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Recent in vitro advances in the ocular antimicrobial agents against Acanthamoeba

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ABSTRACT

This review examines the advancements in antimicrobial drug discovery with in vitro assays for *Acanthamoeba*, highlighting the efficacy of current topical antimicrobial agents. In recent decades, the treatment and diagnosis of Acanthamoeba keratitis (AK) have presented clinical challenges. Clinicians often rely on clinical judgment, risk factors, and patient travel history to guide initial treatment decisions. The clinical presentation of AK frequently coincides with bacterial and fungal keratitis, leading to delays in diagnostic confirmation. This review compiles a list of commonly used antimicrobial agents that may be useful in controlling and preventing *Acanthamoeba* and other microbial infections during the diagnostic waiting period. Due to their unique life cycle, consisting of both trophozoite and cyst stages, amoebae exhibit resistance to various clinical drugs. Current research efforts are focused on identifying alternative and effective treatment options. Despite the ongoing characterization of various cytocidal agents from natural and synthetic sources, chlorhexidine gluconate (CHG) and polyhexamethylene biguanide (PHMB) have emerged as the most effective therapies for AK. Drawing from previous studies, we catalog several commonly used antimicrobial agents that may enhance the efficacy of PHMB and CHG while also preventing other microbial infections. These alternative agents present promising options for treating AK cases. This review evaluates progress in anti-amoebic drug discovery, focusing on antibiotics and cataloging their activity at different stages of *Acanthamoeba*.

1. Introduction

AK is frequently misdiagnosed as herpetic, bacterial, or mycotic keratitis due to the coincides in clinical signs and symptoms with other forms of keratitis (Szentmary et al., 2019). Analysis of the German Acanthamoeba Keratitis Registry has demonstrated that ophthalmologists erroneously diagnosed 47.6% of cases as herpetic keratitis, 25.2% as mycotic keratitis, and 3.9% as bacterial keratitis in patients suffering from AK (Claerhout et al., 2004). Confocal microscopy, amoeba culture, histopathologic staining, and polymerase chain reaction are common methods to diagnose the suspicion of *Acanthamoeba* invasion (Maycock and Jayaswal, 2016). Epithelial debridement is effective in removing shallow damaged cells and *Acanthamoeba*, and in collecting corneal biopsy samples for diagnosis (Brooks et al., 1994). Early diagnosis and targeted treatment significantly enhance the prognosis of AK. However, AK is frequently misdiagnosed as bacterial or other microbial keratitis during clinical examinations. Culture methods and corneal biopsies have limited sensitivity, and their effectiveness can be further diminished when there is a coinfection with another pathogen (Dart et al., 2009).

The genus *Acanthamoeba* can be found in soil, rivers, and lakes. *Acanthamoeba castellanii* is one of the free-living protozoans in the *Acanthamoeba* genus (Brown et al., 1982; Trabelsi et al., 2010). *Acanthamoeba*'s life cycle has two stages: the trophozoite and the cyst. In the trophozoite stage, *Acanthamoeba* can move, feed, and replicate.

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(Visvesvara et al., 2007). As a free-living protozoan, *Acanthamoeba* trophozoites feed on organic microorganisms such as bacteria and yeast in the environment (Marciano-Cabral and Cabral, 2003). If *Acanthamoeba* is exposed to unsuitable environmental surroundings such as nutrient-starvation, increased osmolarity or osmotic downshift, changes in pH and temperature, and dryness, it will transform into the metabolically inactive cyst stage, which is covered by double cell walls, for survival in extreme conditions (Lorenzo-Morales et al., 2010; Muchesa et al., 2014). *Acanthamoeba* feeds on organic environmental microorganisms such as bacteria and fungi, providing shelter and an optimal environment for the proliferation of amoeba-resistant bacteria (Marciano-Cabral and Cabral, 2003; Thomas et al., 2006). *Legionella* spp., *Mycobacterium avium*, and Rickettsia can survive and proliferate in *Acanthamoeba* (Whan et al., 2006; Scheid, 2014; König et al., 2019). Past studies suggest that the genetic transfer between bacteria in *Acanthamoeba* might result in multi-drug resistance due to several bacterial species using *Acanthamoeba* as a fine proliferative environment (Whan et al., 2006). Thus, *Acanthamoeba* is considered as a training ground and mobile media for some bacteria, which means that bacteria may become more harmful and invasive after they interact with *Acanthamoeba* (Molmeret et al., 2005; Casadevall et al., 2019). *Acanthamoeba* is highly resistant to broad antibiotics and the ability may protect co-invasive bacteria from environmental stress (Niyyati et al., 2015). The records from the Department of Ophthalmology at National Cheng Kung University Hospital show that 13.6% of *Acanthamoeba* patients suffer from bacterial infections. In the National Cheng Kung University Hospital, antibiotics are routinely administered to prevent secondary bacterial infections. After corneal epithelium scraping, topical therapy starts with CHG 0.02% or PHMB 0.02% every hour around the clock (Seal, 2003b). Cephalosporin and aminoglycoside are commonly used to limit secondary bacterial infection in the National Cheng Kung University Hospital. In recent years, CHG and PHMB have been demonstrated to be the most effective choice for AK clinical therapy. They have been indicated to be effective against *Acanthamoeba* trophozoites and cysts (Huang et al., 2016). Based on the cyst structure being partly made of cellulose, one potential therapeutic strategy is cyst wall degradation via cellulase enzyme treatment to inhibit cyst formation. Although many researchers have characterized various cysticidal materials from natural and synthetic chemicals, CHG and PHMB are still considered the first-line therapy for AK. In the later stage of AK, a full-thickness transplant surgery may be employed since patients are unresponsive to topical therapy (Joshi and Gurung, 2021; Di Zazzo et al., 2022). The diagnosis of AK can take from 1 to more than 4 weeks (Shah et al., 2021; Przybek-Skrzypecka et al., 2023). Due to the broad indications for antibiotics and antifungal agents and the severe side effects associated with antiamoebic drugs, these agents are commonly employed during the early diagnostic stages of AK. Anti-amoebic drugs are prescribed only after a confirmed diagnosis and are not used for prevention. Experimental evidence suggests potential anti-amoebic or anti-cyst effects of first-line ocular topical antibiotics. Due to *Acanthamoeba*'s unique life cycle, drug characterization evaluations include assays targeting anti-trophozoite, anti-cyst, and anti-encystation activities. The literature search was conducted using the PubMed electronic databases. The search was performed using the keyword "*Acanthamoeba*" and specific terms such as "antibiotic treatment," "antifungus treatment," "clinical anti-microbial treatment," "co-infection treatment," "anti-cyst," "anti-trophozoite," and "anti--encystation." References from identified studies were also reviewed and included if deemed appropriate and important. This review aims to catalog the anti-amoebic activity of other clinical antimicrobial agents to provide new perspectives on treatment strategies and improve clinical outcomes for patients with this disease.

