EVALUATION OF THE EFFICACY OF MULTIPURPOSE CONTACT LENS CARE SOLUTIONS AGAINST *ACANTHAMOEBA* SPECIES

BY

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MAY, 2026

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A THESIS WRITTEN IN THE DEPARTMENT OF ANIMAL AND ENVIRONMENTAL BIOLOGY AND SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF PHILOSOPHY (M.Phil) IN PARASITOLOGY OF THE UNIVERSITY OF BENIN, BENIN CITY

MAY, 2026

CERTIFICATION

This is to certify that the project work was successfully completed by Chiemela Chidozie OKORO in the Department of Animal and Environmental Biology, Faculty of Life Sciences, University of Benin, under my supervision.

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CERTIFICATION OF THESIS

We the undersigned attest and declare that the thesis of Chiemela Chidozie OKORO, titled: **Evaluation of the Efficacy of Multipurpose Contact Lens Care Solutions against *Acanthamoeba* species** has successfully passed the anti-plagiarism test and does not violate any copyright regulations.

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DEDICATION

This thesis is dedicated to my mother, Mrs Patience Abraham and my lovely wife, Mrs Faith Uloaku Okoro.

ACKNOWLEDGEMENT

The completion of this thesis was possible, by far due to the immense contributions of several personages. First and foremost, I would like to acknowledge God almighty for the strength and perfect health to see this project through my dogged and ebulient supervisor, Prof. (Mrs) A.O. Awharitoma, who was always open to questions as well as her invaluable contributions. I would also like to appreciate the effort of my co-supervisor, Assoc. Prof. (Mrs) Edo Taiwo for her immeasurable assistance that made this research come to fruition. I thank my ever-resilient mother, Mrs Patience Abraham and my lovely wife, Mrs Faith Okoro for their support, love and care. My appreciation would also go to some academic staff of the Department of Animal and Environmental Biology who offered help at various times that I needed it. May our good Lord bless you all.

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ABSTRACT

Acanthamoeba species are free-living protozoa commonly found in water sources and are recognized causes of *Acanthamoeba* keratitis, particularly among contact lens wearers. Reticulated bore-hole water is widely used in many communities, yet data on its contamination with *Acanthamoeba* and the effectiveness of multipurpose contact lens solutions against these organisms remain limited. This study investigated the prevalence and species distribution of *Acanthamoeba* in reticulated bore-hole water and evaluated the efficacy of selected multipurpose contact lens solutions in reducing *Acanthamoeba* counts.

A total of 36 reticulated bore-hole water samples were collected and cultured for *Acanthamoeba* species using standard parasitological techniques. For *Acanthamoeba* detection, 500ml of water was passed through a 0.45µm cellulose nitrate membrane filter. Prepared non-nutrient agar plates were seeded with heat-killed *Escherichia coli*. Cultures were incubated at 32ºC for Amoebic growth and examined microscopically. Isolates were identified based on morphological characteristics of trophozoite and cyst stages. The effects of three multipurpose contact lens solutions—Freesol™, Refresh™, and Freshlook™—on *Acanthamoeba* counts were assessed. Data were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test, with statistical significance set at $p < 0.05$.

Acanthamoeba species were detected in 32 (88.9%) of the water samples, yielding a total of 209 isolates and a mean intensity of 6.53 detections per positive sample. Ten species were identified, with *Acanthamoeba royreba* showing the highest prevalence (17.7%), followed by *A. polyphaga* (11.96%), *A. lenticulata* and *A. mauritaniensis* (11.0% each). All contact lens solutions significantly reduced *Acanthamoeba* counts compared with pre-treatment levels ($p < 0.05$). Freesol achieved the greatest reduction (98 ± 9.5), followed by Refresh (101 ± 12.5), while Freshlook showed the least reduction (170 ± 22.0). None of the solutions completely eliminated *Acanthamoeba*. This study showed that reticulated bore-hole water harbored a high prevalence of potentially pathogenic *Acanthamoeba* species. Although multipurpose contact lens solutions significantly reduce *Acanthamoeba* burden, complete elimination was not achieved, highlighting the organism's resistance and the continued risk of exposure. Strict adherence to proper contact lens hygiene and avoidance of bore-hole water use are essential to reduce the risk of *Acanthamoeba* infection.

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND

Acanthamoeba, free-living amoebae commonly found in water sources, can pose health risks to communities reliant on bore-hole water supplies. Members of the genus *Acanthamoeba* are widely

distributed in nature and have been isolated from soil, water, air, and the upper respiratory tract of healthy humans. At least seven species of *Acanthamoeba* are pathogenic to humans, namely *A. castellanii, A. polyphaga, A. culbertsoni, A. astronyxis, A. hatchetti, A. palestinensis,* and *A rhysodes* (Pellegrin *et al.*, 1991).

Among protozoans, *Acanthamoeba* stands out due to its opportunistic behavior. Notably, certain species of *Acanthamoeba* exhibit remarkable resilience, enduring extremely low temperatures as cold as -20ºC (-4ºF). Conversely, temperatures exceeding 42.5°C are lethal to them. A key survival strategy employed by *Acanthamoeba* involves transitioning from the motile trophozoite phase to a cystic phase in unfavorable conditions, allowing them to endure and maintain their virulence over prolonged periods (Bharathi *et al*., 2007; Sowka *et al*., 2016).

A potentially severe corneal disease known as *Acanthamoeba* keratitis, primarily caused by *A. castellani* and *A. polyphaga*, can lead to significant vision loss (Sowka *et al*., 2016). Several risk factors are associated with *Acanthamoeba* keratitis, with the predominant factor in developed nations being the use of contact lenses (Bharathi *et al*., 2007). This ocular infection poses a considerable treatment challenge, requiring an average of more than five months of treatment due to the resistance of the cysts to most antimicrobial agents (Al-Herrawy *et al*., 2017).

Acanthamoeba species are environmental predators of bacteria and fungi, and possess a feeding trophozoite stage that, when subjected to environmental stress, forms a dormant, thickly walled cyst. Both the trophozoite and cyst are resilent to many disinfection solutions and various antimicrobial drugs (Borazjani and Kilvington, 2005).

A contact lens is a thin, curved lens placed directly on the cornea of the eye to correct vision. It is also used for cosmetic purposes. They are an alternative to eyeglasses, providing a way to focus light onto the retina and improve sight. Contact lenses can be used to correct refractive errors like nearsightedness, farsightedness, and astigmatism, or they can be purely cosmetic, like colored lenses (Pagan-Duran, 2025).

Contact lenses are manufactured from a variety of polymer-based materials, with the two primary categories being rigid (hard) and soft lenses. The most widely used hard lens is the rigid gas-permeable (RGP) lens**,** which is composed of durable plastics blended with additional compounds that enhance oxygen permeability. These lenses maintain a stable shape while allowing adequate oxygen transmission to the cornea. Clinically, RGP lenses are particularly advantageous for patients with moderate to severe astigmatism or keratoconus, as they provide superior visual acuity compared to soft lenses in cases of irregular corneal curvature. Furthermore, individuals prone to ocular allergies or protein deposits on lenses may find RGP lenses more suitable (Pagan-Duran, 2025). Despite these benefits, soft contact lenses remain the most commonly selected option, largely due to their greater initial comfort and the wide range of available designs.

Soft contact lenses are available in several forms, each designed to meet specific visual and lifestyle needs. Daily wear contact lenses are worn during waking hours and removed prior to

sleep. Many are manufactured as disposable lenses, intended for single-day use. Others are designed for longer replacement schedules such as weekly, biweekly, or monthly. Disposable daily wear lenses are often recommended for individuals who use contact lenses intermittently, as they reduce the risk of deposit build-up and ocular complications. Extended wear contact lenses, by contrast, may be worn continuously, including during sleep, but must be removed at least once weekly for cleaning and disinfection. Owing to their association with an increased risk of serious ocular infections, extended wear lenses are prescribed less frequently by clinicians.

Specialized options within soft lenses include toric lenses, which are designed to correct astigmatism. Although toric lenses are available in both daily and extended wear modalities, they generally provide less precise correction than rigid lenses and are typically more expensive. Additionally, contact lenses may be manufactured with cosmetic tints, allowing alteration of iris color, and are available in daily wear, extended wear, and toric formats (Pagan-Duran, 2025).

The contact lens (CL) care system involves the use of specialized formulations, such as multipurpose solutions (MPS), designed to clean and disinfect lenses and thereby ensure safe wear. Despite these measures, microbial infections remain a significant complication among CL users, with particularly severe outcomes arising from amoebic keratitis affecting the cornea. The most frequently reported infection is microbial keratitis (MK), which is clinically characterized by ocular discomfort, conjunctival hyperemia, corneal ulceration, and stromal inflammatory infiltrates. A wide spectrum of pathogens—including bacteria, fungi, protozoa, and viruses—has been implicated in the development of MK (Mohd Hasali *et al*., 2024).

A multipurpose solution (MPS) serves as an all-in-one system for lens cleaning, rinsing, disinfection, and storage. Within this framework, ‘disinfection’ refers to the elimination of

microorganisms from the CL surface, whereas cleaning refers to the removal of protein deposits and other debris. Common disinfectant agents incorporated into CL care formulations include quaternary ammonium compounds, biguanides, hydrogen peroxide, povidone-iodine, alcohol, sorbic acid, and thimerosal, many of which are widely used in commercial products (Mohd Hasali *et al*., 2024).

In addition, the preservative components of MPS play a crucial role in preventing CL-related MK and in reducing the risk of contamination of the storage case with *Acanthamoeba* species. An ideal CL care solution must therefore demonstrate broad-spectrum antimicrobial efficacy while maintaining sufficient biocompatibility for safe use on the ocular surface (Bradley *et al*., 2021).

Furthermore, a contact lens solution must adhere to the criteria of ISO 14729, ophthalmic optics - Contact lens care products - Microbiological requirements and test methods for products and regimens for hygienic management of contact lenses. The ISO 14729 antimicrobial activity test (Stand-alone) assesses the microbial load of a solution exposed to 105–106 colony forming units (CFU)/mL of five ISO representative microorganisms at 25%, 50%, 75%, and 100% of the recommended disinfection time (DT) (Gabriel *et al*., 2021). As *Acanthamoeba* spp. are resistant in both their trophozoite and, in particular, their cyst form (Gabriel *et al*., 2021), the most thorough test of antimicrobial effectiveness is to challenge the solution with them.

Keratitis and encephalitis are the two primary chronic illnesses linked to *Acanthamoeba*. Unintentional contact or seize-the-moment scenarios are the most common causes of human infections caused by pathogenic free-living amoebae, such as *Acanthamoeba* (Ghosh, 2018; Sun and Wang, 2017).

The initial location where *Acanthamoeba* protozoa attach themselves during corneal infections is at the lipopolysaccharides or mannosylated glycoproteins on the membranes of corneal epithelial cells. This initial contact involves phagocytosis and the release of toxins. The release of enzymes such as neuraminidase causes the corneal epithelial cells to thin or die as a result of the rupture of Bowman's membrane (Sun and Wang, 2017). Additionally, this procedure impairs the integrity of the epithelial barrier, allowing *Acanthamoeba* protozoa to enter the corneal stroma. *Acanthamoeba* proteases break down corneal stromal collagens, and trophozoites can induce radial keratoneuritis (Sun and Wang, 2017).

Contact lenses are known to act as mechanical vectors, facilitating the spread of *Acanthamoeba* from infected sources to the cornea (Sowka *et al.*, 2016). However, microtrauma to the corneal epithelium is also caused by contact lenses, which makes it easier for these pathogens to enter. Exposure to contaminated materials, either at the same time or at a later time, as well as direct corneal injury (such as corneal abrasions) are other potential infection pathways (Sowka *et al*., 2016).

Contact lenses with scratches or other damage should be thrown away. Wearing contact lenses increases the risk of swimming and washing one's eyes. Chlorine-based disinfectants should not be used routinely for lens cleaning because *Acanthamoeba* is chlorine-resistant (Khan, 2006). Additionally, silicon hydrogel contact lenses are noticeably more adhesive to *Acanthamoeba* than conventional hydrogel lenses.

In addition, *Acanthamoeba* protozoa harm the corneal epithelial layers via three mechanisms: (a) endocytosis, which is comparable to phagocytic activity, in which the protozoa can directly ingest a portion of the corneal cell membrane; (b) spontaneous exocytosis, in which the protozoa can release lysins that further damage the epithelial cell membrane, without the need for an

activation process; and (c) membrane-related activation of exocytosis, in which *Acanthamoeba* protozoa form a coalition of trophozoites that can bind with receptors or ligands on the surface of the epithelial cell, thereby activating proteases. The release of these proteases has the potential to harm epithelial cells (Sun and Wang, 2017).

