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A STUDY ON BIOFILM-MEDIATED MECHANISMS CONTRIBUTING TO MULTIDRUG RESISTANCE IN BACTERIAL PATHOGENS ISOLATED FROM EXTERNAL OCULAR INFECTIONS IN CENTRAL INDIA

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ABSTRACT

Background: External ocular infections are a significant health concern, particularly in pediatric populations and in patients with underlying comorbidities. The increasing prevalence of multidrug-resistant (MDR) bacteria and biofilm-producing pathogens further complicates treatment.

Objective: To identify the bacterial pathogens responsible for external ocular infections, assess their antibiotic susceptibility profiles, and explore the relationship between multidrug resistance and biofilm formation.

Methods: A total of 319 patients (mean age 21 years) presenting with external ocular infections were enrolled. Bacterial culture and antibiotic susceptibility testing were performed using standard microbiological techniques and CLSI 2021 guidelines. Biofilm formation was assessed, and statistical analyses including multivariate logistic regression were conducted.

Results: Positive bacterial growth was obtained from 147 (46.1%) ocular specimens. Gram-positive bacteria accounted for 65.3% of isolates, with coagulase-negative staphylococci (CoNS, 27.9%) and Staphylococcus aureus (19.7%) being the most prevalent. Among Gram-negative bacteria (34.7%), Pseudomonas aeruginosa (10.8%) and Klebsiella pneumoniae (9.1%) predominated. Conjunctivitis was the most common clinical diagnosis (51.7%). Significant associations were found between bacterial infection and comorbid diabetes mellitus (AOR = 0.09, $p = 0.002$) and previous hospitalization (AOR = 0.10, $p = 0.001$). High levels of resistance were observed to penicillin (82.7%) and ampicillin (78.3%) among Gram-positive isolates, while Gram-negative bacteria showed resistance to ampicillin (90%) and tetracycline (73.5%). Ciprofloxacin and gentamicin remained effective against most isolates. MDR was observed in 68.7% of isolates, and 48.4% of MDR strains were strong biofilm producers, indicating a statistically significant correlation ($p < 0.05$).

Conclusion: The high prevalence of MDR and biofilm-producing bacteria in ocular infections underscores the need for ongoing surveillance and prudent antibiotic use. Fluoroquinolones and carbapenems remain effective, but rising resistance trends necessitate timely microbiological evaluation and targeted therapy.

Keywords: Biofilm formation, Multidrug resistance (MDR), Ocular pathogens, External ocular infections

INTRODUCTION

You can see everything around you if you look out of your eye. It's like a body window. Eye sickness is caused by bacteria, which are small living things. This is a big health issue everywhere, but it's even worse in poor places [1]. Headaches in the eyes can damage the structure of the eye and make it harder to see. They can even make you go blind if you don't get the right care and checkup. The most germs are in the cornea, the pupil, and the lens [2]. A lot of people who work outside the home get eye infections like conjunctivitis, blepharitis, and dacryocystitis [2]. Bugs, fungi, viruses, and parasites are all types of pathogenic germs [3]. Most eye diseases around the world are caused by germs [4]. People often use drugs that kill a lot of different types of germs to treat eye diseases. Still, the fact that germs are getting better at fighting drugs is a problem all over the world [5]. Antibiotics that don't kill germs are more likely to show up, spread, and stay around when they are used in the wrong way or for no reason. We now know that biofilms made of germs are a big reason why people get sick and stay sick. It is hard for doctors and scientists to treat biofilms made by bacteria because drugs are 100–1000 times less likely to kill them than bacteria that float on water [6]. Antibiotics change the way bacteria look and work, so they can't reach bacteria that make biofilms. Because of this, antimicrobial drugs work less well against germs. Antacids are easy to get without a prescription in most places in Ethiopia. People may take too many or the wrong drugs because of this, which can help types that aren't killed by antibiotics spread [7]. Also, people in poor countries might not wash their faces enough, which is one reason why more people are getting germ-based eye illnesses. More and more treatments might not work over time, which is bad [8]. The reason for this is that microbes are getting stronger against medicines. In 2012, eye diseases in the Jimma area were looked into, Other studies have shown that the germs that make people sick can change over time and between places. How well drugs work against them can also change [9]. The microorganisms that cause external eye diseases are always changing, and some of them have learnt how to avoid being killed by medicine. In other words, you should always keep an eye on scientific treatment. You should know how to get rid of germs that are bad for your eyes [10]. But it wasn't looked at how bacterial biofilm formed in samples from people in Ethiopia and the Jimma area who had external eye problems. The point of the study was to learn more about the kinds of bacteria that cause eye diseases on the outside, how well drugs can fight them, and whether these bacteria can form biofilms, which are groups of bacteria that stick together [11].