2. Antibacterial agents

Clinicians base their initial treatment of suspected microbial keratitis on clinical acumen, exogenous or endogenous risk factors, the availability of effective topical antimicrobials, and relevant data on individual travel history (Green et al., 2008; Tuft et al., 2022). Bacteria are by far the most common cause of microbial keratitis. Common ocular topical drops include antibiotics for bacterial infections, corticosteroids for inflammation, antihistamines for allergies, and artificial tears for dry eye syndrome (Patel et al., 2013). Ocular antibacterial topical drops can be classified based on the chemical structure of the antibiotics they contain.

Aminoglycoside antibiotics are a group of broad-spectrum drugs that are particularly effective against Gram-negative bacteria. They work by attaching to the bacterial 30S ribosomal subunit, which disrupts protein synthesis and induces bacterial cell death (Krause et al., 2016). Common examples of aminoglycosides include gentamicin, neomycin, and amikacin. Since the first successful therapy was developed in the mid-1980s, neomycin has been used in combination with propamidine to treat AK (Varga et al., 1993). Neomycin is commonly used to eliminate bacteria, which serve as a food source for *Acanthamoeba* (Seal, 2003a). However, neomycin has demonstrated cysticidal activity only against certain *Acanthamoeba* strains, with most strains showing no such activity (Osato et al., 1991; Saunders et al., 1992; Elder et al., 1994). Additionally, studies indicate that neomycin alone is ineffective unless combined with propamidine (Seal, 2003a). Gentamicin has demonstrated efficacy against cysts, with a 0.3% concentration achieving 100% inhibitory efficacy for treatments lasting from 1 to 72 h. However, it has not shown effectiveness against the growth of trophozoites (Thongseesuksai et al., 2020) (Table 1). Conversely, the anti-amoebic activity of amikacin, kanamycin, and tobramycin has not been thoroughly investigated (see Table 1).

Macrolides inhibit bacterial protein synthesis by reversibly binding to the P site of the 50S ribosomal subunit, preventing polypeptide chain elongation and thereby hindering bacterial growth and reproduction (Vazquez-Laslop and Mankin, 2018). In ophthalmology, erythromycin and azithromycin are commonly used macrolides, for conditions such as styes or dry eye syndrome (Foulks et al., 2013). Azithromycin has been shown to inhibit the growth of *Acanthamoeba* at 1, 5, and 10 μg/ml, whereas erythromycin and clarithromycin have no experiments for anti-amoebic activity (Schuster and Visvesvara, 1998).

Ocular fluoroquinolone antibiotics are broad-spectrum antimicrobial agents widely used to treat bacterial eye infections such as conjunctivitis, keratitis, and corneal ulcers. They work by inhibiting bacterial DNA gyrase and topoisomerase IV, enzymes essential for DNA replication and repair, leading to bacterial cell death (Watanabe et al., 2010). These antibiotics are favored for their effectiveness against a broad range of Gram-positive and Gram-negative bacteria. Levofloxacin demonstrated activity in the 312- to 1250-μg/ml range for both *Acanthamoeba* trophozoites and cysts. Moxifloxacin showed no effect on the viability of trophozoites. In contrast, gatifloxacin was highly effective against cysts (Heaselgrave et al., 2019; Thongseesuksai et al., 2020). Research suggests that moxifloxacin could be a valuable adjuvant due to its ability to inhibit encystation (Martin-Navarro et al., 2013). Ciprofloxacin, when used alone at concentrations of 1.5 mg/mL to 6 mg/mL, has demonstrated amoebicidal activity against trophozoites in various strains. It causes damage to the cellular membrane and reduces cell concentration (Ortilles et al., 2017).

Tetracyclines are a class of broad-spectrum antibiotics widely used to treat various bacterial infections. They work by binding to the 30S ribosomal subunit, which prevents aminoacyl-tRNA attachment, thereby inhibiting bacterial protein synthesis and halting bacterial growth (Chopra and Roberts, 2001). Tetracyclines are effective against a broad spectrum of Gram-positive and Gram-negative bacteria and atypical organisms like *Chlamydia* and *Mycoplasma* (Le Roy et al., 2021). Tigecycline, a third-generation tetracycline antibiotic, significantly inhibited the growth of *Acanthamoeba*, achieving 46.4% inhibition at a concentration of 100 μM without affecting cell viability. In contrast, other tetracycline antibiotics, such as tetracycline and doxycycline, showed no inhibitory effects (Jha et al., 2015). Research suggests that tetracycline

Table 1
List of antibacterial agents.

Antibiotic	Category	+/−	Trophozoite	+/−	Cyst	+/−	Encystation
Amikacin	aminoglycoside						
Gentamicin	aminoglycoside	−	Thongseesuksai et al. (2020)	+	Thongseesuksai et al. (2020)		
Kanamycin	aminoglycoside						
Tobramycin	aminoglycoside						
Neomycin	aminoglycoside	−	Elder et al. (1994)	±	(Osato et al., 1991; Saunders et al., 1992; Elder et al., 1994)		
Rifampicin	ansamycin	±	(Das et al., 1991; Ondarza et al., 2006; Taravaud et al., 2017)				
Chloramphenicol	chloramphenicol						
Ciprofloxacin	fluoroquinolone	+	Ortilles et al. (2017)				
Gatifloxacin	fluoroquinolone			+	Thongseesuksai et al. (2020)		
Levofloxacin	fluoroquinolone	+	Heaselgrave et al. (2019)	+	Thongseesuksai et al. (2020)		
Lomefloxacin	fluoroquinolone				Thongseesuksai et al. (2020)		
Norfloxacin	fluoroquinolone						
Moxifloxacin	fluoroquinolone	−	Heaselgrave et al. (2019)			+	Martin-Navarro et al. (2013)
Vancomycin	glycopeptide						
Azithromycin	macrolide	+	Schuster and Visvesvara (1998)				
Clarithromycin	macrolide	−	Schuster and Visvesvara (1998)				
Erythromycin	macrolide	−	Schuster and Visvesvara (1998)				
Metronidazole	nitroimidazole	+	(Ondarza et al., 2006; Taravaud et al., 2017; Dusekova et al., 2021)				
Tetracycline	tetracycline	−	Jha et al. (2015)				
Tigecycline	tetracycline	+	Jha et al. (2015)				
Cefazolin	β-lactam						
Meropenem	β-lactam						
Ampicillin	β-lactam						
Ceftazidime	β-lactam						
Ceftriaxone	β-lactam	−	Makhlouf et al. (2022)	−	Makhlouf et al. (2022)	+	Makhlouf et al. (2022)
Cefotaxime	β-lactam					+	Chen et al. (2020)

+: Amoebacidal effect (kills or harms amoebas), -: No effect (no impact on amoebas), ±: Distinct results (results vary, sometimes there is an effect and sometimes not).

treatment increases susceptibility to amoeba infection in mice, indicating a potentially pathogenic role for *Acanthamoeba* in cases of tetracycline-induced immunosuppression (Markowitz et al., 1978).

Rifampicin, a broad-spectrum antibiotic from the rifamycin class, is widely used to treat bacterial infections, including tuberculosis and leprosy. It works by inhibiting bacterial DNA-dependent RNA polymerase, blocking RNA synthesis, and leading to bacterial cell death (Campbell et al., 2001). Rifampicin was inactive against the growth of *Acanthamoeba* (Ondarza et al., 2006; Taravaud et al., 2017). However, prophylactic treatment with rifampicin (administered daily for 2 days at 75 and 100 mg/kg) provided full protection against *Acanthamoeba* brain infection (Das et al., 1991).

