10.21608/avmj.2024.333203.1457 Assiut University web-site: www.aun.edu.eg

DETECTION AND MOLECULAR CHARACTERIZATION OF AMOEBIC CONTAMINATION TO CONTACT LENSES AS A POTENTIAL PATHOGENIC THREAT CAUSING KERATITIS IN SAUDI ARABIA

HAIAM MOHAMED MAHMOUD FARRAG [1,2*]; SAMIA S. ALKHALIL [1]; MOHAMMED AGEELI HAKAMI [1]; MONA GABER [2]; MARWA KAMAL DARWISH [1,3]; AMIRA ELTAYEB MUSTAFA BADAWI [1] AND ALZAHRAA ABDELRAOUF AHMAD [2]

[1] Department of Clinical Laboratories Sciences, College of Applied Medical Sciences, Shaqra University, Al-Quwaiiyah 19257, Saudi Arabia
[2] Department of Parasitology, Faculty of Medicine, Assiut University, 71515, Assiut, Egypt
[3] Chemistry Department (Biochemistry Branch), Faculty of Science, Suez University, Suez, 43518, Egypt.

Received: 20 October 2024; **Accepted:** 5 November 2024

ABSTRACT

Acanthamoeba spp. is a potentially pathogenic free-living amoeba (FLA) that causes central nervous system infections in both humans and animals and is a significant cause of human keratitis, typically through contaminated water sources, soil, and contact lens solutions. The study aimed to investigate the contamination rate of contact lenses (CL) and their cleaning solutions with FLA, with a specific focus on *Acanthamoeba* as a potential health threat to Saudi Arabian contact lens wearers. A total of 105 samples of previously used contact lenses and their preservative solutions were donated by female students at Shaqra University, Saudi Arabia. Amoebae were isolated through culturing and morphologically identified using standard keys. Molecular identifications based on gene-specific PCR assays were also conducted for all positive cultures. Additionally, genotyping and phylogenetic analysis for *Acanthamoeba* isolates were performed. Of all the samples, 56.19% were infected with *Acanthamoeba* and *Vahlkampfiidae Acanthamoeba spp.* were detected in 76.3% of the positive cultures (n = 45), while *Vahlkampfiidae* contaminated 27 culture samples, either single or mixed infection, including *Naegleria* sp. Morphological identification revealed five *Acanthamoeba* species**,** namely *Acanthamoeba castellenii*, *A. triangularis*, *A. polyphaga* (group II), A. *astronyxis* (group I), *and A. lenticulate* (group III). Sequence analysis of the 18S rRNA gene revealed two strains: *A. castellanii* (T4 genotype) and *A. polyphagia* (T2 genotype). This report highlights the first identification of FLA contamination in contact lenses and cleaning solutions in Saudi Arabia. Efforts are needed to prevent *Acanthamoeba* contamination, and further studies should investigate potential environmental contamination with pathogenic FLA across Saudi Arabian governorates.

Keywords: *Acanthamoeba*, contact lens, contamination, phylogenic analysis, Saudi Arabia.

Corresponding author: Haiam M.M. Farrag
E-mail address: hayammahmoud@su.edu.sa
Present address: Department of Clinical Laboratories Sciences, College of Applied Medical Sciences, Shaqra University, Al-Quwaiiyah 19257, Saudi Arabia and Department of Parasitology, Faculty of Medicine, Assiut University, 71515, Assiut, Egypt

INTRODUCTION

Free-living amoebae (FLA) are opportunistic zoonotic parasites for both humans and animals throughout the world, from contaminated water sources and soil, causing a wide range of infections, such as *acanthamebiasis*, primary amebic meningoencephalitis, granulomatous amebic encephalitis, amebic keratoconjunctivitis, and amebic conjunctivitis. In animals, *Naegleria* can infect inoculated mice and sheep in the laboratory. *Acanthamoeba* can infect both dogs (Pearce *et al*.,1985) and sheep (Van der Lugt and Van der Merve, 1990) in nature, and experimentally. It can also infect the cornea of rabbits, swine, and mice. Researchers reported that FLA cause diseases to animals similar to humans (Simpson *et al.,* 1982; Pearce *et al.,* 1985; Niederkorn *et al.,* 1992; Visvesvara *et al.,* 1993).

Over the past few decades, contact lens (CL) wearing has become widely popular around the world, reaching up to 140 million users. (Moreddu *et al.,* 2019). The contact lens global market is thought to be worth 19.45 billion US dollars by 2024 (Statista, 2022). However, the exact number of contact lens wearers in Saudi Arabia is not estimated. Most studies have focused on contact lens complications, rather than exploring the CL prevalence among Saudi Arabians (Al Hadlaq *et al.,* 2023; Hatami *et al.,* 2021).

Contact lenses have recently gained tremendous popularity among younger generations, who wear them to correct refractive errors and for cosmetic reasons, particularly in females (Tanisha *et al.,* 2008; Bashir *et al.,* 2024). According to research conducted in the Kingdom of Saudi Arabia (KSA), a high number of contact lenses were sold in regular stores without requiring prescriptions (Abahussin *et al.,* 2014). Consequently, the widespread use of contact lenses raised a variable range of complications, up to 39–60.99% among contact lens wearers. Complications range from mild superficial keratitis to vision-threatening events, such as contact-lens-related infectious keratitis, which is a potentially blinding condition that rarely occurs in healthy eyes. It comprises fungal, bacterial, and amoebic keratitis (Alamillo-Velazquez *et al.,* 2021).

One of the main problems of CL wearing is the presence of numerous microbiological lens contaminants, whether potentially pathogenic or nonpathogenic organisms (Liaqat *et al.,* 2019). Free-living amoebae such as *Acanthamoeba*, *Naegleria*, and *Vahlkampfiidae* have been described as water resource contaminants, including brackish water, groundwater, seawater, river water, wastewater, domestic tap water, and swimming pool water. These amoebae, specifically *Acanthamoeba*, can contaminate contact lens cleaning solutions or even grow in CL containers cleaned with contaminated tap water. Therefore, poor CL hygiene, contact with contaminated water or mud, and ineffective contact lens disinfecting solutions lead to amoebic keratitis (AK) (Hassan *et al.,* 2021).

Of note, the presence of minor erosions of the corneal epithelium that may occur with repeated wearing of CL and the subsequent use of contaminated lens solution pose a potential risk for *Acanthamoeba* keratitis, which is a serious problem that can induce corneal stroma invasion and destruction (Visvesvara *et al.,* 2007).

Keratitis caused by *Acanthamoeba* has increased in the past few years (Joslin *et al.,* 2007). Several studies have investigated the link between CL wearers and acanthamoebic keratitis (AK), highlighting the importance of hygienic measures regarding CL and their disinfection. Globally, various studies reported CL contamination rates that vary widely, for example, 1% in Hong Kong up to 65.9% in the Canary Islands, Spain, using different tools of diagnosis, whether culture methods or molecular assays (Boost *et al.,*

2008; Martín-Navarro *et al.,* 2008). However, little is known about the prevalence of *acanthamoeba* keratitis or contact lens contamination in the Middle East, particularly in Arabian countries (Zhang *et al.*, 2023).

Previous studies to detect free-living amoeba (FLA) contamination were primarily based on morphological identification of the isolated amoebae using standard taxonomic keys. Conventionally, the taxonomic classification of *Acanthamoeba* spp. is based on the size and structure of their cysts, comprising three main groups and more than 24 species (Visvesvara *et al.,* 2007). However, such taxonomic classification is currently regarded as ambiguous and unreliable.

An essential feature for species identification is cyst morphology, which could vary depending on changes in culture media conditions. Therefore, a molecular diagnosis has been widely used in the past few years (Khan, 2006; Castrillón and Orozco, 2013).

Acanthamoeba genotyping is an important research topic. There are at least 23 different genotypes (T1–T22) depending on the sequencing of the 18S ribosomal RNA gene. The genetic variation of *Acanthamoeba* may elicit a significant difference in pathogenicity, clinical presentation, and response to treatment. For example, *Acanthamoeba* keratitis has been linked to at least eight genotypes (T2, T3, T4, T5, T6, T10, T11, and T15), with T4 being the most common virulent genotype (Stothard *et al.,* 1998; Putaporntip *et al.,* 2021).

The presence of FLA amoebae, particularly *Acanthamoeba*, in Saudi Arabia has not been well documented in literature, despite its importance for public health. The limited information emphasizes the need to study the contamination rate of contact lenses and their cleaning solutions with FLA, focusing on *Acanthamoeba* as a potential health risk for contact lens wearers in Saudi Arabia, using both morphological and molecular assays.

MATERIALS AND METHODS

Ethics statement

The study was conducted on 105 female student enrollees in the building of Shaqra University at Alqwayeha governorate, Saudi Arabia. They volunteered to provide the CLs and lens cleaning solutions they used for the research, after being informed of the aims and purpose of the study. Written consent was obtained from them before they participated. Ethical approval was acquired from the Scientific Research Ethics Unit at Shaqra University (No: ERC_SU_F_202300006).