MATERIALS AND METHOD

A observational study was conducted from January 2024 to January 2025 at GSVM Medical College, Kanpur in collaboration with CSJMU, involving 319 patients with clinically confirmed external ocular infections. Patients above 4 years of age were included, excluding those who had used antibiotics in the previous five days. Clinical specimens (conjunctival swabs, corneal scrapings, and intraocular fluids) were aseptically collected and analyzed in the microbiology lab. Bacterial identification and antibiotic susceptibility testing were performed using standard culture methods, Kirby-Bauer disk diffusion, and MIC determination as per CLSI 2021 guidelines [12]. Biofilm formation and multidrug resistance patterns were also assessed.

Data Analysis: Statistical analysis was performed using SPSS version excel significance set at $p < 0.05$.

RESULTS

The study included 319 participants ranging in age from 1 month to 95 years, with a mean age of 21 years. Among them, 172 (53.9%) were male. A substantial proportion of the study population consisted of children under the age of 2 years (103; 32.3%), while 74 participants (23.2%) were aged above 45 years. Crude odds ratio (COR) analysis revealed no statistically significant association between sociodemographic variables and the pattern of bacterial isolation. Comorbid conditions among participants included hypertension in 18 cases (5.6%), diabetes mellitus in 17 cases (5.3%), and rheumatoid arthritis in 11 cases (3.4%). Additionally, 21 individuals reported a history of hospitalization due to ocular infection, while 31 participants (9.7%) had previously used topical ocular medications. A history of ocular surgery was documented in 5 cases (1.6%), and 23 participants (7.2%) reported using traditional or herbal remedies for ocular conditions. Contact lens use was reported by 16 participants (5.0%). Multivariate logistic regression analysis identified a statistically significant association between the presence of diabetes mellitus (AOR = 0.09, 95% CI: 0.02–0.43, p = 0.002) and a prior history of hospitalization (AOR = 0.10, 95% CI: 0.03–0.42, p = 0.001) with an increased risk of external bacterial ocular infections. The most frequently diagnosed clinical condition was conjunctivitis, observed in 165 cases (51.7%), followed by blepharoconjunctivitis in 74 cases (23.2%), blepharitis in 52 cases (16.3%), dacryocystitis in 13 cases (4.1%), and other external ocular infections in 15 cases (4.7%). Conjunctivitis was particularly common among younger children. Out of the 319 ocular specimens processed, 147 (46.1%) yielded positive bacterial cultures. All isolates were monomicrobial, with no polymicrobial growth detected. Of these, 96 isolates (65.3%) were Gram-positive and 51 (34.7%) were Gram-negative. The most frequently isolated pathogen was coagulase-negative Staphylococci (CoNS) in 41 cases (27.9%), followed by Staphylococcus aureus in 29 cases (19.7%) and Streptococcus pneumoniae in 13 cases (8.8%). Among the Gram-negative isolates, Pseudomonas aeruginosa (10.8%) and Klebsiella pneumoniae (9.1%) were the most prevalent. The distribution of bacterial isolates showed a predominance in children aged between 1 month and 2 years.

Table 1. Prevalence of bacterial isolate against age groups in eye clinic.

