5 to 10% CO<sub>2</sub> atmosphere. All plates were initially examined after 24 hours and cultures with no growth were further incubated for another 24 hours. Bacteria were identified on the basis of phenotypic and a series of biochemical tests. For *Hemophilus* spp., satellitism test was also performed (11).

### Bacterial Biofilm Tests

Bacteria isolated from fresh agar plates were inoculated into tube filled with sterile trypton soya broth (TSB) with 1% glucose and incubated at 37°C for 24 hours. This culture was diluted 1:100 into fresh media. Then, 200 µL of the suspension was added into sterile 96 wells flat-bottom microtiter plate and incubated at 37°C for 48 hours. The bacterial suspension of each well was gently removed and washed three times with phosphate buffer saline (pH 7.2). Plates were fixed with methanol (99%), and then stained with 220 µL of crystal violet (CV, 0.1% w/v) at room temperature for 15min. Each well was washed three times with PBS to remove unbound CV dye. After drying, 220 µL of ethanol (95%) was added to each well. Finally, the solubilized CV was transferred to new microtiter plate. The optical density (OD) of the biofilm was determined by a microplate reader (HumaReader HS, German) at a wavelength of 570 nm. The experiment was performed in triplicate separately for each strain and the average values was calculated (15).

The cutoff optical density (OD<sub>c</sub>) was proof of the biofilm formation and was defined as the sum of the arithmetic mean of negative controls and a triple value of its standard deviation (OD<sub>c</sub> =  $\bar{x} + 3\sigma$ ). The liquid medium without bacterial suspension incubated in microtiter plate was used as negative control. Biofilm formations of the isolates were classified into four categories as stated in previous study (16): non-adherent (OD < OD<sub>c</sub>), weakly-adherent (OD<sub>c</sub> < OD < 2xOD<sub>c</sub>), moderately-adherent (2xOD<sub>c</sub> < OD < 4xOD<sub>c</sub>), and strongly-adherent (4xOD<sub>c</sub> < OD).

### Antimicrobial susceptibility testing

It was carried out using disk diffusion method as recommended by Clinical and Laboratory Standard Institute (CLSI) guideline (17). From a pure culture, three to five colonies of the test organisms were emulsified in 3 ml of sterile normal saline and the suspension was adjusted to a 0.5 McFarland standards. It was swabbed uniformly onto Muller Hinton agar. Fifteen impregnated antibiotic disks were used in the following concentrations: Amikacin (30 µg), Ampicillin (10µg), Amoxicillin-clavulanic acid (20µg), Cefoxitin (30µg), Ceftazidime (30µg), Ceftriaxone (30µg), Chloramphenicol (30µg), Ciprofloxacin (5µg), Clindamycin (2µg), Erythromycin (15µg), Gentamicin (10µg), Penicillin-G (10IU), Tetracycline (30µg), Trimethoprim-Sulphamethoxazole (1.25/23.75µg) and Tobramycin (10 µg) (all from Oxoid, Hampshire, UK). These drugs were placed and incubated at 37°C for 18-24 hours. The zone of inhibition was measured and interpreted accordingly. Methicillin resistant isolates were determined using cefoxitin disk (30µg) by incubating at 34±1°C as recommended by CLSI (17).

## Data quality assurance

All ophthalmic specimens were collected following standard operating procedure by ophthalmologists. Cross-checking and data cleaning were done on daily basis. All laboratory and clinical data were recorded during the study period as back up. The sterility of culture media was ensured by incubating five percent of each batch of the prepared media at 37°C for 24 hours. For better results, any physical change like cracks, excess moisture or dehydration, color change, hemolysis, contamination, deterioration and expiration dates were checked before using culture media. The quality and performance of culture media, biochemical tests and antimicrobial susceptibility discs were checked using *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923), *P. aeruginosa* (ATCC 27853) and *S. pneumoniae* (ATCC 49619), all obtained from Ethiopian Public Health Institute.

## Data processing and analysis

Data entry, analysis and cleaning were done using Epi-Data 3.1 and SPSS version 21.0 software. Frequency count and percentage were used to present the finding. Prevalence figures were calculated for the total study population and separately by clinical feature of the disease. Bivariate and multivariate logistic regression was used to assess associated variable with bacterial prevalence. P-values less than 0.05 were considered statistically significant.

## Results

### Socio-demographic and clinical feature of study participants

A total of 319 study participants clinically diagnosed with external ocular infection were included in this study; out of which 172(53.9%) were males and the remaining 147(46.1%) were females. The age of the study participants ranged from 1 month to 95 years of age with median of 21 years old. The majority of study subjects were children below the age of two years which accounts for 103(32.3%) followed by ages of above 45 years groups accounting for 74(23.2%). Bivariate analysis (COR) did not show significant association between sociodemographic characteristics and bacterial isolation pattern. Small proportion of study participants had additional chronic disease other than ocular infection like hypertension 18(5.6%), diabetes 17(5.3%) and rheumatoid arthritis 11(3.4%). From the total cases, 21(6.6%) study participants were previously hospitalized for eye infection, and 31(9.7%) had used topical medicine for eye treatment. Five (1.6%) study participants had eye surgery and 23(7.2%) cases had used traditional eye remedy. Only 5(1.6%) study subjects had contact eye lenses. In the multivariate analysis, patients with diabetes (AOR= 0.09, 95% CI: 0.02-0.43, P= 0.002) and previous history of hospitalization (AOR= 0.10, 95% CI: 0.03-0.42, P = 0.001) were significantly associated with occurrence of external ocular bacterial infection.

From all external ocular infected patients, 165(51.7%) of them were with conjunctivitis, 74(23.2%) with blepharoconjunctivitis, 52(16.3%) with blepharitis, 13(4.1%) with dacryocystitis, and 15(4.7%) with other types of external ocular infections. The most dominant external ocular infection among different age groups was conjunctivitis with significant pediatric age group cases.

### Prevalence of Bacterial Isolate

Out of 319 ocular specimens processed for culture, bacteria were isolated from 147 of them giving an overall prevalence of 46.1%. No mixed bacterial isolate was found in this study. Among the bacterial isolates, 96(65.3%) of them were Gram positives whereas the remaining 51(34.7%) were Gram negatives. From Gram positives, CoNS were the most frequent bacterial isolates accounting for 41(27.9%), followed by *S. aureus* and *S. pneumoniae* with 29(19.7%) and 13(8.8%), respectively. From Gram negatives, *P. aeruginosa* was the predominant bacterial isolate accounting for 10(6.8%), and followed by *K. pneumoniae* with 9(6.1%). The spectrum of bacterial isolate varies with age of patients. Most of the bacterial isolates were recovered from cases that were between the age group of one month and two years old (Table 1).