Sample collection and culturing

From April to June 2022, the donated contact lenses were collected from 105 female students enrolled in Shaqra University at Alqwayeha governorate, Saudi Arabia. Approximately 100 µl of concentrated cleaning solutions were recovered by centrifugation of the lens containers, together with lenses. Samples were immediately transferred onto the surface of 1.5% non-nutrient agar medium plates containing 0.12 g NaCl, 0.004 g $CaCl_2 \cdot 2H_2O$, 0.004 g $MgSO_4 \cdot 7H_2O$, 0.136 g KH_2PO_4, 0.142 g Na_2HPO_4, and 15.0 g agar/L of distilled water, seeded with live *Escherichia coli*, ATCC 25922 (ANNE), at pH 6.8. Then, the plates were left in an incubator at 37°C under appropriate conditions. Under an inverted microscope, the plates were monitored daily for seven days and observed for trophozoites' growth and up to 14 days for cysts' formation (Fechtali-Moute *et al.,* 2022). All samples were submitted to 1 or 2 subsequent subcultures according to the recommended subculturing schedules of Schuster (2002).

Morphological identification

For trophozoite collection, 5 mL of Page amoeba saline (PAS) solution was added to newly sub-cultivated agar plates containing amebae. This was done on the 5^{th} to 6^{th} days. While cysts were collected on the 10^{th} to 14^{th} days. The solution was pipetted, and the collected suspension was centrifuged at least three times at 2500 rpm for 10 minutes at

room temperature to minimize the presence of *E. coli* and to remove any additional agar. Then, the supernatant was discarded, and the clean sediment was examined by light microscopy (Olympus, CHA, Japan) in a wet mount, stained with different stains, including 0.1% methylene blue and Giemsa stains (Sigma-Aldrich, Switzerland), and photographed for morphological identification at a magnification of x400 followed by x1000, following the methods described by El-Sayed and Hikal (2015).

The genus level of different types of FLA was determined according to the morphology of trophozoites and cysts using identification keys described by Page (1988). Positive FLA samples were subjected to an additional ex-flagellation test to differentiate the genus *Naegleria* from other members of *Vahlkampfiidae* and the genus *Acanthamoeba* (Garcia, 2001). Further species identification of *Acanthamoeba* cysts was performed using the identification key of Pussard and Pons (1977).

Genus-specific PCR amplification of FLA isolates

Molecular identification at the genus level was performed for positive sub-cultured samples morphologically identified as *Acanthamoeba* spp. and *Vahlkampfiidae*. Genus-specific PCR was done targeting the ASA.S1 (DF3) region of the 18S rRNA gene for *Acanthamoeba* isolates using JDP1 and JDP2 primers (Schroeder *et al.,* 2001) and the ITS gene for *Vahlkampfiidae* isolates using ITS1 and ITS2 primers (Pélandakis and Pernin, 2002). The isolated cysts and trophozoites from subcultures were washed three times to eliminate excess *E. coli*, centrifuged, and the clean sediment was subjected to subsequent genomic DNA extraction using the QIAGEN extraction kit (QIAamp1 DNA Minikit, Germany) following the manufacturer's protocol (Ozcelik *et al.,* 2012). Quantification of DNA concentration was done using a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA).

Genus-specific primers were utilized in a total reaction volume of 25 µl containing 5 µl of DNA template, 1 µl of each primer (20 pmol concentration), 12.5 µl of EmeraldAmp Max PCR Master Mix (Takara, Japan), and 5.5 µl of DNase-free water. The reaction was done in a thermal cycler (Applied Biosystem 2720). The details of reaction conditions are described in Table (1). The amplified PCR products were analyzed by electrophoresis on a 1.5% agarose gel stained with ethidium bromide (Applichem, Germany, GmbH) in 1x TBE buffer and visualized by a gel documentation system (Alpha Innotech, Biometra). The amplicon size was determined using a 100-bp ladder.

Table 1: Details of Genus-specific PCR assay used for molecular identification of isolated FLA.

Gene	Sequences of primers	Amplified fragment (bp)	1ry Denat.	Amplification (35 cycles)			Final ext.	Reference
				2ry denat.	**Ann.**	**Ext.**		
ITS gene	**ITS1F:** GAACCTGCGTAG GGATCATTT **ITS2R:** TTTCTTTTCCTCC CCTTATTA	*Naegleria* 400 bp *Allovahlkampfia* 500 bp *Vahlkampfia* 600 bp *Hartmannella* 800 bp	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	(Pélandakis and Pernin, 2002)
18s rRNA gene	**JDP1:** GGCCCAGATCGT TTACCGTGAA **JDP2:** TCTCACAAGCTG CTAGGGGAGTCA	423-551 bp	94˚C 5 min.	94˚C 30 sec.	60˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	(Schroeder *et al.,* 2001)

Dent = Denaturation , Ann = Annealing , EXT = Extension

Nucleotide sequencing and phylogenetic analysis

Acanthamoeba-positive PCR products were further subjected to sequence analysis and phylogenetic analysis. The amplified products were recovered from the gel using a QIAquick PCR product extraction kit (Qiagen, Valencia). Sequencing of the purified products was done based on the sequence analysis of the 18S rRNA gene on both strands using the BigDye Terminator V3.1 cycle sequencing kit (Perkin-Elmer in facilities of Elim Biopharmaceuticals Company, Hayward, CA, United States). DNA sequences were obtained using an Applied Biosystems 3130 genetic analyzer (HITACHI, Japan). The retrieved nucleotide sequences were deposited in GenBank under accession numbers (OR789577, OR789578 and OR789579 and PP029331, PP029332 and PP029333). Nucleotide sequences from the present study were compared to reference *Acanthamoeba* strains available in GenBank using BLAST® analysis (Altschul *et al.*, 1990). Multiple alignment analyses of the present sequences and homologous reference sequences were performed using Clustal W alignment for calculating dissimilarity percentages (Thompson *et al.*, 1994). The phylogenetic tree was constructed using Kimura 2-parameter models in a distance-based algorithm as neighbour-joining method with 1000 Bootstrap replicates for reliability in MEGAX software (Tamura *et al.*, 2013).

RESULTS

Contamination rates

Out of the 105 tested samples, 59 (56.19%) culture plates were positive for FLA, and 46 (43.81%) samples were negative. The identified types of FLA were *Vahlkampfiidae* (mainly, *Naegleria*) and *Acanthamoeba* spp., based on the morphological criteria of the trophozoites and the cysts, as shown in Table (2).

Morphological identification of the FLA isolates

Identification of Vahlkampfiid trophozoites and cysts was difficult. Out of the 59 positive cultures, 27 were positive for *Vahlkampfiidae* (45.76%), 14 were in the form of a single infection, and 13 were mixed with *Acanthamoeba*. The trophozoite size was approximately 15 to 20 μm in diameter. The cysts were spherical, about 8–15 μm in diameter, with thin cyst walls showing no clear distinction between the endocyst and the exocyst (Figure 1). Based on the ex-flagellation test, 21 subculture samples were identified as *Naegleria* spp. (single and mixed infection). While 11 samples were negative for the flagellation test, were recognized as other Vahlkampfiids (Table 2).

Table 2: Number and types of positive FLA isolates contaminating CL and cleaning solutions.

Distribution of culture infection							
	Single FLA isolates			Mixed FLA isolates			Total Positive cultures
Genus type	*Acanthamoeba* spp.	*Naegleria* spp.	*Vahlkampfiidae* spp.	*A.* spp.+ *N.* spp.	*A.* spp. + *V.* spp	*A.* spp. + *N.* spp.+ *V.* spp	
	32	11	3	5	3	5	**59**

A.= Acanthamoeba, N. = Naegleria, V. = Vahlkampfiidae

Among 45 positive cultures for *Acanthamoeba* spp., 32 were single contaminations (71.11%), and 13 were mixed with different *Vahlkampfiidae*. Based on the morphological criteria of *Acanthamoeba* trophozoites and cysts, as described by standard keys. *Acanthamoeba* species were identified with light microscopy by their unique criteria. The double-walled, uninucleate cyst stages ranged from 11 to 25

µm in diameter. The ectocyst was mostly wrinkled, whereas the endocyst was variable in shape according to the species (Figure 2). Five *Acanthamoeba* species were detected, including *A. castellanii*, *A. polyphaga, and A. triangularis* belonging to group (II), *A. astronyxis* belonging to group (I), and *A. lenticulata* to group (III) (Table 3).