Name of bacterial isolate	**Age in years**					**Total (N= 319)**
	0–2	**3–16**	**17–30**	**31–45**	**>45**	
Gram-positive bacteria						
S. aureus	9 (20.5%)	2 (10.0%)	7 (31.8%)	2 (9.1%)	9 (23.1%)	**29 (19.7%)**
CoNS *	10 (22.7%)	5 (25.0%)	4 (18.2%)	11 (50.0%)	11(28.2%)	**41 (27.9%)**
S. pneumoniae	4 (9.1%)	1 (5.0%)	2 (9.1%)	1 (4.5%)	5 (12.8%)	**13 (8.8%)**
S. pyogenes	1 (2.3%)	3 (15.0%)	1 (4.5%)	(0%)	(0%)	**5 (3.4%)**
S. agalactiae	1 (2.3%)	1 (5.0%)	(0%)	(0%)	3 (7.7%)	**5 (3.4%)**
S. viridians	2 (4.5%%)	(0%)	(0%)	(0%)	1 (2.6%)	**3 (2.0%)**
Gram-negative bacteria						
P. aeruginosa	1 (2.3%)	2 (10.0%)	2 (9.1%)	3 (13.6%)	2 (5.1%)	**10 (6.8%)**
K. pneumoniae	2 (4.5%)	2 (10.0%)	1 (4.5%)	1 (4.5%)	3 (7.7%)	**9 (6.1%)**
P. mirabilis	1 (2.3%)	1 (5.0%)	2 (9.1%)	(0%)	1 (2.6%)	**5 (3.4%)**
P. vulgaris	1 (2.3%)	1 (5.0%)	(0%)	1 (4.5%)	1 (2.6%)	**4 (2.7%)**

Name of bacterial isolate	Age in years					Total (N= 319)
	0–2	3–16	17–30	31–45	>45	
H. influenzae	4 (9.1%)	(0%)	1 (4.5%)	(0%)	(0%)	**5 (3.4%)**
N. meningitidis	1 (2.3%)	1 (5.0%)	(0%)	(0%)	(0%)	**2 (1.4%)**
Total	**44 (29.9%)**	**20(13.6%)**	**22(15.0%)**	**22(15.0%)**	**39 (26.5%)**	**147 (100%)**

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

Table 2. Distribution of bacteria isolates against the different clinical features of external ocular infections in eye clinic.

Name of bacterial isolate	Types of diagnosis					Total (N = 319)
	Conjunctivitic	Blepharitis	Blepharo conjunctivitis	Dacryocystitis	Others	
Gram-positive bacteria						
S. aureus	15 (20.0%)	8 (25.0%)	1 (3.7%)	2 (25.0%)	3(60)	**29(19.7%)**
CoNS *	16 (21.3%)	10 (31.2%)	10 (37.0%)	3 (37.5%)	2(40)	**41 (27.9%)**
S. pneumoniae	9 (12.0%)	2 (6.2%)	0 (%)	2 (25.0%)	(0%)	**13 (8.8%)**
S. pyogenes	4 (5.3%)	(0%)	1 (3.7%)	(0%)	(0%)	**5 (3.4%)**
S. agalactiae	2 (2.7%)	2 (6.2%)	1 (3.7%)	(0%)	(0%)	**5 (3.4%)**
S. viridians#	2 (2.7%)	1 (3.1%)	(0%)	(0%)	(0%)	**3 (2.0%)**
Gram-negative bacteria						
P. aeruginosa	5 (6.7%)	3 (9.4%)	2 (7.4%)	(0%)	(0%)	**10 (6.8%)**
K. pneumoniae	4 (5.3%)	2 (6.2%)	2 (7.4%)	1 (12.5%)	(0%)	**9 (6.1%)**

Name of bacterial isolate	**Types of diagnosis**					**Total (N = 319)**
	Conjunctivitic	**Blepharitis**	**Blepharo conjunctivitis**	**Dacryocystitis**	**Others**	
P. mirabilis	3 (4.0%)	1 (3.1%)	1 (3.7%)	(0%)	(0%)	**5 (3.4%)**
P. vulgaris	2 (2.7%)	(0%)	2 (7.4%)	(0%)	(0%)	**4 (2.7%)**
S. marcescens	3 (4.0%)	(0%)	0 (0.0%)	(0%)	(0%)	**3 (2.0%)**
Citrobacter spp.	3 (4.0%)	1 (3.1%)	1 (3.7%)	(0%)	(0%)	**5 (3.4%)**
Enterobacter spp.	2 (2.7%)	(0%)	1 (3.7%)	(0%)	(0%)	**3 (2.0%)**
E. coli	2 (2.7%)	2 (6.2%)	1 (3.7%)	(0%)	(0%)	**5 (3.4%)**
H. influenzae	2 (2.7%)	(0%)	3 (11.1%)	(0%)	(0%)	**5 (3.4%)**
N. meningitidis	1 (1.3%)	(0%)	1 (3.7%)	(0%)	(0%)	**2 (1.4%)**
Total	**75 (51.0%)**	**32 (21.8)**	**27 (18.4%)**	**8 (5.4%)**	**5(3.4%)**	**147 (100%)**