**Table 1 place here.**

Most of the bacterial isolates were recovered from 75(51.0%) conjunctivitis cases followed by 32(21.8%) blepharitis and 27(18.4%) blepharoconjunctivitis. The least bacterial isolates were found in dacryocystitis cases 8(5.4%). The predominant bacterial isolates among conjunctivitis cases were CoNS which accounted for 16(21.3%) followed by 15(20%) *S. aureus*. In blepharitis, the leading bacterial etiologies were similar with the pattern in conjunctivitis cases with 10(31.2%) CoNS and 8(25%) *S. aureus*. In blepharoconjunctivitis, 10(37%) CoNS followed by 3(11.1%) *H. influenzae* whereas in dacryocystitis, 3(37.5%) CoNS followed by 2(25%) *S. pneumoniae* and 2(25%) *S. aureus* were identified. Among Gram negatives, *P. aeruginosa* and *K. pneumoniae* were the predominant isolates with 5(6.7%) and 4(5.3%) in conjunctivitis cases, respectively (Table 2).

**Table 2 place here.**

### Antimicrobial susceptibility pattern of bacterial isolates

In Gram positive bacteria, twelve antibiotics belonging to nine categories were used to assess susceptibility. *S. aureus* showed high susceptibility to clindamycin 24(82.8%) followed by ciprofloxacin, Amoxicillin-clavulanic acid and Gentamicin each accounting for 26(89.7%), 22(75.9) and 21(72.4%), respectively. On the other hand, this bacterium was highly resistant to penicillin 25(86.2%), erythromycin 24(82.8%) and tetracycline 22(75.9%). CoNS showed almost comparable susceptibilities for the above antimicrobials as that of *S. aureus*. Among *S. aureus* isolates, 4(13.8%) of them were MRSA phenotype. From CoNS isolated in this study, 12(29.3%) of them were also methicillin resistant. *S. pneumoniae* showed complete susceptibility to Amoxicillin-clavulanic acid 13(100%) and superior susceptibility to ciprofloxacin 11(84.6%), however, it was more resistant to penicillin and trimethoprim-sulphamethoxazole each equally accounts for 9(69.2%). Other Gram positive bacterial isolates were highly susceptible to Amoxicillin-clavulanic acid (100%), clindamycin (93.3%) and gentamicin (86.7%) while they were less susceptible to trimethoprim-sulphamethoxazole (31.1%), tetracycline (37.8%) and ampicillin (48.9%) (Table 3).

Among Gram negative bacterial isolate, *P. aeruginosa* showed fairly good susceptibility to ciprofloxacin, 9(90%). Moreover, ceftriaxone and amoxicillin-clavulanic acid were effective in each accounting for 8(80%), and gentamicin and amikacin each accounting for 7(70%). On the other hand, *P. aeruginosa* isolates showed high resistance to ceftazidime 9(90%), tetracycline 8(80%), trimethoprim-sulphamethoxazole and tobramycin each accounting for 7(70%). *K. pneumoniae* showed high susceptibility to ceftriaxone and ciprofloxacin each accounts for 9(100%) whereas, amoxicillin-clavulanic acid, chloramphenicol and amikacin each accounting for 8(88.9%). However, this bacterium was less susceptible to tobramycin, trimethoprim-sulphamethoxazole and tetracycline. Other susceptible Gram negative bacteria isolates from external ocular infected patients was observed against different antibiotics that include: ceftriaxone, 31(96.9%); ciprofloxacin, 32(100%); Amoxicillin-clavulanic acid, 28(93.3%); gentamicin, 25(83.3%); and amikacin, 19(76%). On the other hand, they were less susceptible to tobramycin, trimethoprim-sulphamethoxazole and tetracycline (Table 4).

Table 3 and Table 4. Place here.

Among the total isolates (n=147) from cases of external ocular infections, MDR was recorded in 101 (68.7%) bacterial isolates. From Gram positive isolates, 75(78.1%) out of 96 were MDR whereas from 51 Gram negative isolates, only 26(51.0%) were MDR. Among Gram positive organisms, a high level of multi-drug resistance was found in 25(86.2%) *S. aureus* followed by 34(82.9%) CoNS. Among Gram negatives, 10(100%) *P. aeruginosa* strain showed increased level of MDR followed by 6(66.7%) *K. pneumoniae* and 3(60.0%) *E. coli* (Table 5).

Table 5, place here.

## Biofilm formation profile

From 127 bacterial isolates screened for biofilm formation, 84(66.1%) of them were positive. The intensity of bacterial biofilm production was categorized into four groups i.e. 31(24.4%) of them as strong former, 39(30.7%) as moderate, 14(11.0%) as weak, and 43(33.9%) as non-biofilm former. Among 83 Gram positive isolates, 14(34.1%) CoNS followed by 9(31.0%) *S. aureus* were strong biofilm formers. Generally, about 76% and 72% of each of CoNS and *S. aureus* isolates were biofilm former (strong, moderate or weak forms), respectively. On the otherhand, from the Gram negative isolates, 4(40.0%) *P. aeruginosa* were the most strong biofilm former followed by 2(22.2%) *K. pneumoniae* (Table 6).

## Correlation of antimicrobial resistance and biofilm formation

From the total bacterial isolates, higher biofilm formers were observed among multidrug resistant bacteria. Non-biofilm formers predominated among those bacterial isolates with resistance to one or two antimicrobial agents. But in bacterial isolates where there was MDR features, higher numbers of biofilm formers were the principal ones. Significant rate of strong biofilm formers (48.4%) were seen in bacterial isolates that were resistant to five or more antimicrobial agents. In this study, Chi-square analysis revealed a significant relationship between multidrug resistant and biofilm former bacterial isolates,  $p < 0.05$ , despite that the correlation coefficient (0.491) did not show strength (Figure 1).

Figure 1, place here.