In general, *Acanthamoeba* is susceptible to certain widely used biocides, which can have different modes of action. As an example, chlorhexidine gluconate targets the nucleolar structure, mitochondria, endoplasmic reticulum, and *Acanthamoeba* pseudopodia (Hashim *et al.*, 2013). In addition to causing cyst enlargement and cytoplasmic retreat from the cyst wall, polyhexamethylene biguanide also induced shrinkage from the cyst wall, while chlorhexidine diacetate was found to cause shrinkage from the cyst wall. Lastly, chlorine treatment has resulted in size reduction, permeabilization, and retraction of pseudopods (Walters *et al*., 2021).

The biocides that are available for use in any multipurpose solution pose a particular problem. The biocides included in each multipurpose solution work to achieve the aim of preserving adequate antimicrobial action against possible pathogenic microbes while fostering eye health, in addition to the use of cleaning, wetting, and comfort agents (Borazjani and Kilvington, 2005).

Because of the chance of corneal injury, biocides that are effective against *Acanthamoeba* might not be safe for patients as part of a multi-purpose treatment. Additionally, because *Acanthamoeba* is a unique pathogen in the area of potentially infectious ocular agents, biocides that may be effective against other common microorganisms and can be included in multipurpose solutions may have little to no effect against amoeba in either the trophozoite or cyst formation (Walters *et al*., 2021). Consequently, the purpose of this research was to ascertain the effectiveness of a few of the multipurpose contact lens solutions sold in the Nigerian market at killing *Acanthamoeba*.

1.1.1 Protozoa

Protozoa are the category under which most single-celled organisms lacking a cell membrane fall. These microscopic organisms, which are invisible to the human eye, could be studied thanks to the discovery of the microscope in the 17th century. Antonio van Leeuwenhoek (1632–1723) was the first to accomplish this. Protozoa absorb nutrients mostly by phagocytosis, which involves engulfing bigger particulate matter like bacteria or organic waste, and pinocytosis, which involves ingesting dissolved chemicals via membrane invagination. Protozoa can utilize a diverse array of ecological niches because phagocytosis frequently necessitates cytoskeletal changes and particular receptor-ligand interactions (Khan, 2006; Prescott *et al*., 2014). Parasitic protozoa, which depend on nutrients from the host to support their development and reproduction, depend especially on these feeding techniques.

Protozoa reproduce mostly asexually, with binary fission being the most common method. In this process, a parent cell undergoes mitosis to produce two daughter cells that are genetically identical. Several species reproduce through multiple fission (schizogony), in which a single parent cell gives rise to several offspring, which promotes rapid population growth inside hosts. Furthermore, some protozoa engage in sexual or parasexual mechanisms that promote genetic diversity and adaptability, such as conjugation, syngamy, or genetic recombination (Cox, 2002; Lucius and Roberts, 2017). These reproductive and genetic processes allow parasitic protozoa to survive in harsh conditions, evolve drug resistance, and escape host immune responses.

Due to a process called reductive evolution, parasitic protozoa frequently have smaller genomes. In this process, metabolic pathways that are no longer necessary in the host environment are lost over the course of evolution (Keeling and Slamovits, 2005). However, genome size is not always a reliable indicator of parasitism. For instance, *Trichomonas vaginalis* has an unusually large genome (~176 Mb with about 59,000 protein-coding genes), which is thought to be the result of considerable gene duplication and expansion. Some free-living amoebae, however, have genomes that are even bigger. Among eukaryotes, the free-living amoeba *Entamoeba proteus* has one of the biggest genomes, estimated at about 290,000 Mb (Lucius and Roberts, 2017).

Protozoa are taxonomically and biologically heterogeneous. Members of the Phylum Apicomplexa, such as *Plasmodium* and *Toxoplasma*, possess an endosymbiotic plastid-like organelle known as the apicoplast, derived from a secondary algal endosymbiosis. The apicoplast retains metabolic pathways of plant origin, including fatty acid and isoprenoid synthesis, making it a key target for antiparasitic drugs (McFadden and Yeh, 2017). This evolutionary complexity highlights the difficulty of classifying protozoa using morphology alone.

Protozoa constitute one of the five major classes of human pathogens, alongside bacteria (prokaryotes), fungi, viruses (obligate intracellular parasites), and multicellular parasites (helminths and arthropods) (Khan, 2006). They gain entry into hosts through the skin, respiratory tract, gastrointestinal tract, or genitourinary tract, and may also be transmitted via vectors such as mosquitoes, flies, or ticks. Importantly, protozoa can also be spread through fomites, including surgical instruments, towels, contaminated water, and contact lenses, which is particularly relevant to ocular infections (Khan, 2006; Siddiqui and Khan, 2012).

Once inside the host, protozoa proliferate and establish infection, leading to tissue damage through direct cytopathic effects, nutrient depletion, or immune-mediated pathology. In some cases, excessive host inflammatory responses contribute more to disease severity than the organism itself, underscoring the complex host–parasite interactions involved (Lucius and Roberts, 2017).

1.2 Statement of the Problem

Acanthamoeba keratitis is a sight-threatening ocular infection caused by free-living *Acanthamoeba* species, primarily *A. castellanii* and *A. polyphaga*. This infection is notably associated with the use of contact lenses often cleaned with tap water and is notoriously difficult to treat due to the organism's ability to form resilient cysts that are highly resistant to conventional antimicrobial agents. Despite the increasing use of multipurpose contact lens solutions (MPS) for lens disinfection, evidence suggests that many commercially available products may be inadequate in eliminating both the trophozoite and cystic forms of *Acanthamoeba*. This presents a serious public health concern, especially in regions like Nigeria where regulatory oversight of contact lens products may be limited and where borehole water, a potential source of *Acanthamoeba*, is commonly used. There is therefore a critical need to evaluate the efficacy of multipurpose contact lens care solutions marketed in Nigeria to determine their actual antimicrobial activity against *Acanthamoeba* and ensure that they meet international microbiological safety standards. Failure to address this problem may lead to increased incidence of *Acanthamoeba* keratitis and consequent visual morbidity among contact lens users.

1.3 Aim and Objectives

The aim was to evaluate the efficacy of multipurpose contact lens care solutions against *Acanthamoeba* species.

The objectives of the study were to:

1. isolate and identify *Acanthamoeba* species from reticulated borehole water commonly used in contact lens hygiene.

2. assess the *in vitro* antimicrobial efficacy of selected multipurpose contact lens care solutions against the trophozoite and cyst forms of *Acanthamoeba* spp.

3. compare the efficacy of different commercial multipurpose solutions available in the Nigerian market in eliminating *Acanthamoeba* spp; and

4. determine whether the tested contact lens solutions meet the recommended microbiological standards for disinfection efficacy, particularly against *Acanthamoeba* spp.

1.4 Significance of the Study

1. *Acanthamoeba* keratitis is a rare but devastating ocular infection that can result in permanent visual impairment or blindness. By evaluating the efficacy of multipurpose contact lens care solutions (MPS) available in the Nigerian market, this study will address a key gap in preventing avoidable blindness, especially in regions where tap or borehole water—potentially contaminated with *Acanthamoeba*—is frequently used for contact lens hygiene.

2. The findings will help assess whether locally marketed MPS meet international microbiological safety standards (for example, ISO 14729). Evidence from this research will

guide regulatory bodies in Nigeria to improve quality control, ensuring that only safe and effective products are available to consumers.

3. While many studies focused on bacterial and fungal contamination, few evaluated anti-*Acanthamoeba* efficacy. This study will provide important *in-vitro* data on both trophozoite and cyst resistance to MPS, contributing to global scientific literature on disinfection challenges against free-living amoebae.

4. Eye care professionals will benefit from locally relevant evidence when advising patients on safe contact lens handling, storage, and cleaning practices. This is particularly important in tropical climates where *Acanthamoeba* prevalence is higher.

5. By including an assessment of contact lens users' hygiene practices and awareness of *Acanthamoeba* keratitis, this research will highlight knowledge gaps and the need for targeted health education campaigns to reduce risk factors associated with improper lens care.

1.5 CONCEPTUAL FRAMEWORK

The conceptual framework of this study was based on the interaction between multipurpose contact lens care solutions (MPS) and *Acanthamoeba* species, particularly their trophozoite and cystic forms. The framework posited that the antimicrobial efficacy of MPS varied depending on the formulation and biocidal agents used. The ability of an MPS to effectively kill or inhibit *Acanthamoeba* determines its potential to reduce the risk of *Acanthamoeba* keratitis, especially among contact lens users exposed to contaminated water sources. The study will compare different MPS products to assess whether they meet international disinfection standards and whether they are effective against both life stages of *Acanthamoeba*.

1.6 HYPOTHESIS

Null Hypothesis (H_0):

There is no significant difference in the antimicrobial efficacy of different multipurpose contact lens solutions against *Acanthamoeba* trophozoites and cysts.

Alternative Hypothesis (H_1):

There is a significant difference in the antimicrobial efficacy of different multipurpose contact lens solutions against *Acanthamoeba* trophozoites and cysts.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Protozoa and Parasitic Protists

Protozoans are unicellular eukaryotes that are surrounded by a plasma membrane and frequently have specialized surface features. The diversity of this group is demonstrated by the fact that many parasitic protists have small or diminished genomes as a result of their parasitic way of life and reliance on host metabolic pathways, while some free-living amoebae have very big genomes (Janouškovec *et al*., 2019). Parasitism has evolved independently numerous times among protozoa and related protists; for example, apicomplexan-like parasites are polyphyletic, and many contain cryptic plastid organelles (apicoplasts) of algal origin that are still necessary despite the loss of photosynthesis (Janouškovec *et al.,* 2019). The extraordinary capacity of

parasitic protozoa to adapt, evade the immune system, and live complicated life cycles is based on these evolutionary traits.

The arrangement of mitochondria in parasitic protists is also very different from that in animals and fungi. In contrast to anaerobic parasites like *Trichomonas* and *Entamoeba*, which have mitochondrion-related organelles that have lost their genomes (Voleman and Doležal, 2019), many kinetoplastids and apicomplexans have a single mitochondrion that is closely connected to the cell cycle. Understanding protozoan bioenergetics and possible drug targets depends on this kind of reductive evolution and organellar specialization.

2.2 Pathogenic Free-Living *Amoeba* (FLA)

Free-living amoebae can be opportunistic or non-opportunistic pathogens that live in air, soil, and a variety of natural and artificial water systems (Marciano-cabral and Cabral, 2003; Nageeb *et al*., 2022). Abdul Majid *et al*., (2017), investigated a total of 94 water samples from Laos (31), Myanmar (42), and Singapore (21) to determine if they contained pathogenic FLA, both treated and untreated. Each water sample was filtered and cultured at room temperature on non-nutrient agar inoculated with a live suspension of *Escherichia coli*. Microscopic stains (Giemsa and immunofluorescence) were used to morphologically identify both trophozoites and cysts. The study revealed that *Naegleria*-like features were the most prevalent in both treated and untreated water samples, followed by *Acanthamoeba*-like and *Vermamoeba*-like characteristics. Species-specific primer sets were utilized for molecular identification of *Acanthamoeba*, *Naegleria*, and *Vermamoeba* in order to ascertain the pathogenic isolates. *Vermamoeba vermiformis* was discovered in both treated and untreated water samples, whereas the harmful species of *Acanthamoeba lenticulata* and *A. triangularis* were identified in raw water samples. However, since chlorine disinfection is unable to kill these amoebae in treated water samples, the authors

concluded that the alarming issue may be brought on by the low water quality, inadequate maintenance, and insufficient treatment. They recommended that monitoring and inspection of water quality were necessary to control the growth, hence, further preventing the widespread of FLA infections among the public.

The epidemiological and clinical characteristics of *Acanthamoeba* keratitis were studied by Bharathi *et al*. in 2007, along with the sensitivity and specificity of smears in identifying *Acanthamoeba*. A retrospective evaluation was conducted by the Authors of all culture-confirmed instances of *Acanthamoeba* keratitis occurring between October 1999 and August 2002. Corneal scrapes were examined and cultured using well-known procedures. Results showed that out of 3183 consecutive patients with clinically diagnosed corneal ulcers evaluated, 33 (1.04%) were found to be due to *Acanthamoeba*. Twenty-four out of 33 (72.72%) were less than 51 years of age (P<0.001). All patients were from rural areas (P<0.001) and 26 (78.79%) of them were agricultural workers (P=0.031). All 33 had history of corneal injury (P<0.001) and 28 (84.85%) patients had injury with mud (P<0.001). All 33 (100%) patients had previous medical treatment (P=0.009) and 10 (30.3%) had used traditional eye medicines (P=0.183). A clinical pattern of ring infiltrate was characteristic in 15 (45.45%) patients. The diameter of the corneal ulcer was more than 6 mm in 27 (81.82%) eyes (P<0.001). Twenty-six (78.79%) patients had visual acuity of perception of light on initial presentation (P<0.001) and 24 (72.73%) had the same as their final visual outcome. The sensitivity of 10% potassium hydroxide (KOH) preparation was found to be higher (P<0.001) in the detection of *Acanthamoeba* cysts. Hence, the authors concluded that the incidence of *Acanthamoeba* keratitis amongst the corneal ulcer patients was 1% and it was mainly due to corneal injury by mud. They also determined that KOH

preparation was more of a sensitive diagnostic tool for the detection of *Acanthamoeba*. Delayed diagnosis or misdiagnosis and inappropriate antimicrobial therapy resulted in poor visual outcome.