*Coagulase-negative staphylococcus; Viridans streptococci. Gram-positive ocular bacterial isolates showed the highest sensitivity to ciprofloxacin (89.7% in Staphylococcus aureus) and also responded well to clindamycin. This indicates continued effectiveness of fluoroquinolones and lacosamide. A strong correlation was found between multidrug resistance (MDR) and biofilm formation. While non-MDR strains were poor biofilm producers, 48.4% of MDR isolates formed strong biofilms and were resistant.

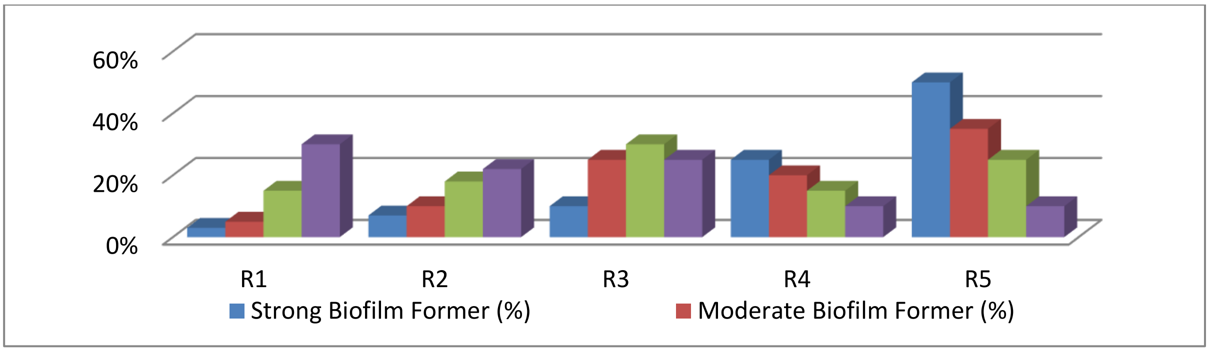

Figure 2: Relationship of antimicrobial resistance and biofilm formation of isolates from eye clinic.

Antibiotic Susceptibility Profile

Antibiotic susceptibility testing was performed on all 147 bacterial isolates using the Kirby-Bauer disk diffusion method as per CLSI 2021 guidelines. The results showed varying degrees of resistance and sensitivity among Gram-positive and Gram-negative ocular pathogens. Gram-positive bacteria, including Staphylococcus aureus and coagulase-negative staphylococci (CoNS), demonstrated high resistance to penicillin (82.7%), ampicillin (78.3%), and erythromycin (65.5%). However, these isolates were largely sensitive to ciprofloxacin (89.7%), gentamicin (81.3%), and clindamycin (76.2%), indicating the continued efficacy of fluoroquinolones and lincosamides. Methicillin resistance was observed in approximately 20% of Staphylococcus spp. On the other hand, Gram-negative isolates such as Pseudomonas aeruginosa and Klebsiella pneumoniae showed high resistance to ampicillin (90%), tetracycline (73.5%), and tobramycin (52%), but were relatively more sensitive to ciprofloxacin (76.4%), gentamicin (69.1%), and imipenem (87.5%), particularly in multidrug-resistant strains. Notably, 68.7% of all isolates were found to be multidrug-resistant (MDR), with a statistically significant correlation observed between MDR and biofilm formation ($p < 0.05$). Strong biofilm production was detected in 48.4% of MDR isolates, whereas non-MDR strains exhibited weak or no biofilm-forming ability. The consolidated antibiotic sensitivity data are presented below.

