ANTIBIOGRAM ON SELECTED ANTIBACTERIAL EYEDROPS WITH VARYING EXPIRATION DATES

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A PROJECT SUBMITTED TO THE FACULTY OF OPTOMETRY, UNIVERSITY OF BENIN, BENIN CITY, IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF DOCTOR OF OPTOMETRY (OD) DEGREE

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CERTIFICATION

This is to certify that this research project titled **ANTIBIOGRAM ON SELECTED ANTIBACTERIAL EYEDROPS WITH VARYING EXPIRATION DATES** was carried out by **ONUOHA EZIUCHE ESE** in the Faculty of Optometry, University of Benin in partial fulfilment of the requirement for the Doctor of Optometry degree in the 2024/2025 Academic Session.

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DEDICATION

I dedicate this project to God almighty, who has preserved my life and kept me throughout my journey at the University of Benin. I also dedicate this project to my super amazing parents, Mr. Samuel and Mrs. Victoria Onuoha for their love, care and consistent moral, emotional and financial support.

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ABSTRACT

Ophthalmic infections are a major cause of ocular morbidity, and effective therapy depends on the potency of antibiotic eyedrops. The study evaluated the in vitro antibacterial activity of four ophthalmic antibiotics namely: Ciprofloxacin (Ciprotab), Moxifloxacin, Gentamicin, and Chloramphenicol, tested across varying expiration intervals and at a single dilution level. Activity was assessed against *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*, and *Staphylococcus aureus* isolated from infected eyes using disk diffusion and agar-well diffusion methods. The findings showed that Moxifloxacin demonstrated the broadest spectrum at both diluted and undiluted concentrations, effective against all bacterial isolates. Ciprofloxacin (Ciprotab) showed activity against *E. coli* and *K. pneumoniae* with the highest inhibition zone (25 mm), but was inactive against *P. aeruginosa*. Gentamicin showed activity against *E. coli and K. pneumoniae* (greater than 10 mm) but was inactive against *S. aureus* and *P. aeruginosa.* Chloramphenicol showed efficacy at both diluted and undiluted concentrations less than six months after expiration (greater 10 mm). However, diluted chloramphenicol samples less than one year before expiration showed no activity against all isolates (0). In conclusion, the study recommended that sensitivity test should be carried out on all bacterial isolates from pathogenic eye(s) before prescribing, to prevent antibiotic resistance. Also, patients should adhere strictly to antibiotic expiration dates and use eyedrops without dilution.

Keywords: *Bacterial isolates, Ophthalmic antibiotics, Antibiogram, Antibacterial activity, Expiration date, Dilution,*

CHAPTER ONE

1.0 INTRODUCTION

An antibiogram is a standardized cumulative summary of antimicrobial susceptibility patterns of local bacterial isolates to a set of antimicrobial agents. It is used to monitor resistance trends and guide empirical treatment decisions. It helps clinicians to select the most effective antimicrobial treatment by presenting data on local resistance patterns (Truong & Hidayat, 2021). In eye care, such data are essential to ensure effective treatment of infections like bacterial conjunctivitis, keratitis, and blepharitis.

Antibacterial eyedrops are sterile ophthalmic preparations containing antimicrobial agents specifically formulated to treat or prevent bacterial infections of the eye. They play a central role in the management of infections such as bacterial conjunctivitis, keratitis, blepharitis, and post-operative ocular infections. Given the sensitivity of ocular tissues and the risk of vision-threatening complications, selecting effective antibiotics and ensuring their continued potency is critical for optimal patient outcomes. Therefore, applying antibiograms to ophthalmic practice can provide evidence-based guidance for antibiotic selection.

Expiration dates are regulatory benchmarks indicating the final date by which a pharmaceutical product is guaranteed to maintain its labeled potency and safety. They do not necessarily reflect the precise point at which a drug loses therapeutic value, especially under ideal storage conditions. However, increasing evidence suggests that many medications, including antibiotics, may retain their chemical and microbiological stability beyond their labeled expiration dates, particularly when stored under recommended conditions (Arioua & Shaw, 2024). While this observation has been more extensively studied in systemic medications, there remains a

significant knowledge gap concerning ophthalmic preparations, which may degrade differently due to their aqueous nature, packaging, and vulnerability to environmental factors. Beyond chemical stability, packaging quality and proper storage influence the shelf-life and safety of ophthalmic drops. Few studies have evaluated the antibacterial efficacy of ophthalmic antibiotics at different expiration stages. . Understanding how expiration influence potency is therefore essential.

In addition to expiration concerns, eye drop dilution is a practice occasionally employed in clinical and non-clinical settings, often arising from economic constraints, limited drug availability, or an attempt to reduce ocular irritation caused by concentrated formulations. In low-resource environments, patients and even healthcare providers may dilute ophthalmic solutions to extend use or share among multiple users, thereby unintentionally altering the antimicrobial concentration and, consequently, its efficacy. Saito Jumei *et al.,* (2019) demonstrated that dilution can significantly affect the physical, chemical, and microbiological stability of ophthalmic solutions, even when aseptic techniques are maintained. This supports the growing concern that dilution, though sometimes used as a cost-saving measure, can lead to subtherapeutic concentrations or contamination risks, ultimately reducing the drug's antibacterial effectiveness and contributing to treatment failure.

Antibacterial eye drops are commonly packaged in multi-dose containers designed for use over a period of days or weeks. This extended use raises concerns regarding their long-term stability due to contamination risks, especially after the bottle is opened. Prolonged use and repeated handling expose the formulation to air and microbes, which may compromise both sterility and active ingredient stability even before reaching the stated expiration date (Tsegaw *et al.,* 2017). In tropical or resource-limited settings, where optimal storage conditions may not be feasible and

expired medications are often reused due to cost or reduced supply, these risks become even more pronounced.

Ophthalmic antibiotics require not only maintained potency but also preserved sterility to prevent treatment failure or the promotion of antimicrobial resistance (AMR) (Nentwich *et al.,* 2007). Given these concerns, there is a pressing need to evaluate the antibacterial efficacy of ophthalmic drops at varying expiration dates under standard room temperature storage conditions.

Therefore, this study aims to determine the antibacterial efficacy of selected ophthalmic antibiotics at varying expiration dates, using antibiogram analysis under controlled room temperature storage. The findings seek to provide evidence-based insights that could inform medication use policies, especially in settings where cost, availability, and waste minimization influence clinical decisions.

1.1 BACKGROUND INFORMATION

1.1.1 ANTIBIOGRAM

An antibiogram is a laboratory tool used to determine the susceptibility of bacterial strains to various antibiotics. It provides a cumulative profile of antimicrobial sensitivity patterns for pathogens isolated within a specific period or setting, often using methods such as the Kirby–Bauer disk diffusion test or minimum inhibitory concentration (MIC) determination (Truong & Hidayat, 2021). In clinical microbiology, it serves as a vital tool for determining which antibiotics are most effective against specific pathogens causing conditions like conjunctivitis, keratitis, blepharitis, and other bacterial eye diseases. Antibiogram is particularly important in the face of increasing antimicrobial resistance. Local antibiograms assist eye care practitioners in identifying prevailing resistance trends among pathogens like *Staphylococcus aureus,*

Pseudomonas aeruginosa, Streptococcus pneumoniae amongst others. This evidence-based approach supports rational antibiotic selection, minimizes treatment failure, and helps prevent the indiscriminate use of broad-spectrum agents that may further drive antimicrobial resistance. It also helps clinicians to select the most effective empirical therapy even before culture results are available.

1.1.2 ANTIBACTERIAL

Antibacterial are agents that inhibit bacterial growth (bacteriostatic) or kill bacteria outright (bactericidal), thus used to treat or prevent bacterial infections. These agents are effective against a wide range of gram-positive and gram-negative bacteria associated with eye infections. Their selection in clinical practice depends on infection severity, bacterial susceptibility, and drug safety profile.

Some commonly used antibacterial classes in eye care include:

1. Fluoroquinolones: Broad-spectrum agents effective against many Gram-positive and Gram-negative ocular pathogens.

2. Aminoglycosides: Especially effective against Gram-negative organisms in ocular infections.

3. Macrolides: Broad-spectrum agents used for certain Gram-positive, some Gram-negative species and atypical organisms in ophthalmic practice."

1.1.3 ANTIBACTERIAL EYEDROPS

Topical eye medication is the main form of treatment for eye conditions as they are administered directly at the site of action. These sterile liquid preparations are intended for application to the eye for therapeutic or diagnostic purposes. In treating bacterial infections, they provide a high

concentration of the drug directly to the infection site, reducing systemic side effects (Mehuys *et al.,* 2020). Antibacterial eye drops contain antimicrobial agents used to prevent or treat bacterial infections of the eye. They are widely employed in managing bacterial conjunctivitis, keratitis, blepharitis, and for postoperative prophylaxis (Cabrera-Aguas *et al.,* 2024).

Some antibacterial eye drops include:

1. Moxifloxacin (a fluoroquinolone)

2. Gentamicin (an aminoglycoside)

3. Ciprofloxacin (a fluoroquinolone with good ocular penetration)

4. Tobramycin (a potent aminoglycoside effective in many ocular infections)

5. Chloramphenicol (a broad-spectrum antibiotic often used as a first-line option in eye care for the treatment of bacterial conjunctivitis).

Effective management of bacterial ocular infections heavily relies on the timely and appropriate use of antibacterial eye drops. The choice of drug depends on the suspected causative organism and local resistance patterns. These drugs are chosen for their spectrum of activity, ocular penetration, and safety profile. However, their efficacy may be influenced by factors such as formulation stability, packaging, storage conditions, and shelf life. Preservatives within the formulations may also lose their efficacy, increasing the risk of secondary infections.

The mechanism of action varies with drug class: fluoroquinolones inhibit bacterial DNA gyrase and topoisomerase IV, aminoglycosides disrupt protein synthesis at the ribosomal level, while chloramphenicol also inhibits protein synthesis but by binding to the 50S ribosomal subunit (Cabrera-Aguas *et al.,* 2024; Mehuys *et al.,* 2020).

In some instances, antibacterial eye drops are intentionally diluted to minimize ocular irritation in sensitive patients or for laboratory assays that require controlled concentrations, and sometimes due to accessibility or cost challenges. However, dilution may significantly influence antibacterial efficacy by lowering the active ingredient concentration below the minimum inhibitory concentration, thereby reducing bactericidal potential and promoting resistance development. Saito *et al.,* (2019) demonstrated that dilution can alter the physical, chemical, and microbiological stability of ophthalmic preparations, while Amiriantz *et al.,* (2024) reported that dilution of an ocular solution markedly decreased its bactericidal activity. These findings reinforce the concern that dilution, though occasionally practiced, may compromise both safety and therapeutic effectiveness.

1.1.4 SHELF-LIFE

This is the period of time during which a drug remains safe for use. To determine the shelf life of a new product, the manufacturer conducts stability studies in specific environmental conditions to analyze any change in the quality of the product. Guidelines consisting of accelerated stability study and real time (also called long-term) study at normal conditions are followed to establish a tentative expiry date (Arioua & Shaw, 2024). Nonetheless, real world factors such as repeated bottle opening, exposure to light, and high ambient temperatures can shorten the actual shelf life of ophthalmic preparations (Montes *et al.,* 2016).

1.1.5 EXPIRATION DATES

Expiration dates refer to the final date at which a pharmaceutical product is guaranteed by the manufacturer to remain within its labeled potency and safety parameters when stored under recommended conditions. These dates are determined through stability testing and are regulated

by bodies such as the U.S. Food and Drug Administration (FDA) and World Health Organization (WHO). Beyond this date, the medication may lose efficacy, change chemically, or become unsafe due to microbial contamination. However, expiration dates do not necessarily indicate the point at which a drug becomes ineffective or harmful, especially if stored under ideal or stable conditions (Arioua & Shaw, 2024) Expired drugs may retain activity, but their chemical integrity, sterility, and efficacy may degrade over time, particularly in tropical or resource-limited settings (Davido *et al.,* 2024).