Between 2009 and 2015, Randag *et al.* (2019) assessed the prevalence of *Acanthamoeba* keratitis in the Netherlands and looked at potential predictors of therapy response. There were 224 individuals in the study, and 224 of their eyes were included. The majority of the patients (95%) wore contact lenses, and 74% wore soft contact lenses. Between 2009 and 2015, there was an increase in cases from 16 to 49. As a consequence, the calculated incidence for soft contact lens users in 2015 was 1 in 21,000. The standards for treatment failure were satisfied by 87 eyes (39%). A multivariable regression analysis revealed a positive correlation between treatment failure and older age at presentation, more severe illness stage, and previous corticosteroid use. An early referral to a corneal expert was linked to improved clinical outcomes. It was determined that, despite the fact that *Acanthamoeba* keratitis was still a rare illness, its prevalence among soft contact lens users had risen to 1 in 21,000 in 2015. Age, more severe stage, corticosteroid usage prior to diagnosis, and indirect referral to a cornea specialist were all significant risk variables for treatment failure, which happened in 39% of cases.

2.3 Nigerian Water Quality Context

In many Nigerian cities, municipal portable water suppy is scarce, and most families depend on wells and private boreholes, frequently augmented by bottled or sachet water. Alabi *et al.,* (2024), evaluated the bacteriological quality of household water collected in the dry and wet seasons across five municipal local government areas (LGAs) in Ibadan, a large city in southwest Nigeria. A total of 447 water samples (dry season, n = 250; wet season, n = 197) were aseptically

collected from a random sample of mapped households within Ibadan's five municipal LGAs. The pH values and total aerobic and coliform bacterial counts were measured, and samples were screened for *Escherichia coli*, *Salmonella*, *Shigella*, and *Yersinia* by standard phenotypic techniques and multiplex polymerase chain reaction. The most common source of water was well (53.2%), followed by borehole (34%). None of the households used municipal tap water. Cumulatively, aerobic (P = 0.0002) and coliform (P = 0.0001) counts as well as pH values (P = 0.0002) changed significantly between seasons, with increasing and decreasing counts depending on the LGA. Non-potable water samples were found to be very common during the dry (86.8%) and wet (74.1%) seasons. *Escherichia coli* spp., as indicators of recent fecal contamination, were isolated from 115 (25.7%) of the household water sources. Thirty-three *Salmonella*, four enteroaggregative *E. coli*, and four enterotoxigenic *E. coli* isolates but no *Shigella* or *Yersinia* isolates were identified.

In addition to the nearby existence of hanging latrines, they also discovered a strong correlation between fecal contamination in boreholes. Over half of the boreholes were found to be well-built during sanitary inspections. There was no correlation between microbial water quality and structural or site flaws. The rainy season poses a serious public health threat to the quality of drinking water, whether it is commercially bottled or untreated groundwater, which emphasizes the need to fix flaws in monitoring and quality control.

The infrastructure and pollution patterns in these Nigerian studies—a significant dependence on untreated groundwater, seasonal deterioration, and inadequate monitoring—are similar to those in other areas where FLA are common in tap water, storage tanks, and household systems (Abdul Majid *et al*., 2017; Nageeb *et al*., 2022;). However, the Nigerian studies mostly looked at bacteriological indicators.

2.4 *Acanthamoeba* Keratitis: Epidemiology, Risk factors and Management

A crucial initial stage in the pathogenesis of *Acanthamoeba* keratitis, a disease that disproportionately affects contact lens users, is the propensity of *Acanthamoeba* to adhere to surfaces. Contact lenses have a direct impact on the integrity of the corneal epithelium through abrasions associated with lens fitting, and an indirect impact through disrupting normal cellular metabolism and physiological functions (Ibrahim *et al*., 2009). The integrity of the epithelial cells is eventually weakened by these alterations, which also cause them to become hypoxic. Prolonged contact lens wear, especially when combined with overnight lens use, results in a notable reduction in oxygenation of the cornea.

A recent systematic review of 61 longitudinal studies, (Marques-Couto *et al.*, 2025), found that the best initial treatment frequently combined therapeutic epithelial debridement (TED) with intensive topical biguanides (polyhexamethylene biguanide [PHMB] or chlorhexidine) and diamidines, along with a topical antibacterial agent. In cases that are resistant or serious, adjunctive treatments like oral miltefosine and topical voriconazole 1% may be taken into consideration ((Marques-Couto *et al.,* 2025).

After unsuccessful drug treatment, possible surgical procedures (keratoplasties) may include: In cases where there is no deep stromal involvement, Deep Anterior Lamellar Keratoplasty (DALK) is a crucial procedure. Optical Penetrating Keratoplasty (OPK) should be used for rehabilitation, and Early Therapeutic Penetrating Keratoplasty (TPK) should be employed as a rescue therapy. The age of the patient, the length of time between the onset of symptoms and the correct diagnosis, the usage of corticosteroids prior to the correct diagnosis, the low initial best corrected visual acuity (BCVA), and the AK stage at presentation are all significant predictive factors (Marques-couto *et al*., 2025).

2.5 Contact-lens Care Products and Performance Standards

Soft contact lenses can be cleaned, disinfected, and stored using multipurpose contact lens solutions. These products must demonstrate antimicrobial efficacy against a panel of common ocular pathogens in accordance with ISO 14729 standards, albeit *Acanthamoeba* is not always included in required testing (Gabriel *et al.,* 2021). The ISO 14729 standard outlines microbiological criteria as well as standardized stand-alone and regimen tests for lens-care disinfection assertions against specified bacterial and fungal panel organisms at manufacturer-stated disinfection times. Despite ISO 14729 compliance being a requirement for market claims, *Acanthamoeba* is not a mandatory test species in the core panel, and anti-amoebic performance can vary significantly across goods even when they adhere to ISO 14729.

However, a recent study advocates for the inclusion of *Acanthamoeba* spp., citing the organism's hardiness and significance to public health. The effectiveness of contact lens solution preservatives against *Acanthamoeba castellanii* and *Acanthamoeba polyphaga* trophozoites and cysts was evaluated by Silvany *et al*., 1991. The effectiveness of preservatives against amoebae was tested at intervals ranging from 30 minutes to 24 hours. Organisms grown non-axenically and axenically were used in the preservative testing. The best preservatives included hydrogen peroxide (3%), benzalkonium chloride (0.001% and 0.004%), polyaminopropyl biguanide (0.0015%), and chlorhexidine (0.001% and 0.005%). Smaller amounts of these same preservatives were less effective. Polyaminopropyl biguanide (0.00005%), polyquaternium-1 (0.001 %), potassium sorbate (0.13%), EDTA (0.1%), thimerosal (0.001 % and 0.004%), and sorbic acid (0.1%) were all tested and found to be ineffective. However, thimerosal 0. 004% was effective when mixed with EDTA in solution. When tested against axenically produced organisms, preservatives were more effective than when tested against co-cultured organisms.

Siddiqui *et al*. (2015) evaluated the antibacterial effects of marketed contact lens disinfecting solutions using eight different contact lens solutions, including ReNu MultiPlus, DuraPlus, Ultimate Plus, OptiFree Express, Kontex Clean, Kontex Normal, Kontex Multisol extra+, and Kontex Soak. The effectiveness of contact lens treatments was evaluated against microorganisms that cause keratitis, such as *Pseudomonas aeruginosa, Serratia marcescens*, *Staphylococcus aureus*, Methicillin-resistant *Staphylococcus aureus*, *Fusarium solani,* and *Acanthamoeba castellanii.* According to the manufacturer's minimum recommended disinfection time, the results showed that DuraPlus, OptiFree Express, and ReNu MultiPlus were all efficient at killing fungal and bacterial pathogens. Although Ultimate Plus was successful against *F. solani* and MRSA, it was not effective against *P. aeruginosa, S. marcescens, or S. aureus*. The fact that none of the locally produced contact lens disinfectants from Pakistan, such as Kontex Clean, Kontex Normal, Kontex Multisol extra+, and Kontex Soak, were effective against any of the keratitis-causing pathogens tested is a cause for concern. None of the eight contact lens disinfection treatments were successful at killing *Acanthamoeba* cysts. As a result, they concluded that the health officials and manufacturers of contact lens disinfection solutions were very concerned about these results because they posed a significant threat to public health. Effective solutions were needed, as well as an emphasis on proper hygiene for contact lens care and special guidelines for developing nations regarding the manufacture and storage of contact lens disinfecting solutions.

Furthermore, the incidence and typical non-compliant behaviours of reusable lens wearers constitutes another huge problem. Yee *et al.* (2021), reviewed the impact of non-compliance to wear of reusable soft contact lenses. They posited that the impact of non-compliance ranged from poor lens comfort through to potentially sight-threatening infective keratitis. The

coronavirus pandemic has refocused the profession on the importance of hand hygiene in particular, and the need for promoting safe contact lens (CL) wear in general. This review summarised typical non-compliant behaviour related to reusable CLs, and examined strategies and opportunities to better support wearers. Patient education was central in encouraging compliant behaviour, although patient recall of information was low, and personal belief systems might result in continuation of non-compliant behaviour despite awareness of the risks. Contact lens care solutions are required for the daily disinfection of lenses, however misuse of multipurpose solutions (MPS) and hydrogen peroxide (H_2O_2)-based care systems could challenge their ability to be fully efficacious. Standard efficacy testing was reviewed, with consideration of how well current protocols model real-world use of CL solutions. Although some recommendations were in place for the inclusion of additional variables such as lens cases, CL materials, organic soil and efficacy against *Acanthamoeba*, opportunity still existed to reevaluate global standards to ensure consistency of testing in all markets. Finally, potential future innovations were discussed which might further support increased safety in reusable lens wear through novel antimicrobial additions to both CL materials and cases.

Studies have indicated wide variability in the efficacy of commercial MPS against *Acanthamoeba* spp. Some solutions containing polyhexamethylene biguanide (PHMB) or chlorhexidine have shown amoebicidal activity, affecting the pseudopodia, nucleoli, and cyst walls of the organism (Hashim *et al*., 2013; Walters *et al*., 2021). However, many formulations that are effective against bacteria and fungi fail to eliminate *Acanthamoeba*, particularly in its cystic form, raising concerns about the adequacy of existing lens care products (Borazjani and Kilvington, 2005).

Additionally, Shoff *et al*. (2008), conducted an investigation to ascertain the effectiveness of contact lens solutions against current clinical and tap water *Acanthamoeba* isolates as well as whether the addition of taurine enhanced *Acanthamoeba* survival against contact lens solutions due to the increase in the incidence of *Acanthamoeba* keratitis and their subsequent resistance to some multipurpose contact solutions. Their results indicated that the majority of the MPSs were ineffective, with a 100% survival rate for all strains after six hours of exposure. With survival of three out of five strains (Clear Care) and one out of five strains (UltraCare) at six hours, hydrogen peroxide systems were more successful. The strain of tap water in the Chicago area was the most resistant. Including taurine in hydrogen peroxide systems made no statistically significant difference in *Acanthamoeba* survival. They came to the conclusion that *Acanthamoeba* strains found in tap water and clinical samples, which represent established human pathogens and/or household strains, are extremely resistant to contact lens solutions.

The risk of AK may be increased in developing nations like Nigeria, where over-the-counter pharmaceutical product regulation may be inconsistent and borehole water, a possible *Acanthamoeba* source, is frequently used. As a result, it is essential to assess the amoebicidal effectiveness of contact lens solutions sold in local markets in order to protect the public and help consumers make informed decisions. According to a recent study, approximately half of patients with *Acanthamoeba* keratitis, which is usually brought on by inadequate contact lens care, experience some visual loss. Eighty-three persons who wore contact lenses and contracted *Acanthamoeba* keratitis were included in the study. The patients' clinical data and self-administered questionnaires for those who wore daily disposable contact lenses were compared to those for those who wore reusable lenses. According to Carnt *et al*. (2022), the latter tripled the likelihood of infection. In both groups of lens users, a number of modifiable risk factors were

discovered. The following were associated with *Acanthamoeba* keratitis among those who wore daily disposable lenses: less frequent professional follow-up (odds ratio [OR]: 10. 12), showering in lenses (OR: 3. 29), lens reuse (OR: 5. 41), and overnight wear (OR: 3. 93).