Vancomycin, a potent glycopeptide antibiotic, is primarily used to treat serious Gram-positive bacterial infections, particularly those caused by methicillin-resistant *Staphylococcus aureus* (Cong et al., 2020). It inhibits cell wall synthesis by binding to the D-alanyl-D-alanine terminus of cell wall precursors, thereby preventing peptidoglycan chain formation and causing bacterial cell death. Chloramphenicol is another broad-spectrum antibiotic that inhibits bacterial protein synthesis by binding to the 50S ribosomal subunit (Brook, 2016). It's effective against a variety of Gram-positive and Gram-negative bacteria. Currently, no experimental assays have been conducted to evaluate the anti-amoebic effects of vancomycin and chloramphenicol.

Metronidazole, a nitroimidazole antibiotic, is utilized in ophthalmic solutions to treat bacterial eye infections by disrupting DNA synthesis in microorganisms, leading to cell death (Edwards, 1993). Commonly used to treat conditions like bacterial vaginosis, trichomoniasis, giardiasis, and *Clostridium difficile* infections (van Prehn et al., 2021; Escrig et al., 2024). In one case, a patient treated with topical neomycin-polymyxin B and metronidazole eye drops, alongside oral antibiotic therapy, successfully controlled a corneal infection (Yeung et al., 2002). Retrospective analysis of medical records indicates that metronidazole has been used as a combination therapy component for managing AK (Sun et al., 2006). The IC90 values for metronidazole against *Acanthamoeba* spp. trophozoites, as reported in the literature, range from 4239 to 4501 μM (Dusekova et al., 2021). Metronidazole demonstrated limited efficacy against *Acanthamoeba polyphaga* trophozoites, exhibiting an IC50 of 17.52 mM and maximum growth inhibition of only 85% at 186.9 mM (Ondarza et al., 2006). Additionally, another study indicated that low doses of metronidazole were inactive against *Acanthamoeba castellanii* trophozoites at concentrations 100 μM (Taravaud et al., 2017). While not typically considered a first-line treatment for ocular *Acanthamoeba* infections, these findings, along with clinical evidence of its use in combination therapies, suggest that metronidazole may have a role in the treatment of amoebic infections (Xuguang et al., 2003).

Beta-lactam antibiotics, such as carbapenems and cephalosporins, are commonly used in eye drops to treat eye-bacterial infections, including bacterial conjunctivitis and keratitis (Egrilmez and Yildirim-Theveny, 2020; Moledina et al., 2023). These antibiotics inhibit bacterial cell wall synthesis, leading to cell lysis and death (Mora-Ochomogo and Lohans, 2021). Ceftriaxone, a third-generation cephalosporin, inhibited the encystment of *Acanthamoeba castellanii* at a 100 μg/mL concentration. However, ceftriaxone has shown no amoebicidal activity against trophozoites at the same concentration. (Makhlouf et al., 2022). Cefotaxime, another third-generation intravenous cephalosporin antibiotic, decreases the encystation percentage of *Acanthamoeba* at a concentration of 128 μg/mL (Chen et al., 2020). The activity of cefazolin, meropenem, ampicillin, and ceftazidime, against *Acanthamoeba* spp. has not been investigated.

3. Antifungal agents

In addition to bacteria, fungi are also significant pathogens in microbial keratitis. (Green et al., 2008). Fungal keratitis (FK) is a significant cause of corneal blindness, characterized by ocular surface damage, inflammation, and invasive, vision-threatening symptoms (Xie et al., 2006). The fungi implicated in FK are broadly classified into filamentous fungi and yeast or yeast-like fungi. Filamentous fungi, such as *Fusarium* spp. and *Aspergillus* spp., are predominantly found in tropical climates, while yeast-like fungi, such as *Candida* spp., are more common in

temperate regions (Xie et al., 2008; Tuft and Tullo, 2009). The antifungal treatment of fungal keratitis typically involves polyenes and azoles, two major drug classes with a broad spectrum of activity. These drugs are vital in managing ocular fungal infections, including keratitis, endophthalmitis, conjunctivitis, and blepharitis. Polyenes and azoles target both filamentous and yeast-like fungi, making them essential components of ophthalmic antifungal therapy (Lakhani et al., 2019). Their broad-spectrum efficacy against a variety of fungal species has established these agents as important treatments for severe fungal eye infections.

Azole antifungals inhibit the 14α-demethylation of lanosterol or 24-methylenedihydro-lanosterol in fungal cells, disrupting the biosynthesis of ergosterol, a key component of the fungal cell membrane (Monk et al., 2020). Azoles used for invasive fungal infections are divided into two main classes: imidazoles and triazoles. Imidazoles, such as miconazole, econazole, and ketoconazole, are commonly employed in clinical settings to treat fungal infections. Triazoles, including fluconazole, itraconazole, voriconazole, and posaconazole, contain three nitrogen atoms in their ring structure. Both imidazoles and triazoles target lanosterol 14α-demethylase, impairing fungal cell membrane integrity (Peyton et al., 2015). Thomson et al. demonstrated that econazole did not inhibit *Acanthamoeba* growth at 24 h. In contrast, miconazole exhibited IC50 values of 47.7 μM and 46.85 μM. At 96 h, all tested antifungals—econazole, miconazole, and voriconazole demonstrated growth inhibition against various *Acanthamoeba* isolates, with IC_{50} ranges of 90–140 μM, 8.2–21 μM, and 0.2–3.1 μM, respectively (Thomson et al., 2017). Ketoconazole (IC50 = 1.5 mg/mL) and miconazole (IC_{50} = 2 mg/mL) both inhibited *Acanthamoeba polyphaga* trophozoites in a dose-dependent manner, achieving >90% inhibition at 32 mg/mL (Ondarza et al., 2006). Fluconazole exhibited an MIC50 range of 64–256 μg/mL and a minimal cysticidal concentration range of 256–512 μg/mL (Megha et al., 2022). Itraconazole inhibited *Acanthamoeba* growth in both the 7-day and 12-h tests. The concentrations required to inhibit 90% of growth were 4.1 μg/mL for the 7-day test and 11.6 μg/mL for the 12-h test. (Nakaminami et al., 2017; Rao et al., 2024). Posaconazole effectively inhibited the growth of *Acanthamoeba castellanii* trophozoites, with concentrations that inhibited 50% of EC_{50} ranging from 0.003 to 0.065 μM (Shing et al., 2020). Among triazoles, voriconazole and posaconazole showed cysticidal activity (minimal cysticidal concentration 33.1–46.2 μg/mL; 43.7–52.5μg/mL, respectively), while itraconazole did not exhibit anti-cyst activity (Iovieno et al., 2014) (Table 2).