In resource- limited environments, expired drugs are sometimes used due to financial and accessibility challenges, raising safety concerns about reduced efficacy and sterility (Montes *et al.,* 2016) Using expired eye drops may lead to treatment failure, and prolonged infection. In some cases, degraded products can even cause irritation or toxicity to ocular tissues, making it important to study the effectiveness of eyedrops beyond expiration.

1.1.6 ZONE OF INHIBITION

The zone of inhibition refers to the clear circular area surrounding the spot of an antimicrobial agent on an inoculated agar plate where bacterial growth is suppressed; this is a standard measure of antimicrobial activity and is used to assess bacterial susceptibility (CLSI, 2024). Zone-diameter outcomes are influenced by agent diffusion, agar composition, inoculum density and other methodological variables. When comparing formulations with different expiration dates or dilution levels, a consistent reduction in zone diameter may indicate decreased antimicrobial potency, though specific thresholds and clinical implications require cautious interpretation.

1.1.7 DRUG SUSCEPTIBILITY

Drug susceptibility refers to the extent to which a microorganism is inhibited or destroyed by a specific concentration of an antimicrobial agent. The Clinical and Laboratory Standards Institute (CLSI) categorizes susceptibility results as Susceptible (S), Intermediate (I), or Resistant (R) based on standardized interpretive criteria (CLSI, 2025). In this study, the susceptibility of bacterial isolates to selected antibacterial eye drops was evaluated using the disk diffusion method. Variations observed in the zones of inhibition across different expiration dates and dilution levels provide insight into whether the duration since manufacture or reduced concentration affects antibacterial potency and therapeutic reliability.

1.1.8 BACTERIA

Bacteria are single-celled microorganisms with diverse structural and metabolic traits that enable them to survive in a variety of environments, including the ocular surface. They are broadly classified by their cell wall composition, staining characteristics, and morphology. Pathogenic species possess virulence factors such as toxins, enzymes, and adhesion molecules that facilitate host invasion and immune evasion (Madigan *et al.,* 2023). The ocular surface, though naturally protected by the tear film's antimicrobial components like lysozyme and lactoferrin, can become vulnerable through trauma, contact lens wear, or systemic disease, creating opportunities for bacterial colonization and infection (Willcox, 2019).

1.1.8.1 SELECTED BACTERIA

Common bacterial pathogens implicated in ocular infections include *Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Escherichia coli.* These organisms vary in their pathogenic potential, antimicrobial susceptibility, and ability to develop resistance, making them clinically significant in the management of bacterial eye infections

(Teweldemedhin *et al.,* 2017). Understanding the characteristics of these pathogens is essential for selecting appropriate antibacterial therapy and guiding empirical treatment decisions in ophthalmic practice.

1.1.8.2 GRAM-POSITIVE BACTERIA

1. *Staphylococcus aureus*: This gram-positive cocci has a thick peptidoglycan cell wall that retains the violet gram stain. It is a common cause of conjunctivitis, blepharitis and post-surgical ocular infections, and can also result in sight-threatening keratitis (Cabrera-Aguas *et al.,* 2024). Methicillin-resistant *S. aureus* (MRSA) strains have emerged in both community and healthcare settings, complicating empirical therapy and highlighting the need for local antibiograms to guide treatment (Ghita *et al.,* 2023).

1.1.8.3 GRAM-NEGATIVE BACTERIA

1. *Pseudomonas aeruginosa:* A gram-negative organism that possess an outer membrane containing lipopolysaccharides that enhance resistance to antibiotics and disinfectants. *Pseudomonas aeruginosa* is an opportunistic pathogen strongly associated with contact-lens related keratitis and can lead to rapid corneal destruction. It often shows intrinsic and acquired resistance mechanisms and frequently requires agents with confirmed anti-pseudomonal activity (high-generation fluoroquinolones, fortified aminoglycosides) guided by antibiogram results (Ghita *et al.,* 2023).

2. *Escherichia coli:* Although primarily an intestinal commensal, E. coli can occasionally invade extraintestinal sites, including ocular tissues, under favorable conditions such as

immunocompromised status or trauma. While uncommon, it may contribute to conjunctival or intraocular infections. Susceptibility varies by strain and local resistance patterns.

3. *Klebsiella pneumoniae:* A gram-negative rod with a polysaccharide capsule that enhances resistance to host defenses and antimicrobial agents. It can occasionally be implicated in ocular infections, such as conjunctivitis or corneal involvement. Reports of multidrug-resistant strains underscore the need for careful antibiotic selection and reliance on susceptibility data.

Collectively, these four organisms encompassing both Gram-positive and Gram-negative classes demonstrate diverse resistance patterns, emphasizing the importance of local antibiograms to guide effective ocular therapy (Teweldemedhin *et al.,* 2017; Ghita *et al.,* 2023).

1.1.9 RELEVANCE TO OPTOMETRY

Optometrists are frontline eye care providers who frequently prescribe antibiotics for bacterial ocular infections. Ensuring these medications remain effective is critical not only for symptom relief but also for preventing complications such as corneal ulcers and vision loss (Cabrera-Aguas *et al.,* 2024). Given the rising challenge of antimicrobial resistance and the economic constraints faced by patients in under-resourced areas, it is essential that optometrists base their clinical decisions on evidence regarding the safety and efficacy of medications, including those nearing or surpassing expiration dates or subjected to dilution. Understanding the efficacy profile of these drugs at different expiration stages can directly improve patient outcomes.

1.1.10 RESEARCH GAP

Although research exists on the stability and potency of systemic antibiotics beyond their labeled expiration dates, few studies have focused specifically on ophthalmic antibiotics, which are widely used for bacterial eye infections (Salih & El-Mahdi, 2018). Their antibacterial efficacy at

various expiration stages or after a single dilution remains largely unexamined. Most studies treat expiration as a binary state (expired versus unexpired) and do not explore staged degradation or region-specific factors such as tropical climate storage conditions. Dilution practices of antibacterial eyedrops, sometimes applied for cost savings or to reduce ocular irritation, are rarely evaluated in stability assessments. Saito *et al.* (2019) demonstrated that dilution of ophthalmic solutions can significantly affect their physical, chemical, and microbiological stability. This study aims to address these gaps by evaluating the in vitro antibacterial activity of selected commercial antibacterial eye drops at multiple expiration intervals and after a single dilution using standard antibiogram techniques.

1.2 STATEMENT OF THE PROBLEM

Bacterial ocular infections continue to be a cause of visual impairment, particularly in low-resource settings where access to quality ophthalmic antibiotics is limited (Nentwich *et al.,* 2007). Expired or diluted antibacterial eye drops may be used due to financial or supply constraints. This may reduce potency, compromise preservatives, and increase the risk of treatment failure (Arioua & Shaw, 2024; Hanssens *et al.,* 2018).

Ophthalmic formulations are less studied than systemic antibiotics, leaving uncertainty about their stability and antibacterial activity (Davido *et al.,* 2024). Evaluating the effects of expiration and dilution is therefore essential to ensure safe and effective eye care.

1.3 AIM

The aim of this study is to assess antibiogram on selected antibacterial eyedrops with varying expiration dates and one single dilution.

1.4 OBJECTIVES

1. To evaluate the antimicrobial efficacy of selected antibacterial eyedrops across various expiration intervals.

2. To determine and compare the in vitro antibacterial activity of selected commercial antibacterial eyedrops at their labelled (neat) strength and at a diluted concentration of 25 mg/mL.

3. To compare the antibacterial activity of the experimented eyedrops with standard antibiogram results of the test organisms.

1.5 RESEARCH QUESTIONS

1. Does variation in expiration intervals affect the antibacterial efficacy of selected eyedrops?

2. Does dilution affect the antibacterial efficacy of the selected antibacterial eyedrops?

3. Do expiration intervals and dilution combined have an effect on the antibacterial efficacy of the selected eyedrops?

1.6 SIGNIFICANCE OF THE STUDY

1. This study addresses an important gap in knowledge about the antibacterial efficacy and safety of ophthalmic antibiotics at different expiration dates.

2. By evaluating potency and sterility across varying expiration intervals, the research will inform evidence-based guidelines, reduce unnecessary medication waste, enhance patient safety, and support antimicrobial stewardship.

3. This study holds particular importance for both well-resourced and resource-limited settings where expired drugs may be used due to economic constraints.

4. The findings will guide clinicians, pharmacists, and policymakers in making informed decisions about dispensing, storage, and patient counseling, ultimately promoting cost-effective, safe, and sustainable eye care.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 The Concept and Application of an Antibiogram

Truong and Hidayat (2021) discussed the development and proper use of antibiograms as vital tools for monitoring antimicrobial resistance and guiding empirical therapy. The review emphasized that an antibiogram is a summary of local bacterial isolates and their susceptibility patterns, helping clinicians choose effective antibiotics. They highlighted that accurate data collection, standardized susceptibility testing, and adherence to CLSI guidelines are key to generating reliable results. Their findings stressed that antibiograms should be updated regularly, stratified by patient group or specimen type, and interpreted by both microbiologists and clinicians to ensure relevance. They concluded that when properly developed and used, antibiograms strengthen antimicrobial stewardship programs, improve treatment outcomes, and help track resistance trends in healthcare settings. Furthermore, the paper underscored the importance of educating healthcare workers on proper interpretation to avoid misuse. It was also noted that integrating antibiogram data into hospital policies can enhance infection control practices and promote rational antibiotic use. However, the review also acknowledged certain limitations. Truong and Hidayat noted that antibiograms are inherently retrospective and may not capture rapid changes in resistance patterns, highlighting that variability in laboratory techniques, sampling biases, and incomplete reporting of isolates can affect the reliability of data.

Additionally, they cautioned that antibiograms alone cannot replace clinical judgment and must be interpreted alongside patient-specific factors such as comorbidities, infection severity, and pharmacokinetics. Despite these limitations, the study reinforced that regularly updated and accurately interpreted antibiograms remain essential tools for guiding empirical therapy and supporting antimicrobial stewardship, including in specialized areas such as ophthalmology where local susceptibility patterns may differ.

Similarly, Nilay *et al.,* (2024) conducted a prospective cross-sectional study to evaluate antibacterial drug utilization and establish an antibiogram pattern among patients with infectious diseases. They examined prescription trends, resistance profiles, and the alignment between empirical antibiotic use and laboratory susceptibility data. They found that broad-spectrum antibiotics, particularly cephalosporins and fluoroquinolones, were frequently prescribed empirically before culture confirmation. The resulting antibiogram showed increasing resistance among *Escherichia coli* and *Klebsiella pneumoniae,* indicating reduced susceptibility to several first-line antibiotics. This study revealed a concerning gap between empirical therapy and actual sensitivity results, suggesting that antibiotic selection often overlooked resistance data. Nilay *et al.,* concluded that continuous antibiogram monitoring and its integration into hospital prescribing policies are essential to promote evidence-based therapy, reduce antibiotic misuse, and curb antimicrobial resistance in healthcare systems. Additionally, this work highlighted the importance of updating antibiograms regularly to reflect evolving resistance patterns and to guide clinicians in making more precise antibiotic choices. The authors emphasized that proper use of antibiogram data could improve patient outcomes, reduce unnecessary exposure to broad-spectrum antibiotics, and help limit the emergence of multi-drug resistant organisms. They also suggested that such findings have broader implications for infection control practices,

stewardship programs, and public health policies. Collectively, these insights reinforce the relevance of generating ophthalmic-specific antibiograms to guide appropriate antibacterial eye drop selection, particularly in the face of emerging ocular pathogens.