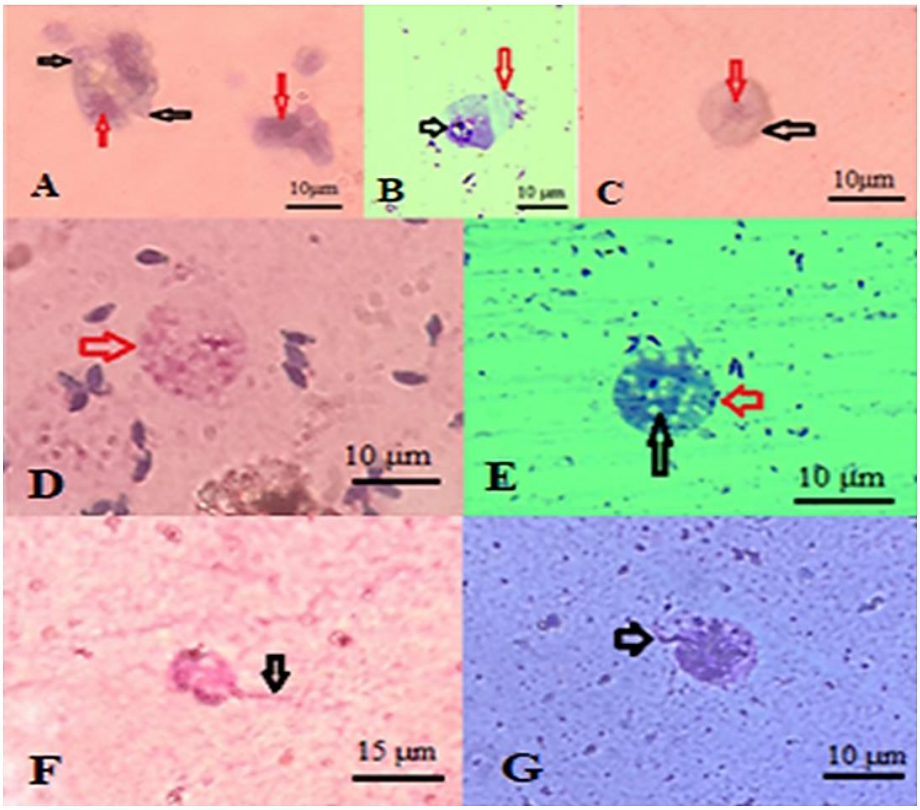

Figure 1 (A): *Vahlkampfiidae* trophozoite (x1000) stained with Giemsa stain, showing multiple pseudopodia (black arrows) and a single nucleus (red arrows). **(B)** *Naegleria* sp. trophozoite (x1000) stained with methylene blue, showing bluntly rounded pseudopodium at the anterior end (red arrow), granular endoplasm, and a single vesicular nucleus (black arrow). **(C)** *Vahlkampfiidae* cyst (x1000) stained with Giemsa stain, showing a thin cyst wall with no pores (black arrow) and a solitary nucleus (red arrow). *Naegleria* sp. cysts (x1000) stained with Giemsa **(D)** and methylene blue **(E)**, showing a thin cyst wall with multiple pores (red arrow), granular endoplasm, and a single nucleus (black arrow). *Naegleria* flagellate stage, stained with Giemsa (x400) and methylene blue (x1000) (**F** and **G** respectively), both show a pair of flagella raised from a temporary pear-shaped body (black arrows).

Table 3: Numbers and types of ***Acanthamoeba*** species detected by the morphological identification of cyst stage.

Acanthamoeba **species**	No. of positive samples		Total
	Single isolates	**Mixed samples**	
A. castellanii	19	2	21
A. triangularis	7	4	11
A. polyphaga	6	2	8
A. astronyxis	0	3	3
A. lenticulata	0	2	2
Total	**32**	**13**	**45**

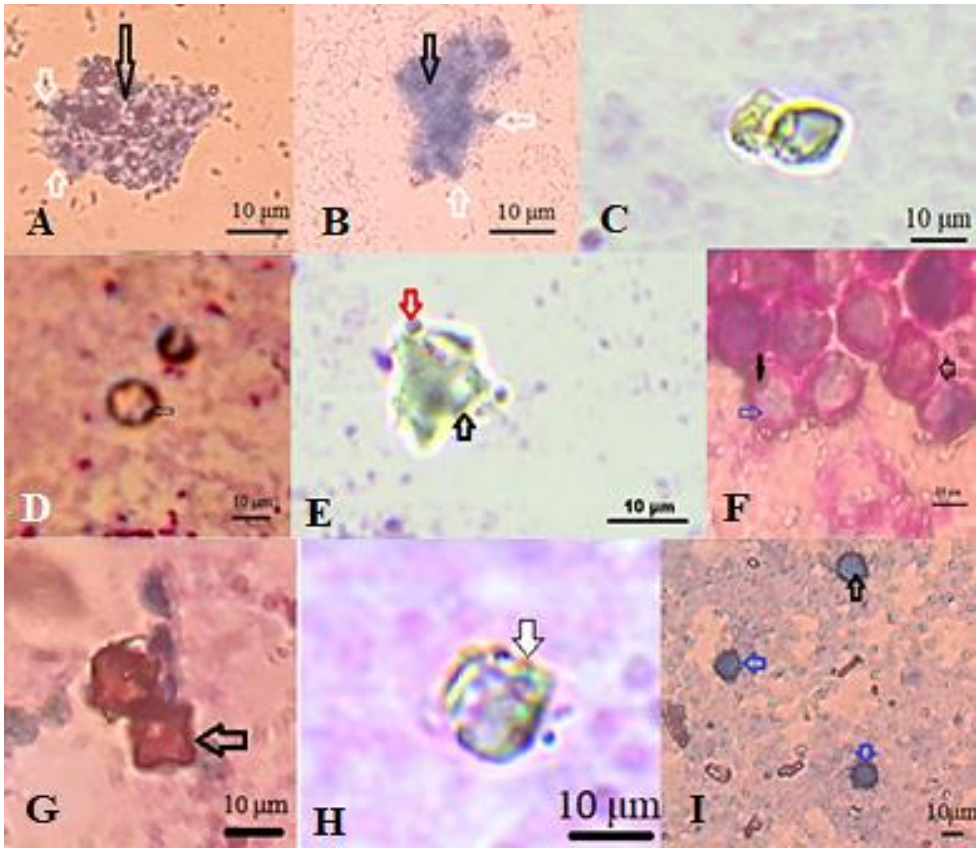

Figure 2: *Acanthamoeba* trophozoite (x1000) stained with methylene blue (A and **B**) showing characteristic thorn-like acanthopodia (white arrows) with highly vacuolar endoplasm (black arrows). (**C**) The excystment process of *Acanthamoeba* dislodges the cyst through the operculum, stained with methylene blue. (**D**) *A. astronyxis* cyst (x1000), stained with Giemsa stain. The cyst size is 18 µm. The ectocyst is smoothly circular. The endocyst ended with five rays' stellate (black arrow). (**E**) *A. triangularis* cyst (x1000) stained with methylene blue; cyst size is 13 µm. The endocyst is triangular with broad rays (black arrow). The ectocyst is thick and not rounded (red arrow). (**F**) *A. castellanii* cysts (x1000) stained with Giemsa stain; the ectocyst is wrinkled and thin (black arrow) and nearly rounded endocyst (blue arrow); the cyst diameter is 18µm. *A. polyphaga* cyst (x1000) stained with iodine (**G**) and methylene blue (**H**); The endocyst is irregularly quadrangular, with the sides somewhat concave outwardly. The ectocyst is irregular and loosely applied to the endocyst. The average diameter of cysts is 14 µm. (**I**) *A. lenticulata* cyst (x1000) stained with methylene blue, the endocyst is nearly rounded (black arrow). The ectocyst closely follows the endocyst contour and pleated, forming a saw-teeth appearance in optical shots around the endocyst (blue arrow). The cyst diameter is 11µm.

Molecular identification of FLA isolates

Genus-specific PCR was done to confirm contamination of CL and cleaning solutions with FLA, showing positive amplification of *Acanthamoeba* and *Vahlkampfiidae* isolates, producing PCR products of the expected fragment size for these isolates (400-551 bp). PCR amplification of the ITS region revealed that the morphologically identified *Vahlkampfiidae* belonged to the genus *Naegleria*, producing positive bands at 400 bp (Figure 3A). However, those identified as *Acanthamoeba* were confirmed by amplifying the 18S rRNA gene, producing PCR products at 551 bp. (Figure 3B).