Polyene antifungal drugs, distinguished by their cyclic amphiphilic macrolide structure, target sterols in cell membranes—specifically ergosterol in fungal cells. This interaction leads to the formation of pores in the membrane, increasing permeability and causing cellular leakage (Vandeputte et al., 2012). Ocular polyene antifungal agents, such as natamycin and amphotericin B, are commonly employed in treating eye fungal infections, demonstrating broad efficacy against various fungal pathogens (Reginatto et al., 2023). These drugs are crucial in managing severe fungal keratitis and other ocular fungal eye infections. amphotericin B (IC_{50} = 5.5 mg/mL) and natamycin (IC_{50} = 1.8 mg/mL) exhibited anti-amoebic activity against various clinical isolates of *Acanthamoeba* (Nakaminami et al., 2017; Abdelnasir et al., 2020). Additionally, natamycin exhibited in vitro cysticidal activity, effectively targeting the cystic of Acanthamoeba in clinical isolates. The MIC50 and MIC90 values were 16 μg/mL and 128 μg/mL, respectively. (Osato et al., 1991; Redd et al., 2021). Amphotericin B demonstrated in vitro efficacy against *Acanthamoeba*, exhibiting IC_{50} values of 2.3–10.5 μg/mL and 0.2–155 μg/mL in 7-day and 12-h assays, respectively (Nakaminami et al., 2017).

Allylamines, like azoles, disrupt the ergosterol biosynthesis pathway but act by inhibiting squalene epoxidase, an upstream enzyme in the pathway (Balfour and Faulds, 1992). This inhibition leads to an accumulation of intracellular squalene and a deficiency of ergosterol in the fungal cell membrane, impairing cell function and viability. Terbinafine, a prominent allylamine antifungal, is primarily used to treat fungal keratitis due to its broad efficacy against various fungal pathogens (Leyden, 1998). However, no reports revealed the activity of allylamines against *Acanthamoeba*.

4. Conclusion

Over the past few decades, research has identified numerous potential therapeutic compounds and their synthetic analogs. The co-administration of an antiamoebic agent with ATPase inhibitors, β-glucanase enzyme, or cellulase enzymes has demonstrated the ability to eradicate the viability of both cysts and trophozoites (Moon et al., 2015; Abjani et al., 2016; Rased et al., 2022; Shih et al., 2024). Furthermore, nanocomposites and self-assembled structures have demonstrated enhanced pharmacological efficacy against *Acanthamoeba* (Rajendran et al., 2020; Akbar et al., 2023; Ahmed et al., 2024). Patuletin nano-formulations exhibit potent anti-*Acanthamoeba* activity while maintaining minimal cytotoxicity against human cells (Siddiqui et al., 2024). Itraconazole, when incorporated into the self-assembled micellar structure of AB2 midterms, has shown superior in vitro efficacy in killing *Acanthamoeba* compared to monotherapy with the drug alone (Rao et al., 2024). However, many of these compounds have not progressed to clinical trials. Furthermore, developing and producing new drugs for *Acanthamoeba* infections face numerous practical limitations. Conducting studies on drug efficacy and structural differences between clinical ocular antimicrobial agents may offer a new direction for future research to improve therapeutic agents. Therefore, attempting to use or modify existing drugs may be a more practical approach for treating AK.

Here, we highlight the potential anti-amoebic properties of several topical antimicrobial agents, including antibacterial and antifungal drugs. Given the lack of effective treatments with minimal cytotoxicity for AK, we focus on summarizing in vitro amoebicidal evidence of antimicrobial agents for the future investigation. Antifungal agents exhibit more in vitro evidence and show more activity against

Table 2
List of antifungal agents.

Antibiotic	Category	+/−	Trophozoite	+/−	Cyst	+/−	Encystation
Econazole	Imidazole	+	Thomson et al. (2017)				
Miconazole	Imidazole	+	(Nakaminami et al., 2017; Thomson et al., 2017)				
Ketoconazole	Imidazole	+	Ondarza et al. (2006)				
Fluconazole	Triazole	+	Megha et al. (2022)	±	(Iovieno et al., 2014; Megha et al., 2022)		
Itraconazole	Triazole	+	(Nakaminami et al., 2017; Rao et al., 2024)	–	Iovieno et al. (2014)		
Voriconazole	Triazole	+	(Martin-Navarro et al., 2015; Ortilles et al., 2017; Thomson et al., 2017; Shing et al., 2020)	+	(Martin-Navarro et al., 2013; Iovieno et al., 2014; Ortilles et al., 2017)		
Posaconazole	Triazole	+	Shing et al. (2020)	+	Iovieno et al. (2014)		
Amphotericin B	Polyene	+	(Nakaminami et al., 2017; Abdelnasir et al., 2020)	–	Iovieno et al. (2014)		
Natamycin	Polyene	+	Abdelnasir et al. (2020)	+	(Osato et al., 1991; Redd et al., 2021)		
Terbinafine	Allylamine						

+: Amoebacidal effect (kills or harms amoebas), -: No effect (no impact on amoebas), ±: Distinct results (results vary, sometimes there is an effect and sometimes not).

Acanthamoeba trophozoites and cysts compared to antibacterial agents. Additionally, few antibacterial agents are known to disrupt the process of encystation, but no studies have yet demonstrated this effect for antifungal agents. These drugs are commonly used for microbial keratitis. Some of them have been used to treat as adjuvant in AK treatment (Varga et al., 1993; Seal, 2003a). Combined treatments are widely employed in clinical settings to prevent and control complex microbial infections. Numerous studies have demonstrated success in treating AK using PHMB with various antimicrobial drugs, highlighting that PHMB monotherapy may be less effective than combination therapy (Seal, 2003b). Cases of AK complicated by fungal infections were diagnosed at Kaohsiung Medical University, Taiwan. Treatment with PHMB and fluconazole yielded positive results with no recurrence of infection (Tien and Sheu, 1999). Topical voriconazole in conjunction with PHMB treatments, has been suggested as an effective option with minimal side effects for AK management (Musayeva et al., 2020). Combining therapies can improve the effectiveness of AK treatment and reduce the risk of microbial co-infection. Consequently, studying the effects of ocular antimicrobial agents on *Acanthamoeba* is crucial. Numerous studies have been conducted on this topic over the past years. This review summarizes several promising candidates with anti-amoebic potential, including anti-trophozoite, anti-cyst, and anti-encystation activities for future research.

The diagnosis of *Acanthamoeba* ocular infection presents significant challenges (Przybek-Skrzypecka et al., 2023). The time required for current diagnostic methods, including culture, in vivo confocal microscopy, and polymerase chain reaction (PCR), varies considerably (Huang et al., 2023). A retrospective observational cohort study of AK patients admitted to the Manchester Royal Eye Hospital between 2003 and 2017 revealed that the time to diagnosis ranged from 7 to 29 days (Przybek-Skrzypecka et al., 2023). Another retrospective review conducted at the Wilmer Eye Institute included 45 eyes from 43 patients. The study revealed that 31% of patients were diagnosed within 28 days of symptom onset, whereas the remaining 69% were diagnosed after 28 days (Shah et al., 2021). Given the side effects of PHMB and CHG, these alternative well-tolerated and commonly used antimicrobial agents may offer viable options for managing unconfirmed AK cases enhancing the first-line AK therapy, and preventing other forms of microbial keratitis.