2.2 Antibacterial Eyedrops and the Need for Antibiogram Evaluation

Ghita *et al.,* (2023) conducted a study in a Romanian ophthalmology clinic to determine the susceptibility of ocular surface bacteria to commonly used antibiotics. The research analyzed conjunctival swabs collected before cataract surgeries to establish local resistance patterns and guide appropriate antibiotic selection for prophylaxis and treatment. The study identified *Staphylococcus epidermidis* and *Staphylococcus aureus* as the predominant bacterial isolates, with notable resistance to beta-lactams such as ampicillin and cefuroxime. However, most isolates remained sensitive to fluoroquinolones and aminoglycosides, including ciprofloxacin, tobramycin, and gentamicin. The findings revealed a concerning trend of increasing resistance among ocular surface flora to several first-line antibiotics, emphasizing the importance of continuous microbial surveillance. The authors concluded that regular antibiogram evaluation of ocular isolates is essential to ensure effective antibiotic use and prevent postoperative infections. They stressed that empirical use of topical antibiotics without updated susceptibility data could lead to treatment failures and contribute to the growing problem of antimicrobial resistance in ophthalmic practice.

Cabrera-Aguas *et al.,* (2024) reviewed the issue of antimicrobial resistance in ocular infections, emphasizing the growing threat it poses to effective eye care. Their study analyzed global and regional trends in bacterial resistance patterns, identifying *Staphylococcus aureus, Staphylococcus epidermidis,* and *Pseudomonas aeruginosa* as the most common and

increasingly resistant ocular pathogens. The review showed that empirical use of topical antibiotics without updated susceptibility data often resulted in reduced treatment success and prolonged infections. It also highlighted how resistance to commonly used ophthalmic antibiotics, including fluoroquinolones and macrolides, has steadily increased due to misuse and over-prescription. The authors concluded that regular antibiogram evaluation of ocular isolates is critical for guiding rational antibiotic selection, optimizing patient outcomes, and curbing antimicrobial resistance in ophthalmic practice.

Teweldemedhin *et al.,* (2017) conducted a hospital-based cross-sectional study at a tertiary care center in Ethiopia to identify bacterial isolates associated with ocular infections and determine their antibiotic susceptibility profiles. The study found *Staphylococcus aureus* and *Pseudomonas aeruginosa* to be the most prevalent pathogens, showing pronounced resistance to β-lactam antibiotics such as ampicillin and amoxicillin–clavulanate, while fluoroquinolones like ciprofloxacin and ofloxacin remained the most effective agents. The authors emphasized the clinical importance of generating localized antibiogram data to guide empirical therapy, reduce inappropriate antibiotic selection, and improve patient outcomes. Importantly, the study demonstrated how variability in bacterial susceptibility can directly influence treatment success, underscoring the need for periodic resistance surveillance in ophthalmic settings. Although the research did not assess the effects of expiration or dilution on antibacterial activity, it highlighted the significance of maintaining drug potency and proper formulation integrity to ensure consistent therapeutic response. The authors also pointed out that poor adherence to antibiotic guidelines and over-the-counter use of ophthalmic antibiotics could contribute to the rising resistance trends observed. Furthermore, they recommended continuous laboratory monitoring and clinician education to ensure rational antibiotic use in eye care. These insights support the

relevance of evaluating how factors such as expiration intervals and dilution could alter the efficacy of antibacterial eye drops, particularly in resource-limited settings where outdated or modified formulations may still be used in clinical practice

The Clinical and Laboratory Standards Institute (2025) provided the standardized reference for antimicrobial susceptibility testing, outlining the interpretive criteria used to classify bacterial isolates as susceptible, intermediate, or resistant. These guidelines form the global benchmark for preparing and interpreting antibiograms, ensuring consistency and reliability across microbiological evaluations. The CLSI manual also specifies standardized methods for disc diffusion, broth dilution, and quality control measures, which are essential for reproducibility and accuracy. In this study, adherence to CLSI standards guarantees that the observed antibacterial activity patterns of the tested eyedrops are scientifically valid, comparable across laboratories, and reflective of true clinical relevance.

2.3 Studies on Drug Expiration and Dilution with Relevance to Ophthalmic Use

Cohen (2000) reported that many medications retain significant potency for years beyond their labeled expiration dates. The article, published in The Wall Street Journal, highlighted both anecdotal and documented evidence indicating that chemical degradation often occurs more slowly than manufacturers' expiration dates suggest. Although the report was not specific to ophthalmic preparations, it provided important insight into the general stability of pharmaceuticals and raised questions about the actual risks associated with using expired medications. Cohen emphasized that while some drugs remain therapeutically effective past expiration, factors such as formulation, storage conditions, and drug type can influence the extent of potency retention. This highlights the lack of ophthalmic-specific data, revealing a gap in

understanding the potency of expired eye drops, which may have direct clinical implications for patient safety and treatment effectiveness. This work supports the rationale for examining expiration effects in ophthalmic preparations, particularly eye drops, where efficacy and sterility are critical for patient safety.

Davido *et al.,* (2024) reviewed the efficacy of expired antibiotics in the context of repeated drug shortages. The study highlighted that, while many antibiotics retain partial activity beyond their labeled expiration dates, the degree of potency loss varied by drug class and formulation. The authors noted that liquid preparations and suspensions generally degraded faster than solid dosage forms, leading to reduced antibacterial activity over time. Although the review focused primarily on systemic antibiotics rather than ophthalmic preparations, it underscored the clinical risks associated with using expired drugs, including potential treatment failure and the promotion of antimicrobial resistance. These findings highlight the need for studies specifically assessing the effect of expiration on ophthalmic antibiotic solutions, which are often in liquid form and may be more susceptible to potency loss. Davido *et al.,* concluded that empirical use of expired antibiotics should be approached with caution and emphasized the importance of systematic testing to verify retained potency. This work supports the rationale for studies that specifically evaluate the efficacy of expired or diluted eye drops.

McBride *et al.,* (1991) investigated the stability of gentamicin sulfate and tobramycin sulfate in extemporaneously prepared ophthalmic solutions stored at 8°C. The study found that both antibiotics largely retained their antibacterial potency during the observation period, though minor reductions were noted with prolonged storage. These findings demonstrated that even freshly prepared ophthalmic solutions can experience gradual decreases in efficacy over time, highlighting the need for monitoring potency, particularly when medications are used beyond

recommended periods. While specific to ophthalmic solutions, the study is somewhat dated, indicating a need for updated research on contemporary formulations and a closer examination of how expiration intervals impact effectiveness in real-world use. The research further emphasized that the method of preparation and the sterility of the compounding process play critical roles in maintaining the efficacy of ophthalmic antibiotics. Any deviations in compounding, storage, or handling could result in variable potency, which may compromise therapeutic outcomes. The study also pointed to the importance of routine microbiological testing to detect potential contamination during storage, particularly for extemporaneously prepared solutions that are intended for multiple doses. However, McBride *et al.,* did not specifically evaluate the effects of expiration dates or dilution on antibacterial potency, leaving a gap relevant to current clinical practice where eye drops may be used past labeled expiration or altered in concentration for specific purposes. This highlights the need for studies that assess how time since manufacture and modification of standard formulations influence the therapeutic effectiveness of ophthalmic antibiotics. Such investigations are particularly important for ensuring patient safety and optimizing treatment outcomes, as even minor reductions in potency could affect clinical efficacy against ocular pathogens. Therefore, these findings provide foundational knowledge on stability but also underscore the necessity of targeted research on expiration and dilution effects in contemporary ophthalmic preparations.

Montes *et al.,* (2016) evaluated the potency and sterility of fortified tobramycin, vancomycin, and moxifloxacin ophthalmic solutions over 14 days at three different temperatures: 4°C, 24°C, and 35°C. The study found that most solutions maintained effective antibacterial activity and sterility throughout the observation period, although slight decreases in potency were noted at higher temperatures. These findings highlighted that even compounded ophthalmic antibiotics

can experience measurable changes in efficacy over time. The authors emphasized that regular monitoring of antibacterial activity and sterility is essential to ensure the therapeutic reliability of ophthalmic preparations. However, the study did not examine the effects of expiration intervals, leaving a gap regarding the real-world effectiveness of ophthalmic antibiotics, particularly in settings where eye drops may be used over extended periods or under variable storage conditions. The authors also noted that slight reductions in potency, if unmonitored, could potentially compromise treatment outcomes, especially in vulnerable populations or in infections with resistant pathogens. Additionally, the study suggested that compounded eye drops require careful handling and storage to maintain quality, emphasizing the importance of clinician awareness and adherence to best practices. These observations highlight the broader need for research on how time since manufacture, storage, and preparation methods influence therapeutic efficacy. Collectively, the study reinforces the rationale for investigating both expiration and dilution effects on ophthalmic drugs to ensure patient safety and optimal clinical outcomes.

Saito Jumpei *et al.,* (2019) investigated the physical, chemical, and microbiological stability of diluted atropine eye drops over a six-month period. The study evaluated different concentrations of atropine prepared from commercially available stock solutions and monitored their stability under standard storage conditions. The results showed that the diluted solutions remained chemically stable, physically intact, and free from microbial contamination throughout the study period. Although the study did not assess antibacterial activity, it highlighted that dilution of ophthalmic preparations can be safely performed without compromising sterility or chemical integrity. Importantly, the findings demonstrated that dilution does not inherently increase the risk of microbial growth, which is critical for patient safety when using modified or compounded eye drops. The study also emphasized the need for careful monitoring of both concentration and

storage conditions to maintain therapeutic reliability. Additionally, it suggested that ophthalmic solutions can be customized in concentration for specific clinical purposes without compromising quality. However, the authors noted that the impact of dilution on pharmacological or antibacterial efficacy remains underexplored, representing a clear gap in knowledge. These insights support the rationale for examining how both expiration and dilution may influence the potency and safety of antibacterial eye drops, particularly in settings where modifications to standard formulations are common.

Hanssens *et al.,* (2018) evaluated the shelf life and efficacy of diagnostic eye drops, assessing how potency and effectiveness changed over time under standard storage conditions. The study analyzed commonly used ophthalmic preparations, measuring their chemical stability and antibacterial activity at different points in their labeled shelf life. Results indicated that while most preparations maintained adequate efficacy throughout the recommended period, some solutions exhibited measurable reductions in potency closer to their expiration dates. The authors highlighted that even small declines in potency could affect diagnostic accuracy or therapeutic outcomes, particularly in sensitive populations. The study also emphasized the importance of following manufacturer guidelines for shelf life and recommended storage conditions to ensure maximal drug effectiveness. These findings underscore the need for routine monitoring of ophthalmic preparations in clinical settings and reinforce the relevance of studying expiration and dilution effects. This work provides strong justification for evaluating how variations in expiration intervals and preparation methods may influence the effectiveness of antibacterial eye drops in the present study.

2.4 Methodological Insights from Previous Studies

Kumar *et al.,* (2025) evaluated the antimicrobial properties of various ophthalmic solutions, including antibiotic eye drops and antiseptic preparations, against common ocular pathogens using the disk diffusion method on agar plates. The study measured zones of inhibition to assess antibacterial activity, providing a clear example of how microbiological assays can reliably determine the potency of ophthalmic solutions. The findings highlighted differences in efficacy among the tested preparations, showing that some solutions were more effective against specific pathogens than others. While the research focused on in vitro potency assessment, it did not examine factors such as dilution, expiration intervals, or long-term storage conditions, leaving these areas unexplored. Nonetheless, the study provides a practical reference for conducting disc diffusion experiments, which aligns with the approach applied in the present study to evaluate selected antibacterial eye drops. These observations reinforce the need for further research to investigate how real-world variables like expiration and dilution may influence the clinical effectiveness of ophthalmic antibiotics.