It is argued that daily disposables are not risk-free, and that we may reduce this risk by discontinuing the practice of reusing daily disposable items and scheduling more frequent optometry visits. Among users of both daily disposable and reusable contact lenses, two important risk factors were also found to be leaving lenses on while sleeping and showering in lenses, with the former increasing the risk of infection by 3.3 times (Carnt *et al*., 2022),

Therefore, specific trophozoite/cyst tests that are relevant to AK research are frequently used in conjunction with ISO methods. McAnally *et al*. (2021) study employed the International Standards Organization protocol 14729 and 18259, which involved inoculating the microorganisms into each MPS both with and without contact lenses, and maintaining them for the manufacturer's recommended disinfection time of 24 hours and 7 days following challenge with *Serratia marcescens* or *Fusarium* spp. The number of live microorganisms was determined by conducting plate counts on plates that had been incubated for two to seven days. Without contact lenses, the majority of MPSs displayed much greater disinfection effectiveness. Overall, when combined with any contact lens, the OPTI-FREE products (Puremoist, Express, and Replenish) maintained the highest disinfection efficacies among the tested microorganisms at the manufacturer's specified disinfection duration, when compared to other MPSs. RevitaLens and renu Advanced were next in line.

The most effective disinfection against a variety of ocular pathogens was found in myristamidopropyl dimethylamine and polyquaternium-1, which are dual biocides in

multipurpose solutions. Additionally, at advised disinfection durations, early and later in-vitro experiments have consistently demonstrated that several maintained, all-in-one MPS have little efficacy against *Acanthamoeba*, especially the cyst stage (Hussain *et al*., 2021). Although the effectiveness of hydrogen-peroxide systems (particularly 3% one-step or 0. 6% two-step peroxide regimens) varies depending on the organism stage, strain, presence of a lens/case, and exposure time, comparative assessments have shown that they are more consistently amoebicidal than many preserved MPS. According to recent study, peroxide systems may be superior to povidone-iodine systems for treating both trophozoites and cysts when lenses are present during the challenge (Johnston *et al.,* 2009).

Lens material and the presence of a lens or case during testing influenced observed efficacy. Some studies noted greater adherence of *Acanthamoeba* to silicone hydrogel surfaces compared with conventional hydrogel, and showed that including the lens during challenge can reduce measured disinfectant performance. Lens cases are frequent reservoirs of microbial contamination; behaviours such as topping-up solution and infrequent case replacement facilitate biofilm formation and survival of pathogens, including *Acanthamoeba* (Joslin *et al*., 2007).

2.6 Comparative Efficacy of Care Systems against *Acanthamoeba*

When used at the manufacturer's recommended disinfection times, several in vitro studies have repeatedly shown that preserved, all-in-one multipurpose solutions (MPS) have little effect against *Acanthamoeba*, especially against the extremely resistant cyst stage. Although many MPS satisfied the ISO 14729 efficacy criteria for bacteria and fungi, they frequently fall short of producing clinically significant drops in *Acanthamoeba* viability, which raises worries about

their protective ability when used in real-world contact lenses (Borazjani and Kilvington, 2005; Shoff *et al*., 2008; Hussain *et al*., 2021).

On the other hand, hydrogen-peroxide–based care systems, which commonly use 3% peroxide in one-step or two-step procedures, have consistently shown superior amoebicidal effectiveness, achieving consistent inactivation of trophozoites and, with enough exposure time, partial to full cyst kill. Comparative study showed that peroxide systems, especially those based on povidone-iodine and preserved MPS, perform better, particularly when lenses are present during a microbial challenge (Hiti *et al*., 2005; Johnston *et al*., 2009; Walters *et al*., 2021). Peroxide systems have improved effectiveness because of their potent oxidative mechanism, which breaks down cyst wall integrity, cellular membranes, and enzymes—characteristics that are frequently unaffected by preserved biocides.

In a 2022 head-to-head study, Walters *et al.* (2022), employed standardized plate-count techniques under simulated real-world use scenarios, it was discovered that only hydrogen-peroxide systems maintained robust, broad-spectrum antimicrobial activity against tested organisms, including *Acanthamoeba*, while preserved MPS exhibited significant decreases in effectiveness. These results were further corroborated by more recent systematic testing, which ranked peroxide- and hypochlorite-based disinfection methods as the most effective, especially when preceded by a specialized cleaning or rubbing step that lowers organic load and surface-adherent organisms (Pastrana *et al*., 2025).

Notably, study that uses lens cases and contact lenses during testing consistently revealed lesser disinfection effectiveness than experiments using just solutions. This decrease is a result of active biocides being adsorbed onto lens materials, organic waste neutralizing the biocides, and

the protective benefits of biofilms inside lens cases (Joslin *et al*., 2007; McAnally *et al*., 2021). Particularly, silicone hydrogel lenses have been demonstrated to have a higher *Acanthamoeba* adherence than some traditional hydrogel materials, which may raise the microbial load that disinfection systems must handle (Ibrahim *et al*., 2009; Walters *et al.,* 2022).

Lens cases themselves represent a persistent reservoir for contamination, especially when users engage in unsafe practices such as topping-up old solution, infrequent case replacement, or exposure to tap or borehole water. Biofilm formation within cases significantly enhances *Acanthamoeba* survival and resistance to disinfectants, underscoring the importance of regimen-based testing and comprehensive user education (Joslin *et al.,* 2007; Thomas and Ashbolt, 2011). Collectively, these findings highlight that disinfection efficacy observed under idealized laboratory conditions may substantially overestimate real-world performance, reinforcing the need for stringent testing protocols and improved preventive strategies.

Figure 2.1A typical contact Lens and a case (Source:

https://www.istockphoto.com/photos/contact-lens-case)

Figure 2.2 A typical contact Lens and a case (Source:

https://www.istockphoto.com/photos/contact-lens-case)

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

Water samples were collected from bore-hole water sources, such as reticulated bore-hole water from storage tanks in areas where contact lens usage was prevalent (such as, urban and semi-urban parts of Benin City).

Benin City, is positioned at latitude 6° 20' North, and longitude 5° 37' East (Fig. 3.1) (Idibie *et al.,* 2018). It is strategically located along the main highways linking Lagos to Asaba and as well as the eastern states. Situated on a branch of the Benin River, the city is well-connected by air, with access to the ports of Koko and Sapele in the Niger River Delta, as well as road links to Sapele, Siluko, Okene, and Ubiaja (Idibie *et al.,* 2018).

3.2 Research Design/ Sample Collection

The study is a cross-sectional investigation. Water samples from boreholes, were collected from various locations in Benin City. Samples were collected separately into sterile, dry, and clean 75cl polypropylene containers. The samples were then taken to the laboratory for processing. The primary aim was to evaluate and compare the amoebicidal efficacy of various multipurpose contact lens care solutions available in the Nigerian market against *Acanthamoeba* spp. *in vitro*. The study involved both isolation of *Acanthamoeba* from environmental sources and testing of contact lens solutions against cultured trophozoites and cysts.

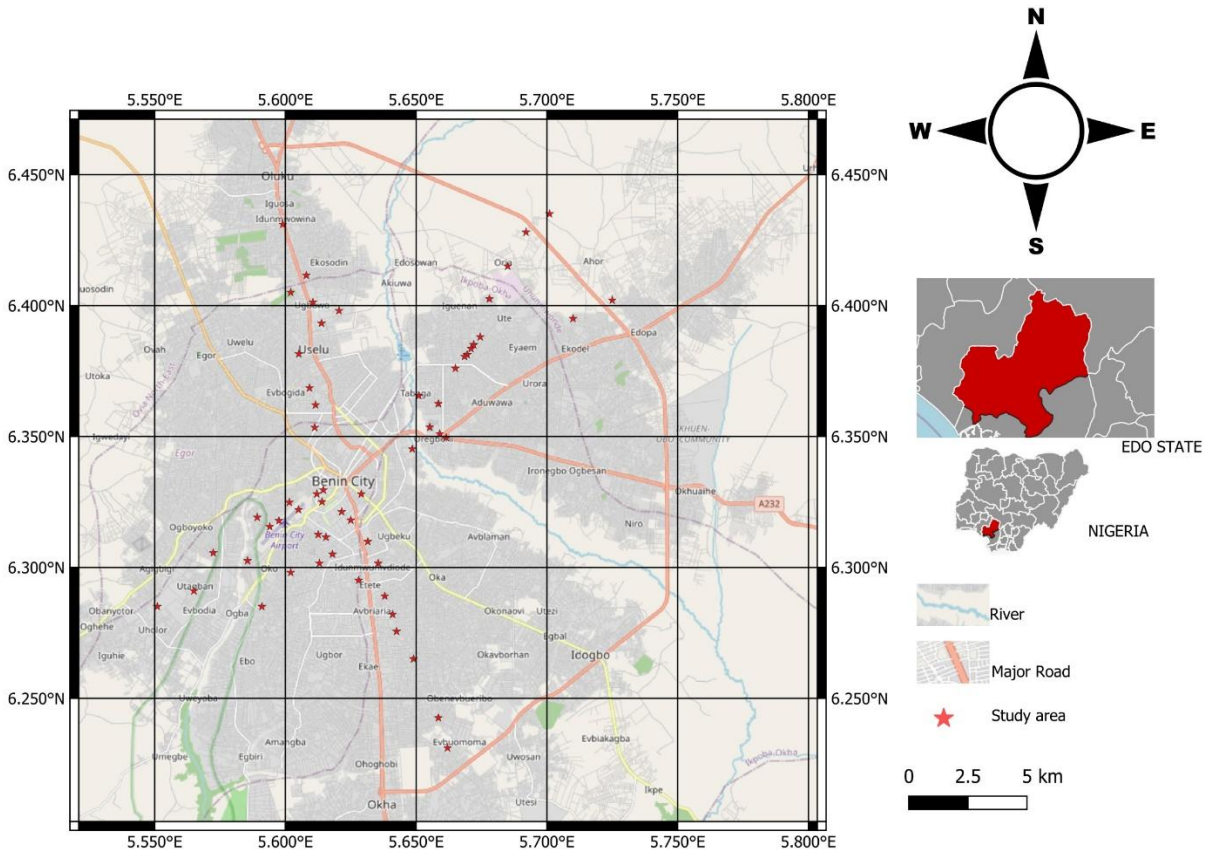

Figure 3.1 Map of the study area showing the sampled stations within the Benin Metropolis, Edo State, Nigeria (Software: QGIS.org)

3.3 Sampling Technique

A purposive sampling technique was employed for the selection of water sources. Borehole water commonly used for domestic activities and contact lens hygiene were deliberately selected from different locations within Benin City. This approach was adopted to maximize the likelihood of isolating environmentally relevant *Acanthamoeba* species associated with human exposure. A total of 36 reticulated borehole water samples were collected during the study period. Each water source was sampled once to avoid duplication.

3.4 Materials

Sterile polypropylene containers

Nitrocellulose membrane filter (0.45 μm pore size and 47 mm diameter).

Stainless steel filter

Suction pump

Non-nutrient Agar

Heat-killed *Escherichia coli.*

3.5 Procedure for Detection of *Acanthamoeba* species

In the process of identification of *Acanthamoeba* species, 500 mL of water underwent filtration through a 0.45μm-pore-sized cellulose nitrate membrane. The membrane was covered with parafilm and placed in the incubator at 32ºC to facilitate the growth of free-living amoebae. After a 3-day incubation period, the plates were examined under the microscope to detect *Acanthamoeba* trophozoites. Throughout this phase, the plates were maintained at 26 ºC. If, after

the initial incubation, no parasite was observed, the plates were continuously monitored for up to 14 days under the same conditions (Al-Herrawy *et al*.,2014).

Morphological Identification of motile trophozoites and double-walled cysts was identified using standard morphological criteria, including pseudopodia shape and cyst wall structure; comparing same from sample plates as a guide (Al-Herrawy *et al*.,2014 and 2017). The distinctive features of the cysts, including their shape, size, the number and arrangement of the cyst pores, were instrumental in identifying different species within the genus *Acanthamoeba*.

3.6 Preparation of *Acanthamoeba* for Testing

Isolated *Acanthamoeba* trophozoites were harvested from cultured plates and standardized to approximately 10^5 organisms/mL**.** To induce encystment, trophozoites were transferred to encystment media (Neff's saline) and incubated at room temperature for 72 hours.

3.7 Test Solutions

Three commercially available multipurpose contact lens solutions marketed in Nigeria namely: Freshlook™ (Polyhexanide 0.0001%), Refresh™(Sodium Chloride) and Freesol™ (Polyhexamthylene Biguanide-Hydrochloride Etidronatetetra sodium) were selected based on market availability and consumer use. Solutions were tested undiluted as per manufacturer instructions.