CRediT authorship contribution statement

Chun-Hsien Chen: Writing – original draft, Validation, Investigation, Formal analysis, Data curation, Conceptualization. **Jian-Ming Huang:** Writing – review & editing, Validation, Supervision. **Yu-Jen Wang:** Writing – review & editing, Validation. **Chih-Ming Tsai:** Writing – review & editing, Validation. **Wei-Chen Lin:** Writing – review & editing, Validation, Funding acquisition, Conceptualization.

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References

Abdelnasir, S., Anwar, A., Kawish, M., Anwar, A., Shah, M.R., Siddiqui, R., Khan, N.A., 2020. Metronidazole conjugated magnetic nanoparticles loaded with amphotericin B exhibited potent effects against pathogenic Acanthamoeba castellanii belonging to the T4 genotype. AMB Express 10, 127. https://doi.org/10.1186/s13568-020-01061-z.

Abjani, F., Khan, N.A., Yousuf, F.A., Siddiqui, R., 2016. Targeting cyst wall is an effective strategy in improving the efficacy of marketed contact lens disinfecting solutions against Acanthamoeba castellanii cysts. Cont Lens Anterior Eye 39, 239–243. https://doi.org/10.1016/j.clae.2015.11.004.

Ahmed, U., Gew, L.T., Siddiqui, R., Khan, N.A., Alharbi, A.M., Alhazmi, A., Anwar, A., 2024. Metal oxide nanoparticles exhibit anti-Acanthamoeba castellanii properties by inducing necrotic cell death. Acta Parasitol. 69, 1717–1723. https://doi.org/10.1007/s11686-024-00891-2.

Akbar, N., Kawish, M., Jabri, T., Khan, N.A., Shah, M.R., Siddiqui, R., 2023. Cinnamic acid and lactobionic acid based nanoformulations as a potential antiamoebic therapeutics. Exp. Parasitol. 246, 108474. https://doi.org/10.1016/j.exppara.2023.108474.

Balfour, J.A., Faulds, D., 1992. Terbinafine. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in superficial mycoses. Drugs 43, 259–284. https://doi.org/10.2165/00003495-199243020-00010.

Brook, I., 2016. Antimicrobials therapy of anaerobic infections. J. Chemother. 28, 143–150. https://doi.org/10.1179/1973947815Y.0000000068.

Brooks Jr., J.G., Coster, D.J., Badenoch, P.R., 1994. Acanthamoeba keratitis. Resolution after epithelial debridement. Cornea 13, 186–189.

Brown, T.J., Cursons, R.T., Keys, E.A., 1982. Amoebae from antarctic soil and water. Appl. Environ. Microbiol. 44, 491–493. https://doi.org/10.1128/AEM.44.2.491-493.1982.

Campbell, E.A., Korzheva, N., Mustaev, A., Murakami, K., Nair, S., Goldfarb, A., Darst, S. A., 2001. Structural mechanism for rifampicin inhibition of bacterial rna polymerase. Cell 104, 901–912. https://doi.org/10.1016/s0092-8674(01)00286-0.

Casadevall, A., Fu, M.S., Guimaraes, A.J., Albuquerque, P., 2019. The 'amoeboid predator-fungal animal virulence'hypothesis. Journal of Fungi 5, 10.

Chen, C.H., Huang, C.L., He, M.S., Huang, F.C., Lin, W.C., 2020. Characterisation of the beta-lactam resistance enzyme in Acanthamoeba castellanii. Int. J. Antimicrob. Agents 55, 105823. https://doi.org/10.1016/j.ijantimicag.2019.10.004.

Chopra, I., Roberts, M., 2001. Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. Microbiol. Mol. Biol. Rev. 65, 232–260. https://doi.org/10.1128/MMBR.65.2.232-260.2001. ; second page, table of contents.

Claerhout, I., Goegebuer, A., Van Den Broecke, C., Kestelyn, P., 2004. Delay in diagnosis and outcome of Acanthamoeba keratitis. Graefes Arch. Clin. Exp. Ophthalmol. 242, 648–653. https://doi.org/10.1007/s00417-003-0805-7.

Cong, Y., Yang, S., Rao, X., 2020. Vancomycin resistant Staphylococcus aureus infections: a review of case updating and clinical features. J. Adv. Res. 21, 169–176. https://doi.org/10.1016/j.jare.2019.10.005.

Dart, J.K., Saw, V.P., Kilvington, S., 2009. Acanthamoeba keratitis: diagnosis and treatment update 2009. Am. J. Ophthalmol. 148, 487–499. https://doi.org/10.1016/j.ajo.2009.06.009 e482.

Das, S.R., Asiri, S., el-Soofi, A., Baer, H.P., 1991. Protective and curative effects of rifampicin in Acanthamoeba meningitis of the mouse. J. Infect. Dis. 163, 916–917. https://doi.org/10.1093/infdis/163.4.916.

Di Zazzo, A., Varacalli, G., De Gregorio, C., Coassin, M., Bonini, S., 2022. Therapeutic corneal transplantation in Acanthamoeba keratitis: penetrating versus lamellar keratoplasty. Cornea 41, 396–401. https://doi.org/10.1097/ICO.0000000000002880.

Dusekova, A., Garajova, M., Lukac, M., Mrva, M., 2021. Derivatisation of metronidazole enhances cytotoxic effect against Acanthamoeba genotype T4 isolates and leads to cytomorphological changes in trophozoites. Acta Trop. 216, 105830. https://doi.org/10.1016/j.actatropica.2021.105830.

Edwards, D.I., 1993. Nitroimidazole drugs–action and resistance mechanisms. I. Mechanisms of action. J. Antimicrob. Chemother. 31, 9–20. https://doi.org/10.1093/jac/31.1.9.

Egrilmez, S., Yildirim-Theveny, S., 2020. Treatment-resistant bacterial keratitis: challenges and solutions. Clin. Ophthalmol. 14, 287–297. https://doi.org/10.2147/OPTH.S181997.

Elder, M.J., Kilvington, S., Dart, J.K., 1994. A clinicopathologic study of in vitro sensitivity testing and Acanthamoeba keratitis. Investig. Ophthalmol. Vis. Sci. 35, 1059–1064.

Escrig, J.I., Miyamoto, Y., Aznar, A.D., Eckmann, L., Debnath, A., 2024. Antigiardial and antiamebic activities of fexinidazole and its metabolites: new drug leads for giardiasis and amebiasis. Antimicrob. Agents Chemother. 68, e0073123. https://doi.org/10.1128/aac.00731-23.

Foulks, G.N., Borchman, D., Yappert, M., Kakar, S., 2013. Topical azithromycin and oral doxycycline therapy of meibomian gland dysfunction: a comparative clinical and spectroscopic pilot study. Cornea 32, 44–53. https://doi.org/10.1097/ICO.0b013e318254205f.

Green, M., Apel, A., Stapleton, F., 2008. Risk factors and causative organisms in microbial keratitis. Cornea 27, 22–27. https://doi.org/10.1097/ICO.0b013e318156caf2.

Heaselgrave, W., Hamad, A., Coles, S., Hau, S., 2019. In vitro evaluation of the inhibitory effect of topical ophthalmic agents on Acanthamoeba viability. Transl Vis Sci Technol 8, 17. https://doi.org/10.1167/tvst.8.5.17.