Caruso *et al.,* (2022) conducted a comparative evaluation of six commercially available antiseptic ophthalmic formulations to determine their antimicrobial, antiamoebic, and antiviral efficacy. The study employed disk diffusion and minimum inhibitory concentration (MIC) methods to assess the activity of each formulation against Gram-positive bacteria, Gram-negative bacteria, Acanthamoeba castellanii, and two respiratory viruses (HAdV-2 and HCoV-OC43). Results indicated that the formulations varied in potency, with certain products demonstrating broad-spectrum antimicrobial effects. While the study provided valuable insights into the comparative efficacy of ophthalmic antiseptics and demonstrated the utility of in vitro assays such as disk diffusion and MIC for evaluating ocular preparations, it did not examine standard antibacterial eye drops, nor did it investigate the effects of expiration, dilution, or storage

conditions on potency. These limitations highlight a gap in understanding how clinically used antibacterial ophthalmic solutions may be influenced by such variables. The findings nonetheless underscore the importance of assessing antibacterial activity under controlled conditions, offering a methodological precedent for evaluating eye drops. Consequently, this study supports the rationale for research specifically targeting the efficacy of antibacterial eye drops under varying expiration intervals and dilution levels, particularly in settings where ensuring potency and sterility is critical for patient safety.

2.5 Microbial Contamination of Ophthalmic Preparations

Tsegaw *et al.,* (2017) investigated bacterial contamination of multi-dose eye drops at the Department of Ophthalmology, University of Gondar Teaching Hospital. The study collected samples from commonly used multi-dose ophthalmic preparations and assessed microbial growth using standard microbiological techniques. Results showed that a notable proportion of samples were contaminated, with organisms including *Staphylococcus aureus, Pseudomonas* species, and other opportunistic bacteria. The findings highlighted the potential risk of infection associated with repeated use of multi-dose eye drops, particularly in settings where proper handling and storage practices may be inconsistent. The study further emphasized that contamination could compromise both patient safety and the therapeutic effectiveness of eye drops, especially when used over extended periods or by multiple users. Tsegaw *et al.,* noted that proper aseptic techniques, adherence to expiration dates, and careful storage are essential to minimize microbial risks. The authors acknowledged limitations including a relatively small sample size, collection from a single hospital, and lack of investigation into the influence of storage duration or

environmental conditions on contamination rates. Despite these limitations, the work highlighted a critical gap in routine sterility monitoring and quality assurance for multi-dose ophthalmic preparations. These results underline the importance of evaluating both sterility and antibacterial efficacy in eye drops and support the rationale for examining how expiration and dilution may affect their safety and effectiveness in real-world clinical settings.

Nentwich *et al.,* (2007) examined microbial contamination of multi-use ophthalmic solutions in Kenya. The study sampled commonly used eye drops from hospital and clinic settings and assessed bacterial growth using standard microbiological methods. Findings revealed that several preparations were contaminated with organisms such as *Staphylococcus species* and *Pseudomonas aeruginosa*, indicating that repeated use of multi-use eye drops can pose a significant risk of infection. The study highlighted the importance of proper handling, storage, and monitoring of ophthalmic solutions to maintain sterility. The authors further noted that contamination rates varied depending on the type of preparation and handling practices, suggesting that user behavior and clinic protocols significantly influence microbial risk. Limitations of the study included a relatively small number of samples, collection from a limited number of healthcare facilities, and lack of evaluation of the effect of storage duration or environmental conditions on contamination. Despite these limitations, the findings underscore the broader issue of sterility in multi-use ophthalmic products, particularly in low-resource settings where routine monitoring may be lacking. The study emphasizes the need to assess both potency and sterility in eye drops, providing a strong rationale for investigations into the effects of expiration and dilution on the safety and efficacy of ophthalmic preparations.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 RESEARCH DESIGN

This study was done using an experimental laboratory-based study design.

3.2 RESEARCH LOCATION

The experimental procedures were carried out in the Microbiology Laboratory of the Department of Microbiology, University of Benin, Ugbowo, Benin City, Edo State.

3.3 STUDY POPULATION

The study population consisted of selected antibacterial ophthalmic medications used in eye care which included moxifloxacin, gentamicin, chloramphenicol and ciprofloxacin.

3.4 STUDY PERIOD

This study was carried out for a duration of 2 months.

3.5 SAMPLING TECHNIQUE

This study employed a purposive sampling technique, based on availability and expiration date variation.

3.6 RESEARCH MATERIALS

Selected antibacterial agents commonly used in optometric practice (Moxifloxacin, Chloramphenicol, Ciprofloxacin, and Gentamicin) at varying expiration dates:

1. Greater than equal to one year,
2. Greater than equal to six months to less than equal to one year,
3. Greater than equal to three months to less than equal than equal to six months
4. Expired less than six months

Nutrient agar, Mueller-Hinton agar

Bacterial isolates obtained from ocular swabs of infected patients (*S. aureus*, *P. aeruginosa*, *E. coli*, *K. pneumoniae*).

Sterile filter paper discs

Sterile cotton swabs

Sterile inoculating loops

Sterile corkborer

Markers

Disposable pipettes

Universal sterile bottle

Incubator, autoclave,

Sterile Petri dishes

Calipers/ruler for measuring zones of inhibition

Personal Protective Equipment (PPE): Gloves, lab coats, masks, and safety goggles for safe laboratory practices

Data Recording Materials: notebooks, data recording sheets, biro.

3.7 INCLUSION AND EXCLUSION CRITERIA

3.7.1 INCLUSION CRITERIA

1. Antibacterial Eye Drops must be commercially available and approved for ophthalmic use with clear expiration labeling

2. Eye drop samples from the same brand for each active ingredient to ensure consistency.

3. Sealed and unopened antibacterial eye drops to maintain sterility before testing.

4. Viable clinical bacterial strains known to be implicated in ocular infections and maintained under standard laboratory conditions

3.7. 2 EXCLUSION CRITERIA

1. Eye drops with unclear labeling or damaged packaging as they may have been contaminated or degraded.

2. Brands of eye drops with unknown manufacturing or expiration dates.

3. Eye drops with combination therapies (containing both antibacterial and corticosteroid agents), to avoid confounding results.

4. Bacterial isolates that are not commonly associated with ocular infections or that cannot be reliably cultured under standard lab conditions.

5. Preservative-free eye drops (due to risk of contamination)

3.8 ETHICAL CONSIDERATION

1.This study involved no human or animal subjects. All materials used were commercially available and were handled under bio safety guidelines. Ethical approval to conduct this study was obtained from the Research and Ethics Committee of the Faculty of Optometry, University of Benin.

2. All microbial agents and eye drops were handled using proper bio safety measures to prevent infection or contamination.

3. Bacterial cultures were disposed according to biohazard regulations to avoid environmental contamination.

4. Transparency in data collection, analysis, and reporting was maintained. All forms of fabrication, falsification, or plagiarism was avoided.

5. Neutrality and avoidance of bias among brands of eye drops used unless supported by scientific data.

3. 9 DESCRIPTION OF PROCEDURE

3.9.1 AGAR WELL DIFFUSION METHOD

Preparation of Samples:

All the samples were prepared based on the concentration, that is the neat (10 mL) and the diluted (0.25 mL).

Preparation of Media:

Thirty-seven grams (37g) of Mueller Hinton agar was weighed, prepared, and sterilized using an autoclave at a temperature of 121°C for 15 minutes.

Preparation of Bacterial Isolates:

All the 24-hour cultures were inoculated on nutrient broth for resuscitation.

Procedure:

Sterilized Mueller Hinton agar (molten) was poured into sterilized Petri dishes and allowed to solidify. After solidification, test bacterial isolates were streaked on the agar. Then, a sterilized corkborer was used to bore holes on the agar plates, and they were labelled accordingly. In one hole, a sterile pipette was used to transfer 0.5 mL of the 10 mL neat sample concentration into one hole, while another sterile pipette was used to transfer 0.5 mL from the 0.25 mL (diluted) concentration into another hole. All the plates were allowed to stand for 2–4 hours for proper diffusion.

After this, all the plates were incubated at a temperature of 37°C for 24 hours, after which the zones of inhibition were measured using a graduated measuring ruler (in millimetres). The minimum zone value was taken as the Minimum Inhibitory Concentration (M.I.C.).

3.9.2 ANTIBACTEIAL ACTIVITY OF BACTERIAL ISOLATES

DISK DIFFUSION METHOD

Procedure:

37 gramme of mueller hinton agar was prepared, sterilized and allowed to cool, after which they were poured on sterilized petri dishes and allowed to solidify.

Upon solidification the test isolate *Staphylococcus aureus, Escherichia coli, klebsiella, and pseudomonas aeruginosa* were streaked on the agar plates

After this, a sterilized forceps was used to pick antibiotic disc and place on top of each agar plates firmly without pressing

All the plates were incubated at a temperature of 37°C for 24 hours after which zone of inhibition was measured using a graduated ruler in Millimeter (mm)

The multiple antibiotic Resistance Index was taken as the number of bacterial resistance divided by the total number antibiotic used.

That is: % MAR index = No of bacteria resistance to antibiotics / total number of antibiotics used X 100/1

3.10 LIMITATION OF THE STUDY

1. Time Frame: The study was conducted within a period of less than three months, which limited the scope for more extensive data collection and analysis.

2. Availability of Materials: There was limited access to antibacterial eyedrops with varying expiration dates, restricting the range of samples that could be evaluated.

3. Brand Consistency: To avoid variability in results, the study focused on a single brand of eyedrops. This approach, however, made sourcing sufficient samples more challenging.

3.11 DATA ANALYSIS:

1. Data has been presented in tables for easy visualization and comparison.

2. Data collected from experiment was entered into Microsoft Excel 2016 spreadsheet. 3. The Statistical Package for the Social Sciences (SPSS), Version 25.0 (IBM Corp., Armonk, New York, USA), was then used to conduct statistical analysis. Descriptive statistics including frequency, mean, standard deviation, and percentages were utilized to analyze the data. Also, inferential analysis was performed to test relationships. To provide a visual representation of the data, the findings were presented using tables. These tools were also employed to examine associations between expiration interval, single dilution and antimicrobial efficacy.

CHAPTER 4

RESULTS

TABLE 4.1: Bacterial Isolates Used in Study (3 gram-negative and 1 gram-positive)

Bacteria Isolates	Gram Staining
Escherichia coli	Gram-negative
Klebsiella pnemoniae	Gram-negative
Psuedomonas aeruginosa	Gram-negative
Staphylococcus aureus	Gram-positive

Value for sensitivity; ≥ 10mm

Value for resistance; ≤ 10mm

TABLE 4.2: **Mean Inhibitory Zones (mm) of 10 Commercially Available Reference Antibiotics Against Three Gram-Negative Bacterial Isolates**

S/N	**Antibiotic**	***Escherichia coli* (Mean Inhibitory Zone)**	***Pseudomonas aeruginosa* (Mean Inhibitory Zone)**	***Klebsiella pneumoniae* (Mean Inhibitory Zone)**
1	Augmentin (AU)	0	0	0
2	Cefepime (CEP)	11	0	0
3	Ceftazidime (CTC)	15	15	13
4	Ceftriaxone (TRX)	12	0	0
5	Cefuroxime (CEF)	13	0	13
6	Ciprofloxacin (CPX)	14	0	11
7	Gentamicin (CN)	15	13	12
8	Ofloxacin (OFX)	0	14	12
9	Pefloxacin (PEF)	0	11	15
10	Streptomycin (S)	13	11	13

Augmentin (AU/AUG) was found to be completely ineffective, with a mean inhibitory zone of 0 against all three species. Against the Gram-negative isolates, Ceftazidime (CTC) and Gentamicin (CN) showed the largest zones against *E. coli* (mean = 15mm). *P. aeruginosa* demonstrated significant resistance; it was inhibited by only five of the antibiotics tested and was notably

resistant (mean = 0) to Cefepime, Ceftriaxone, and Ciprofloxacin. *K. pneumoniae* was most susceptible to Pefloxacin (PEF) (mean = 15mm) and resistant to three other antibiotics.