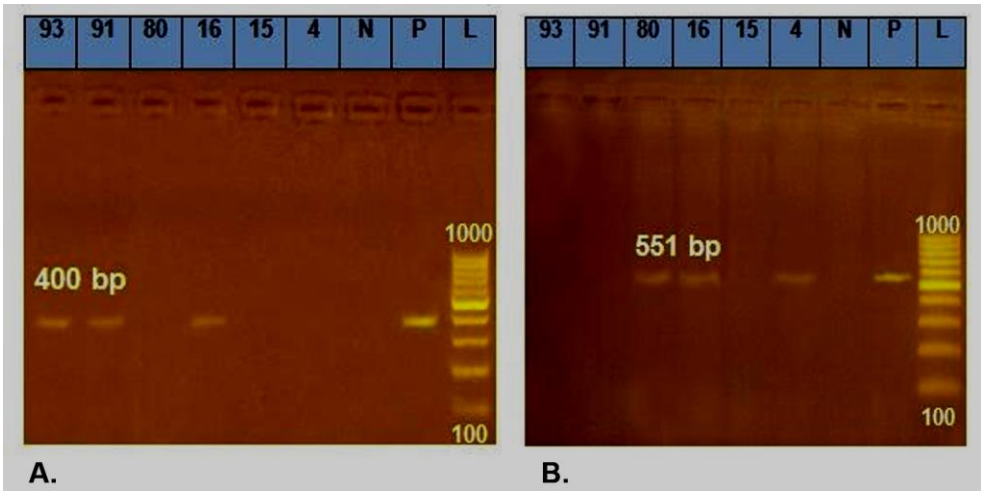

Figure 3: Representative photograph of 1.5% agarose gel stained with ethidium bromide showing the PCR amplicons of positive culture isolates targeting ITS region for *Vahlkampfiidae* and 18S rRNA gene for *Acanthamoeba* showing a single band of 400 bp. (*Naegleria*) (A) and 551 bp DNA products specific for *Acanthamoeba* (B) Lane (P) = positive control; Lane (N) = negative control; (L) = 100 bp DNA ladder.

Sequencing and phylogenetic analysis

Partial nucleotide sequence analyses of the ASA.S1 (DF3) region of the 18S rRNA gene of *Acanthamoeba* spp. positive isolates were done. In the present study, six isolates were successfully sequenced, and the phylogenetic analyses revealed that five isolates (Sa1, Sa2, Sa4, Sa5, Sa6) were identified as *A. castellanii* (accession numbers OR789577, OR789578, PP029331, PP029332, and PP029333), belonging to the pathogenic *Acanthamoeba* genotype T4. Only one isolate (Sa3) was identified as *A. polyphaga* (accession number OR789579), which shared a high similarity with *Acanthamoeba* genotype T2. The sequence homology search for most of the retrieved *Acanthamoeba* isolates in the present study (Sa1, Sa2, Sa4-6) showed 100% similarities with other available strains of *A. castellanii* in the NCBI. database with 99% bootstrap confidence forming a separate subtree branch (Figure 4).

Percent Identity

Divergence

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29		
	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	95.2	96.2	95.9	95.6	95.6	95.6	94.9	95.9	95.9	94.9	97.8	97.5	95.9	94.9	1	KF318462 A. castellanii ATCC 30011
0.0		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	95.2	96.2	95.9	95.6	95.6	95.6	94.9	95.9	95.9	94.9	97.8	97.5	95.9	94.9	2	U07413 A. castellanii ATCC 50374
0.0	0.0		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	95.2	96.2	95.9	95.6	95.6	95.6	94.9	95.9	95.9	94.9	97.8	97.5	95.9	94.9	3	AF260724 A. castellanii 4CL
0.0	0.0	0.0		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	95.2	96.2	95.9	95.6	95.6	95.6	94.9	95.9	95.9	94.9	97.8	97.5	95.9	94.9	4	KT185626 A. castellanii
0.0	0.0	0.0	0.0		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	95.2	96.2	95.9	95.6	95.6	95.6	94.9	95.9	95.9	94.9	97.8	97.5	95.9	94.9	5	MF399034 A. castellanii PA24
0.0	0.0	0.0	0.0	0.0		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	95.2	96.2	95.9	95.6	95.6	95.6	94.9	95.9	95.9	94.9	97.8	97.5	95.9	94.9	6	MT292607 Acanthamoeba genotype T4
0.0	0.0	0.0	0.0	0.0	0.0		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	95.2	96.2	95.9	95.6	95.6	95.6	94.9	95.9	95.9	94.9	97.8	97.5	95.9	94.9	7	MT109098 Acanthamoeba genotype T4
0.0	0.0	0.0	0.0	0.0	0.0	0.0		100.0	100.0	100.0	100.0	100.0	100.0	100.0	95.2	96.2	95.9	95.6	95.6	95.6	94.9	95.9	95.9	94.9	97.8	97.5	95.9	94.9	8	MN700299 Acanthamoeba genotype T4
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		100.0	100.0	100.0	100.0	100.0	100.0	95.2	96.2	95.9	95.6	95.6	95.6	94.9	95.9	95.9	94.9	97.8	97.5	95.9	94.9	9	MN700288 Acanthamoeba genotype T4
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		100.0	100.0	100.0	100.0	100.0	95.2	96.2	95.9	95.6	95.6	95.6	94.9	95.9	95.9	94.9	97.8	97.5	95.9	94.9	10	MN096650 Acanthamoeba Genotype T4D
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		100.0	100.0	100.0	100.0	95.2	96.2	95.9	95.6	95.6	95.6	94.9	95.9	95.9	94.9	97.8	97.5	95.9	94.9	11	OR789577 A. castellanii Sa1
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		100.0	100.0	100.0	95.2	96.2	95.9	95.6	95.6	95.6	94.9	95.9	95.9	94.9	97.8	97.5	95.9	94.9	12	OR789578 A. castellanii Sa2
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		100.0	100.0	95.2	96.2	95.9	95.6	95.6	95.6	94.9	95.9	95.9	94.9	97.8	97.5	95.9	94.9	13	PP029331 A. castellanii Sa4
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		100.0	95.2	96.2	95.9	95.6	95.6	95.6	94.9	95.9	95.9	94.9	97.8	97.5	95.9	94.9	14	PP029332 A. castellanii Sa5
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		95.2	96.2	95.9	95.6	95.6	95.6	94.9	95.9	95.9	94.9	97.8	97.5	95.9	94.9	15	PP029333 A. castellanii Sa6
3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4		94.6	93.0	92.7	94.6	94.6	94.0	94.9	94.9	94.0	94.9	94.6	93.0	92.1	16	MN153019 A. lenticulata AFR1
3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.7		94.3	94.6	97.5	97.8	97.1	97.8	97.8	97.1	96.5	95.9	94.3	94.0	17	AF019063 A. palestinensis 2802
1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	2.4	2.4		97.5	94.3	94.0	93.3	94.3	94.3	93.3	96.8	98.4	99.7	97.1	18	GU573861 A. jacobsi F13/SB1
2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	3.5	2.7	1.4		94.3	94.6	94.0	94.9	94.9	94.0	97.1	97.8	97.5	98.7	19	AF019067 A. culbertsoni LillyA-1
3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	2.7	2.4	3.1		98.7	98.1	98.4	98.4	98.1	96.2	95.9	94.3	93.7	20	GU573876 A. polyphaga C17/PHTP
3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	2.3	2.8	2.7	1.3		98.7	99.0	99.0	98.7	96.2	95.6	94.0	94.0	21	AF019051 A. polyphaga OX-1
3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	2.0	2.4	2.4	1.0	0.3		98.4	98.4	100.0	95.6	94.9	93.3	93.3	22	DQ490964 A. polyphaga AC012
3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	1.7	2.4	2.4	1.0	0.3	0.0		100.0	98.4	96.5	95.9	94.3	94.3	23	JQ669661 Acanthamoeba genotype T2/6C
3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	1.7	2.4	2.4	1.0	0.3	0.0	0.0		98.4	96.5	95.9	94.3	94.3	24	KX688012 Acanthamoeba genotype T2
3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	2.0	2.4	2.4	1.0	0.3	0.0	0.0	0.0		95.6	94.9	93.3	93.3	25	OR789579 A. polyphaga Sa3
0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	2.4	2.0	0.7	1.7	2.4	2.4	2.0	2.0	2.0	2.0		98.4	96.8	96.5	26	S81337 A. griffini H37
0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	1.7	1.7	0.7	1.4	1.7	2.0	1.7	1.7	1.7	1.7	0.0		98.4	97.1	27	KP711387 A. micheli BRO-2
1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	2.4	2.4	0.3	1.4	2.4	2.8	2.4	2.4	2.4	2.4	0.7	0.7		97.1	28	MN153026 A. jacobsi AFR13
2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	3.5	2.8	1.7	0.7	3.1	2.8	2.4	2.4	2.4	2.4	1.7	1.4	1.7		29	GQ996533 A. healyi
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29		

Figure 4: The distance matrix of *Acanthamoeba* sequences of the ASA.S1 region of the 18S rDNA gene of the present study and reference sequences available in GenBank.