Figure 3.1 Three Multi-purpose Contact Lens Solutions: Freshlook™ (Polyhexanide 0.0001%), Refresh™(Sodium Chloride) and Freesol™ (Polyhexamthylene Biguanide-Hydrochloride Etidronatetetra sodium)

3.8 Antimicrobial Efficacy Testing

Antimicrobial efficacy was evaluated using the Stand-Alone Test in accordance with ISO 14729 guidelines, designed to assess the intrinsic anti-*Acanthamoeba* activity of the test solutions independent of mechanical lens cleaning or rubbing. Equal volumes of standardized *Acanthamoeba* trophozoite or cyst suspensions were mixed with each test solution in sterile tubes to ensure uniform organism exposure. The mixtures were maintained at predefined contact times of 15, 30, 60, and 240 minutes, reflecting both manufacturer-recommended disinfection periods and extended exposure conditions (International Organization for Standardization, 2001).

At the conclusion of each contact time, aliquots were aseptically withdrawn, neutralized where necessary, and inoculated onto non-nutrient agar (NNA) plates overlaid with *Escherichia coli* to support amoebal recovery and growth. The inoculated plates were incubated under appropriate conditions and monitored daily for evidence of trophozoite emergence or cyst excystment (Kilvington and Lonnen, 2009). The presence or absence of regrowth was used as an indicator of organism survival, thereby allowing determination of the time-dependent antimicrobial effectiveness of each test solution against both trophozoite and cyst forms of *Acanthamoeba*.

3.9 Viability Assessment

After incubation, viable trophozoites or cysts were assessed microscopically. Vital staining with methylene blue exclusion test was utilized to confirm organism viability.

3.10 Limitation of Study

The rising cost of laboratory procedures incurred during this study constituted a limitation. The reluctance of borehole owners to allow the collection of water samples was a constant challenge.

3.12 Data Analysis

Data were analyzed using SPSS following entry into Microsoft Excel. Outcomes were reported as mean log reductions in viable trophozoites and cysts. Differences in efficacy among solutions across time points were assessed using Analysis of Variance (ANOVA), with statistical significance defined as $p < 0.05$. Values are expressed as mean ± standard deviation (SD).

3.13 Ethical Considerations

This study involves no human or animal subjects. However, standard biosafety protocols were followed during handling of microorganisms and chemical agents to ensure laboratory safety (CDC, 2024).

CHAPTER FOUR

4.0 RESULTS

4.1 Overall Prevalence of *Acanthamoeba* Species in Reticulated Bore-hole Water.

A total of 36 water samples were examined for *Acanthamoeba* species in this study and 32 (88.9%) were infected; and a mean total of 209 *Acanthamoeba* were recorded from the 32 positive samples, yielding a mean intensity of 6.53 detections per positive sample**.** Trophozoites and cystic forms became visible approximately a week in some samples and about 3 weeks in others. Ten *Acanthamoeba* species including *A. astronyxis, A. castellanii, A. comandoni, A. culbertsoni, A. lenticulate, A. mauritaniensis, A. polyphaga, A. quina, A. triangularis,* and *A. royreba* were detected in water samples. *Acanthamoeba royreba* exhibited the highest prevalence (17.70%; 37 isolates), followed by *A. polyphaga* (11.96%; 25 isolates), *A. lenticulata* and *A. mauritaniensis* (each 11.00%; 23 isolates each). The lowest prevalence (1.44%; 3 isolates) of parasitic infection was recorded for *A. astronyxis*. The prevalence of *Acanthamoeba* species in reticulated bore-hole water is presented in Figure 4.1. Plate 4.1, represents the cyst stages of detected *Acanthamoeba* species from Borehole after culture before treatment.

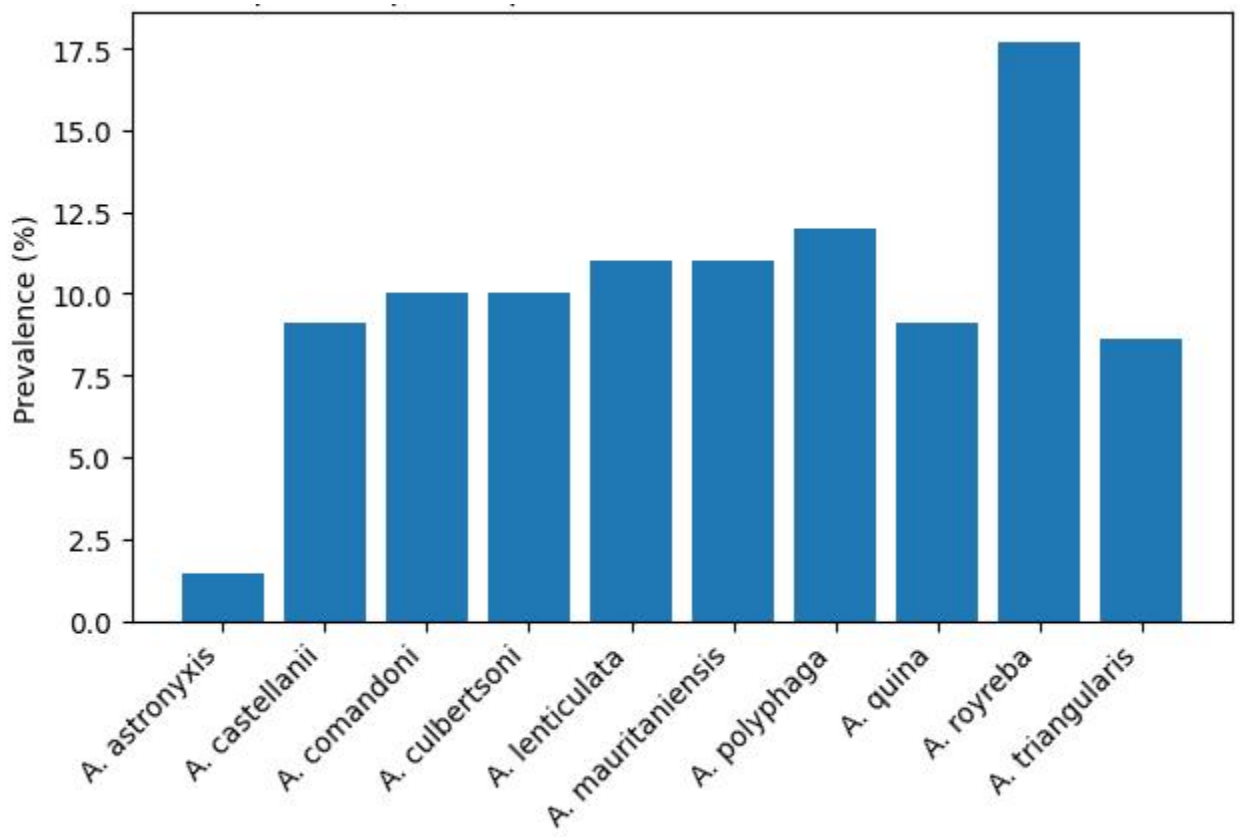

Figure 4.1 Prevalence of *Acanthamoeba* species from Reticulated Borehole Water Samples

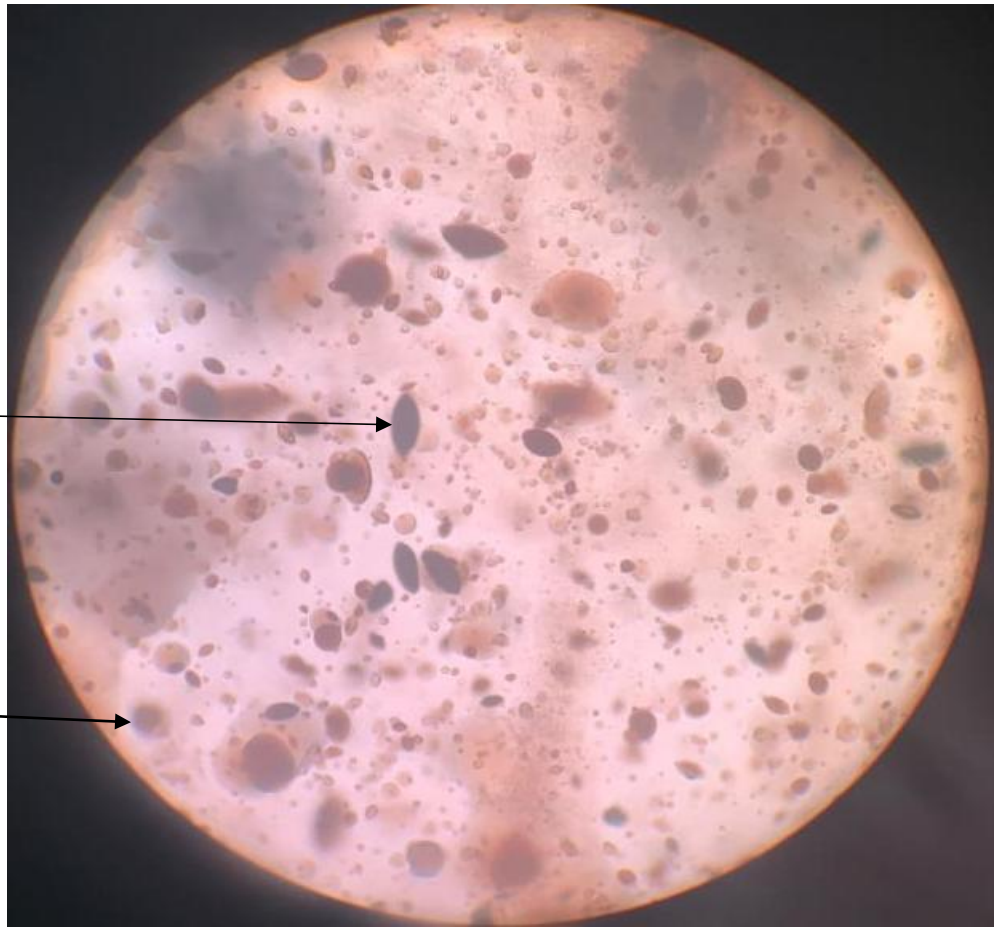

Plate 4.1. Cyst Stages of Detected *Acanthamoeba* species from Reticulated Bore-hole Water after Culture before Treatment

4.2. Prevalence of *Acanthamoeba* Species after Treatment with Three Multipurpose Contact Lens Solutions

From the 32 infected water samples, a mean total of 209 (100%) *Acanthamoeba* species were detected. Treatment with multipurpose contact lens solutions resulted in varying degrees of reduction in *Acanthamoeba* prevalence. Exposure to Freesol™ reduced the number of detectable *Acanthamoeba* species to 98**(**46.89%)**,** representing a statistically significant reduction compared with pre-treatment levels ($p = 0.010$) (Fig. 4.2). Treatment with Refresh™ reduced the number of detectable species from 209 to 101**(**48.33%**),** which was also statistically significant ($p = 0.014$) (Fig. 4.3) Treatment with Freshlook™ reduced the number of detectable *Acanthamoeba* species to 170**(**81.34%) (Figure 4.4). Overall, treatment with all three multipurpose contact lens solutions resulted in a significant reduction in *Acanthamoeba* species burden (Figure 4.5). Plate 4.2 represents cyst stages of detected *Acanthamoeba* species from reticulated Borehole water after culture and after treatment with Freesol™. The distribution of *Acanthamoeba* counts before and after treatments is represented in Table 4.1.