TABLE 4.3: Mean Inhibitory Zones (mm) of 8 Commercially Available Reference Antibiotics Against One Gram-Positive Bacterial Isolate

S/N	**Antibiotic**	***Staphylococcus aureus* (Mean Inhibitory Zone)**
1	Augmentin (AUG)	0
2	Ceftazianine (CAZ)	10
3	Cefuroxime (CRX)	0
4	Cefixime (CXC)	18
5	Erythromycin (ERY)	0
6	Ofloxacin (OFL)	18
7	Gentamicin (GEN)	0
8	Ceftriaxone (CTR)	0

Augmentin (AU/AUG) was found to be completely ineffective, with a mean inhibitory zone of 0 against *S. aureus.* Cefixime (CXC) and Ofloxacin (OFL) were most effective, both producing a mean inhibitory zone of 18mm. *S. aureus w*as resistant (mean = 0) to five antibiotics, including Augmentin, Ceftriaxone, erythromycin, cefuroxime and Gentamicin.

TABLE 4.4: *in vitro* Antibacterial Activity of CIPROTAB Eyedrop (≥ 1 year)

Sample	**Sensitivity**	***Escherichia coli***	***Klebsiella pnemoniae***	***Psuedomonas aeruginosa***	***Staphylococcus aureus***
Undiluted Eyedrops	Resistant	No	Yes	Yes	Yes
Undiluted Eyedrops	Sensitive	Yes	No	No	No
Diluted Eyedrops	Resistant	No	Yes	Yes	Yes
Diluted Eyedrops	Sensitive	Yes	No	No	No

CIPROTAB eyedrops aged ≥1 year demonstrated selective antibacterial activity. Both diluted and undiluted samples were found to be active against *Escherichia coli*, and showed resistance to *Klebsiella pnemoniae*, *Psuedomonas aeruginosa*, and *Staphylococcus aureus*.

TABLE 4.5: *in vitro* Antibacterial Activity of CIPROTAB Eyedrop (≥ 6 Months ≤ 1 Year)

Sample	**Sensitivity**	***Escherichia coli***	***Klebsiella pnemoniae***	***Psuedomonas aeruginosa***	***Staphylococcus aureus***
Undiluted Eyedrops	Resistant	No	No	Yes	No
Undiluted Eyedrops	Sensitive	Yes	Yes	No	Yes
Diluted Eyedrops	Resistant	No	No	Yes	No
Diluted Eyedrops	Sensitive	Yes	Yes	No	Yes

The samples showed sensitivity to *E. coli, K. pnemoniae, and S. aureus.* Resistance was recorded only against *P. aeruginosa.*

TABLE 4.6: *in vitro* Antibacterial Activity of CIPROTAB Eyedrop (≥ 3 Months ≤ 6 Months)

Sample	**Sensitivity**	***Escherichia coli***	***Klebsiella pnemoniae***	***Psuedomonas aeruginosa***	***Staphylococcus aureus***
Undiluted Eyedrops	Resistant	No	No	Yes	No
Undiluted Eyedrops	Sensitive	Yes	Yes	No	Yes
Diluted Eyedrops	Resistant	No	No	Yes	No
Diluted Eyedrops	Sensitive	Yes	Yes	No	Yes

For both diluted and undiluted samples, sensitivity was observed against *E. coli, K. pnemoniae, and S. aureus.* In contrast, resistance was observed against *P. aeruginosa.*

Table 4.7: in vitro Antibacterial Activity of MOXIFLOXACIN Eyedrop (≥ 1 year)

Sample	**Sensitivity**	***Escherichia coli***	***Klebsiella pnemoniae***	***Psuedomonas aeruginosa***	***Staphylococcus aureus***
Undiluted Eyedrops	Resistant	Yes	No	No	No
Undiluted Eyedrops	Sensitive	No	Yes	Yes	Yes
Diluted Eyedrops	Resistant	Yes	No	Yes	No
Diluted Eyedrops	Sensitive	No	Yes	No	Yes

The activity of MOXIFLOXACIN eyedrops aged ≥ 1 year varied by dilution. The undiluted sample was active against *K. pnemoniae, P. aeruginosa, and S. aureus,* but resistant to *E. coli*. The diluted sample was active against *K. pnemoniae* and *S. aureus,* but showed no effect against *E. coli and P. aeruginosa.*

Table 4.8: *in vitro* Antibacterial Activity of MOXIFLOXACIN Eyedrop (≥ 3 Months ≤ 6 Months)

Sample	**Sensitivity**	***Escherichia coli***	***Klebsiella pnemoniae***	***Psuedomonas aeruginosa***	***Staphylococcus aureus***
Undiluted Eyedrops	Resistant	No	No	No	No
Undiluted Eyedrops	Sensitive	Yes	Yes	Yes	Yes
Diluted Eyedrops	Resistant	No	No	No	No
Diluted Eyedrops	Sensitive	Yes	Yes	Yes	Yes

MOXIFLOXACIN eyedrops aged ≥ 3 Months ≤ 6 Months showed broad-spectrum activity. Both diluted and undiluted samples were found to be active against all four bacterial isolates tested: *E. coli, K. pnemoniae, P. aeruginosa, and S. aureus.* No resistance was observed for any isolate.

Table 4.9: *in vitro* Antibacterial Activity of GENTAMYCIN Eyedrop (≥ 1 year)

Sample	**Sensitivity**	***Escherichia coli***	***Klebsiella pnemoniae***	***Psuedomonas aeruginosa***	***Staphylococcus aureus***
Undiluted Eyedrops	Resistant	Yes	No	Yes	Yes
Undiluted Eyedrops	Sensitive	No	Yes	No	No
Diluted Eyedrops	Resistant	Yes	No	Yes	Yes
Diluted Eyedrops	Sensitive	No	Yes	No	No

The results for GENTAMYCIN eyedrops aged ≥ 1 year, were consistent for diluted and undiluted samples. The eyedrops were active only against *K. pnemoniae* and showed no activity against to *E. coli, P. aeruginosa, and S. aureus.*

Table 4.10: *in vitro* Antibacterial Activity of GENTAMYCIN Eyedrop (≥ 6 Months ≤ 1 Year)

Sample	**Sensitivity**	***Escherichia coli***	***Klebsiella pnemoniae***	***Psuedomonas aeruginosa***	***Staphylococcus aureus***
Undiluted Eyedrops	Resistant	No	No	Yes	Yes
Undiluted Eyedrops	Sensitive	Yes	Yes	No	No
Diluted Eyedrops	Resistant	No	No	Yes	Yes
Diluted Eyedrops	Sensitive	Yes	Yes	No	No

GENTAMYCIN eyedrops (≥ 6 Months ≤ 1 Year) at both diluted and undiluted samples were active against *E. coli and K. pnemoniae*, while *P. aeruginosa and S. aureus* were resistant.

Table 4.11: *in vitro* Antibacterial Activity of CHLORAMPHENICOL Eyedrop (≥ 6 Months ≤ 1 Year)

Sample	**Sensitivity**	***Escherichia coli***	***Klebsiella pnemoniae***	***Psuedomonas aeruginosa***	***Staphylococcus aureus***
Undiluted Eyedrops	Resistant	No	No	Yes	Yes
Undiluted Eyedrops	Sensitive	Yes	Yes	No	No
Diluted Eyedrops	Resistant	Yes	Yes	Yes	Yes
Diluted Eyedrops	Sensitive	No	No	No	No

The antibacterial activity of the CHLORAMPHENICOL eyedrops was dependent on dilution. The undiluted sample was active against *E. coli and K. pnemoniae* but showed no activity against *P. aeruginosa and S. aureus.* In contrast, the diluted sample was resistant to all four bacterial isolates, showing no activity.

Table 4.12: *in vitro* Antibacterial Activity of CHLORAMPHENICOL Eyedrop (Expired ≤ 6 Months)

Sample	**Sensitivity**	***Escherichia coli***	***Klebsiella pnemoniae***	***Psuedomonas aeruginosa***	***Staphylococcus aureus***
Undilduted Eyedrops	Resistant	Yes	No	No	No
Undiluted Eyedrops	Sensitive	No	Yes	Yes	Yes
Diluted Eyedrops	Resistant	Yes	No	No	No
Diluted Eyedrops	Sensitive	No	Yes	Yes	Yes

Both diluted and undiluted samples were active against *K. pnemoniae, P. aeruginosa, and S. aureus*. Only *E. coli* showed resistance.

TABLE 4.13: Mean Inhibitory Zones (mm) of 13 Reference Antibiotics Against Four Bacterial Isolates (3 gram-negative and 1 gram-positive)

Antibiotic	***Escherichia coli* (Mean Inhibitory Zone)**	**Pseudomonas aeruginosa (Mean Inhibitory Zone)**	***Klebsiella pneumoniae* (Mean Inhibitory Zone)**	***Staphylococcus aureus* (Mean Inhibitory Zone)**
Augmentin (AU/AUG)	0	0	0	0
Cefepime (CEP)	11	0	0	-
Ceftazidime (CTC)	15	15	13	-
Ceftazianine (CAZ)	-	-	-	10
Ceftriaxone (TRX/CTR)	12	0	0	0
Cefuroxime (CEF/CRX)	13	0	13	0
Cefixime (CXC)	-	-	-	18
Ciprofloxacin	14	0	11	-

(CPX)				
Gentamicin (CN/GEN)	15	13	12	0
Ofloxacin (OFX/OFL)	0	14	12	18
Pefloxacin (PEF)	0	11	15	-
Streptomycin (S)	13	11	13	-
Erythromycin (ERY)	-	-	-	0

Augmentin (AU/AUG) was found to be completely ineffective, with a mean inhibitory zone of 0 against all four species. Against the Gram-negative isolates, Ceftazidime (CTC) and Gentamicin (CN) showed the largest zones against *E. coli* (mean = 15mm). *P. aeruginosa* demonstrated significant resistance; it was inhibited by only five of the antibiotics tested and was notably resistant (mean = 0) to Cefepime, Ceftriaxone, and Ciprofloxacin. *K. pneumoniae* was most susceptible to Pefloxacin (PEF) (mean = 15mm) and resistant to three other antibiotics. For the Gram-positive *S. aureus*, Cefixime (CXC) and Ofloxacin (OFL) were most effective, both producing a mean inhibitory zone of 18. *S. aureus* was resistant (mean = 0) to five antibiotics, including Augmentin, Ceftriaxone, and Gentamicin.

TABLE 4.14: Mean Inhibitory Zones (mm) of Neat (undiluted) and Diluted Chloramphenicol Eyedrops by Expiration Status

Time Period	**Bacteria Isolates**	**Diameter of Zone of Inhibition of Undiluted Eye drop (Mean)**	**Diameter of Zone of Inhibition of Diluted Eye drop (Mean)**
≥ 6 Months ≤ 1 Year	*Escherichia coli*	14	0
≥ 6 Months ≤ 1 Year	*Klebsiella pnemoniae*	12	0
≥ 6 Months ≤ 1 Year	*Psuedomonas aeruginosa*	0	0
≥ 6 Months ≤ 1 Year	*Staphylococcus aureus*		0
Expired ≤ 6 Months	*Escherichia coli*	0	0
Expired ≤ 6 Months	*Klebsiella pnemoniae*	18	18
Expired ≤ 6 Months	*Psuedomonas aeruginosa*	14	18
Expired ≤ 6 Months	*Staphylococcus aureus*	12	10

The Chloramphenicol eyedrop's activity was highly dependent on its expiry status. The in-date sample (≥ 6 Months ≤ 1 Year until expiry) was active against *E. coli* (mean = 14) and *K. pneumoniae* (mean = 12) when undiluted, but this activity was completely lost upon dilution

(mean = 0 for all). In contrast, the sample that was expired by up to 6 months showed no activity against *E. coli* but was active against *K. pneumoniae* (mean = 18), *P. aeruginosa* (mean = 14-18mm), and *S. aureus* (mean = 10-12mm).