## Discussion

In this study, a total of 319 patients suffering from external ocular infections were included in four months period in 2017. The overall prevalence of bacterial external ocular infections rate was 46.1%. This result is comparable with previous study conducted in Southern Ethiopia (18). However, the result is lower than the prevalence reported from elsewhere ranging between 74% and 88% (4,19,20). But it is higher than the study conducted in Bangalore (11). The different rate of bacterial isolation from one place to another or within the same place might be due to different factors including geographic variation, sample collection and transportation method, culture media used, differences in study period, and varied cases in inclusion criteria.

Conjunctivitis was the predominant external ocular infection accounting for 51.7% followed by blepharoconjunctivitis (23.2%), blepharitis (16.3%), dacryocystitis (4.1%), and other eye infection accounting for the remaining 4.7%. This is similar with previous studies conducted in Ethiopia (18) and India (19) where conjunctivitis was the leading causes of external ocular infections. In contrast to this study; however, one finding in southwest Ethiopia (4) reported blepharoconjunctivitis as the predominant types of external ocular infection. The differences within the study might be due to differences in study period, and smaller sample size.

In this study, the most common isolates observed were Gram positive cocci (65.3%). The finding is indicative of Gram positive cocci as primary cause of external ocular infections in Jimma area and it is comparable with other previous studies (9,21,22). From the Gram positives, CoNS (27.9%) was the most predominant isolates followed by *S. aureus* (19.7%) and *S. pneumoniae* (8.8%). Similar studies conducted in Ethiopia (23,24) and India (25) showed similar pattern of isolation. The increased predominance of CoNS and *S. aureus* in external ocular infections indicate that these are responsible for a variety of anterior and posterior segment of eye infections emerging probably from the surface of the skin. Over the past 15 years, there has been an increase in the documentations of ocular infections due to CoNS (26). These bacteria can be important nosocomial pathogen and the cause of health-care related infections extending to the inner surfaces of the eye, partly as the result of the increasing use of medical devices (27). As a result, CoNS may become the most common cause of postoperative eye infection in recent years (28-31) and thoughtfulness about this bacterium may be required at the eye health settings.

The prevalence of Gram negative bacterial isolate among patients suffering from external ocular infection was 34.7%, with *P. aeruginosa* (6.8%) as the leading Gram negative agent followed by *K. pneumoniae* (6.1%) in this study. Similar studies in different geographic locations (4,32-34) also reported that *P. aeruginosa* is the most frequent isolate. Transient contamination of patient hand may be the source of infection of external eye structures.

Among the clinical features of external ocular infections, the predominant bacterial isolates identified among cases of bacterial conjunctivitis and blepharitis were CoNS followed by *S. aureus*. In the case of dacryocystitis, in addition to CoNS and *S. aureus*, *S. pneumoniae* was also the predominant strains recovered. This is in agreement with several studies conducted in Ethiopia and elsewhere (18,20,21,22,24,35-40). Hence, most ocular cases may be managed by considering these members of bacterial etiologies.

This study showed high rate of antimicrobial resistance to different agents in both Gram positive and Gram negative bacteria, which is consistent with findings from Ethiopia (4,23) and Uganda (36). In this *in vitro* study, high frequency of resistance to ampicillin, penicillin, erythromycin, trimethoprim sulphamethoxazole, tobramycin and tetracycline has been observed. From these antimicrobial categories, medicinal preparations usable in ophthalmic purposes, including tetracycline and tobramycin, needs particular concern at the study location. The observed resistance of the bacteria might be due to

accessibility of the antimicrobial drugs over the counter in Ethiopia. In addition, indiscriminate use of antimicrobial drugs and empirical treatment by the health professionals without susceptibility testing results for severe external ocular infections, and shortage of microbiological services for culture and susceptibility testing or unavailability of updated guideline regarding the selection of drugs are some of the factors which can lead to the development of the increased resistance rate.

In this study, the overall resistance rate of isolated *Staphylococcus* spp. to commonly prescribed antibiotics such as to ampicillin, tetracycline, erythromycin and penicillin was between 73% and 85%. Among these antimicrobials, tetracycline preparation is available in external ophthalmic treatments which may warrant prudent use of it as most *S. aureus* and CoNS were resistant. Consistent results were reported from different studies elsewhere (4,9,18,39). *P. aeruginosa*, on the otherhand, was highly resistant to ceftazidime (90%) and tetracycline (80%). This is consistent with a study done in Ethiopia (4) where *P. aeruginosa* was highly resistant to tetracycline. Other Gram negative isolates were highly resistant to tetracycline and tobramycin in the range between 40% and 80%. Epidemiological factors, study period and geographic location may be among the factors contributing to highly variable resistance rates. The necessity of bacterial culture and susceptibility for suspected cases of external ocular infected patients may be compulsory to select the most effective ophthalmic antimicrobial preparations.

All Gram positive bacterial isolates were susceptible to amoxicillin-clavulanic acid, ciprofloxacin and gentamicin within the susceptibility range of 69%-100%. This is comparable with previous studies done in Ethiopia (18,23,40) and India (41). On the otherhand, most Gram negative bacteria from our study were susceptible to Amoxicillin-clavulanic acid, ciprofloxacin, ceftriaxone and gentamicin within the range of 66%-100%. This is in agreement with previous studies done in different locations (18,23).

From all *S. aureus* strain isolated, 13.8% of them were MRSA strains. The finding is comparable with other study reported in India (37). However, in this study significant proportion of CoNS isolates were also MRSA. The most frequent ocular infections resistant to methicillin were found among conjunctivitis cases. In this study, those MRSA isolates were highly susceptible to chloramphenicol (75%), but resistant to clindamycin, tetracycline and gentamicin. Other similar studies reported chloramphenicol as clinically effective in MRSA (>81%) isolates from conjunctivitis cases and highly resistant to clindamycin, tetracycline and gentamicin (42,43).