In addition, the phylogenetic tree showed that they were clustered in the same clade with other types of *Acanthamoeba* spp., *including A. griffini* (S81337), *A. Micheli* (KP711387), and *A. Jacobsi* (GU573860, GU573861), sharing > 95% similarity with high bootstrap support. While *the Acanthamoeba* Sa3 isolate showed a 100% similarity with *A. polyphaga* isolates (AF019051, DQ490964) that clustered with *A. palestinensis* isolates (KC694192, KC694193, and AF019063) in the same clade, sharing about 97% identity with 92% bootstrap confidence (Figure 5).

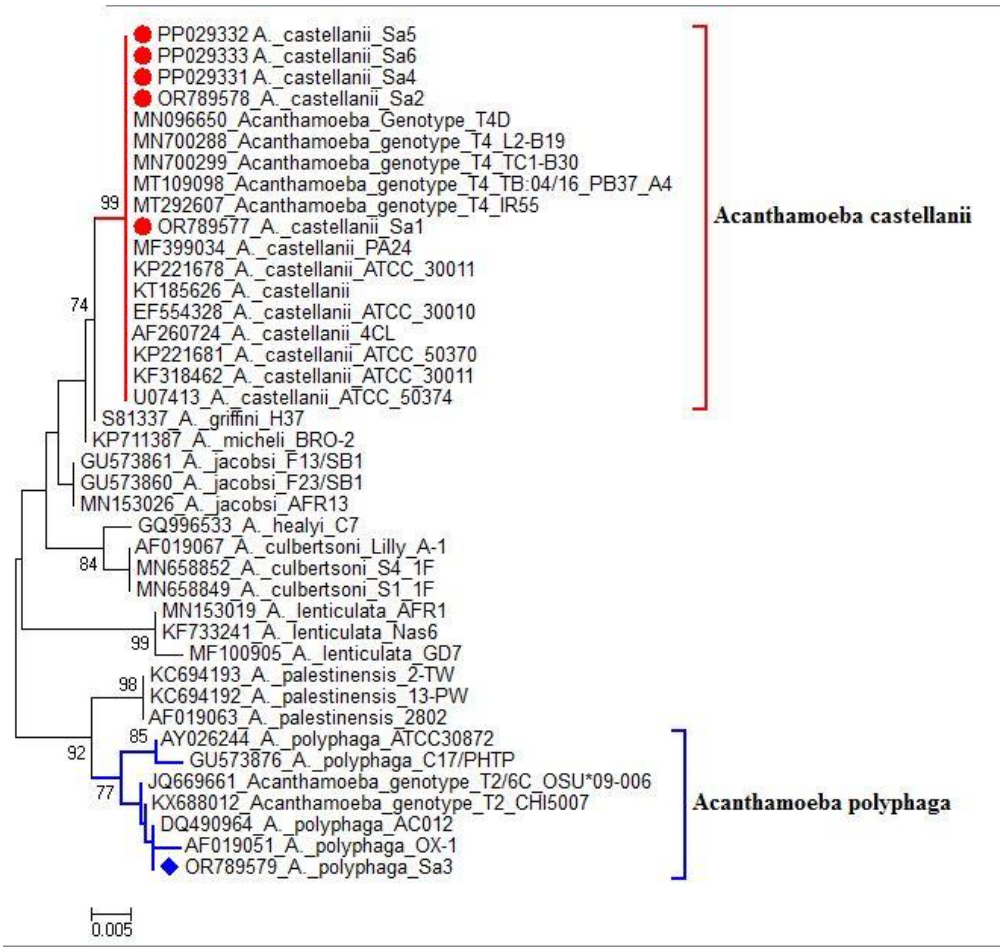

Figure 5: Neighbour-joining phylogenetic tree showing the evolutionary relationship among the studied *Acanthamoeba* isolates and reference sequences retrieved from the GenBank. The phylogenetic tree was reconstructed using the neighbor-joining algorithm with 1000 bootstrap replicates. The scale bar demonstrates the number of substitutions.

DISCUSSION

Contact lens wear is a significant risk factor for infectious keratitis in developed countries, and is considered a second cause in developing countries, following trauma. Microbial keratitis remains a sight-threatening illness for contact lens wearers, with a visual loss rate of up to 28.6% (Alamillo-Velazquez *et al.*, 2021).

FLA is a commonly distributed protozoa in the environment that lives in freshwater sources and soils. One of its members, *Acanthamoeba*, is one of the causative pathogens causing sinus infections, cutaneous lesions, vision-threatening keratitis, and a rare fatal encephalitis (Visvesvara *et al.*, 2007).

To our knowledge, this is the first report to detect potentially pathogenic free-living

amoebae contaminating contact lenses and lens cleaning solutions in Saudi Arabian contact lens wearers, especially with *Acanthamoeba* spp., the most common causative parasitic agent of keratitis.

Our study revealed a high contamination rate of 56.19% of the samples examined were contaminated with FLA, the majority of which belonged to *Acanthamoeba spp.* This might be due to *Acanthamoeba's* ability to adhere to the irregular and rough surface of CLs through their acanthopodia. Furthermore, CL and preservative solutions could be contaminated with FLA from domestic tap water, pool water, or even dust (Lee et al., 2018).

These findings support previous studies that have found that the plastic nature of soft contact lenses has the potential to attract free-living amoebas, owing to their relative resistance to antibiotics and antiseptics, which augments the accumulation of microorganisms and biofilm formation, which could be an appealing niche for free-living amoebae (Gray et al., 1995; Rivera and Adao, 2009; Niyyati *et al*., 2014).

Identification of CL contamination was dependent on microscopic examination for detecting developmental stages of FLA directly or after using different stains, despite advances in immunological and molecular methods for identification (Guarner *et al*., 2007). The non-stained wet preparation allowed us to monitor trophozoite motility. This procedure, however, makes it appear transparent and hides many internal structures. Furthermore, the presence of bacterial and fungal growth on the agar surface complicates amoebae detection (Ithoi *et al*., 2011). Thus, staining is crucial for identifying amoebas and providing detailed structures of their different stages. According to Nageeb *et al.* (2022), the detection of FLA based on morphological identification is still a problematic and time-consuming approach. Consequently, molecular techniques are now considered a reliable method for FLA identification.

In the present study, the visual observation of the different developmental stages of *Naegleria* and *Acanthamoeba* was performed according to the existence of acanthopodia or lobopodia and the shape of the cysts (Eldeek *et al.,* 2019). Based on morphological data provided herein, 27 positive culture samples showed *Vahlkampfiidae* infection, whether single or mixed infection, including *Naegleria* sp. cysts and trophozoites in most of these cultures (77.78%), as demonstrated by the ex-flagellation test and confirmed by PCR assay.

Out of 59 FLA-positive cultures, *Acanthamoeba* spp. trophozoites and cysts were detected in 45 cultures (76.3%). According to the morphological criteria of both Page (1988) and Pussard and Pons (1977), five *Acanthamoeba* species were identified**,** namely *A. castellenii*, *A. triangularis*, *A. polyphaga* (group II), *A. astronyxis* (group I), *and A. lenticulate* (group I). No similar studies were conducted in Saudi Arabia, as far as we know. However, *Acanthamoeba* species have been recognized and isolated from different water resources in a few studies in the studied area (Toula and Sayed Elahl, 2017).

The present results are consistent with previous reports in Egypt (Hassan *et al.,* 2021). Isolation of *Acanthamoeba* spp. from CL and CL cleaning solutions was widely investigated in different parts of the world, showing the potential risk of CL contamination and the hazardous transmission of such pathogens causing AK in contact lens wearers (Carnt and Stapleton, 2016; Gomes *et al.,* 2016; Abdel-Ghaffar *et al.,* 2019; Li *et al.,* 2019; Susanto *et al.,* 2020). Despite the importance of diagnosis by light microscopy, as previously mentioned, molecular diagnosis has the sensitivity to distinguish between the subspecies. It can detect even a tiny number of amoebae, less than one per microliter

(Khosravinia *et al.,* 2021). Therefore, the isolated amoebae were molecularly characterized by genus-specific PCR methods using highly specific primers for *Vahlkampfiidae* and *Acanthamoeba*, which confirmed CL contamination with *Naegleria* spp. and *Acanthamoeba* spp. Further, *Acanthamoeba* isolates were subjected to sequencing and phylogenetic analysis to confirm the topology of the retrieved sequences and prove the pathogenicity. Results of sequencing of the DF3 region of the 18S ribosomal DNA gene of most *Acanthamoeba* isolates were compatible with *A. castellanii* belonging to genotype T4 and one isolate identified as *A. polyphaga* that was highly similar to genotype T2 isolates in the NCBI database. This was in accordance with similar strains isolated from previous studies in Egypt (Hassan *et al.,* 2021), Iran (Khosravinia *et al.,* 2021), Austria (Walochnik *et al.,* 2000), and Argentina (Casero *et al.,* 2017).