TABLE 4.15: Mean Inhibitory Zones (mm) of Neat (undiluted) and Diluted Ciprotab Eyedrops by Expiration Status

Time Period	**Bacteria Isolates**	**Diameter of Zone of Inhibition of Undiluted Eye drop (Mean)**	**Diameter of Zone of Inhibition of Diluted Eye drop (Mean)**
≥ 1 year	*Escherichia coli*	20	20
≥ 1 year	*Klebsiella pnemoniae*	0	0
≥ 1 year	*Psuedomonas aeruginosa*	0	0
≥ 1 year	*Staphylococcus aureus*	0	0
≥ 3 Months ≤ 6 Months	*Klebsiella pnemoniae*	12	10
≥ 3 Months ≤ 6 Months	*Psuedomonas*	0	0

	aeruginosa		
≥ 3 Months ≤ 6 Months	*Staphylococcus aureus*	14	10
≥ 3 Months ≤ 7 Months	*Escherichia coli*	18	18
≥ 6 Months ≤ 1 Year	*Escherichia coli*	14	10
≥ 6 Months ≤ 1 Year	*Klebsiella pnemoniae*	25	25
≥ 6 Months ≤ 1 Year	*Psuedomonas aeruginosa*	0	0
≥ 6 Months ≤ 1 Year	*Staphylococcus aureus*	14	14

For Ciprotab (Ciprofloxacin) eyedrop, the most consistent finding was the complete resistance of *P. aeruginosa* (mean = 0) to all samples, regardless of expiry status or dilution. The sample with 6-12 months until expiry showed the broadest spectrum, inhibiting *E. coli*, *K. pneumoniae*, and *S. aureus*. The sample with ≥ 1 year until expiry was only active against *E. coli*.

TABLE 4.16: Mean Inhibitory Zones (mm) of Neat (undiluted) and Diluted Gentamicin Eyedrops by Expiration Status

Time Period	**Bacteria Isolates**	**Diameter of Zone of Inhibition of Undiluted Eye drop (Mean)**	**Diameter of Zone of Inhibition of Diluted Eye drop (Mean)**
≥ 1 year	*Escherichia coli*	0	0
≥ 1 year	*Klebsiella pnemoniae*	10	10
≥ 1 year	*Psuedomonas aeruginosa*	0	0
≥ 1 year	*Staphylococcus aureus*	0	0
≥ 6 Months ≤ 1 Year	*Escherichia coli*	14	16
≥ 6 Months ≤ 1 Year	*Klebsiella pnemoniae*	14	12
≥ 6 Months ≤ 1 Year	*Psuedomonas aeruginosa*	0	0
≥ 6 Months ≤ 1 Year	*Staphylococcus aureus*	0	0

All tested samples, both those with ≥ 1 year and 6-12 months until expiry, were completely ineffective (mean = 0) against *P. aeruginosa* and *S. aureus*. The drops did, however, retain activity against *E. coli* and *K. pneumoniae*.

TABLE 4.17: Mean Inhibitory Zones (mm) of Neat (undiluted) and Diluted Moxifloxacin Eyedrops by Expiration Status

Time Period	**Bacteria Isolates**	**Diameter of Zone of Inhibition of Undiluted Eye drop (Mean)**	**Diameter of Zone of Inhibition of Diluted Eye drop (Mean)**
≥ 1 year	*Escherichia coli*	0	0
≥ 1 year	*Klebsiella pnemoniae*	14	14
≥ 1 year	*Psuedomonas aeruginosa*	12	0
≥ 1 year	*Staphylococcus aureus*	10	10
≥ 3 Months ≤ 6 Months	*Escherichia coli*	18	14
≥ 3 Months ≤ 6 Months	*Klebsiella pnemoniae*	14	12
≥ 3 Months ≤ 6 Months	*Psuedomonas aeruginosa*	14	12
≥ 3 Months ≤ 6 Months	*Staphylococcus aureus*	14	12

The Moxifloxacin eyedrop demonstrated broad-spectrum activity. The sample with 3-6 months until expiry was effective against all four bacterial isolates. The sample with ≥ 1 year until expiry was inactive against *E. coli* (mean = 0) and lost its activity against *P. aeruginosa* when diluted.

Comparison of Antibiotic Disc and Eyedrop Findings

The reference Gentamicin disc (CN) was effective against *Pseudomonas aeruginosa*, producing a mean inhibitory zone of 13mm. However, the Gentamicin eyedrop formulation was completely ineffective (mean = 0) against *P. aeruginosa* in all samples tested, regardless of its expiry status. A point of consistency for Gentamicin was its ineffectiveness against *Staphylococcus aureus* (mean = 0) in both the reference disc and all eyedrop tests.

In direct contrast to the Gentamicin finding, the Ciprofloxacin (CPX) and Ciprotab data showed a major consistency. Both the reference Ciprofloxacin disc and all Ciprotab eyedrop formulations were completely ineffective (mean = 0) against *Pseudomonas aeruginosa*. This consistent result across both testing methods strongly supports the finding that this particular *P. aeruginosa* isolate possesses a high level of inherent resistance to Ciprofloxacin.

A final comparison, within the fluoroquinolone class, highlights a key difference in spectrum. The reference Ofloxacin (OFX) disc was found to be ineffective (mean = 0) against *Escherichia coli*. However, the Moxifloxacin eyedrop, a different fluoroquinolone, was highly effective against *E. coli* (mean = 18mm and 14mm) in both neat and diluted sample with less than 6 months until expiry.

CHAPTER FIVE

5.0 DISCUSSION

This study assessed the in vitro antibacterial activity of four ophthalmic antibiotics namely: Ciprofloxacin (Ciprotab), Moxifloxacin, Gentamicin, and Chloramphenicol, tested at varying expiration intervals and at a single dilution level. Their activity was evaluated against *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Staphylococcus aureus* isolated from infected eyes.

Moxifloxacin in both neat and diluted concentrations showed the broadest activity against all tested bacteria isolates. This was followed by Ciprotab eyedrops in both neat and diluted concentrations. Diluted chloramphenicol greater than one year before expiration showed no activity against all tested bacteria isolates. This was followed by diluted Moxifloxacin at more than one year to expiration showing activity only against *K. pnemoniae* and *S. aureus*.

Gentamycin greater than one year to expiration showed the least activity in both neat and diluted concentrations, active against only *K. pnemoniae*.

5.1 Reference Antibiotic Activity pattern to test organisms

The results from Tables 4.2 and 4.3 show distinct variations in the antibacterial activity of reference antibiotics against both Gram-negative and Gram-positive bacterial isolates. The Gram-negative bacteria, which included *Escherichia coli, Pseudomonas aeruginosa,* and *Klebsiella pneumoniae,* showed the highest sensitivity to Ceftazidime (13-15mm), followed by Gentamicin (12-15mm), while showing complete resistance to Augmentin (0). The Gram-

positive bacterium *Staphylococcus aureus* showed the highest sensitivity to Cefixime (18 mm) and Ofloxacin (18 mm). The lowest sensitivity was shown against ceftazianine (10mm).

These findings agree with Teweldemedhin *et al.,* (2017), who reported that aminoglycosides and fluoroquinolones retain good activity *against E. coli, K. pneumoniae,* and *P. aeruginosa* ocular isolates, whereas many β-lactams show limited effect. Similarly, Ghita *et al.,* (2023) found that *S. aureus* retained susceptibility to fluoroquinolones and certain cephalosporins but exhibited resistance to many β-lactams and macrolides, consistent with the present results. The broader Gram-negative susceptibility also aligns with Cabrera-Aguas et al. (2024), who reviewed global trends and reported preserved activity of aminoglycosides and fluoroquinolones against common ocular pathogens.

However, the high resistance of *S. aureus* to Gentamicin observed here contrasts with Davido *et al.,* (2024), who reported moderate susceptibility of ocular *S. aureus* isolates to aminoglycosides. This difference may be due to geographic variation or local strain differences. Likewise, the selective resistance of *P. aeruginosa* matches expected intrinsic mechanisms, such as efflux pumps and outer membrane barriers, consistent with global reports (Cabrera-Aguas *et al.,* 2024).

This finding underscores the importance of continuous surveillance of antibiotic susceptibility patterns to guide rational antibiotic use and minimize resistance development.

5.2 The Anti-microbial Efficacy of Selected Antibacterial across varying expiration date intervals

5.2.1 CIPROTAB

Tables 4.4, 4.5, and 4.6 show that the antimicrobial efficacy of CIPROTAB eyedrops varied across different pre-expiration intervals. Samples less than equal to one year before expiration showed the highest activity against *Escherichia coli, Klebsiella pneumoniae, and Staphylococcus aureus,* (14-25mm) but showed no activity against *Pseudomonas aeruginosa.* In contrast, samples tested more than one year before expiration showed activity only against *E. coli* (20mm). These findings agree with Kumar *et al.,* (2025) and Teweldemedhin *et al.,* (2017), which show that fluoroquinolones like ciprofloxacin retain activity against common ocular Gram-negative bacteria, while resistance in *Pseudomonas aeruginosa* is frequently observed. The reduced efficacy in older samples may reflect batch variability, storage conditions, or intrinsic bacterial resistance (Davido *et al.,* 2024).

5.2.2 MOXIFLOXACIN

Tables 4.7 and 4.8 show that Moxifloxacin tested less than equal 6 months before expiration showed the highest activity with no resistance across all bacterial isolates. In contrast, samples with greater than or equal to one year to expiration showed activity against *Klebsiella pneumoniae, Staphylococcus aureus, and Pseudomonas aeruginosa*, and no activity against *Escherichia coli*. The diluted sample demonstrated activity against *K. pneumoniae and S. aureus* only, indicating slight variations in response between the neat and undiluted forms. These findings suggest that the antimicrobial efficacy of Moxifloxacin eye drops remains generally stable within their shelf life, although minor fluctuations in bacterial sensitivity may occur

depending on the remaining time to expiration and sample concentration. This pattern corresponds with Montes *et al.,* (2016) and Kumar *et al.,* (2025), which indicate that moxifloxacin retains broad-spectrum antibacterial activity within its shelf life. The observed reduction in efficacy for older or diluted samples aligns with CLSI (2025) guidance that lowering drug concentration can drop levels below the MIC for some organisms.

5.2.3 GENTAMICIN

Tables 4.9 and 4.10 show that Gentamicin eyedrops with expiration date less than equal to one year showed the highest activity at both neat and diluted concentration against *Klebsiella pneumoniae* and *Escherichia coli,* but no activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Samples with more than one year to expiration showed no activity against *Staphylococcus aureus, Escherichia coli,* and *Pseudomonas aeruginosa* in both neat and diluted forms, while remaining sensitive only to *Klebsiella pneumoniae.* The reduced sensitivity observed in samples with longer remaining shelf life may reflect batch variability or formulation factors rather than true loss of potency over time. These results agree with Teweldemedhin *et al.,* (2017) and Ghita *et al.,* (2023), who reported Gentamicin's retained activity against many Gram-negative ocular pathogens. The reduced spectrum observed in sample 4.9 also partially aligns with Davido *et al.,* (2024), emphasizing that product composition and bacterial resistance can influence efficacy independently of expiration interval.

5.2.4 CHLORAMPHENICOL

Table 4.11 and 4.12 shows that Chloramphenicol eyedrops within the expiration range of less than equal to one year showed antibacterial activity in the neat samples, active against *Klebsiella pneumoniae* and *Escherichia coli*, and no activity against *Staphylococcus aureus* and

Pseudomonas aeruginosa. However, when diluted, Chloramphenicol showed no activity against all tested bacterial isolates (0). In contrast, the expired chloramphenicol eyedrops (less than 6 months past expiration) were active against *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* at both neat and diluted concentration while showing no activity against *E. coli.* This supports the findings of Cohen (2000), who reported that many medicine can retain potency beyond their labeled expiration dates. Hanssens *et al.,* (2018) further support expectation of minor declines near expiration, depending on formulation and storage.