Huang, F.C., Liu, T.S., Li, S.C., Shih, M.H., Shin, J.W., Lin, W.C., 2016. The effect of the disulfideisomerase domain containing protein in the defense against polyhexamethylene biguanide of highly tolerant Acanthamoeba at the trophozoite stage. Int J Parasitol Drugs Drug Resist 6, 251–257. https://doi.org/10.1016/j.ijpddr.2016.11.001.

Huang, L., Suhler, E.B., Rosenberg, C., Ta Kim, D., Winthrop, K.L., Doan, T., Lin, P., 2023. Acanthamoeba-associated retinitis successfully treated with intravitreal and systemic antimicrobials. Am J Ophthalmol Case Rep 32, 101902. https://doi.org/10.1016/j.ajoc.2023.101902.

Iovieno, A., Miller, D., Ledee, D.R., Alfonso, E.C., 2014. Cysticidal activity of antifungals against different genotypes of Acanthamoeba. Antimicrob. Agents Chemother. 58, 5626–5628. https://doi.org/10.1128/AAC.02635-14.

Jha, B.K., Seo, I., Kong, H.H., Suh, S.I., Suh, M.H., Baek, W.K., 2015. Tigecycline inhibits proliferation of Acanthamoeba castellanii. Parasitol. Res. 114, 1189–1195. https://doi.org/10.1007/s00436-014-4302-1.

Joshi, L.S., Gurung, R., 2021. Acanthamoeba keratitis - a case report. Nepal. J. Ophthalmol. 13, 133–136. https://doi.org/10.3126/nepjoph.v13i1.29912.

König, L., Wentrup, C., Schulz, F., Wascher, F., Swanson, M.S., Buchrieser, C., Horn, M., 2019. Symbiont-mediated defense against Legionella pneumophila in amoebae. mBio 10.

Krause, K.M., Serio, A.W., Kane, T.R., Connolly, L.E., 2016. Aminoglycosides: an overview. Cold Spring Harb Perspect Med 6. https://doi.org/10.1101/cshperspect.a027029.

Lakhani, P., Patil, A., Majumdar, S., 2019. Challenges in the polyene- and azole-based pharmacotherapy of ocular fungal infections. J Ocul Pharmacol Ther 35, 6–22. https://doi.org/10.1089/jop.2018.0089.

Le Roy, C., Touati, A., Balcon, C., Garraud, J., Molina, J.M., Bercot, B., de Barbeyrac, B., Pereyre, S., Peuchant, O., Bebear, C., 2021. Identification of 16S rRNA mutations in Mycoplasma genitalium potentially associated with tetracycline resistance in vivo but not selected in vitro in M. genitalium and Chlamydia trachomatis. J. Antimicrob. Chemother. 76, 1150–1154. https://doi.org/10.1093/jac/dkab016.

Leyden, J., 1998. Pharmacokinetics and pharmacology of terbinafine and itraconazole. J. Am. Acad. Dermatol. 38, S42–S47. https://doi.org/10.1016/s0190-9622(98)70483-9.

Lorenzo-Morales, J., Martin-Navarro, C.M., Lopez-Arencibia, A., Santana-Morales, M.A., Afonso-Lehmann, R.N., Maciver, S.K., Valladares, B., Martinez-Carretero, E., 2010. Therapeutic potential of a combination of two gene-specific small interfering RNAs against clinical strains of Acanthamoeba. Antimicrob. Agents Chemother. 54, 5151–5155. https://doi.org/10.1128/AAC.00329-10.

Makhlouf, Z., Akbar, N., Khan, N.A., Shah, M.R., Alharbi, A.M., Alfahemi, H., Siddiqui, R., 2022. Antiamoebic properties of ceftriaxone and zinc-oxide-cyclodextrin-conjugated ceftriaxone. Antibiotics (Basel) 11. https://doi.org/10.3390/antibiotics11121721.

Marciano-Cabral, F., Cabral, G., 2003. Acanthamoeba spp. as agents of disease in humans. Clin. Microbiol. Rev. 16, 273–307. https://doi.org/10.1128/cmr.16.2.273-307.2003.

Markowitz, S.M., Sobieski, T., Martinez, A.J., Duma, R.J., 1978. Experimental Acanthamoeba infections in mice pretreated with methylprednisolone or tetracycline. Am. J. Pathol. 92, 733–744.

Martin-Navarro, C.M., Lopez-Arencibia, A., Arnalich-Montiel, F., Valladares, B., Pinero, J.E., Lorenzo-Morales, J., 2013. Evaluation of the in vitro activity of commercially available moxifloxacin and voriconazole eye-drops against clinical strains of Acanthamoeba. Graefes Arch. Clin. Exp. Ophthalmol. 251, 2111–2117. https://doi.org/10.1007/s00417-013-2371-y.

Martin-Navarro, C.M., Lopez-Arencibia, A., Sifaoui, I., Reyes-Batlle, M., Valladares, B., Martinez-Carretero, E., Pinero, J.E., Maciver, S.K., Lorenzo-Morales, J., 2015. Statins and voriconazole induce programmed cell death in Acanthamoeba castellanii. Antimicrob. Agents Chemother. 59, 2817–2824. https://doi.org/10.1128/AAC.00066-15.

Maycock, N.J., Jayaswal, R., 2016. Update on Acanthamoeba keratitis: diagnosis, treatment, and outcomes. Cornea 35, 713–720. https://doi.org/10.1097/ICO.0000000000000804.

Megha, K., Sharma, M., Sharma, C., Gupta, A., Sehgal, R., Khurana, S., 2022. Evaluation of in vitro activity of five antimicrobial agents on Acanthamoeba isolates and their toxicity on human corneal epithelium. Eye (Lond) 36, 1911–1917. https://doi.org/10.1038/s41433-021-01768-8.

Moledina, M., Roberts, H.W., Mukherjee, A., Spokes, D., Pimenides, D., Stephenson, C., Bassily, R., Rajan, M.S., Myerscough, J., 2023. Analysis of microbial keratitis incidence, isolates and in-vitro antimicrobial susceptibility in the East of England: a 6-year study. Eye (Lond) 37, 2716–2722. https://doi.org/10.1038/s41433-023-02404-3.

Molmeret, M., Horn, M., Wagner, M., Santic, M., Kwaik, Y.A., 2005. Amoebae as training grounds for intracellular bacterial pathogens. Appl. Environ. Microbiol. 71, 20–28.

Monk, B.C., Sagatova, A.A., Hosseini, P., Ruma, Y.N., Wilson, R.K., Keniya, M.V., 2020. Fungal Lanosterol 14alpha-demethylase: a target for next-generation antifungal design. Biochim Biophys Acta Proteins Proteom 1868, 140206. https://doi.org/10.1016/j.bbapap.2019.02.008.

Moon, E.K., Hong, Y., Chung, D.I., Goo, Y.K., Kong, H.H., 2015. Potential value of cellulose synthesis inhibitors combined with PHMB in the treatment of Acanthamoeba keratitis. Cornea 34, 1593–1598. https://doi.org/10.1097/ICO.0000000000000642.