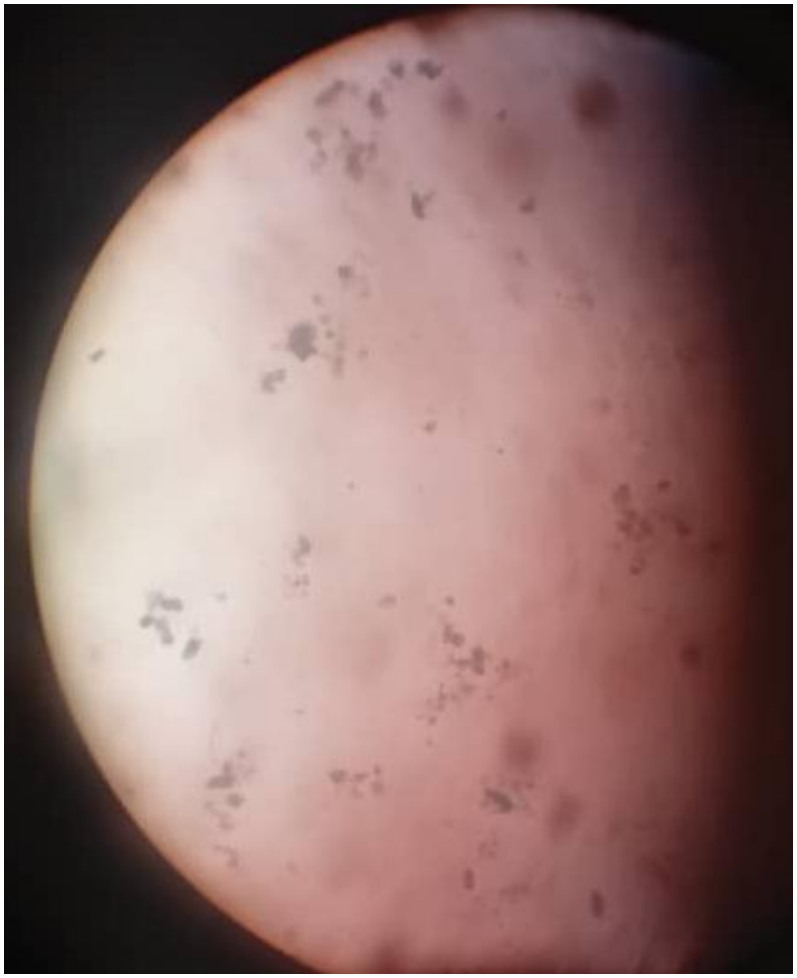

Plate 4.2. Cyst Stages of Detected *Acanthamoeba* species from Reticulated Bore-hole Water after Culture and after Treatment with Freesol™

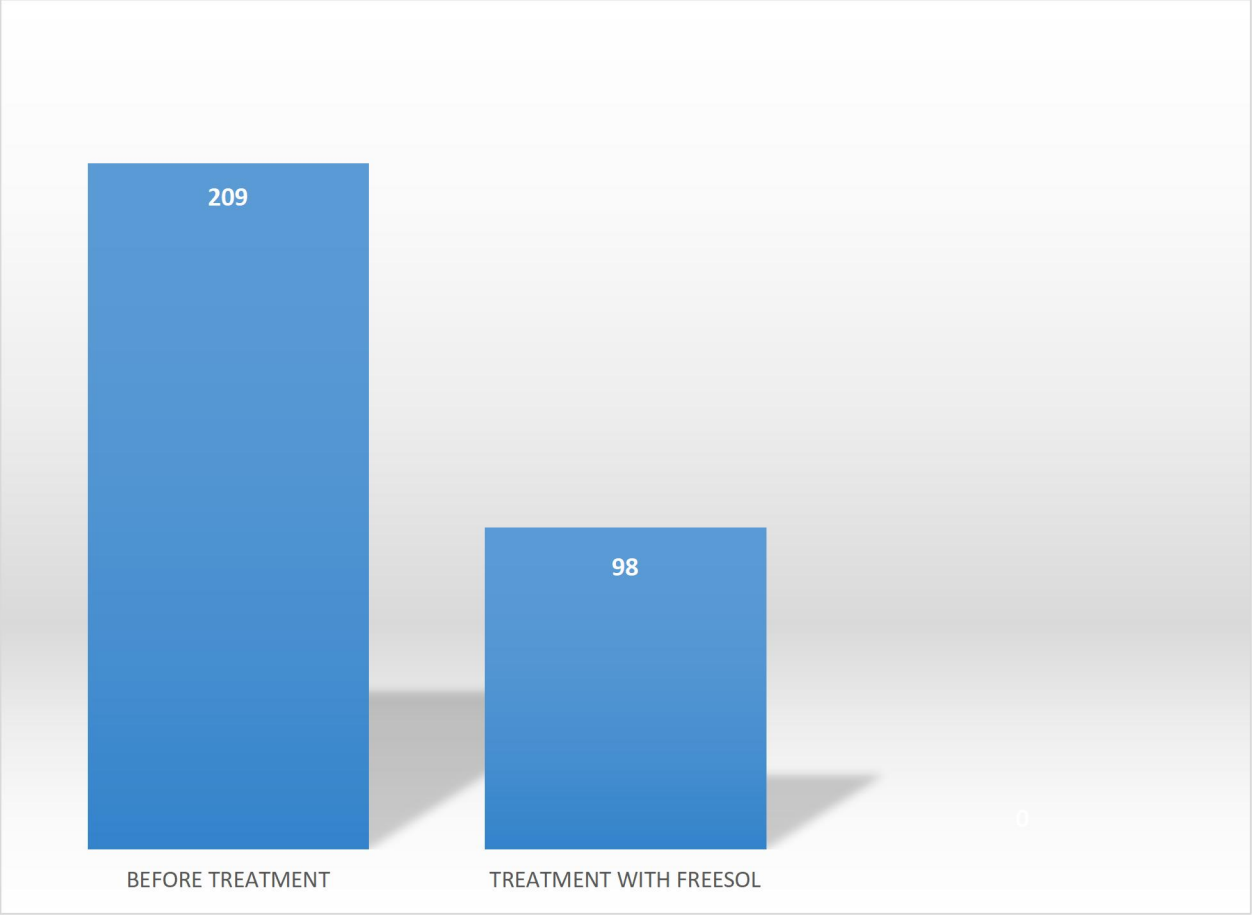

Figure 4.2 Distribution of the Number of *Acanthamoeba* species before and after Treatment with Freesol™ (Polyhexamthylene Biguanide-Hydrochloride Etidronatetetra sodium)

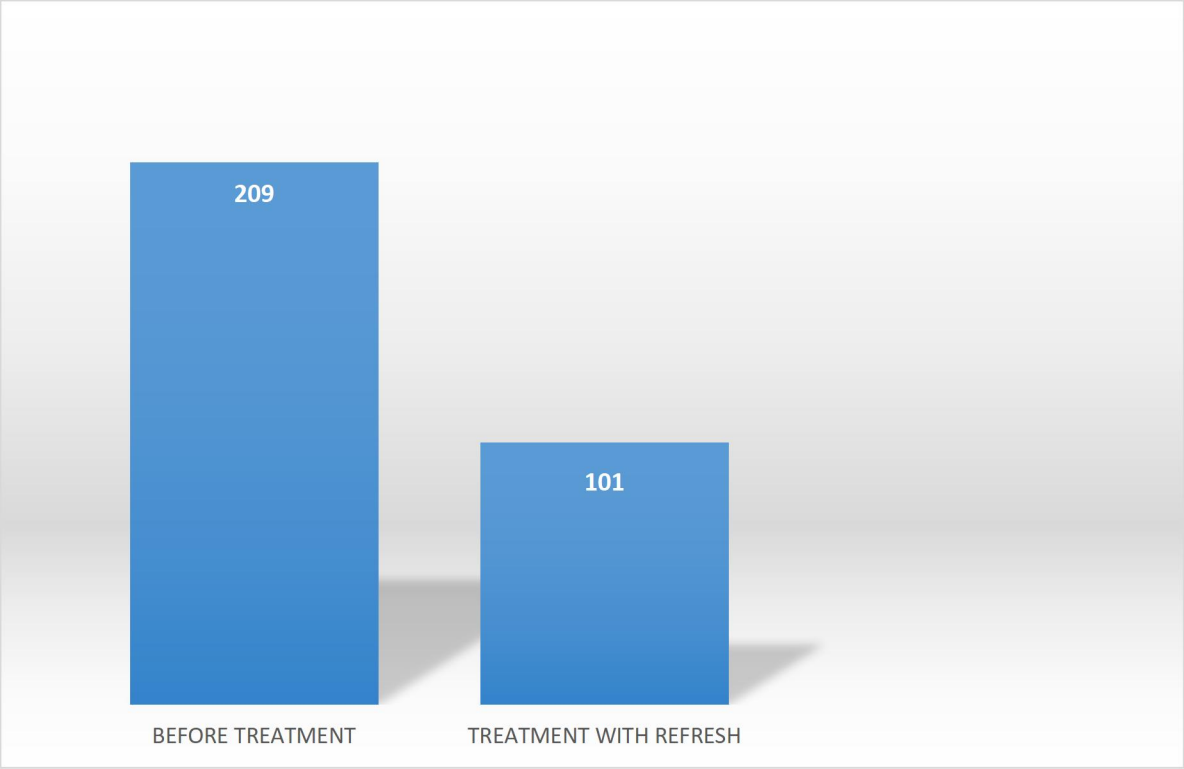

Figure 4.3. Distribution of the Number of *Acanthamoeba* species before and after Treatment with Refresh™ (Sodium Chloride)

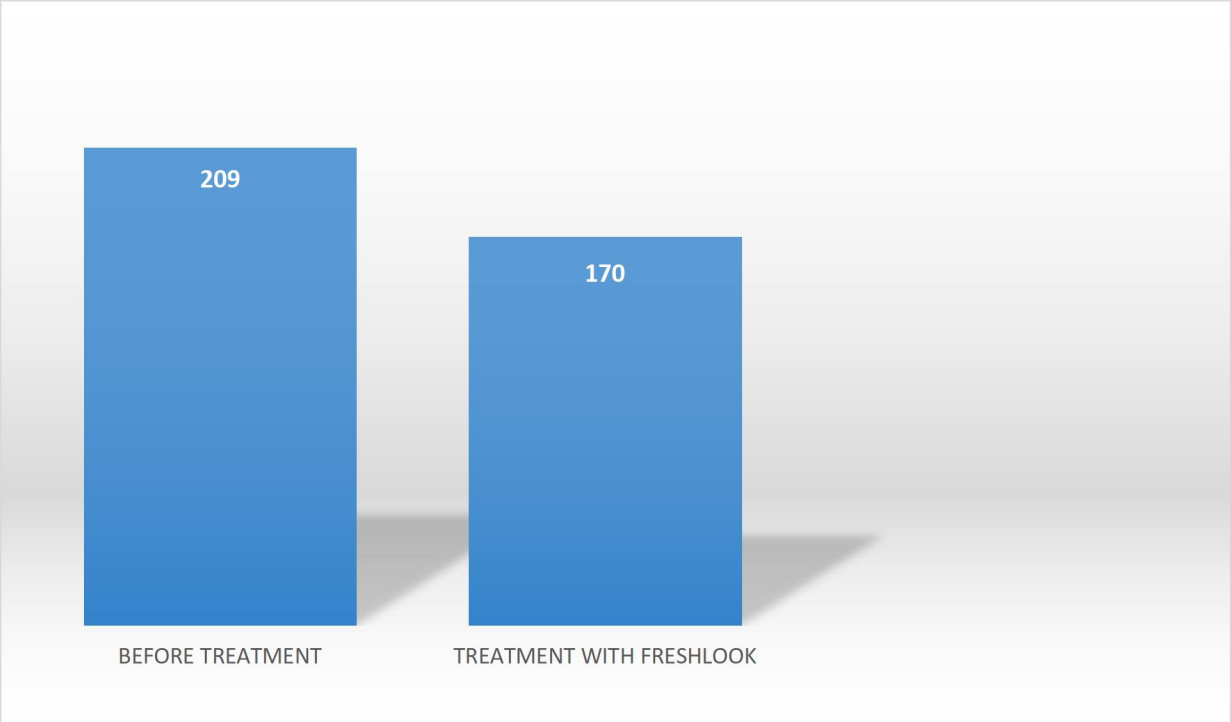

Figure 4.4. Distribution of the Number of *Acanthamoeba* species before and after Treatment with Freshlook™ (Polyhexanide 0.0001%)

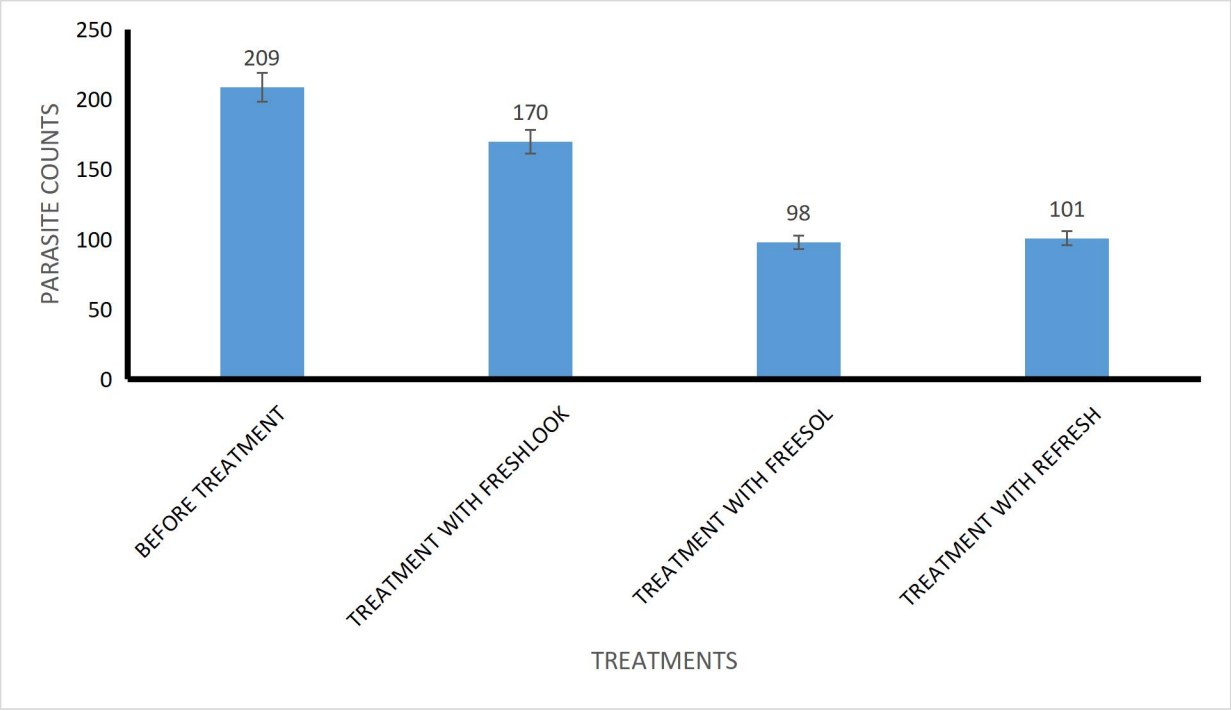

Figure 4.5. Distribution of the Number of *Acanthamoeba* species before and after Treatment with all three Multipurpose Contact Lens Solutions

Means with different superscript letters (a–d) are significantly different from one another as determined by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test at $p < 0.05$.