About two third of bacterial isolates in this study showed ability to produce biofilm ranged from weak to strong adherence abilities. This feature can contribute to antimicrobial drug resistance development and play a vital role in pathologic processes over a long period by withstanding the effect of immune defence mechanisms. Bacterial biofilm formation feature is little noticed and studied in Ethiopia. Biofilm formation rate found in this study is comparable with study conducted in Chicago (44). However, comparatively lower biofilm formation rate was reported in Saudi Arabia (45). *P. aeruginosa* (80%), *K. pneumoniae* (77.8%), CoNS (75.6%), *S. aureus* (72.4%) and *E. coli* (60%) were among the leading biofilm former. This biofilm producing features may be responsible for many recalcitrant or refractory infections due to Gram negative bacilli and Gram positive cocci, and are notoriously difficult to eradicate. It is a well-known pathogenic mechanism in most bacterial strains as they exhibit resistance to antibiotics by various means like restricting penetration of antibiotic into biofilms, decreasing growth rate and expressing resistance genes. It is noted that biofilm formation allows microorganisms to survive and thrive in hostile environment, to disperse so that forming new niches and to give them significant advantages in protection against environmental fluctuations. Bacteria in biofilms display increased cell-to-cell communication while becoming less sensitive to chemical and physical stresses, and this may further complicate patient treatment outcome (46, 47).

Multiple drug resistance (MDR) to three or more antimicrobial agents were observed in 101(68.7%) of the tested isolates. This is a significant proportion that can influence patient cure from external ocular infection. Comparable findings is reported in studies conducted from southern and northern Ethiopia (18,24). It has been suggested that indiscriminate and prolonged use of a wide range of antibiotics, and lack of personal hygiene might be a major factor leading to emergence of multidrug resistant strains.

In the current study, strains capable of forming biofilms were more frequently observed to be MDR phenotype. Simultaneous occurrence of most MDR and biofilm forming strains show that biofilm phenotype may play a great role in antimicrobial resistance, despite the correlation was not appreciable in this study. Other studies (48,49) reported biofilm formation is higher in MDR bacteria. Phenotypic changes in the bacterial shape, physiological changes within cells, low diffusion of antibiotics across the biofilm matrix, elevated expression of efflux and quorum-sensing may be some of the reasons for this high MDR property. Most of the Gram positives and Gram negative bacteria isolated in this examination that are biofilm positives were found to be resistant to aminoglycosides, penicillins, fluoroquinolones, folate pathway inhibitors, chloramphenicol and tetracycline. Similar previous studies have also shown biofilm formation is higher in those described antimicrobial categories (50-52). In this study, as biofilm forming features of bacteria were assessed in *in vitro* set up, the result might be different from the real biofilm formed on external ocular site infection of the patients. Moreover, the absence of routinely performed and standardized antibiofilm susceptibility protocols worldwide may undermine the influence of bacterial biofilm in patient morbidity. Anaerobic bacterial culture and *Chlamydia trachomatis* test were not included in this research due to resource constraint. In addition, anti-biofilm drug susceptibility test was not done for biofilm former bacterial isolates due to lack of antimicrobial constituents for agar dilution method.

## Conclusion

Both Gram positive and Gram negative bacteria were responsible for external ocular infections with the most predominant isolates: CoNS followed by *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. Increased resistance rate was observed to ampicillin, penicillin, erythromycin, trimethoprim-sulphamethoxazole, tobramycin and tetracycline. Ciprofloxacin and gentamicin were the most effective agents against a good number of isolates from external ocular infections. MDR bacterial isolate was prevalent and methicillin resistance was detected in about a fifth of staphylococci isolates. *P. aeruginosa* (80%) was the leading biofilm former followed by *Klebsiella pneumoniae* (77.8%) and CoNS (75.6%). Almost all biofilm formers were MDR.

## Declarations

**Ethics approval and consent to participate:** Ethical clearance was approved and obtained from Institutional Research and Ethical Review Committee Board under the Institute of Health, Jimma University. Permission was also obtained from Jimma University Medical Center clinical director and ophthalmology department. Written informed consent and assent was obtained from the study subjects immediately before data collection.

**Consent for publication:** It was obtained from each participant or guardian as incorporated in the 'participant information sheet' before inclusion in this study.

**Availability of data and material:** The data sets used and/or analysed during this study are available from the corresponding author on reasonable request.

**Competing interests:** The authors declare that they have no competing interests.

**Funding:** Financial support was obtained from School of graduate studies, Jimma University, Ethiopia.

**Authors' contributions:** KD: Designed the study, collected data, performed the culture and experiments, analyzed the data and prepared draft write up of the manuscript. TK: Participated in conception and design of the study, controlled all the laboratory experiments, data analysis and interpretations in addition to preparation of the drafted write up of the manuscript. YA: participated in data analysis and interpretation. SB: Participated in patient segregation, data collection, data analysis and interpretations of the findings. All authors reviewed and approved the final manuscript.

**Acknowledgements:** The authors would like to acknowledge Jimma University for providing laboratory space and facilities to conduct this research work. We gratefully acknowledge Jimma University Medical Center Ophthalmology Department staff and all study participants for their cooperation during data collection.