5.3 Determining and comparing the in vitro antibacterial activity of selected commercial antibacterial eyedrops at their labelled (neat) strength and at a diluted concentration of 25 mg/mL.

5.3.1 CIPROTAB (≥1 year)

Table 4.4 shows that shows that CIPROTAB eyedrops greater than one year to expiration showed antibacterial activity against *Escherichia coli* only in both the neat and diluted preparations while no activity was observed against *Pseudomonas aeruginosa, K. pnemoniae* and *S. aureus.* The identical sensitivity pattern in both neat and diluted samples suggests that the active concentration of ciprofloxacin in this sample remained above the minimum inhibitory concentration (MIC) for the tested organism, indicating sustained antimicrobial potency. These findings agree with Kumar *et al.,* (2025) and CLSI (2025), which indicate that when the starting concentration of fluoroquinolones is well above MIC, moderate dilution may not affect detectable antibacterial activity. Reduced activity against other organisms reflects intrinsic resistance rather than concentration effects

5.3.2 CIPROTAB (≥ 6 months ≤ 1 Year)

Table 4.5 CIPROTAB eyedrops less than equal to one year showed antibacterial activity against *Klebsiella pneumoniae, E. col*i and *Staphylococcus aureus* at both neat and diluted preparations while there was no effect against *Pseudomonas aeruginosa.* Ciprotab greater than one year to expiration showed the highest activity against *Escherichia coli* at both neat and undiluted concentrations. This aligns with Teweldemedhin *et al.,* (2017) and Montes *et al.,* (2016), supporting that ciprofloxacin maintains sufficient potency within closer-to-expiry intervals. Dilution did not reduce activity because the drug concentration remained above MIC for most organisms.

5.3.3 CIPROTAB (≥ 3 months ≤6 Months)

Table 4.6 shows that CIPROTAB eyedrops with expiration dates less than six months showed antibacterial activity against *Escherichia coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus,* while resistance was confined to *Pseudomonas aeruginosa* at both the neat and diluted samples. This pattern is supported by Kumar *et al.,* (2025) and CLSI (2025), showing that ophthalmic ciprofloxacin is chemically stable and maintains antibacterial potency when stored properly, even under modest dilution.

5.3.4 MOXIFLOXACIN (≥1 Year)

Table 4.7 shows that MOXIFLOXACIN eyedrops greater than one year displayed antibacterial activity influenced by dilution. The neat sample showed activity against *Klebsiella pneumoniae*, *Pseudomonas aeruginosa,* and *Staphylococcus aureus*, but showed no activity against *Escherichia coli*. In contrast, the diluted sample showed activity only against *K. pneumoniae* and *S. aureus*, while no activity was shown against both *E. coli* and *P. aeruginosa*. This shift in the

susceptibility pattern after dilution suggests that reducing the concentration of the solution lowers its potency below the minimum inhibitory concentration (MIC) for certain organisms, especially the more resistant Gram-negative bacteria. Montes *et al.,* (2016) and CLSI (2025) explain that reduced drug concentration can fall below minimum inhibitory concentration for more resistant organisms, such as *E. coli or P. aeruginosa*, producing a concentration-dependent effect.

5.3.5 MOXIFLOXACIN (≥ 3 months ≤ 6 months)

Table 4.8 shows that MOXIFLOXACIN eyedrops less than equal to six months demonstrated antibacterial activity across all tested organisms in both the neat and diluted samples. *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* were sensitive with no resistance recorded. Supported by Montes *et al.,* (2016), showing moxifloxacin retains full antimicrobial activity, and dilution does not reduce efficacy when the concentration remains above minimum inhibitory concentration.

5.3.6 GENTAMICIN (≥ 1 year)

GENTAMYCIN eyedrops greater than one year was active only against *Klebsiella pneumoniae* at both the neat and diluted concentrations. This consistent result across dilution levels indicates that the concentration did not influence antimicrobial activity, as both neat and diluted forms produced the same limited response profile. This agrees with Teweldemedhin *et al.,* (2017) and Davido *et al.,* (2024), indicating that Gentamicin eyedrops may show reduced spectrum, and when concentration is already near the lower limit for minimum inhibitory concentration, dilution does not further change the profile.

5.3.7 GENTAMICIN (≥ 6 months ≤ 1 year)

Table 4.10 shows that GENTAMYCIN eyedrops less than equal to one year expiration showed antibacterial activity against *Escherichia coli* and *Klebsiella pneumoniae*, but no activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus* at both neat and diluted concentrations. This consistency across concentrations indicates that the drug's active component remained sufficiently potent during this storage period and that dilution did not reduce the concentration below the minimum inhibitory concentration (MIC) for the susceptible organisms. The observed activity against *E. coli* and *K. pneumoniae* confirms gentamycin's retained effectiveness against many Gram-negative bacteria within the first year of storage. This pattern supports McBride *et al.,* (1991) and Teweldemedhin *et al.,* (2017), showing that Gentamicin maintains antibacterial potency within closer-to-expiration intervals, and dilution does not drop concentration below MIC for susceptible organisms.

5.3. 8 CHLORAMPHINICOL (≥ 6 months ≥ 1 year)

Table 4.11 shows that CHLORAMPHENICOL eyedrops less than one year expiration interval showed a dilution-dependent variation in antibacterial activity. The neat samples were active against *Escherichia coli* and *Klebsiella pneumoniae* but showed no activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. In contrast, the diluted samples showed no activity against all four tested bacterial isolates (0). This pattern suggests that the antimicrobial potency of chloramphenicol was weakened by reduction in concentration through dilution lowering the drug's level below the minimum inhibitory concentration (MIC), rendering it ineffective against all tested organisms. These observations agree with Cohen (2000) and Davido *et al.,* (2024),

highlighting that concentration reduction through dilution can lower drug levels below minimum inhibitory concentration, especially for drugs with marginal pre-dilution potency.

5.3. 9 CHLORAMPHENICOL (Expired ≤ 6 months)

Table 4.12 shows that CHLORAMPHENICOL eyedrops that had expired less than six months displayed retained antimicrobial activity across multiple bacterial species, suggesting that short-term post-expiration does not immediately lead to total loss of efficacy. Both the neat and diluted samples showed antibacterial activity against *Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Staphylococcus aureus*, and showed no activity against *Escherichia coli*. The uniformity of results across both concentrations implies that the remaining active ingredient concentration, even after dilution, was still above the minimum inhibitory concentration (MIC) required for these susceptible organisms, reflecting partial preservation of chemical integrity despite expiration. Supported by Cohen (2000) and Hanssens *et al.,* (2018), showing that short-term post-expiration use does not immediately abolish antibacterial activity, and remaining concentrations can still inhibit susceptible organisms.

5.4 Comparison of antibacterial activity of experimented eyedrops with reference antibiogram results of test organisms.

5.4. 1 GENTAMICIN (≥ 1 year)

The comparison between the antibacterial activity of the experimented Gentamicin eyedrop in Table 4.9 and the reference antibiogram result in Table 4.13 shows a clear difference in their effectiveness against the tested bacterial isolates. The experimented Gentamicin eyedrop with greater than equal to one year showed reduced activity, being active only against *Klebsiella*

pneumoniae and showed no activity against *Escherichia coli, Pseudomonas aeruginosa,* and *Staphylococcus aureus.* In contrast, the reference Gentamicin antibiotic displayed broader antibacterial activity with mean inhibitory zones of 15 mm for *Escherichia coli,* 13 mm for *Pseudomonas aeruginosa,* and 12 mm for *Klebsiella pneumoniae,* but no inhibition against *Staphylococcus aureus*. This indicates that while the reference Gentamicin remains active against a range of gram-negative organisms, the experimented eyedrop sample has reduced potency, showing activity only against one species. The observed difference suggests that experimented eyedrops may have narrower antibacterial spectra than reference antibiotics, highlighting the importance of verifying the potency of commercial ophthalmic preparations to ensure therapeutic effectiveness. This aligns with Ghita *et al.,* (2023) and Teweldemedhin *et al.,* (2017), which show Gentamicin retains activity mainly against Gram-negative ocular isolates, while resistance in *S. aureus* is common. Differences reflect inherent bacterial resistance and formulation variability rather than expiration interval.

5.4. 2 GENTAMICIN (≥ 6 months ≤ 1 year)

The comparison between the experimented Gentamicin eyedrops in Table 4.10 and the reference Gentamicin in Table 4.13 shows a clear difference in antibacterial potency. The experimented eyedrop between period less than one year was active against *Escherichia coli* and *Klebsiella pneumoniae* but showed no activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus.* In contrast, the reference Gentamicin showed broader activity with inhibition zones of 15 mm for *E. coli*, 13 mm for *P. aeruginosa*, and 12 mm for *K. pneumoniae,* while *S. aureus* remained resistant. This indicates that the experimented sample had reduced efficacy compared to the reference antibiotics. This agrees with Tsegaw *et al.,* (2017) regarding partial retention of

Gentamicin activity and contrasts with McBride *et al.,* (1991), where compounded solutions under controlled conditions maintained full activity.

5.4.3 CIPROTAB (≥1 year)

The comparative analysis of Ciprotab (Table 4.4) and reference ciprofloxacin (Table 4.13) shows that the experimental eyedrop showed antimicrobial activity comparable to the reference antibiotics. Both produced strong inhibition against *E. coli* and *K. pneumoniae*. This parallel pattern indicates the tested formulation maintained pharmacologic integrity under the study conditions and that the reduced effect against *P. aeruginosa* reflects known bacterial resistance mechanisms rather than product failure. Clinically, Ciprotab appears suitable for treating infections caused by ciprofloxacin-susceptible ocular pathogens, but its limited activity against *P. aeruginosa* supports the use of alternative or fortified therapy for pseudomonal keratitis (Lauffenburger & Cohen, 1993). Consistent with Kumar *et al.,* (2025) and Cabrera-Aguas *et al.,* (2024), showing fluoroquinolones retain broad-spectrum activity; intrinsic *P. aeruginosa* resistance explains the weak inhibition.

5.4. 4 CIPROTAB (≥ 6months ≤ 1 year)

The comparison between the antibacterial activity of the Ciprotab formulation (Table 4.5) and the reference ciprofloxacin (Table 4.13) shows a similar but varied susceptibility pattern. Both experimented eyedrop and reference antibiotic were active against *Escherichia coli* and *Klebsiella pneumoniae* with showed no activity against *Pseudomonas aeruginosa.* This observation is supported by Idu *et al.,* (2003), who reported similar susceptibility of *E. coli* and *K. pneumoniae* to ciprofloxacin in ocular isolates.

This indicates that Ciprotab retained comparable antibacterial potency to the reference ciprofloxacin within its shelf life. However, alternative or fortified therapy may be required for infections caused by resistant organisms such as *P. aeruginosa.* The shared resistance to *P. aeruginosa* reflects intrinsic bacterial defense mechanisms rather than product degradation, consistent with known limitations of ciprofloxacin against this organism (Lauffenburger & Cohen, 1993)

5.4.5 CIPROTAB (≥ 3 months ≤ 6 months)

The comparison between the in vitro antibacterial activity of Ciprotab eyedrops (Table 4.6) and the reference ciprofloxacin results (Table 4.13) shows similar activity patterns but differences in potency. Both demonstrated activity against *Escherichia coli* and *Klebsiella pneumoniae* while *Pseudomonas aeruginosa* remained resistant in both cases, showing that Ciprotab was still effective at less than six months before expiry. The persistent resistance of *P. aeruginosa* aligns with reports attributing reduced susceptibility to efflux mechanisms and genetic mutations. Clinically, Ciprotab remains effective against common ocular pathogens within its shelf life; however, alternative agents should be considered for *pseudomonal* infections to minimize treatment failure and prevent resistance development. Montes *et al.,* (2016) report that fluoroquinolone eyedrops maintain pharmacologic stability up to six months before expiry; persistent *P. aeruginosa* resistance requires alternative therapy.