TABLE 4.1. *Acanthamoeba* Counts Before and After Treatments

PARAMETERS	PARASITES COUNTS (Mean ± S.D)
BEFORE TREATMENT	209 ± 7.88^{d}
TREATMENT WITH FRESHLOOK™	170 ± 22.0^{c}
TREATMENT WITH FREESOL™	98 ± 9.5^{a}
TREATMENT WITH REFRESH™	101 ± 12.5^{b}

Means with different superscript letters (a–d) are significantly different from one another as determined by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test at $p < 0.05$.

Acanthamoeba counts differed significantly across treatment groups ($p < 0.05$). The highest mean organism count was observed before treatment (209 ± 7.88), followed by treatment with Freshlook™ (170 ± 22.0). Freesol™ treatment resulted in the greatest reduction in parasite counts (98 ± 9.5), which was significantly lower than all other groups. Refresh™ also produced a

significant reduction (101 ± 12.5), though parasite counts remained higher than those observed with Freesol™.

CHAPTER FIVE

5.0 DISCUSSION

This study examined the prevalence of *Acanthamoeba* species from bore-hole water sources from Benin Metropolis commonly used in contact lens hygiene.

Compared to others studies on tap/groundwater studies globally, where the prevalence of *Acanthamoeba* varied from about 10 to 45% depending on the source and methodology used (Carnt *et al.,* 2020). Aykur *et al*., (2021), recorded a prevalence of 12.16% of *Acanthamoeba* species; a very high prevalence (88.9%) of *Acanthamoeba* species in bore-hole water samples was recorded. This indicates that bore-hole water may serve as significant reservoirs for free-living amoebae capable of causing ocular infections such as *Acanthamoeba* keratitis, particularly among contact lens users. The high contamination rate observed in this study suggest that these organisms are widely distributed in the aquatic environment and capable of surviving in groundwater commonly used for domestic purposes. The mean intensity of 6.53 detections per positive sample further supported the widespread presence of these protozoans in the sampled water sources. The observation that trophozoites and cysts became visible after approximately one to three weeks of culture indicate that some strains required longer incubation periods for growth (Marciano-Cabral *et al*.,2003), reflecting differences in species adaptability and environmental survival strategies. The presence of both trophozoite and cyst stages is significant because cysts are highly resistant forms that can withstand adverse environmental conditions and many disinfectants (Khan, 2009).

The species profile identified in this study (*A. royreba, A. polyphaga, A. lenticulata, A. mauritaniensis, A. culbertsoni, A. triangularis, A. astronyxis*) aligned with water studies from Egypt and other regions, which often found *A. polyphaga, A. lenticulata*, and *A. triangularis* in

tap/tank water and drinking systems (Al-Herrawy *et al*, 2017). The predominance of these species may be attributed to their known ecological adaptability and ability to thrive in various water environments. In particular, some of these species have been previously associated with human infections, suggesting potential public health risks. Seven species of *Acanthamoeba* were reported by Nageeb *et al.,* (2022), with *A. triangularis*, *A. polyphaga*, *A. lenticulata*, and *A. culbertsoni* being thermo- and osmo-tolerant, and *A. astronyxis*, *A. comandoni*, and *A. echinulata* being non-thermo- and non-osmo-tolerant. The group most commonly linked to *Acanthamoeba* keratitis (AK) and other human illnesses is the T4-related genotype, to which several of these belong (*A. polyphaga, A. lenticulata*, *A. culbertsoni, A. triangularis*) (Hassan *et al.,* 2021). This shows a genuine risk to public health for those who wear contact lenses with such water. Prior studies have frequently isolated *A. royreba* from water and soil environments and shown its exceptional tolerance to disinfectants (Booton *et al.,* 2005), which is consistent with its dominance (17. 7%). The low incidence of *A. astronyxis* may be attributable to its poor culturability in laboratory settings or to species-specific environmental preferences (Visvesvara *et al.,* 2007). This study also evaluated the effectiveness of three multipurpose contact lens solutions in reducing *Acanthamoeba* contamination. The results demonstrated that all three solutions significantly reduced the number of detectable *Acanthamoeba* species, although their effectiveness varied.

Before treatment, a total mean count of 209 *Acanthamoeba* species was recorded from the infected samples. After treatment, significant reductions were observed ($p < 0.05$), in the multipurpose contact lens solutions indicating that solutions possessed anti-amoebic activity. However, none of the tested solutions completely eliminated the organisms.

Among the tested solutions, Freesol™ exhibited the highest efficacy, reducing the parasite mean count to 98 (46.89%), representing the greatest reduction among the three products tested. This effectiveness may be attributed to the presence of polyhexamethylene biguanide (PHMB), which is known to possess strong antimicrobial activity against protozoa, bacteria, and fungi. PHMB has been widely reported as an effective agent against *Acanthamoeba*, particularly the trophozoite stage. Furthermore, the anti-amoebic action of PHMB has been attributed to its ability to disrupt cytoplasmic membranes and interfere with cellular metabolism in protozoa (Lonnen *et al.,* 2012). Similarly, Refresh™ solution also demonstrated significant anti-amoebic activity, reducing the parasite mean count to 101 (48.33%). Although effective, its performance was slightly lower than that of Freesol™. This difference may be related to variations in the formulation and concentration of active antimicrobial agents present in the solution.

In contrast, Freshlook™ showed the least effectiveness, reducing the parasite count to 170 (81.34%). Although the reduction was statistically significant, the relatively high number of surviving organisms suggests that this solution may have limited efficacy against certain *Acanthamoeba* species or cyst stages. RefreshTM's efficacy may be explained by its composition, which is mostly intended to rinse and comfort lenses rather than kill protozoa. Even if polyhexanide is present in Freshlook™, its somewhat reduced effectiveness could indicate that the amoebicidal potency is significantly influenced by concentration, formulation, exposure time, and cyst maturation (Shoff *et al.,* 2008). This is important because cysts are known to be highly resistant to many disinfecting agents used in contact lens solutions. This finding also aligns with numerous investigations that show that *Acanthamoeba* cysts are extremely resistant to widely used contact lens disinfectants (Johnston *et al*., 2009; Kilvington *et al.,* 2009). Many MPS, especially at conventional PHMB concentrations (about 0. 0001%), have been found in studies to

have little effect against *Acanthamoeba*, particularly cysts (Kilvington *et al.,* 2013; Kolar *et al.,* 2015 Bahr *et al.,* 2025).

Typically, hydrogen peroxide and povidone iodine systems kill trophozoites at higher rates and sometimes have superior cysticidal action compared to just PHMB MPS (Kilvington *et al*., 2013; Kolar *et al.,* 2015; Pastrana *et al.,* 2025; Bahr *et al.,* 2025). Additionally, *in vitro* studies indicate that versatile treatments can lower the motility and viability of trophozoites, but they are frequently ineffective in killing cysts, which may live and encyst in lens cases and solution leftovers (Verani *et al.,* 2009; Thomas *et al.,* 2010; Ahearn *et al.,* 2011; S. Kolar *et al.,* 2015). Furthermore, the remaining *Acanthamoeba* counts after disinfection in this study indicate organism resistance, with an increase in cystic forms. This is consistent with experimental studies which showed that only strong biocides (such as 3% H2O2) can completely eliminate cysts during regular disinfection times (Kilvington *et al.,* 2013; Kolar *et al.,* 2015).

The persistence of *Acanthamoeba* following therapy has major consequences for contact lens–associated keratitis, a disease that is difficult to diagnose and has bad visual results when treatment is postponed (Dart *et al*., 2009). Existing advice that bore-hole water should never be used for contact lens cleaning, rinsing, or storage is supported by the high environmental prevalence seen in this study (CDC, 2019).

There was a significant difference ($p < 0.05$) among treatment groups, an indication that the effectiveness of the solutions varied. Freesol™ achieved the greatest reduction in parasite counts, followed by Refresh™, while Freshlook™ had the least reduction. The combination of extremely polluted Bore-hole water and a partially effective MPS fits well with established risk patterns for

AK, which include tap or ground water contaminating lenses, cases, and solutions, as well as insufficient disinfection (Verani *et al*. 2009; Gomes *et al*. 2016; Cope *et al.* 2016; Carnt *et al.* 2020; Hassan *et al.,* 2021).

Epidemiological studies have consistently linked the exposure of lenses/cases to tap water and subpar solution performance to AK outbreaks and sporadic instances, even when solutions satisfy bacterial standards (Verani *et al*., 2009; Gomes *et al*., 2016; Cope *et al.*, 2016; Carnt *et al.,* 2020).

The discovery of species like *A. polyphaga* and *A. lenticulata* in water, which have also been found on lenses and in AK cases elsewhere, supports the idea that this water might cause lens contamination and infection (Gomes *et al.,* 2016; Hassan *et al.,* 2021; Nageeb *et al*., 2022).

From a public health standpoint, the results emphasize the need for: improved water quality surveillance**,** public education on contact lens hygiene, and Stricter regulatory evaluation of contact lens solutions against *Acanthamoeba* cysts.

The superior performance of PHMB-containing solutions suggests they may offer a relative advantage**,** although they should be used alongside rigorous hygiene practices.

Limitations of this study include the modest sample size and reliance on morphological identification, which may underestimate cryptic species diversity. Molecular techniques such as PCR-based genotyping would enhance species-level resolution (Booton *et al.,* 2005). Additionally, future studies should assess cyst viability post-treatment and explore extended exposure times or combination disinfection strategies**.**

5.1 SUMMARY

This study investigated the prevalence and burden of *Acanthamoeba* species in reticulated borehole water samples. A total of 36 water samples were examined, of which 32 (88.9%) tested positive for *Acanthamoeba*, indicating widespread contamination of the sampled water sources. From the positive samples, a total of 209 *Acanthamoeba* detections were recorded, yielding a mean intensity of 6.53 detections per positive sample. Both trophozoite and cyst forms were identified, confirming the presence of viable and potentially infectious stages of the organism.

The high prevalence indicates that reticulated borehole water may be a major environmental reservoir for *Acanthamoeba*, which could pose a risk to public health, especially for those with weakened immune systems and those who wear contact lenses. Furthermore, the selected multipurpose contact lens care products used in Nigeria have showed reduced effectiveness against *Acanthamoeba* species. These results emphasized the necessity of enhanced water treatment methods, regular monitoring of water sources, and greater public education about safe water consumption in order to lower the risk of infections caused by *Acanthamoeba*. However, there is dearth of studies on *Acanthamoeba* in Nigeria, emphasizing the necessity for more investigation.

5.2 CONTRIBUTION TO KNOWLEDGE

The study has contributed to knowledge in the following ways:

1. Revealed a substantial prevalence of *Acanthamoeba* species in Bore-hole water sources in Benin City.
2. Revealed that the common multi-purpose contact lens care solutions available in Nigeria showed limited control over *Acanthamoeba* species.

5.3 CONCLUSION AND RECOMMENDATION

This study confirmed that reticulated bore-hole water harbours a high burden of diverse *Acanthamoeba* species**,** many of which are clinically relevant. Although multipurpose contact lens solutions significantly reduced amoebic counts, none achieved complete elimination, underscoring the organism's resilience. These findings highlight the importance of safe water use, improved disinfectant efficacy, and strict adherence to contact lens hygiene to reduce the risk of *Acanthamoeba* infection. When wearing contact lenses, the advice is to avoid use of bore-hole water for their cleaning and storage.

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APPENDIX

ISO 14729:2001(en)
Ophthalmic optics — Contact lens care products — Microbiological requirements and test methods for products and regimens for hygienic management of contact lenses

Foreword

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International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 3.

Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this International Standard may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

International Standard ISO 14729 was prepared by Technical Committee ISO/TC 172, *Optics and optical instruments*, Subcommittee SC 7, *Ophthalmic optics and instruments*.