## References

1. Ramesh S, Ramakrishnan R, Bharathi MJ, Amuthan M, Viswanathan S. Prevalence of bacterial pathogens causing ocular infections in South India. *Indian J Pathol Microbiol.* 2010;52:281-6.
2. Mariotti SP, Pascolini D, Rose-Nussbaumer J. Trachoma: global magnitude of a preventable cause of blindness. *Br J Ophthalmol.* 2009;93:563-8.
3. Bertino Jr JS. Impact of antibiotic resistance in the management of ocular infections: the role of current and future antibiotics. *Clin Ophthalmol.* 2009;3:507-21.
4. Tesfaye T, Beyene G, Gelaw Y, Bekele S, Saravanan M. Bacterial profile and antimicrobial susceptibility pattern of external ocular infections in Jimma University Specialized Hospital, Southwest Ethiopia. *Am J Infect Dis Microbiol.* 2013;1:13-20.
5. McDonald M, Blondeau JM. Emerging antibiotic resistance in ocular infections and the role of fluoroquinolones. *J Cataract Refract Surg.* 2010;36:1588-98.
6. Sharma S. Antibiotics and Resistance in Ocular Infections—Indian Perspective. *Indian J Med Microbiol.* 2011;29:218-22.
7. Alhede M, Kragh KN, Qvortrup K, Allesen-Holm M, van Gennip M, Christensen LD, et al. Phenotypes of non-attached *Pseudomonas aeruginosa* aggregates resemble surface attached biofilm. *PLoS One.* 2011;6:e27943.
8. Zegans ME, Becker HI, Budzik J, O'Toole G. The role of bacterial biofilms in ocular infections. *DNA Cell Biol.* 2002;21:415-20.
9. Anagaw B, Biadlegne F, Belyhun Y, Mulu A. Bacteriology of ocular infections and antibiotic susceptibility pattern in Gondar University Hospital, north west Ethiopia. *Ethiop Med J.* 2011;49:117-23.
10. Lee K-M, Lee H-S, Kim M-S. Two cases of corneal ulcer due to methicillin-resistant *Staphylococcus aureus* in high risk groups. *Korean J Ophthalmol.* 2010;24:240-4.
11. Hemavathi, Sarmah P, Shenoy P. Profile of microbial isolates in ophthalmic infections and antibiotic susceptibility of the bacterial isolates: a study in an eye care hospital, Bangalore. *J Clin Diagn Res.* 2014;8:23-5.
12. Baron EJ, Miller JM, Weinstein MP, Richter SS, Gilligan PH, Thomson RB, et al. A guide to utilization of the microbiology laboratory for diagnosis of infectious diseases: 2013 recommendations by the Infectious Diseases Society of America (IDSA) and the American Society for Microbiology (ASM). *Clin Infect Dis.* 2013;57:e22-e121.
13. Harris AM, Bramley AM, Jain S, Arnold SR, Ampofo K, Self WH, et al. Influence of Antibiotics on the Detection of Bacteria by Culture-Based and Culture-Independent Diagnostic Tests in Patients Hospitalized With Community-Acquired Pneumonia. *Open Forum Infect Dis.* 2017;4:ofx014. doi:10.1093/ofid/ofx014
14. Sharma S. Diagnosis of infectious diseases of the eye. *Eye (Lond).* 2012;26:177-84.
15. Christensen GD, Simpson W, Younger J, Baddour L, Barrett F, Melton D, et al. Adherence of coagulase-negative staphylococci to plastic tissue culture plates: a quantitative model for the adherence of staphylococci to medical devices. *J Clin Microbiol.* 1985;22:996-1006.
16. Stepanović S, Vuković D, Dakić I, Savić B, Švabić-Vlahović M. A modified microtiter-plate test for quantification of staphylococcal biofilm formation. *J Microbiol Methods.* 2000;40:175-9.

17. Wkler MA, Cockerill FR, Bush K, Dudely M, Etiopoule GM, Hardy DJ, et al. Clinical and laboratory standard institute. Performance standards for antimicrobial disc susceptibility tests; Approved standard. 10th edition, 2015
18. Amsalu A, Abebe T, Mihret A, Delelegne D, Tadesse E. Potential bacterial pathogens of external ocular infections and their antibiotic susceptibility pattern at Hawassa University Teaching and Referral Hospital, Southern Ethiopia. *Afr J Microbiol Res.* 2015;9:1012-19.
19. Bharathi MJ, Ramakrishnan R, Shivakumar C, Meenakshi R, Lionalraj D. Etiology and antibacterial susceptibility pattern of community-acquired bacterial ocular infections in a tertiary eye care hospital in south India. *Indian J Ophthalmol.* 2010;58:497-507.
20. Okesola A, Salako A. Microbiological profile of bacterial conjunctivitis in Ibadan, Nigeria. *Ann Ib Postgrad Med.* 2010;8:20-4.
21. Ubani U. Common bacterial isolates from infected eyes. *Journal of the Nigerian Optometric Association.* 2009;15:40-7.
22. Sherwal BL, Verma AK. Epidemiology of Ocular Infection Due to Bacteria and Fungus—A Prospective Study. *JK Science; J Med Education Res.* 10. 2008.
23. Shiferaw B, Gelaw B, Assefa A, Assefa Y, Addis Z. Bacterial isolates and their antimicrobial susceptibility pattern among patients with external ocular infections at Borumeda hospital, Northeast Ethiopia. *BMC Ophthalmol.* 2015;15:103. doi: 10.1186/s12886-015-0078-z.
24. Assefa Y, Moges F, Endris M, Zereay B, Amare B, Bekele D, et al. Bacteriological profile and drug susceptibility patterns in dacryocystitis patients attending Gondar University Teaching Hospital, Northwest Ethiopia. *BMC Ophthalmol.* 2015;15:34. doi: 10.1186/s12886-015-0016-0.
25. Perkins R, Kundsinn R, Pratt M, Abrahamsen I, Leibowitz H. Bacteriology of normal and infected conjunctiva. *J Clin Microbiol.* 1975;1:147-9.
26. Giraldez MJ, Resua CG, Lira M, Oliveira ME, Magariños B, Toranzo AE, et al. Contact lens hydrophobicity and roughness effects on bacterial adhesion. *Optom Vis Sci.* 2010;87:E426-31.
27. Piette A, Verschraegen G. Role of coagulase-negative staphylococci in human disease. *Vet Microbiol.* 2009;134:45-54.
28. Benz MS, Scott IU, Flynn HW, Unonius N, Miller D. Endophthalmitis isolates and antibiotic sensitivities: a 6-year review of culture-proven cases. *Am J Ophthalmol.* 2004;137:38-42.
29. Wong TY, Chee S-P. The epidemiology of acute endophthalmitis after cataract surgery in an Asian population. *Ophthalmol.* 2004;111:699-705.
30. Srinivasan R, Tiroumal S, Kanungo R, Natarajan MK. Microbial contamination of the anterior chamber during phacoemulsification. *J Cataract Refract Surg.* 2002;28:2173-6.
31. Kunimoto DY, Das T, Sharma S, Jalali S, Majji AB, Gopinathan U, et al. Microbiologic spectrum and susceptibility of isolates: part II. Posttraumatic endophthalmitis. *Am J Ophthalmol.* 1999;128:242-4.
32. Iwalokun BA, Oluwadun A, Akinsinde KA, Niemogha MT, Nwaokorie FO. Bacteriologic and plasmid analysis of etiologic agents of conjunctivitis in Lagos, Nigeria. *J Ophthalmic Inflamm Infect.* 2011;1:95-103.
33. Hong J, Chen J, Sun X, Deng S, Chen L, Gong L, et al. Paediatric bacterial keratitis cases in Shanghai: microbiological profile, antibiotic susceptibility and visual outcomes. *Eye (Lond).* 2012;26:1571-8.
34. Marasini S, Swift S, Dean S, Ormonde S, Craig J. Spectrum and Sensitivity of Bacterial Keratitis Isolates in Auckland. *J Ophthalmol.* 2016;2016:3769341. doi: 10.1155/2016/3769341.
35. Musa AA, Nazeerullah R, Sarite SR. Bacterial profile and antimicrobial susceptibility pattern of anterior blepharitis in Misurata region, Libya. *Dent Med Res.* 2014;2:8-13.
36. Mshangila B, Paddy M, Kajumbula H, Ateenyi-Agaba C, Kahwa B, Seni J. External ocular surface bacterial isolates and their antimicrobial susceptibility patterns among pre-operative cataract patients at Mulago National Hospital in Kampala, Uganda. *BMC Ophthalmol.* 2013;13:71. doi: 10.1186/1471-2415-13-71.
37. Moinuddin K, Nazeer HA, Vani K, Prakash B. Bacteriology and antimicrobial susceptibility pattern of external ocular infections in rural tertiary care teaching hospital. *Indian J Microbiol Res.* 2016;3:203-8.
38. Modarres S, Lasheii A, Oskooi NN. Bacterial etiologic agents of ocular infection in children in the Islamic Republic of Iran. *Eastern Mediterranean Health J.* 1998;4:44-9.
39. Briscoe D, Rubowitz A, Assia EI. Changing bacterial isolates and antibiotic sensitivities of purulent dacryocystitis. *Orbit.* 2005;24:29-32.
40. Teweldemedhin M, Saravanan M, Gebreyesus A, Gebreegziabiher D. Ocular bacterial infections at Quiha Ophthalmic Hospital, Northern Ethiopia: an evaluation according to the risk factors and the antimicrobial susceptibility of bacterial isolates. *BMC Infect Dis.* 2017;17:207. doi: 10.1186/s12879-017-2304-1.
41. Mulla Summaiya A, Khokhar Neeta D, Revdiwala Sangita B. Ocular infections: rational approach to antibiotic therapy. *National J Med Res.* 2012;2:22-4.