There are eight genotypes (T2-6, T10, T11, and T15) that have been associated with AK, with the commonest genotype being T4 (Stothard *et al.,* 1998; Montiel *et al.,* 2014). In the literature, the most common pathogenic species reported were *Acanthamoeba polyphaga* and *Acanthamoeba castellanii*, which were previously classified as T4 genotypes (de Lacerda and Lira, 2021) which were identical to the isolated species in our study.

It seems that the T4 genotype is predominantly associated with corneal infections and AK, which may be attributed to its widespread distribution in the environment, cyst resistance to antiseptics, and expression of many cytotoxic products, compared to other genotypes. In addition, T4 showed significantly higher binding ability and severely damaged the host cells (Golestani *et al.,* 2018; Hassan *et al.,* 2021). Consequently, exploring the genetic diversity of *Acanthamoeba* is essential, as different genotypes exhibit wide, variable clinical presentations with varying responses to treatment (Montiel *et al.,* 2014).

In the current study, an *A. polyphaga* isolate (Sa3) was grouped with genotype T2 in the reconstructed phylogenetic tree. Although genotype T4 was recognized as the main genotype associated with keratitis and genotype T3 was considered a secondary cause of corneal infection, the T2 genotype has been identified as a rare cause of AK. (Maghsood *et al.,* 2005; Xuan *et al.,* 2017). Other studies, however, concluded that the T2 genotype may be nonpathogenic to humans due to its low cytotoxicity and low binding capacity to the host cells (Alsam *et al.,* 2003).

In the current study, all participants included in our research were young female students who are considered a risk group for contact lens-related keratitis, as documented by many studies (Garate *et al.,* 2005; Rezeaian *et al.,* 2012; Khosravinia *et al.,* 2021). Contact lenses are predisposing factors in amebic keratitis and even fungal keratitis due to the usual use of home remedies or boiled water to maintain the lens, and the ineffectiveness of the contact lens disinfectant solutions in removing *Acanthamoeba* cysts. The contact lenses, mainly the soft type, are entirely attached to the cornea and impair its cellular oxygenation, leading to desquamation of the superficial cells, feeding of *Acanthamoeba* on the keratocytes, and providing easier invasion to the corneal stroma (Hammersmith, 2006).

To the best of the authors' knowledge, few reports were conducted across Saudi Arabia demonstrating the contamination level of FLA, whether in the environment, reactional water resources, or human population. Nevertheless, *Acanthamoeba* and *Naegleria*, and even Anti-*Acanthamoeba* antibodies in human populations (Alouffi *et al.,* 2021), were detected in some studies investigating water facilities and environmental contamination with FLA in Jeddah, Makkah, and Riyadh cities in Saudi Arabia (Al-Herrawy and Al-Rasheid, 1998; Toula and Sayed Elahl, 2017; Bakri *et* These studies

have revealed that FLA, particularly *Acanthamoeba*, significantly contaminates water sources such as domestic tap water in houses and mosques, swimming pools, ponds, and air conditioning systems. The heightened risk of environmental contamination may be attributed to the prevalent use of water storage tanks and wells in Saudi Arabia and the hot climate, which results in increased air conditioning usage among residents. Raising awareness about these opportunistic pathogens is essential for reducing the risk of FLA infections among contact lens wearers and the general population in the future.

To date, no studies in Saudi Arabia have been conducted on the molecular characterization and genotyping of *Acanthamoeba* species contaminating the CL and contact lens solutions and their potential risk with AK. This study presents the first data on the frequency, morphological, and molecular characterization of potentially pathogenic FLA, particularly *Acanthamoeba*, isolated from used CL and contact lens solutions in Alqwayeha Governorate, Saudi Arabia.

CONCLUSIONS

Our study revealed a high contamination rate (56.19%) of the examined samples with the potentially pathogenic *Acanthamoeba* and *Naegleria*, as evident by morphological study and PCR assay. Morphological identification revealed five *Acanthamoeba* species**,** namely, *A. castellenii, A. triangularis, and A. polyphaga* belonged to group II; *A. astronyxis* belonged to group I, and *A. lenticulate* belonged to group III. However, genotyping of the *Acanthamoeba* isolates revealed two main strains: *A. castellanii and A. polyphaga,* which belong to the T4 and T2 genotypes, respectively, which are pathogenic genotypes and could be responsible for human amebic keratitis. Therefore, our findings emphasize the significance of practicing good hygiene habits and following ophthalmologist instructions for contact lens wearers. It is crucial to use only lens solutions from reliable sources. Health education and proper hygiene measures are highly recommended to prevent contamination and its harmful effects.

ACKNOWLEDGMENT

The authors extend their appreciation to the Deanship of Scientific Research at Shaqra University for funding this research work through the project number (SU-ANN-2023011).

AUTHOR CONTRIBUTIONS

All co-authors participated in the research in equal proportions.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Abahussin, M.; AlAnazi, M.; Ogbuehi, K.C. and Osuagwu, U.L. (2014): Prevalence, use and sale of contact lenses in Saudi Arabia: Survey on university women and non-ophthalmic stores. Cont Lens Anterior Eye 37(3): 185-90. https://doi.org/10.1016/j.clae.2013.10.001

Abdel-Ghaffar, M.; El Sobky, M.; Harba, N.; Rady, A. and Abokhalil, N. (2019): Comparison Between Different Methods for Diagnosis of *Acanthamoeba* Infection and Isolation of Genotype T9 from a Contact Lens Cases. J. Egypt. Soc. Parasitol. 49, 265–276. https://doi.org/10.21608/jesp.2019.68130

Al Hadlaq, A.M. and Al Harbi, M.M. (2023): The Epidemiological Profile and Predisposing Factors of Microbial Keratitis Over a 10-Year Period at a Tertiary Hospital in Central Saudi Arabia. Cureus 15. https://doi.org/10.7759/cureus.45433

Alamillo-Velazquez, J.; Ruiz-Lozano, R.E.; Hernandez-Camarena, J.C. and Rodriguez-Garcia, A. (2021): Contact

Lens-Associated Infectious Keratitis: Update on Diagnosis and Therapy [Internet]. Infectious Eye Diseases - Recent Advances in Diagnosis and Treatment. IntechOpen Available from: http://dx.doi.org/10.5772/intechopen.100261

Al-Herrawy, A.Z. and Al-Rasheid, K.A. (1998): Identification of *Acanthamoeba* strains isolated from a freshwater course in Saudi Arabia. J. Egypt. Public Health Assoc. 73, 621–633.

Alouffi, A.S.; Dawoud, T.M.; Almaary, K.S.; Mubarak, A.S.; Jarallah, K. and Matin, A. (2021): First report of detection of IgA anti-*acanthamoeba* antibodies among saudi population and amoeba isolation from their surroundings. Trop. Biomed. 38, 73–80. https://doi.org/10.47665 / tb.38.1.013

Alsam, S.; Kim, K.S.; Stins, M.; Rivas, A.O.; Sissons, J. and Khan, N.A. (2003): Acanthamoeba interactions with human brain microvascular endothelial cells. Microb. Pathog. 35, 235–241.

Altschul, S.F.; Gish, W.; Miller, W.; Myers, E.W. and Lipmanl, D.J. (1990): Basic Local Alignment Search Tool J Mol Biol. 215,403-410.

Bakri, R.; Mohamed, R.; Alghanmi, M.; EL-Malky, M. and El-Bali, M. (2021): Isolation, Morphotyping, Molecular Characterization and Prevalence of Free-Living Amoebae from Different Water sources in Makkah city, Saudi Arabia. J. Umm Al-Qura Univ. Med. Sci. 7, 5–8. https://doi.org/10.54940/ms76736861

Bashir, M.; Alghamdi, A.H.; Ahmed, A.A.; Taishan, S.S.; Taishan, W.S.; Alghamdi, M.G. and Alghamdi, A.S. (2024): The Prevalence of Contact Lens Wear and Improper Use Among Students of Al-Baha University, Saudi Arabia. The Open Ophthalmol. J. 18(1), e18743641306553. http://dx.doi.org/10.2174/0118743641306553240313061238

Boost, M.; Cho, P.; Lai, S. and Sun, W.M. (2008): Detection of *Acanthamoeba* in tap water and contact lens cases using polymerase chain reaction. Optom. Vis. Sci. 85, 526–530.

Carnt, N. and Stapleton, F. (2016): Strategies for the prevention of contact lens-related *Acanthamoeba* keratitis: a review. Ophthalmic Physiol. Opt. 36, 77–92. https://doi.org/https://doi.org/10.1111/opo.12271

Castrillón, J.C. and Orozco, L.P. (2013): Acanthamoeba spp. como parásitos patógenos y oportunistas. Rev. Chil. Infectología. 30, 147–155.

de Lacerda, A.G. and Lira, M. (2021): Acanthamoeba keratitis: a review of biology, pathophysiology and epidemiology. Ophthalmic Physiol. Opt. 41, 116–135. https://doi.org/10.1111/opo.12752

Eldeek, H.E.M.; Attia, R.A.H.; Nageeb, M.M. and Sakla, A.A. (2019): Comparative evaluation of multiple staining techniques for identification of different developmental stages of *Acanthamoeba* and *Naegleria*. J. Egypt. Soc. Parasitol. (JESP), (2), 409- 422.