Table 3: Antibiotic Sensitivity Profile of Gram-Positive and Gram-Negative Isolates

Antibiotic	Gram-Positive Sensitivity (%)	Gram-Negative Sensitivity (%)
Ciprofloxacin	(89.7%)	(76.4%)
%Gentamicin	(81.3%)	(69.1%)
Erythromycin	(34.5%)	—
Tetracycline	(38.9%)	(26.5%)
Ampicillin	(21.7%)	(10%)
Tobramycin	(47.5%)	(48%)
Clindamycin	(76.2%)	—
Vancomycin	(100%)	—
Imipenem/Meropenem	—	(87.5%)
Methicillin (MRSA/MRCoNS)	(20.0%) (resistance rate)	—

DISCUSSION

An observational study was conducted over a period of four months, involving 319 patients with clinically diagnosed external ocular infections. The study aimed to determine the prevalence, microbial etiology, antimicrobial susceptibility patterns, and the correlation between multidrug resistance (MDR) and biofilm formation in ocular pathogens. The culture positivity rate was 46.1%, which aligns with findings from similar studies conducted in Southern Ethiopia, though higher or lower rates have been reported in other regions due to differences in study duration, case selection, sampling methods, and diagnostic capabilities. The most frequently diagnosed clinical condition among the participants was conjunctivitis, accounting for 51.7% of cases, followed by blepharoconjunctivitis (23.2%), blepharitis (16.3%), dacryocystitis (4.1%), and other external ocular infections (4.7%) [13]. Conjunctivitis was particularly prevalent among infants and young children. The findings are consistent with previous studies from various regions, which also report conjunctivitis as the leading cause of external ocular infections. Of the 147 positive cultures, Gram-positive bacteria were more commonly isolated (65.3%) compared to Gram-negative bacteria (34.7%). The most prevalent Gram-positive organisms included Coagulase-negative Staphylococci (CoNS, 27.9%), Staphylococcus aureus (19.7%), and Streptococcus pneumoniae (8.8%). Among the Gram-negative isolates, Pseudomonas aeruginosa (10.8%) and Klebsiella pneumoniae (9.1%) were predominant [14]. These organisms have been consistently identified as major contributors to external ocular infections in multiple studies from India, Ethiopia, and other parts of the world. Antimicrobial susceptibility testing revealed significant resistance to commonly used antibiotics. Gram-positive isolates, particularly S. aureus and CoNS, demonstrated high resistance rates to penicillin, ampicillin, erythromycin, tetracycline, and trimethoprim-sulfamethoxazole, with resistance rates ranging from 73% to 85%. Conversely, ciprofloxacin showed the highest sensitivity, with 89.7% of S. aureus isolates being susceptible. Clindamycin also displayed good efficacy against Gram-positive pathogens. These findings underscore the continued utility of fluoroquinolones and lincosamides in the management of ocular infections, although resistance to other frontline antibiotics remains a growing concern [15].

For Gram-negative bacteria, antibiotics such as ciprofloxacin, gentamicin, amoxicillin-clavulanic acid, and ceftriaxone were more effective, with susceptibility rates between 66% and 100%. However, resistance to tobramycin and tetracycline was observed in 40% to 80% of Gram-negative isolates, especially in P. aeruginosa and E. coli. These variable resistance patterns may be influenced by regional antibiotic usage practices, the unregulated sale of antibiotics, and limited microbiological diagnostics in peripheral health settings. A notable feature of this study was the investigation of biofilm formation and its correlation with antibiotic resistance [16]. Biofilms are structured communities of bacteria enclosed in a self-produced matrix that adheres to surfaces and protects the organisms from host immune responses and antibiotics. Approximately two-thirds of the isolates were biofilm producers, with a significant portion being either moderate or strong biofilm formers. Strong biofilm production was particularly common among P. aeruginosa (80%), K. pneumoniae (77.8%), CoNS (75.6%), S. aureus (72.4%), and E. coli (60%). Statistical analysis showed a strong correlation between biofilm formation and multidrug resistance. MDR isolates defined as resistant to three or more classes of antibiotics were significantly more likely to form biofilms. In fact, 48.4% of MDR isolates in the study were strong biofilm producers. This supports existing literature indicating that biofilm production enhances bacterial survival in hostile environments, contributing to chronic infections and treatment failure. The protective biofilm matrix acts as a physical barrier, slows antibiotic penetration, and enables quorum sensing, all of which promote resistance development and persistence [17]. The study also identified a high prevalence of MDR strains, with 68.7% of isolates being resistant to three or more antibiotic classes. This finding mirrors trends reported in both Central India, as well as in parts of India, highlighting the widespread and escalating problem of antimicrobial resistance in ocular pathogens. Contributing factors may include self-medication, incomplete antibiotic courses, lack of microbiological testing, and widespread availability of over-the-counter antibiotics. The results emphasize the need for region-specific antibiotic stewardship programs, routine microbiological testing including biofilm detection, and updated treatment guidelines for ocular infections. Strengthening diagnostic laboratory capacity and regulating antibiotic use are essential steps to curb the spread of MDR and biofilm-producing bacteria [18].