Mora-Ochomogo, M., Lohans, C.T., 2021. beta-Lactam antibiotic targets and resistance mechanisms: from covalent inhibitors to substrates. RSC Med. Chem. 12, 1623–1639. https://doi.org/10.1039/d1md00200g.

Muchesa, P., Mwamba, O., Barnard, T.G., Bartie, C., 2014. Detection of free-living amoebae using amoebal enrichment in a wastewater treatment plant of Gauteng Province, South Africa. BioMed Res. Int. 2014, 575297. https://doi.org/10.1155/2014/575297.

Musayeva, A., Riedl, J.C., Schuster, A.K., Wasielica-Poslednik, J., Pfeiffer, N., Gericke, A., 2020. Topical voriconazole as supplemental treatment for Acanthamoeba keratitis. Cornea 39, 986–990. https://doi.org/10.1097/ICO.0000000000002315.

Nakaminami, H., Tanuma, K., Enomoto, K., Yoshimura, Y., Onuki, T., Nihonyanagi, S., Hamada, Y., Noguchi, N., 2017. Evaluation of in vitro antiamoebic activity of antimicrobial agents against clinical Acanthamoeba isolates. J Ocul Pharmacol Ther 33, 629–634. https://doi.org/10.1089/jop.2017.0033.

Niyyati, M., Mafi, M., Haghighi, A., Hakemi Vala, M., 2015. Occurrence of potentially pathogenic bacterial-endosymbionts in Acanthamoeba spp. Iran. J. Parasitol. 10, 181–188.

Ondarza, R.N., Iturbe, A., Hernandez, E., 2006. In vitro antiproliferative effects of neuroleptics, antimycotics and antibiotics on the human pathogens Acanthamoeba polyphaga and Naegleria fowleri. Arch. Med. Res. 37, 723–729. https://doi.org/10.1016/j.arcmed.2006.02.007.

Ortilles, A., Belloc, J., Rubio, E., Fernandez, M.T., Benito, M., Cristobal, J.A., Calvo, B., Goni, P., 2017. In-vitro development of an effective treatment for Acanthamoeba keratitis. Int. J. Antimicrob. Agents 50, 325–333. https://doi.org/10.1016/j.ijantimicag.2017.03.033.

Osato, M.S., Robinson, N.M., Wilhelmus, K.R., Jones, D.B., 1991. In vitro evaluation of antimicrobial compounds for cysticidal activity against Acanthamoeba. Rev. Infect. Dis. 13 (Suppl. 5), S431–S435. https://doi.org/10.1093/clind/13.supplement_5.s431.

Patel, A., Cholkar, K., Agrahari, V., Mitra, A.K., 2013. Ocular drug delivery systems: an overview. World J. Pharmacol. 2, 47–64. https://doi.org/10.5497/wjp.v2.i2.47.

Peyton, L.R., Gallagher, S., Hashemzadeh, M., 2015. Triazole antifungals: a review. Drugs Today 51, 705–718. https://doi.org/10.1358/dot.2015.51.12.2421058.

Przybek-Skrzypecka, J., Walkden, A., Brahma, A., Chidambaram, J., Carley, F.M., 2023. Impact of first healthcare provider on Acanthamoeba keratitis course: how to overcome poor prognosis in Acanthamoeba keratitis treatment? A single tertiary center, observational study. Clin. Ophthalmol. 17, 3975–3982. https://doi.org/10.2147/OPTH.S438990.

Rajendran, K., Anwar, A., Khan, N.A., Aslam, Z., Raza Shah, M., Siddiqui, R., 2020. Oleic acid coated silver nanoparticles showed better in vitro amoebicidal effects against naegleria fowleri than amphotericin B. ACS Chem. Neurosci. 11, 2431–2437. https://doi.org/10.1021/acschemneuro.9b00289.

Rao, K., Abdullah, M., Ahmed, U., Wehelie, H.I., Shah, M.R., Siddiqui, R., Khan, N.A., Alawfi, B.S., Anwar, A., 2024. Self-assembled micelles loaded with itraconazole as anti-Acanthamoeba nano-formulation. Arch. Microbiol. 206, 134. https://doi.org/10.1007/s00203-024-03854-3.

Rased, N.M., Johari, S., Zakeri, H.A., Ma, N.L., Razali, S.A., Hashim, F., 2022. Combinatorial treatment with beta-glucanase enzyme and chlorhexidine induces cysticidal effects in Acanthamoeba cyst. Parasitol. Res. 121, 3105–3119. https://doi.org/10.1007/s00436-022-07650-0.

Redd, T.K., Talbott, M., Cevallos, V., Lalitha, P., Seitzman, G.D., Lietman, T.M., Keenan, J.D., 2021. In vitro comparison of the Acanthamoeba cysticidal activity of povidone iodine, natamycin, and chlorhexidine. Ophthalmol Sci 1, 100025. https://doi.org/10.1016/j.xops.2021.100025.

Reginatto, P., Agostinetto, G.J., Fuentefria, R.D.N., Marinho, D.R., Pizzol, M.D., Fuentefria, A.M., 2023. Eye fungal infections: a mini review. Arch. Microbiol. 205, 236. https://doi.org/10.1007/s00203-023-03536-6.

Saunders, P.P., Proctor, E.M., Rollins, D.F., Richards, J.S., 1992. Enhanced killing of Acanthamoeba cysts in vitro using dimethylsulfoxide. Ophthalmology 99, 1197–1200. https://doi.org/10.1016/s0161-6420(92)31823-8.

Scheid, P., 2014. Relevance of free-living amoebae as hosts for phylogenetically diverse microorganisms. Parasitol. Res. 113, 2407–2414.

Schuster, F.L., Visvesvara, G.S., 1998. Efficacy of novel antimicrobials against clinical isolates of opportunistic amebas. J. Eukaryot. Microbiol. 45, 612–618. https://doi.org/10.1111/j.1550-7408.1998.tb04557.x.

Seal, D., 2003a. Treatment of Acanthamoeba keratitis. Expert Rev. Anti Infect. Ther. 1, 205–208. https://doi.org/10.1586/14787210.1.2.205.

Seal, D.V., 2003b. Acanthamoeba keratitis update-incidence, molecular epidemiology and new drugs for treatment. Eye (Lond) 17, 893–905. https://doi.org/10.1038/sj.eye.6700563.

Shah, Y.S., Stroh, I.G., Zafar, S., Zhang, N., Sriparna, M., Shekhawat, N., Ghos, Z., Srikumaran, D., Woreta, F.A., 2021. Delayed diagnoses of Acanthamoeba keratitis at a tertiary care medical centre. Acta Ophthalmol. 99, 916–921. https://doi.org/10.1111/aos.14792.

Shih, K.Y., Chang, Y.T., Wang, Y.J., Huang, J.M., 2024. Ouabain, ATPase inhibitor, potentially enhances the effect of polyhexamethylene biguanide on Acanthamoeba castellanii. Int J Parasitol Drugs Drug Resist 25, 100550. https://doi.org/10.1016/j.ijpddr.2024.100550.