42. Muluye D, Wondimeneh Y, Moges F, Nega T, Ferede G. Types and drug susceptibility patterns of bacterial isolates from eye discharge samples at Gondar University Hospital, Northwest Ethiopia. *BMC Res Notes*. 2014;7:292.
43. Wang N, Yang Q, Tan Y, Lin L, Huang Q, Wu K. Bacterial spectrum and antibiotic resistance patterns of ocular infection: differences between external and intraocular diseases. *J Ophthalmol*. 2015;2015:813979. doi: 10.1155/2015/813979.
44. Jassim SH, Sivaraman KR, Jimenez JC, Jaboori AH, Federle MJ, de la Cruz J, et al. Bacteria colonizing the ocular surface in eyes with Boston Type 1 Keratoprosthesis: Analysis of Biofilm-forming capability and vancomycin tolerance. *Invest Ophthalmol Vis Sci*. 2015;56:4689-96.
45. El-Ganiny AM, Shaker GH, Aboelazm AA, El-Dash HA. Prevention of bacterial biofilm formation on soft contact lenses using natural compounds. *J Ophthalmic Inflamm Infect*. 2017;7:11. doi: 10.1186/s12348-017-0129-0.
46. Tan SY-E, Chew SC, Tan SY-Y, Givskov M, Yang L. Emerging frontiers in detection and control of bacterial biofilms. *Curr Opin Biotech*. 2014;26:1-6.
47. Taraszkiwicz A, Fila G, Grinholc M, Nakonieczna J. Innovative strategies to overcome biofilm resistance. *Biomed Res Int*. 2013;2013:150653. doi: 10.1155/2013/150653.
48. Fernández-Rubio ME, Rebolledo-Lara L, Martínez-García M, Alarcón-Tomás M, Cortés-Valdés C. The conjunctival bacterial pattern of diabetics undergoing cataract surgery. *Eye (Lond)*. 2010;24:825-34.
49. Kivanç S, Kivanç M, Bayramlar H. Microbiology of corneal wounds after cataract surgery: biofilm formation and antibiotic resistance patterns. *J Wound Care*. 2016;25:12,14-9.
50. Sánchez CJ, Mende K, Beckius ML, Akers KS, Romano DR, Wenke JC, et al. Biofilm formation by clinical isolates and the implications in chronic infections. *BMC Infect Dis*. 2013;13:47. doi: 10.1186/1471-2334-13-47.
51. Kwon AS, Park GC, Ryu SY, Lim DH, Lim DY, Choi CH, et al. Higher biofilm formation in multidrug-resistant clinical isolates of *Staphylococcus aureus*. *Int J Antimicrob Agents*. 2008;32:68-72.
52. Reiter KC, DA Silva Paim TG, DE Oliveira CF, D'Azevedo PA. High biofilm production by invasive multiresistant staphylococci. *APMIS*. 2011;119:776-81.

## Tables

Due to technical limitations, Table(s) 1 and 2 are only available as a download in the supplemental files section.

**Table 3:** Antimicrobial susceptibility patterns of Gram-positive isolates from external ocular infection at JUMC eye clinic, March-1/2017-June-30/2017.

Bacterial Isolate	Antimicrobial agents tested		AMC No(%)	AMP No(%)	CIP No(%)	AK No(%)	C No(%)	CLN No(%)	TE No (%)	SXT No(%)	ERY No(%)	CN No(%)
	Total	Pattern										
<i>S. aureus</i>	29	S	22(75.9)	5(17.2)	26(89.7)	20(68.9)	16(55.2)	24(82.8)	7(24.1)	9(31.0)	5(17.2)	21(72.4)
		R	7(24.1)	24(82.8)	3(10.3)	9(31.0)	13(44.8)	5(17.2)	22(75.9)	20(69.0)	24(82.8)	8(27.4)
CoNS	41	S	37(90.2)	7(17.1)	32(78.0)	30(73.2)	23(56.1)	33(80.5)	12(29.3)	13(31.7)	11(26.8)	32(78.0)
		R	4(9.8)	34(82.9)	9(22.0)	11(26.8)	18(43.9)	8(19.5)	29(70.7)	28(68.3)	30(73.2)	9(22.0)
<i>S.pneumoniae</i>	13	S	13(100)	5(38.5)	11(84.6)	10(76.9)	10(76.9)	8(61.5)	5(38.5)	4(30.8)	7(53.8)	10(76.9)
		R	0(0.0)	8(61.5)	2(15.4)	3(23.1)	3(23.1)	5(38.5)	8(61.5)	9(69.2)	6(46.2)	3(23.1)
<i>S. pyogenes</i>	5	S	5(100)	2(40.0)	4(80.0)	4(80.0)	4(80.0)	4(80.0)	2(40.0)	2(40.0)	3(60.0)	4(80.0)
		R	0(0.0)	3(60.0)	1(20.0)	1(20.0)	1(20.0)	1(20.0)	3(60.0)	3(60.0)	2(40.0)	1(20.0)
<i>S.agalactiae</i>	5	S	5(100)	2(40.0)	5(100)	4(80)	4(80.0)	5(100)	2(40.0)	3(60.0)	4(80.0)	4(80.0)
		R	0(0.0)	3(60.0)	0(0.0)	1(20.0)	1(20.0)	0(0.0)	3(60.0)	2(40.0)	1(20.0)	1(20.0)
<i>S.viridians</i>	3	S	3(100)	2(66.7)	3(100)	3(100)	3(100)	3(100)	2(66.7)	1(33.3)	2(66.7)	3(100)
		R	0(0.0)	1(33.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(33.3)	2(66.7)	1(33.3)