5.4.6 MULTIPLE ANTIBIOTIC RESISTANCE (MAR) INDEX

All isolates showed multiple antibiotic resistance (MAR > 0.2). *S. aureus* had the highest MAR (0.625), followed by *P. aeruginosa* (0.50). These values suggest exposure to antibiotic-rich environments or circulation of multi-resistant strains (Cheesbrough, 2004).

Difference in number of eyedrops

The difference in the number of eyedrop samples tested for each class was due to limitations in product availability and the restricted duration of the study. Because a single brand was used for all antibacterial eyedrop to maintain formulation consistency, obtaining multiple units across all desired expiration intervals was difficult. Some formulations were more readily available than others, resulting in unequal sample numbers (three for some brands and two for others). The short research period of less than three months further limited the ability to source additional samples. Nevertheless, the available samples were carefully selected to ensure representativeness and methodological consistency across tests.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

This study evaluated the in vitro antibacterial activity of four commonly used ophthalmic antibiotics ciprofloxacin (Ciprotab), moxifloxacin, gentamicin, and chloramphenicol across different expiration intervals and dilutions, using the disk diffusion and agar-well diffusion method against *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Staphylococcus aureus.* The research sought to determine how expiry and dilution influence antibacterial potency and to compare the activity of commercial preparations with reference antibiotic discs.

The results showed that the antibacterial efficacy of ophthalmic antibiotics is affected by the nature of the active ingredient, expiry status, and concentration. Overall, the fluoroquinolones—moxifloxacin and ciprofloxacin demonstrated broader and more consistent antibacterial activity than gentamicin and chloramphenicol. Moxifloxacin retained strong inhibitory effects against both Gram-positive and Gram-negative organisms when within six months to expiry, while ciprofloxacin remained active against *E. coli* but was ineffective against *P. aeruginosa*. These outcomes shows that both drug stability and bacterial resistance patterns determine therapeutic success.

Gentamicin, though effective in disc form, showed poor activity as a commercial eyedrop, likely due to reduced drug stability in solution, or formulation factors such as preservative interactions that may have limited its bioactive concentration. Chloramphenicol up to 1year expiration displayed inconsistent activity, with neat, in-date samples showing some effectiveness, whereas

diluted samples lost activity entirely. This highlights the importance of chemical stability and the detrimental effect of improper dilution.

Dilution was found to markedly reduce antibacterial potency across all tested antibiotics, indicating that reduced concentrations may lead to sub-therapeutic dosing and promote resistance. Although the expired sample retained partial activity, the inconsistency observed makes it unsafe to rely on expired ophthalmic preparations in clinical use. Therefore, expiry dates remain essential for ensuring drug safety and efficacy.

6.2 RECOMMENDATION

1. Do not dilute ophthalmic antibiotic preparations before use.

Dilution resulted in a significant reduction in antibacterial potency for nearly all formulations tested. Unless dilution is specifically indicated and validated under sterile, controlled compounding conditions, neat products should be used to ensure therapeutic concentration.

2. Avoid the use of expired ophthalmic antibiotics in clinical practice.

The use of expired eye drops poses both therapeutic and microbiological risks, including treatment failure and resistance development. Strict inventory control and timely disposal of expired drugs are therefore recommended.

3. Encourage evidence-based empirical therapy.

All bacterial isolates from pathogenic eye(s) before prescribing, to prevent antibiotic resistance. Clinicians should base empirical antibiotic selection on local antibiogram data rather than routine prescription habits. Establishing and updating local susceptibility patterns will help guide the rational choice of antibiotics and reduce the emergence of resistant ocular pathogens.

4. Improve formulation testing and stability monitoring.

Discrepancies observed between antibiotic discs and eyedrop formulations highlight the need for regular formulation stability testing. Future evaluations should include chemical assays (such as HPLC) to determine active ingredient concentration alongside in vitro antimicrobial tests for a comprehensive assessment of drug potency.

5. Enhance patient and public awareness.

Public health education should emphasize that the expiry date on medications, particularly ophthalmic products, is a critical safety measure. Patients should be informed about the risks associated with storing eye drops improperly, sharing multi-dose bottles, or extending use beyond the manufacturer's recommended period.

6. Support further research on drug stability under local environmental conditions.

Since temperature, humidity, and light exposure can influence degradation rates, further studies should investigate the stability of commonly used ophthalmic antibiotics under tropical conditions. This would provide data to guide storage recommendations and policy formulation in resource-limited regions.

7. Develop local guidelines on ophthalmic antibiotic stewardship.

Establishing national or institutional frameworks for rational antibiotic use in eye care will help preserve drug efficacy, reduce resistance, and improve clinical outcomes. Such guidelines should integrate laboratory surveillance data, clinician training, and patient education.

8. Strengthen pharmacy and quality assurance practices.

Hospital and community pharmacies should implement stringent quality control and stock management systems to ensure that only effective, in-date, and properly stored ophthalmic antibiotics reach patients. Pharmacists should also educate patients on correct storage and the dangers of using expired or contaminated eye drops.

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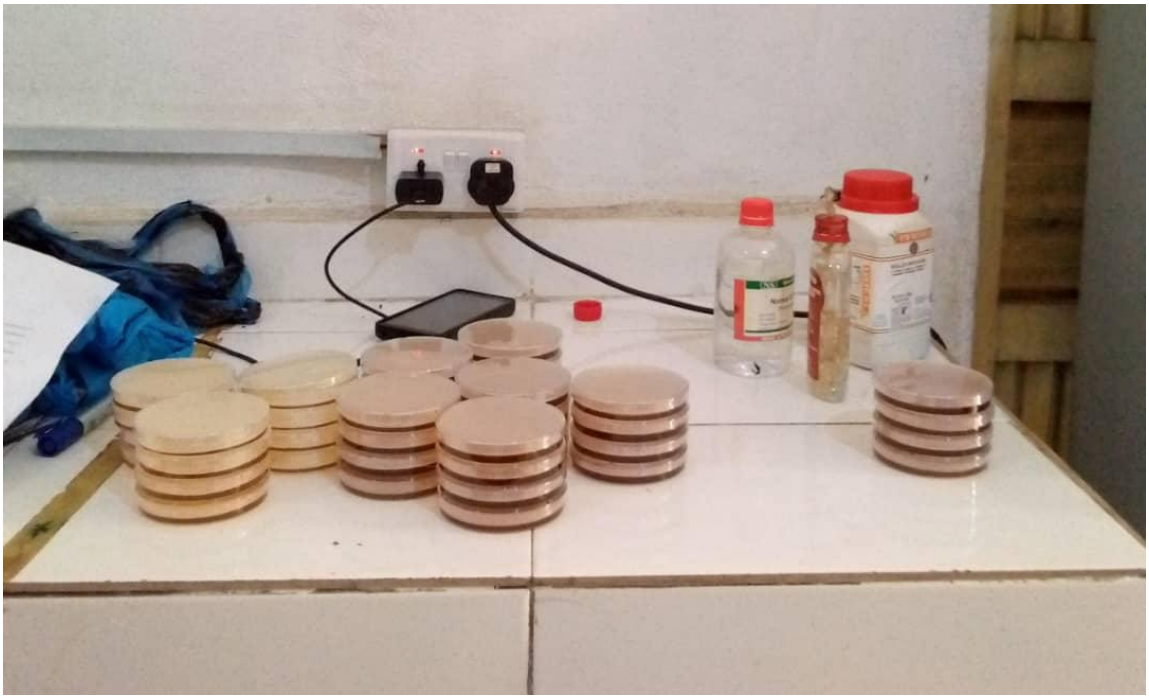
APPENDIX

Universal sterile bottles containing four bacteria Isolates, sterile cotton swabs and corkborer.

Neat and diluted antibacterial eyedrop samples 1 to 9.

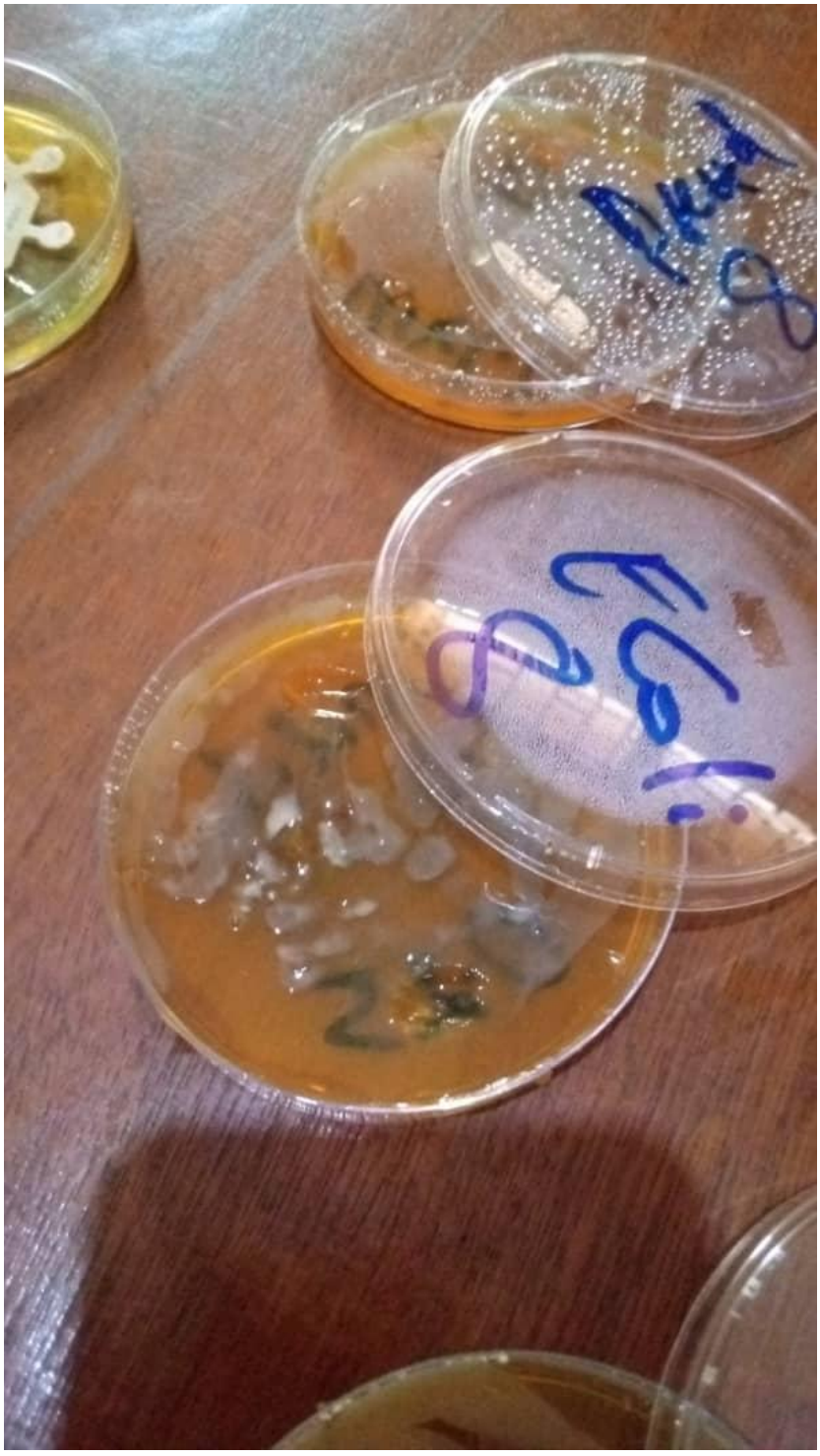
Investigator participating in experimental work.



Mueller Hinton agar in petri dishes, process of solidification.

Petri dishes were labelled to separate neat from diluted agents (0.25).

Antibiotics tested against 3 gram negative and 1 gram positive on left side of image.



***Escherichia coli* showing resistance to antibacterial eyedrop (sample 8). Table 4.12**