Introduction

Products for contact lens disinfection by chemical means are intended to reduce microbial contamination introduced during lens wear and removal, cleaning and storage and are required to contain antimicrobial agents capable of achieving this.

It is essential that all liquid contact lens care products are sterile until opened. Dry products (tablets, granules, etc.) should be subject to control of microbial contamination and should be dissolved in a suitable diluent immediately prior to use. Multidose contact lens care products must be adequately preserved or be packaged in a container designed and labelled to minimize the risk of injury resulting from in-use contamination.

Contact lenses are normally subject to a regimen of cleaning and contact lens disinfection between periods of wear. Aqueous solutions containing cleaning and/or disinfecting agents are commonly used for this purpose. These products may be marketed as solutions or as tablets for dissolution immediately prior to use in a suitable diluent such as saline.

The past 20 years of experience in the use and regulation of contact lens disinfecting products has shown distinct disinfecting antimicrobial criteria for this class of medical devices. Ocular toxicology concerns, process convenience and product comfort on the eye, have meant an evolution of products which maintain a low incidence of contact lens associated ocular infection when used as instructed by the manufacturer. This International Standard gives these distinct contact lens disinfecting antimicrobial criteria along with annexes to explain why viruses (annex C) and *Acanthamoeba* (annex D) are not included as challenges. Organic soil is not required for evaluation of contact lens care disinfecting products but may be used; an informative

annex (annex E) is included to discuss organic soil in the context of contact lenses and contact lens care products.

1 Scope

This International Standard specifies two test methods for evaluating the antimicrobial activity of products to be marketed for contact lens disinfection by chemical means and for products that are part of a contact lens care regimen.

This International Standard is not applicable to the hygienic management of trial lenses.

ISO 14729 requires a three log reduction for bacteria and a one log reduction for yeast and mold at disinfection time (DT) to meet primary criteria. If an MPS cannot meet this requirement, a manufacturer may demonstrate that the MPS meets secondary criteria, which requires evidence of disinfection when utilizing the directions for use. Further, as previous investigations have indicated that the presence of contact lenses can reduce the antimicrobial efficacy of MPSs, ISO 18259 was developed for manufacturers to assess the impact of contact lenses and contact lens cases on the antimicrobial efficacy of their solutions. It is important that MPSs are tested both with and without lenses present in order to fully understand real-world applications and efficacies of any MPS. These tests should additionally be undertaken in the manufacturer-provided contact lens cases, as biocide uptake by contact lens cases can introduce significant reductions to the presence and activity of MPSs. Although we have previously shown that commonly available MPSs demonstrate strong biocidal efficacy against ocular pathogens such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans*, previous investigations have yet to fully examine the more novel contact lenses available after challenge with resistant infectious microorganisms, such as the *Fusarium* and *Serratia* species. Further, the

most recent additions to the contact lens product market have not been fully scrutinized in this way.

NOTE General disinfection product standards are not applicable to contact lens care products, e.g. EN 1040:1997 and EN 1275:1997.

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

- ISO 8320-1:—[1)], Contact lenses and contact lens care products — Vocabulary — Part 1: **Contact lenses.**
- ISO 8320-2:—[1)], Contact lenses and contact lens care products — Vocabulary — Part 2:

Contact lens care products.

3 Terms and definitions

For the purposes of this International Standard, the definitions given in ISO 8320 apply together with the following.

3.1 contact lens disinfecting product product that possesses cidal activity (kills, destroys and/or inactivates) meeting the primary criteria of the stand-alone test specified in this International Standard

3.2 contact lens disinfecting regimen contact lens care regimen designed to meet both the secondary criteria of the stand-alone test and the regimen test as specified in this International Standard.

3.3 contact lens disinfection chemical or physical process to reduce the number of viable microorganisms as specified in the performance requirement sections of this International Standard.

STANDARD SAFETY PRACTICES IN THE

Microbiology Laboratory

Laboratorians working with infectious agents are subject to laboratory- acquired infections through accidents or unrecognized incidents. The degree of hazard depends upon the virulence of the biological agent concerned and host resistance. Laboratory-acquired infections occur when microorganisms are inadvertently ingested, inhaled, or introduced into the tissues. The primary

laboratory hazard associated with enteric pathogens such as *Shigella* and *E. coli* O157:H7 is accidental ingestion. Biosafety Level 2 (BSL-2) practices

are suitable for work involving these agents, which are a moderate potential hazard to personnel and the environment. BSL-2 requirements:

• Laboratory personnel have specific training in handling pathogenic agents and are directed by competent scientists;

• Access to the laboratory is limited when work is being conducted;

• Extreme precautions are taken with contaminated sharp items;

• Certain procedures in which infectious aerosols or splashes may be created are conducted using protective clothing and equipment.

A. Standard Microbiological Safety Practices

The safety guidelines listed below apply to all microbiology laboratories regardless of biosafety level.

Limiting access to laboratory

Biohazard signs or stickers should be posted near all laboratory doors and on all equipment (incubators, hoods, refrigerators, freezers) used for laboratory work. Children under 12 years of age and pets are not allowed in laboratory areas. All laboratories should be locked when not in use. All freezers and refrigerators located in corridors should be locked.

Handwashing

Each laboratory should contain a sink for handwashing. Frequent handwashing is one of the most effective procedures for avoiding laboratory- acquired infections. Hands should be washed with an appropriate germicidal soap before exiting the laboratory or after handling infectious materials.

Eating

Eating, drinking, and smoking are not permitted in the work areas. Food must be stored and eaten outside of the work area in designated areas used for that purpose only. Do not lay personal articles such as handbags or eyeglasses on the workstations.

Standard Safety Practices in the Microbiology Laboratory

Mouth pipetting

Mouth pipetting should be strictly prohibited in the laboratory. Rubber bulbs or mechanical devices should be used.

Sharps

A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels. Dispose of sharps in designated containers. To minimize finger sticks, used disposable needles must not be bent,

sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.

Non-disposable sharps, including syringes, should be placed in a labeled discard pan for decontamination before cleaning. Broken glassware should not be handled directly by hand but should be removed by mechanical means such as a brush and dustpan, tongs, or forceps.

Aerosols

Perform all procedures carefully to minimize the creation of splashes or aerosols. Techniques that tend to produce aerosols should be avoided. Cool inoculating wires and loops by holding them still in the air for 5 to 10 seconds before touching colonies or clinical material. Loops containing infectious material should be dried in the hot air above the burner before flaming. Vortexing and

centrifugation should be done in closed containers. Gauze should be used to remove the tops on blood specimens and should be placed around the top of blood culture bottles to minimize aerosol production during removal of the needle.

Needles should never be cut or removed from the syringe before autoclaving. All body fluids should be centrifuged in carriers with safety caps only.

When procedures with a high potential for creating infectious aerosols are conducted or when there is a risk of splashing or spraying the face with infectious or other hazardous materials, laboratory work should be conducted in a safety cabinet or with face protection (goggles, mask, face shield or other splatter guards). Procedures that pose a risk may include centrifuging, grinding, blending,

vigorous shaking or mixing, sonic disruption, opening containers of infectious materials whose internal pressures may be different from ambient pressures, inoculating animals intranasally, and harvesting infected tissues from animals or eggs. Face protection should also be used when working with high concentrations or large volumes of infectious agents.

Decontaminating bench tops and other surfaces Bench tops should be wiped with a disinfectant (a phenolic disinfectant, 1% sodium hypochlorite, or 70% alcohol) routinely after working with infectious agents or clinical specimens or after spills, splashes, or other contamination by infectious materials. Solutions of disinfectants should be maintained at the work station (see Disinfectants below).

Standard Safety Practices in the Microbiology Laboratory

Disposal of contaminated materials

All discarded plates, tubes, clinical samples or other contaminated materials are to be placed in disposal containers at each bench. Special disposal boxes must be used for sharps such as syringes or broken glass to minimize the risk of injury.

Avoid overfilling such containers. Containers of contaminated material should be carefully transported to the autoclave room and autoclaved before disposal.

Autoclaving

An autoclave must be available for the BSL-2/3 laboratory and must be operated only by personnel who have been properly trained in its use. To verify that each autoclave is working properly, spore strips or other biological indicators designed to test for efficiency of sterilization should be included in autoclave loads on a regular basis. Each autoclave load should be monitored with temperature-sensitive tape, thermograph, or other means (e.g., biological indicators).

General laboratory policies

All areas of the laboratory must be kept clean and orderly. Dirt, dust, crowding, or clutter is a safety hazard and is not consistent with acceptable biological research. Floors should be kept clean and free of unnecessary clutter. They should be washed with a germicidal solution on a regular basis and after any spills of infectious material have occurred.

Refrigerators and freezers

Refrigerators and freezers should be regularly inspected for the presence of broken vials or tubes containing infectious agents. Wear gloves and proper attire when removing and discarding broken material. Refrigerators and freezers should be regularly cleaned with a disinfectant and defrosted to prevent possible contami nation and temperature failure.

Fire prevention

Keep burners away from lamps and flammable materials. Bulk flammable material must be stored in the safety cabinet. Small amounts of these materials, such as ethyl acetate, ethyl alcohol, and methanol, can be stored in safety containers. Turn off burners when not in use. Know the location of fire extinguishers, fire blankets, and showers. Fire safety instructions and evacuation routes should be posted.

B. Special Practices

Transport of biohazardous materials

Transport of biohazardous materials from one building to another increases the

risk of breakage and spills. If transport is necessary, the primary infectious agent

container (regardless of size) must be placed in an unbreakable second container

that can be sealed (e.g., screw-top tube, plastic bag).

Disinfectants

Organisms may have different susceptibilities to various disinfectants. As a surface disinfectant, 70% alcohol is generally effective for the Enterobacteriaceae, but other organisms are more resistant. However, 70% alcohol is not the disinfectant of choice for decontaminating spills. Phenolic disinfectants, although expensive, are usually effective against many organisms. Always read disinfectant labels for manufacturers' recommendations for dilution and for

exposure times for efficacy, especially before use on BSL-3 organisms such as Mycobacterium tuberculosis. A good general disinfectant is a 1:100 (1%) dilution of household bleach in water; at this dilution, bleach can be used for wiping surfaces of benches, hoods and other equipment. A 1:10 (10%) dilution of bleach is corrosive and will pit stainless steel and should not be used routinely; however, it may be used to clean up spills of cultured or concentrated infectious material where heavy contamination has occurred. Dilutions of sodium hypochlorite should be made daily from a stock solution.

Decontamination of spills

The following procedure is recommended for decontaminating spills. Isolate the area to prevent anyone from entering. Wear gloves and protective clothing (gown or lab coat; mask if the spill may contain a respiratory agent or if the agent is unknown). Absorb or cover the spill with disposable towels. Saturate the towels with an appropriately diluted intermediate or high level disinfectant (e.g., a phenolic formulation or household bleach). Place disinfectant-soaked towels over the area and leave them in place for at least 15 minutes before removing and discarding them. Wipe area using clean disinfectant-soaked towels and allow area to air dry. Place all disposable materials used to decontaminate the spill into a biohazard container. Handle the material in the same manner as other infectious waste.

Accidents

All injuries or unusual incidents should be reported immediately to the supervisor. When cuts or puncture wounds from potentially infected needles or glassware occur, the affected area should be promptly washed with disinfectant soap and water. In the event of a centrifuge accident in which safety carriers have not been used, other personnel in the area should be warned immediately and the area isolated to prevent anyone from entering.

C. PROTECTIVE CLOTHING AND EQUIPMENT

Laboratory coats

Protective coats, gowns, smocks, or uniforms designated for laboratory use must be worn while working in the laboratory. This protective clothing should be removed and left in the laboratory before leaving for non-laboratory areas. All protective clothing is either disposed of in the laboratory or laundered by the institution; it should never be taken home by personnel.

Gloves

Regardless of the type of infectious material, gloves should be worn when performing potentially hazardous procedures (e.g., slide agglutination) in which there is a risk of splashing or skin contamination or when the laboratory worker has cuts or broken skin on his or her hands. Gloves should always be worn when handling clinical specimens, body fluids, and tissues from humans and animals. These tissues should be assumed to be positive for hepatitis B virus, HIV, other

bloodborne pathogens, or Mycobacterium tuberculosis. Gloves must be removed when contaminated by splashing or spills or when work with infectious materials is completed. Gloves

should not be worn outside the laboratory. Do not use the telephone or open doors with gloves that have been used in laboratory procedures.

Dispose of all used gloves by discarding them with other disposable materials and autoclaving. Hands should be washed immediately after removing gloves.

Barrier precautions

Clinical specimens, body fluids, and tissues from humans and animals should be assumed to be positive for hepatitis B virus, HIV, other bloodborne pathogens, or Mycobacterium tuberculosis. These materials should be handled in a safety cabinet or using other barrier precautions such as goggles, mask, face shield or other splatter guards whenever there is a potential for creating an aerosol.