Key: CoNS = Coagulase negative Staphylococci; S = Susceptible; R = Resistance; AMC = Amoxicillin-clavulanic acid; AMP = Ampicillin; CIP = Ciprofloxacin; AK = Amikacin; C = Chloramphenicol; CLN = Clindamycin; TE = Tetracycline; SXT = Trimethoprim-sulphamethoxazole; ERY = Erythromycin; CN = Gentamicin; FOX = Cefoxitin; P = Penicillin G; No = Number; NT = Not tested. Few Intermediate susceptibility of isolate results were included to Susceptible category.

**Table 4:** Antimicrobial susceptibility patterns of Gram-negative isolates from external ocular infection at JUMC eye clinic, March-1/2017-June-30/2017.

Bacterial isolate	Total	Pattern	Antimicrobial agents tested										
			AMC No(%)	AMP No(%)	CIP No(%)	CRO No(%)	C No(%)	CAZ No(%)	TE No(%)	SXT No(%)	CN No(%)	TOB No(%)	AK No(%)
<i>P. aeruginosa</i>	10	S	8(80.0)	5(50.0)	9(90.0)	8(80.0)	5(50.0)	1(10.0)	2(20.0)	3(30.0)	8(80.0)	3(30.0)	8(80.0)
		R	2(20.0)	5(50.0)	1(10.0)	2(20.0)	5(50.0)	9(90.0)	8(80.0)	7(70.0)	2(20.0)	7(70.0)	2(20.0)
<i>K. pneumoniae</i>	9	S	8(88.9)	6(66.7)	9(100)	9(100)	8(88.9)	6(66.7)	4(44.4)	4(44.4)	8(88.9)	4(44.4)	8(88.9)
		R	1(11.1)	3(33.3)	0(0.0)	0(0.0)	1(11.1)	3(33.3)	5(55.6)	5(55.6)	1(11.1)	5(55.6)	1(11.1)
<i>P. mirabilis</i>	5	S	5(100)	4(80.0)	5(100)	4(80.0)	4(80.0)	4(80.0)	3(60.0)	3(60.0)	5(100)	2(40.0)	5(100)
		R	0(0.0)	1(20.0)	0(0.0)	1(20.0)	1(20.0)	1(20.0)	2(40.0)	2(40.0)	0(0.0)	3(60.0)	0(0.0)
<i>P. vulgaris</i>	4	S	4(100)	3(75.0)	4(100)	4(100)	4(100)	3(75.0)	3(75.0)	3(75.0)	3(75.5)	1(25.0)	3(75.0)
		R	0(0.0)	1(25.0)	0(0.0)	0(0.0)	0(0.0)	1(25.0)	1(25.0)	1(25.0)	1(25.0)	3(75.0)	1(25.0)
<i>S. marcescens</i>	3	S	3(100)	1(66.7)	3(100)	3(100)	3(100)	3(100)	2(66.7)	0(0.0)	3(100)	1(33.3)	2(66.7)
		R	0(0.0)	1(33.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(33.3)	3(100)	0(0.0)	2(66.7)	1(33.3)
<i>Citrobacter spp</i>	5	S	4(80.0)	4(80.0)	5(100)	5(100)	4(80.0)	4(80.0)	3(60.0)	5(100)	4(80.0)	3(60.0)	4(80.0)
		R	1(20.0)	1(20.0)	0(0.0)	0(0.0)	1(20.0)	1(20.0)	2(40.0)	0(0.0)	1(20.0)	2(40.0)	1(20.0)
<i>Enterobacter spp</i>	3	S	3(100)	2(66.7)	3(100)	3(100)	3(100)	3(100)	1(33.3)	3(100)	2(66.7)	1(33.3)	2(66.7)
		R	0(0.0)	1(33.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(66.7)	0(0.0)	1(33.3)	2(66.7)	1(33.3)
<i>E. coli</i>	5	S	4(80)	3(60.0)	5(100)	5(100)	3(60.0)	4(80.0)	1(20.0)	4(80.0)	5(100)	2(40.0)	4(80.0)
		R	1(20.0)	2(40.0)	0(0.0)	0(0.0)	2(40.0)	1(20.0)	4(80.0)	2(40.0)	0(0.0)	3(60.0)	1(20.0)
<i>H. influenza</i>	5	S	4(80.0)	3(60.0)	5(100)	5(100)	4(80.0)	4(80.0)	3(60.0)	3(60.0)	4(80.0)	NT	NT
		R	1(20.0)	2(40.0)	0(0.0)	0(0.0)	1(20.0)	1(20.0)	2(40.0)	2(40.0)	1(20.0)	NT	NT
<i>N. meningitides</i>	2	S	NT	NT	2(100)	2(100)	1(50.0)	NT	NT	1(50.0)	NT	NT	NT
		R	NT	NT	0(0.0)	0(0.0)	1(50.0)	NT	NT	1(50.0)	NT	NT	NT

Key: S = Susceptible; R = Resistance; AMC = Amoxicillin-clavulanic acid; AMP = Ampicillin; CIP = Ciprofloxacin; AK = Amikacin; C = Chloramphenicol; TE = Tetracycline; SXT = Trimethoprim-sulphamethoxazole; CN = Gentamicin; CRO = Ceftriaxone; CAZ = Ceftazidime; TOB = Tobramycin; No = Number; NT = Not tested. Few Intermediate susceptibility of bacterial isolate results were included to Susceptible category.