El-Sayed, N.M. and Hikal, W.M. (2015): Several staining techniques to enhance the visibility of *Acanthamoeba* cysts. Parasitol Res. 114(3):823–30. https://doi.org/10.1007/s00436-014-4190-4 PMID:25346196.

Fechtali-Moute, Z.; Loiseau, P.M. and Pomel, S. (2022): Stimulation of *Acanthamoeba castellanii* excystment by enzyme treatment and consequences on trophozoite growth. Front Cell Dev Biol. 10:982897. doi: 10.3389/fcell.2022.982897

Garate, M.; Cubillos, I. and Marchant. J. et al (2005): Biochemical characterization and functional studies of *Acanthamoeba* mannose-binding protein. Infect Immun. 73(9):5775-81.

Garcia, L.S. (2001): Diagnostic medical parasitology. ASM Press.

Golestani, M.H.; Rasti, S.; Hooshyar, H.; Delavari, M. and Mousavi, S.G.A. et al (2018): Molecular Identification and Genotyping of *Acanthamoeba* Isolated from Environmental Sources in

Kashan, Central Iran. Jundishapur J Microbiol. 11(4): e55582. https://doi.org/10.5812/jjm.55582

Gomes, T.D.S.; Magnet, A.; Izquierdo, F.; Vaccaro, L.; Redondo, F.; Bueno, S.; Sánchez, M.L.; Angulo, S.; Fenoy, S.; Hurtado, C. and Aguila, C. Del (2016): Acanthamoeba spp. in Contact Lenses from Healthy Individuals from Madrid, Spain. PLoS One. 11, 1–12. https://doi.org/10.1371/journal.pone.0154246

Gray, T.B.; Cursons, R.T.; Sherwan, J.F. and Rose, P.R. (1995): Acanthamoeba, bacterial, and fungal contamination of contact lens storage cases. Br. J. Ophthalmol. 79, 601.

Guarner, J.; Bartlett, J.; Shieh, W.J.; Paddock, C.D. and Visvesvara, G.S. et al (2007): Histopathologic spectrum and immunohistochemical diagnosis of amebic meningoencephalitis. Mod. Pathol. 20, 12:1230-37.

Hammersmith, K.M. (2006): Diagnosis and management of *Acanthamoeba* keratitis. Curr Opin Ophthalmol. 17(4):327-31.

Hassan, F.A.M.; Tolba, M.E.M.; Abed, G.H.; Omar, H.M. and Abdel-Hakeem, S.S. (2021): Contact lenses contamination by *Acanthamoeba* spp. In Upper Egypt. PLoS ONE. 16(11): e0259847. https://doi.org/10.1371/journal.pone.0259847

Hatami, H.; Ghaffari, N.; Jolfayi, A.; Ebrahimi, A., Golmohammadi, S.; Zangiabadian, M. and Nasiri, M.J. (2021): Contact Lens Associated Bacterial Keratitis: Common Organisms, Antibiotic Therapy, and Global Resistance Trends: A Systematic Review. Front. Ophthalmol. 1:759271. doi: 10.3389/fopht.2021.759271

Ithoi, I.; Ahmad, A.F.; Mak. J.W.; Nissapatorn, V.; Lau, Y.L. and Mahmud, R. (2011): Morphological characteristics of developmental stages of *Acanthamoeba* and *Naegleria* species before and after staining by various techniques. Southeast Asian J. Trop. Med. Publ. Hlth. 42:1327-38.

Joslin, C.E.; Tu, E.Y. and Shoff, M.E. et al (2007): The association of contact lens solution use and A*canthamoeba* keratitis. Am J Ophthalmol. 144(2): 169-180. doi: 10.1016/j.ajo.2007.05.029

Khan, N.A. (2006): Acanthamoeba: biology and increasing importance in human health. FEMS Microbiol. Rev. 30, 564–595. https://doi.org/10.1111/j.1574-6976.2006.00023.x

Khosravinia, N.; Abdolmajid, F.A.T.A.; Moghaddas, E.; Farash, B.R.H.; Sedaghat, M.R. and Eslampour, A.R. (2021): Diagnosis of *Acanthamoeba* keratitis in Mashhad, Northeastern Iran: A Gene-Based PCR Assay. Iranian Journal of Parasitology. 16 (1), 111. https://doi.org/10.18502/ijpa.v16i1.5530 PMID:33786053

Lee, S.M.; Lee, J.E.; Lee, D.I. and Yu, H.S. (2018): Adhesion of *Acanthamoeba* on cosmetic contact lenses. J. Korean Med. *Sci*. 33.

Li, W.; Wang, Z.; Qu, J.; Zhang, Y. and Sun, X. (2019): Acanthamoeba keratitis related to contact lens use in a tertiary hospital in China. BMC Ophthalmol. 19, 1–6. https://doi.org/10.1186/s12886-019-1210-2

Liaqat, I.; Saleem, M.Q.A.; Tahir, H.M.; Arshad, M. and Arshad, N. (2019): Identification of virulence factors in contact lens associated bacteria: A physiological approach. Cont Lens Anterior Eye. 42(2): 159-64. https://doi.org/10.1016/j.clae.2018.10.002

Maghsood, A.H.; Sissons, J.; Rezaian, M.; Nolder, D.; Warhurst, D. and Khan, N.A. (2005): Acanthamoeba genotype T4 from the UK and Iran and isolation of the T2 genotype from clinical isolates. J. Med. Microbiol. 54, 755–759.

Martín-Navarro, C.M.; Lorenzo-Morales, J.; Cabrera-Serra, M.G.; Rancel, F.; Coronado-Alvarez, N.M.; Pinero, J.E. and Valladares, B. (2008): The

potential pathogenicity of chlorhexidine-sensitive *Acanthamoeba* strains isolated from contact lens cases from asymptomatic individuals in Tenerife, Canary Islands, Spain. J. Med. Microbiol. 57, 1399–1404.

Montiel, A.F.; Fernández, L.B.; Navarro, M.C.M.; Valladares, B.; Velez, L.R.; Laiz, M.R. and Morales, L.J. (2014): Influence of *Acanthamoeba* Genotype on Clinical Course and Outcomes for Patients with *Acanthamoeba* Keratitis in Spain. J. Clin. Microbiol. 52, 1213–1216.

Moreddu, R.; Vigolo, D. and Yetisen, A.K. (2019): Contact lens technology: from fundamentals to applications. Adv. Healthc. Mater. 8, 1900368.

Nageeb, M.M.; Eldeek, H.E.M.; Attia, R.A.H.; Sakla, A.A.; Alkhalil, S.S. and Farrag, H.M.M. (2022): Isolation and morphological and molecular characterization of waterborne free-living amoebae: Evidence of potentially pathogenic *Acanthamoeba* and *Vahlkampfiidae* in Assiut, Upper Egypt. PLoS ONE. 17(7): e0267591. https://doi.org/10.1371/journal.pone.0267591

Niederkorn, J.Y.; Ubelaker, J.E. and McCulley, J.P. (1992): Susceptibility of corneas from various animal species to in vitro binding and invasion by *Acanthamoeba castellanii.* Invest Ophthalmol Vis Sci 33:104–112.

Niyyati, M.; Rahimi, F.; Lasejerdi, Z. and Rezaeian, M. (2014): Potentially pathogenic free-living amoebae in contact lenses of the asymptomatic contact lens wearers. Iran. J. Parasitol. 9, 14–19.

Ozcelik, S.; Coskun, K.A.; Yunlu, O.; Alim, A. and Malatyali, E. (2012): The prevalence, isolation and morphotyping of potentially pathogenic free-living amoebae from tap water and environmental water sources in Sivas. Turkiye Parazitol Derg. 36 (4):198–203. https://doi.org/10.5152/tpd.2012.48 PMID: 23339938.

Page, F.C. (1988): A new key to freshwater and soil *Gymnamoebae*. Freshwater Biological Association, Ambleside, Cumbria; P. 92–97.

Pearce, J.R.; Powell, H.S.; Chandler, F.W. and Visvesvara, G.S. (1985): Amebic meningoencephalitis caused by *Acanthamoeba castellani* in a dog. J Am Vet Assoc 187: 951–952.

Pélandakis, M. and Pernin, P. (2002): Use of Multiplex PCR and PCR Restriction Enzyme Analysis for Detection and Exploration of the Variability in the Free-Living Amoeba *Naegleria* in the Environment. Applied and environmental microbiology. 68(4):2061–2065.