This study provides valuable insights into the bacteriological profile, antibiotic resistance patterns, and biofilm-forming ability of pathogens isolated from external ocular infections in Central India. The culture positivity rate of 46.1% aligns with other regional studies on ocular infections, although variability in positivity rates may depend on sample types, diagnostic timing, and prior antibiotic use [19]. The predominance of Gram-positive bacteria (65.3%), particularly coagulase-negative staphylococci (CoNS) and Staphylococcus aureus, is consistent with previous studies conducted in similar geographic and clinical settings. CoNS, often dismissed as contaminants, have now emerged as significant pathogens in external ocular infections, especially in immunocompromised individuals and patients with ocular devices. The high frequency of S. aureus also reaffirms its well-established role in conjunctivitis, blepharitis, and dacryocystitis [20]. Among Gram-negative isolates, Pseudomonas aeruginosa and Klebsiella pneumoniae were dominant. These organisms are notorious for their intrinsic resistance mechanisms and ability to survive in hospital environments, often leading to severe keratitis or endophthalmitis. The high resistance observed to penicillin, ampicillin, erythromycin, and tetracycline among both Gram-positive and Gram-negative isolates is concerning and reflects the widespread and often unregulated use of antibiotics in the region. This is in agreement with earlier reports from India and other LMICs that document rising resistance trends due to empirical or over-the-counter antibiotic usage [21]. Of notable clinical concern is the high prevalence of multidrug resistance (MDR) in 68.7% of isolates. This underscores the urgent need for routine microbiological surveillance and evidence-based antibiotic stewardship in ophthalmic practice. Interestingly, fluoroquinolones (ciprofloxacin) and aminoglycosides (gentamicin) retained comparatively better efficacy against both bacterial groups, reaffirming their continued role as frontline agents for empiric therapy in external ocular infections. However, increasing resistance trends even to these agents in other parts of India suggest that susceptibility patterns must be monitored regularly [22]. A pivotal aspect of this study was the investigation of biofilm formation among ocular isolates. Strong biofilm production was significantly associated with MDR, particularly in P. aeruginosa (80%), K. pneumoniae, and S. aureus strains. This correlation supports existing literature that highlights biofilms as a key virulence factor contributing to chronic infections and therapeutic failure. Biofilms act as a physical barrier, limiting antibiotic penetration and facilitating horizontal gene transfer, which promotes the persistence of resistant phenotypes [23]. Our findings resonate with earlier studies suggesting that biofilm-forming strains are often 10–1000 times more resistant to antimicrobials than their planktonic counterparts [24]. Additionally, the demographic analysis indicated a higher burden of infection among children below two years of age and individuals with comorbid conditions like diabetes mellitus, a known risk factor for poor ocular immune defenses and altered ocular surface microbiota [25]. This highlights the importance of targeted preventive strategies and patient education in high-risk groups.

CONCLUSION

People with eye diseases on the outside were found to have a lot of different kinds of bugs. Getting sick on the outside of the eyes was caused by both Gram-positive and Gram-negative germs. The next most common types of bacteria found in the samples were Streptococcus pneumoniae, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Staphylococcus aureus. More and more bacteria were becoming resistant to ampicillin, penicillin, erythromycin, trimethoprim-sulphamethoxazole, tobramycin, and tetracycline. There were some examples of eye diseases that ciprofloxacin and gentamicin worked better against. These diseases happen outside of the eye. It was mostly MDR bacteria that were not killed by methicillin. About 20% of the staphylococci samples were also not killed by methicillin. Finally,CoNS made the least amount of biofilm. P. aeruginosa made the most.

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