**Table 5:** MDR pattern of bacteria isolated from external ocular infected patients at JUMC eye clinic, March-1/2017-June-30/2017.

Bacterial isolates	Total	Antibiotic resistance pattern					
		Ro	R1	R2	R3	R4	≥ R5
<i>S. aureus</i>	29	0(0.0)	0(0.0)	4(13.8)	5(17.2)	7(24.1)	13(44.8)
CoNS	41	0(0.0)	2(4.9)	5(12.2)	8(19.5)	10(24.4)	16(39.0)
<i>S. pneumonia</i>	13	0(0.0)	1(7.7)	3(23.1)	3(23.1)	6(46.2)	0(0.0)
<i>S. pyogenes</i>	5	0(0.0)	1(20.0)	0(0.0)	2(40.0)	1(20.0)	1(20.0)
<i>S. agalactiae</i>	5	1(20.0)	1(20.0)	1(20.0)	1(20.0)	0(0.0)	1(20.0)
<i>S. viridians</i>	3	0(0.0)	2(66.7)	0(0.0)	1(33.3)	0(0.0)	0(0.0)
<i>P. aeruginosa</i>	10	0(0.0)	0(0.0)	0(0.0)	3(30.0)	2(20.0)	5(50.0)
<i>K. pneumonia</i>	9	1(11.1)	1(11.1)	1(11.1)	4(44.4)	2(22.2)	0(0.0)
<i>P. mirabilis</i>	5	0(0.0)	2(40.0)	2(40.0)	0(0.0)	0(0.0)	1(20.0)
<i>P. vulgaris</i>	4	0(0.0)	2(50.0)	1(25.0)	1(25.0)	0(0.0)	0(0.0)
<i>S. marcescens</i>	3	0(0.0)	0(0.0)	2(66.7)	1(33.3)	0(0.0)	0(0.0)
<i>Citrobacter species</i>	5	1(20.0)	2(40.0)	0(0.0)	2(40.0)	0(0.0)	0(0.0)
<i>Enterobacter species</i>	3	0(0.0)	1(33.3)	1(33.3)	1(33.3)	0(0.0)	0(0.0)
<i>E. coli</i>	5	0(0.0)	1(20.0)	1(20.0)	1(20.0)	1(20.0)	1(20.0)
<i>H. influenza</i>	5	1(20.0)	2(40.0)	1(20.0)	1(20.0)	0(0.0)	0(0.0)
<i>N. meningitides</i>	2	0(0.0)	2(100)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
<b>Total</b>	<b>147</b>	<b>4(2.7)</b>	<b>20(13.6)</b>	<b>22(15.0)</b>	<b>34(23.1)</b>	<b>29(19.7)</b>	<b>38(25.9)</b>

CoNS = Coagulase negative Staphylococci, Ro = bacterial isolate susceptible to all antimicrobial agents, R1 = bacterial isolate resistance to 1 antimicrobial agent, R2 = bacterial isolate resistance to 2 antimicrobial agents of different classes, R3 = bacterial isolate resistance to 3 antimicrobial agents of different classes, R4 = bacterial isolate resistance to 4 antimicrobial agents of different classes; ≥R5 = bacterial isolate resistance to 5 and above antimicrobial agents of different classes.

**Table 6:** Biofilm forming capability and adherence classification of bacteria from external ocular infected patients, March-1/2017- June-30/2017

Bacterial isolates	Biofilm formation classification				
	Total*	Strong	Moderate	Weak	Non-adherent
<i>S. aureus</i>	29	9(31.0)	10(34.5)	2(6.9)	8(27.6)
CoNS	41	14(34.1)	13(31.7)	4(9.8)	10(24.4)
<i>S. pyogenes</i>	5	0(0.0)	1(20.0)	1(20.0)	3(60.0)
<i>S. agalactiae</i>	5	0(0.0)	1(20.0)	1(20.0)	3(60.0)
<i>S. viridians</i>	3	0(0.0)	0(0.0)	0(0.0)	3(100)
<i>P. aeruginosa</i>	10	4(40.0)	3(30.0)	1(10.0)	2(20.0)
<i>K. pneumoniae</i>	9	2(22.2)	3(33.3)	2(22.2)	2(22.2)
<i>P. mirabilis</i>	5	1(20.0)	1(20.0)	1(20.0)	2(40.0)
<i>P. vulgaris</i>	4	0(0.0)	0(0.0)	2(50.0)	2(50.0)
<i>S. marcescens</i>	3	0(0.0)	1(33.3)	0(0.0)	2(66.7)
<i>Citrobacter species</i>	5	0(0.0)	3(60.0)	0(0.0)	2(40.0)
<i>Enterobacter species</i>	3	0(0.0)	1(33.3)	0(0.0)	2(66.7)
<i>E. coli</i>	5	1(20.0)	2(40.0)	0(0.0)	2(40.0)
<b>Total</b>	<b>127</b>	<b>31(24.4)</b>	<b>39(30.7)</b>	<b>14(11.0)</b>	<b>43(33.9)</b>

\*: *S. pneumoniae* (n=13), *H. influenzae* (n=5) and *N. meningitidis* (n=2) altogether, their biofilm formation capability was not assessed for they are delicate and fastidious by nature.

## Figures

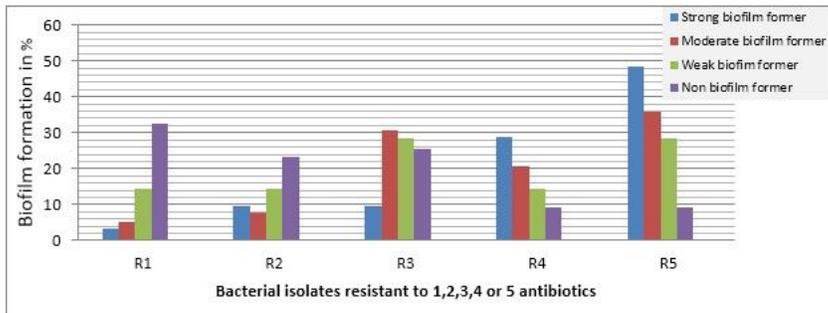


Figure 1

Correlation of antimicrobial resistance and biofilm formation of isolates from JUMC eye clinic, March-1/2017-June-30/2017.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [supplement1.pdf](#)
- [supplement1.pdf](#)