Shing, B., Singh, S., Podust, L.M., McKerrow, J.H., Debnath, A., 2020. The antifungal drug isavuconazole is both amebicidal and cysticidal against Acanthamoeba castellanii. Antimicrob. Agents Chemother. 64. https://doi.org/10.1128/AAC.02223-19.

Siddiqui, R., Khatoon, B., Kawish, M., Sajeev, S., Faizi, S., Shah, M.R., Alharbi, A.M., Khan, N.A., 2024. The potential of nanocomposites (patuletin-conjugated with gallic acid-coated zinc oxide) against free-living amoebae pathogens. Int. Microbiol. https://doi.org/10.1007/s10123-024-00584-w.

Sun, X., Zhang, Y., Li, R., Wang, Z., Luo, S., Gao, M., Deng, S., Chen, W., Jin, X., 2006. Acanthamoeba keratitis: clinical characteristics and management. Ophthalmology 113, 412–416. https://doi.org/10.1016/j.ophtha.2005.10.041.

Szentmary, N., Daas, L., Shi, L., Laurik, K.L., Lepper, S., Milioti, G., Seitz, B., 2019. Acanthamoeba keratitis - clinical signs, differential diagnosis and treatment. J Curr Ophthalmol 31, 16–23. https://doi.org/10.1016/j.joco.2018.09.008.

Taravaud, A., Loiseau, P.M., Pomel, S., 2017. In vitro evaluation of antimicrobial agents on Acanthamoeba sp. and evidence of a natural resilience to amphotericin B. Int J Parasitol Drugs Drug Resist 7, 328–336. https://doi.org/10.1016/j.ijpddr.2017.09.002.

Thomas, V., Herrera-Rimann, K., Blanc, D.S., Greub, G., 2006. Biodiversity of amoebae and amoeba-resisting bacteria in a hospital water network. Appl. Environ. Microbiol. 72, 2428–2438.

Thomson, S., Rice, C.A., Zhang, T., Edrada-Ebel, R., Henriquez, F.L., Roberts, C.W., 2017. Characterisation of sterol biosynthesis and validation of 14alpha-demethylase as a drug target in Acanthamoeba. Sci. Rep. 7, 8247. https://doi.org/10.1038/s41598-017-07495-z.

Thongseesuksai, T., Wongwai, P., Boonmars, T., Sanpool, O., Laummaunwai, P., 2020. Evaluating the in vitro efficacy of gatifloxacin, levofloxacin and gentamicin against Acanthamoeba cysts. Int. Ophthalmol. 40, 361–368. https://doi.org/10.1007/s10792-019-01188-4.

Tien, S.H., Sheu, M.M., 1999. Treatment of Acanthamoeba keratitis combined with fungal infection with polyhexamethylene biguanide. Kaohsiung J. Med. Sci. 15, 665–673.

Trabelsi, H., Sellami, A., Dendena, F., Sellami, H., Cheikh-Rouhou, F., Makni, F., Ben, D. S., Ayadi, A., 2010. Free-living Amoebae (FLA): morphological and molecular identification of Acanthamoeba in dental unit water. Parasite 17, 67–70. https://doi.org/10.1051/parasite/2010171067.

Tuft, S., Somerville, T.F., Li, J.O., Neal, T., De, S., Horsburgh, M.J., Fothergill, J.L., Foulkes, D., Kaye, S., 2022. Bacterial keratitis: identifying the areas of clinical uncertainty. Prog. Retin. Eye Res. 89, 101031. https://doi.org/10.1016/j.preteyeres.2021.101031.

Tuft, S.J., Tullo, A.B., 2009. Fungal keratitis in the United Kingdom 2003-2005. Eye (Lond) 23, 1308–1313. https://doi.org/10.1038/eye.2008.298.

van Prehn, J., Reigadas, E., Vogelzang, E.H., Bouza, E., Hristea, A., Guery, B., Krutova, M., Noren, T., Allerberger, F., Coia, J.E., Goorhuis, A., van Rossen, T.M., Ooijevaar, R.E., Burns, K., Scharvik Olesen, B.R., Tschudin-Sutter, S., Wilcox, M.H., Vehreschild, M., Fitzpatrick, F., Kuijper, E.J., Guideline Committee of the European Study Group on Clostridioides, d., 2021. European Society of Clinical Microbiology and Infectious Diseases: 2021 update on the treatment guidance document for Clostridioides difficile infection in adults. Clin. Microbiol. Infect. 27 (Suppl. 2), S1–S21. https://doi.org/10.1016/j.cmi.2021.09.038.

Vandeputte, P., Ferrari, S., Coste, A.T., 2012. Antifungal resistance and new strategies to control fungal infections. Int J Microbiol 2012, 713687. https://doi.org/10.1155/2012/713687.

Varga, J.H., Wolf, T.C., Jensen, H.G., Parmley, V.C., Rowsey, J.J., 1993. Combined treatment of Acanthamoeba keratitis with propamidine, neomycin, and polyhexamethylene biguanide. Am. J. Ophthalmol. 115, 466–470. https://doi.org/10.1016/s0002-9394(14)74448-4.

Vazquez-Laslop, N., Mankin, A.S., 2018. How macrolide antibiotics work. Trends Biochem. Sci. 43, 668–684. https://doi.org/10.1016/j.tibs.2018.06.011.

Visvesvara, G.S., Moura, H., Schuster, F.L., 2007. Pathogenic and opportunistic free-living amoebae: Acanthamoeba spp., Balamuthia mandrillaris, Naegleria fowleri, and Sappinia diploidea. FEMS Immunol. Med. Microbiol. 50, 1–26. https://doi.org/10.1111/j.1574-695X.2007.00232.x.

Watanabe, R., Nakazawa, T., Yokokura, S., Kubota, A., Kubota, H., Nishida, K., 2010. Fluoroquinolone antibacterial eye drops: effects on normal human corneal epithelium, stroma, and endothelium. Clin. Ophthalmol. 4, 1181–1187. https://doi.org/10.2147/OPTH.S13672.

Whan, L., Grant, I.R., Rowe, M.T., 2006. Interaction between Mycobacterium avium subsp. paratuberculosis and environmental protozoa. BMC Microbiol. 6, 63.

Xie, L., Zhai, H., Zhao, J., Sun, S., Shi, W., Dong, X., 2008. Antifungal susceptibility for common pathogens of fungal keratitis in Shandong Province, China. Am. J. Ophthalmol. 146, 260–265. https://doi.org/10.1016/j.ajo.2008.04.019.

Xie, L., Zhong, W., Shi, W., Sun, S., 2006. Spectrum of fungal keratitis in north China. Ophthalmology 113, 1943–1948. https://doi.org/10.1016/j.ophtha.2006.05.035.

Xuguang, S., Lin, C., Yan, Z., Zhiqun, W., Ran, L., Shiyun, L., Xiuying, J., 2003. Acanthamoeba keratitis as a complication of orthokeratology. Am. J. Ophthalmol. 136, 1159–1161. https://doi.org/10.1016/s0002-9394(03)00635-4.

Yeung, E.Y., Huang, S.C., Tsai, R.J., 2002. Acanthamoeba keratitis presenting as dendritic keratitis in a soft contact lens wearer. Chang Gung Med. J. 25, 201–206.