Pussard, M. and Pons, R. (1977): Morphologie de la paroi kystique et taxonomie du genre *Acanthamoeba* (Protozoa, Amoebida) Protistologica. 13: 557–598.

Putaporntip, C.; Kuamsab, N. and Nuprasert, W. et al (2021): Analysis of *Acanthamoeba* genotypes from public freshwater sources in Thailand reveals a new genotype, T23 *Acanthamoeba* bangkokensis sp. nov. Sci Rep 11, 17290. https://doi.org/10.1038/s41598-021-96690-0

Rezeaian, M.; Farnia, S. and Niyyati, M. et al (2012): Amoebic keratitis in Iran (1997-2007). Iran J Parasitol. 2(3):1-6.

Rivera, W.L.; Adao, D.E.V. (2009): 18S ribosomal DNA genotypes of *Acanthamoeba* species isolated from contact lens cases in the Philippines. Parasitol. Res. 105, 1119–1124.

Schroeder, J.M.; Booton, G.C.; Hay, J.; Niszl, I.A.; Seal, D.V.; Markus, M.B.; Fuerst, P.A. and Byers, T.J. (2001): Use of subgenic 18S ribosomal DNA PCR and sequencing for genus and genotype identification of *Acanthamoebae* from humans with keratitis and from sewage sludge. J Clin Microbiol. 39 (5):1903–1911.

Schuster, F.L. (2002): Cultivation of pathogenic and opportunistic free-

living amebas. Clin Microbiol Rev. 15(3): 342-54. doi:10.1128/ CMR.15.3.342-354.2002.PMID: 120 97243; PMCID: PMC118083.

Simpson, C.P.; Willaert, E.; Neal, F.C.; Stevens, A.R. and Young. M.D. (1982): Experimental *Naegleria fowleri* meningoencephalitis in sheep: Light and electron microscopic studies. Am J Vet Res 43:154–157.

Statista, (2022): Value of the lunchbox market worldwide from 2017 to 2024.

Stothard, D.R.; Diedrich, S.J.M.; Awwad. M.H.; Gast, R.J.; Ledee, D.R.; Zaragoza, R.S.; Dean, C.L.; Fuerst, P.A. and Byers, T.J. (1998): The Evolutionary History of the Genus *Acanthamoeba* and the Identification of Eight New 18S rRNA Gene Sequence Types. J. Eukaryot. Microbiol. 45,45–54.

Susanto, I.K.; Wahdini, S. and Sari, I.P. (2020): Potential transmission of *acanthamoeba* spp. From contact lens solution and tap water in jakarta, indonesia. Open Access Maced. J. Med. Sci. 8, 333–337. https://doi.org/10.3889/oamjms.2020.4551

Tamura, K.; Stecher, G.; Peterson, D.; Filipski, A. and Kumar, S. (2013): MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol. 30,2725–2729.

Tanisha, I.; Reddy, S.C. and Phuah, S.J. (2008): Knowledge and practice of contact lens wear and care among Medical Students of University of Malaya. Med J Malaysia. 63:1121-1124.

Thompson, J.D.; Higgins, D.G. and Gibson, T.J. (1994): CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research. 22(22):4673-4680.

Toula, F.H. and Sayed Elahl, S.A. (2017): Isolation and identification of free living amoebae from water sources with respect to *Acanthamoeba*, *Naegleria* in Jeddah city, Saudi Arabia. Int. J. Pharm. Res. *Allied Sci*. 6, 1–8.

Van der Lugt, J.J. and Van der Merwe, H.E. (1990): Amoebic meningoencephalitis in a sheep. J S Afr Vet Assoc 61:33–36.

Visvesvara, G.S.; Moura, H. and Schuster, F.L. (2007): Pathogenic and opportunistic freeliving amoebae: *Acanthamoeba* spp., *Balamuthia mandrillaris*, *Naegleria fowleri*, and *Sappinia diploidea*. FEMS Immunol Med Microbiol. 50:1-26.

Visvesvara, G.S.; Schuster, F.L. and Martínez, A.J. (1993): Balamuthia mandrillaris, N. G., N. Sp., agent of amebic meningoencephalitis in humans and other animals. J Eukaryot Microbiol 40:504–514.

Walochnik, J.; Obwaller, A. and Aspöck, H. (2000): Correlations between morphological, molecular biological, and physiological characteristics in clinical and nonclinical isolates of *Acanthamoeba* spp. Appl. Environ. Microbiol. 66, 4408–4413. https://doi.org/10.1128/AEM.66.10.4408-4413.2000

Xuan, Y.; Shen, Y.; Ge, Y.; Yan, G. and Zheng, S. (2017): Isolation and identification of *Acanthamoeba* strains from soil and tap water in Yanji, China. Environ. Health Prev. Med. 22, 1–6.

Zhang, Y.; Xu, X.; Wei, Z.; Cao, K.; Zhang, Z. and Liang, Q. (2023): The global epidemiology and clinical diagnosis of Acanthamoeba keratitis. J. Infect. Pub. Health. 16(6), 841-852. https://doi.org/ 10.1016/j.jiph.2023.03.020

كشف وتحديد الالتصاق الجرثومي للعدسات اللاصقة كنموذج لأنواع التهاب القرنية الجرثومي في المملكة العربية السعودية

هيام محمد محمود فراج[٢،١] ، سامية سعد الخليل[١] ، محمد عقيلي حكمي [١] ، منى جابر [٢] ، مروة كمال درويش [٣،١] ، أميرة الطيب مصطفى بدوي[١]، الزهراء عبد الرؤف أحمد [٢]

[١] قسم المختبرات الطبية بكلية العلوم الطبية التطبيقية بجامعة شقراء، المملكة العربية السعودية.

[٢] قسم الطفيليات الطبية بكلية الطب جامعة أسيوط، جمهورية مصر العربية.

[٣] قسم الكيمياء كلية العلوم، جامعة قناة السويس، جمهورية مصر العربية.

Email: hayammahmoud@su.edu.sa Assiut University web-site: www.aun.edu.eg

Acanthamoeba spp. هي أميبا حية حرة مسببة للأمراض بشكل متزايد، وهي معترف بها كسبب مهم لالتهاب القرنية لدى كلا من البشر والحيوانات، وعادة ما تنتقل من خلال مصادر المياه الملوثة أو محاليل العدسات اللاصقة. كان الهدف من الدراسة هو التحقيق في معدل تلوث العدسات اللاصقة ومحاليل تنظيفها بالأميبا الحية الحرة، مع التركيز بشكل خاص على الأكانثاميبا كتهديد صحي محتمل لمرتدي العدسات اللاصقة في المملكة العربية السعودية.

تم التبرع بإجمالي ١٠٥ عينة من العدسات اللاصقة المستخدمة سابقًا ومحاليلها الحافظة من قبل طالبات في جامعة شقراء، المملكة العربية السعودية. تم عزل الأميبا من خلال الزراعة المعملية وتحديدها مورفولوجياً باستخدام مفاتيح قياسية. كما تم إجراء تحديدات جزيئية بناءً على اختبار تفاعل البوليميراز المتسلسل الخاص بالجنس لجميع المستعمرات المعملية الإيجابية. بالإضافة إلى ذلك، تم إجراء تحديد النمط الجيني والتحليل التطوري لعزلات الأكانثاميبا. من بين جميع العينات، كانت ٥٦,١٩٪ مصابة بالأكانثاميبا *Vahlkampfiidae*. وتم الكشف عن Acanthamoeba spp. في ٧٦,٣٪ من المستعمرات المعملية الإيجابية (ن = ٤٥)؛ بينما تسببت *Vahlkampfiidae* في تلويث ٢٧ عينة من المستعمرات المعملية إما بعدوى واحدة أو مختلطة؛ بما في ذلك Naegleria sp. كشف التعريف المورفولوجي عن خمسة أنواع من Acanthamoeba، وهي Acanthamoeba كاثتاليني وترانجلارز ,وبوليفاجا و التي تنتمي للمجموعة الثانية, و Acanthamoeba استرونيكسس والتي تنتمي للمجموعة الأولى وAcanthamoeba لينتيكيولاتا (المجموعة الثالثة). كما كشف تحليل تسلسل جين ١٨S rRNA عن سلالتين: Acanthamoeba كاثتاليني ذات النمط الجيني (T4), و Acanthamoeba بوليفاجا ذات النمط الجيني (T2). يسلط هذا البحث الضوء على أول تحديد لتلوث الأميبا الحرة في العدسات اللاصقة ومحاليل التنظيف في المملكة العربية السعودية. هناك حاجة إلى بذل الجهود لمنع تلوث Acanthamoeba، وينبغي إجراء المزيد من الدراسات للتحقيق في التلوث البيئي المحتمل بـ FLA المسببة للأمراض في جميع المحافظات السعودية.