# Chemical and Microbial Analysis of Water Resources in United Arab Emirates

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# Abstract

*Acanthamoeba* (AC) is a microscopic, free-living amoeba found worldwide in the environment. It can infect the eyes through contact lens use, cuts, skin wounds, or inhaling into the lungs. Three diseases caused by (AC) are *Acanthamoeba* keratitis: which is an infection of the eye cornea, Granulomatous Amoebic Encephalitis (GAE): which is a severe infection of the brain and spinal cord; and Disseminated infection: which is an infection of the skin, sinuses, lungs, and other organs independently or in combination. When exposed to unfavourable conditions, *Acanthamoeba* develops into a dormant cyst that can resist temperature extremes (-20 to 56°C). In their trophozoite form, *Acanthamoeba* can survive and multiply at a pH range from 4 to 12. With these unique characteristics, AC can provide the bacteria with a safe haven from environmental harsh conditions in addition to becoming a renewable source of bacterial contamination. This study is the first to investigate the existence of AC in the United Arab Emirates (UAE). Therefore, this study aims at isolating AC from UAE water samples, identifying AC species by molecular methods, and investigating the mode of association with bacterial communities. Fifty-seven samples from 4 emirates were filtered through nitrocellulose membrane, which was placed afterwards on non-nutrient agar (NNA) supplemented with heat-killed *Escherichia coli* (*E.coli*), incubated for two weeks, and examined under a converted microscope. Most of the plates were found to contain *Acanthamoeba* trophozoite and or cysts. *Acanthamoeba* was grown axenically in Peptone Yeast Glucose (PYG) medium and identified by 18s rDNA PCR as *Acanthamoeba* spp. Testing samples for microbial contents found that Emirate-3 (130 species) was the highest in bacterial

species contents, followed by Emirate -4 (121 species), and finally, Emirate-1(91 species) had the least in bacteria species numbers. It is well known that AC feeds on some bacterial species and hides others from harsh environments. To investigate the interaction activity of AC with the bacterial spp isolated from UAE water samples, the toxicity of a group of bacteria, isolated from different water sources on Human KB Epithelial Cells was assessed. Survivability studies of *Acanthamoeba* cysts were assessed by various levels of chlorination exposure. Results indicated that chlorination at a concentration of 5ppm and exposure for 30 minutes achieved about 72% efficiency of AC disinfection; however, as chlorination levels increase, the disinfectant effect becomes more obvious. At 6 ppm, 91.7% of AC were killed, while at concentrations above 12 ppm, all AC were dead. A quick methodology for identifying AC directly from nitrocellulose membrane was developed, which would consume a short time (two hours) to isolate AC without having to wait for weeks to grow AC on nutrient cultures.

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# Abbreviations

| | |
|---|---|
| (µ, m) M | (micro, milli) Molar |
| (µ, m, k) g | (micro, milli, kilo) gram |
| °C | Celsius |
| 18s rRNA | 18S ribosomal Ribonucleic acid |
| AC | *Acanthamoeba* |
| AK | *Acanthamoeba keratitis* |
| APS | Ammonium persulfate |
| ARB | *Amoeba*-Resisting Bacteria |
| ATCC | American Type Culture Collection |
| CFU | Colony forming units |
| CNS | Central nervous system |
| CSF | Cerebrospinal fluid |
| dNTP | Deoxynucleotides |
| DO | Dissolved oxygen |
| EtOH | Ethanol |
| FBS | Fetal bovine serum |
| FEWA | Federal electricity & water authority |
| FLA | Free living *amoebae* |
| GAE | Granulomatous amebic encephalitis |
| GMS | Gomori methamine silver |

| | |
|---|---|
| GP & GN | Gram-negative & Gram-positive |
| HCl | Hydrochloric acid |
| PCA | Plate count agar |
| IgG | Immunoglobulin G is a type of antibody |
| LB | Luria-Bertani |
| MDL | Method detection limit |
| MP | Maximum parsimony |
| MRSA | Methicillin-resistant *Staphylococcus aureus* |
| NaCl | Sodium chloride |
| NNA | Non-nutrient agar |
| PBS | Phosphate-buffered saline |
| PCR | Polymerase Chain Reaction |
| PYG medium | Peptone Yeast Glucose (Dextrose) medium |
| QCC | Abu Dhabi quality and conformity council |
| RE | Restriction enzymes |
| SDS | Sodium dodecyl sulphate |
| UAE | United Arab Emirates |
| UTIs | Urinary tract infections |
| WHO | World Health Organization |

# Chapter One

# Literature Review

## 1.1 Introduction

When you fill a glass with tap water, go swimming in the ocean or cook freshly caught fish from the local lake, most of us take it for granted that these are all safe practices. The reality is behind the scenes; scientists work diligently to ensure that the quality of the world's most valuable resource is as high as possible. From lakes and streams to coastal waters and estuaries, water quality monitoring is a critical practice in countries across the globe. Water quality and water monitoring are essential tools in managing water resources; they provide important information describing the physical, chemical and or biological status of water resources, determining trends and changes over time, and identifying emerging water quality issues. They also provide the means to identify policies and measures to enhance water quality and consumable water, reduce and control water pollution from specific sources, evaluate the efficacy of pollution control and regulation policies and their implementation and deal with water quality emergencies. Water quality and water monitoring are poorly implemented in many countries, especially developing countries, due to a lack of appropriate instruments, financial resources, and technical capacities(Kirschke et al., 2020). They are mainly based on ineffective traditional approaches and are jeopardised by a lack of scientific knowledge and technical skills. As a result, water quality data are scarce, unreliable and unsystematic. Water quality monitoring is also becoming a complex issue due to new water quality challenges.

The World Water Assessment Programme of the United Nations reports that every day a stunning two million tons of human waste is disposed into water resource(Unitednations, 2003).

Keeping tab water at the best quality is critical at its core and serves many vital objectives. Regular monitoring of water quality is a crucial part of identifying any existing problems or issues that may emerge in the future. For example, data has been used to reveal that over the past few years, increases in fertilisers used for food production had increased global nitrogen pollution in rivers by up to 20% (Irlinger, 2008).

## 1.2 Water in the United Arab Emirates (UAE)

The United Arab Emirates (UAE) is located in the Middle East. It is located in the South-Eastern region of the Asian continent. It overlooks the Arabian Gulf on the

North and Northwest, the Eastern part of the Arabian Peninsula, and on the West and South borders with the Kingdom of Saudi Arabia, figure 1.1

The United Arab Emirates is a constitutional federation of seven Emirates: Abu Dhabi, Dubai, Ajman, Fujairah, Ras al Khaimah, Sharjah and Umm al Quwain. The UAE area is approximately 83,600 sq km of land, plus some islands in the Arab Gulf and 27,624.9 sq.km of regional water. Abu Dhabi Emirate accounts for 84% of the country's total landmass. In 2020, the total population of the UAE was 9,282,410.

The management of water resources is a topic of growing importance worldwide. As UAE grows and thrives, the water demand continues to rise. Natural geographical and geological factors have always made water readily available in some regions and rare in others. The United Arab Emirates is in a desert climate where freshwater resources are naturally limited. United Arab Emirates water resources can be classified into conventional and non-conventional water resources:

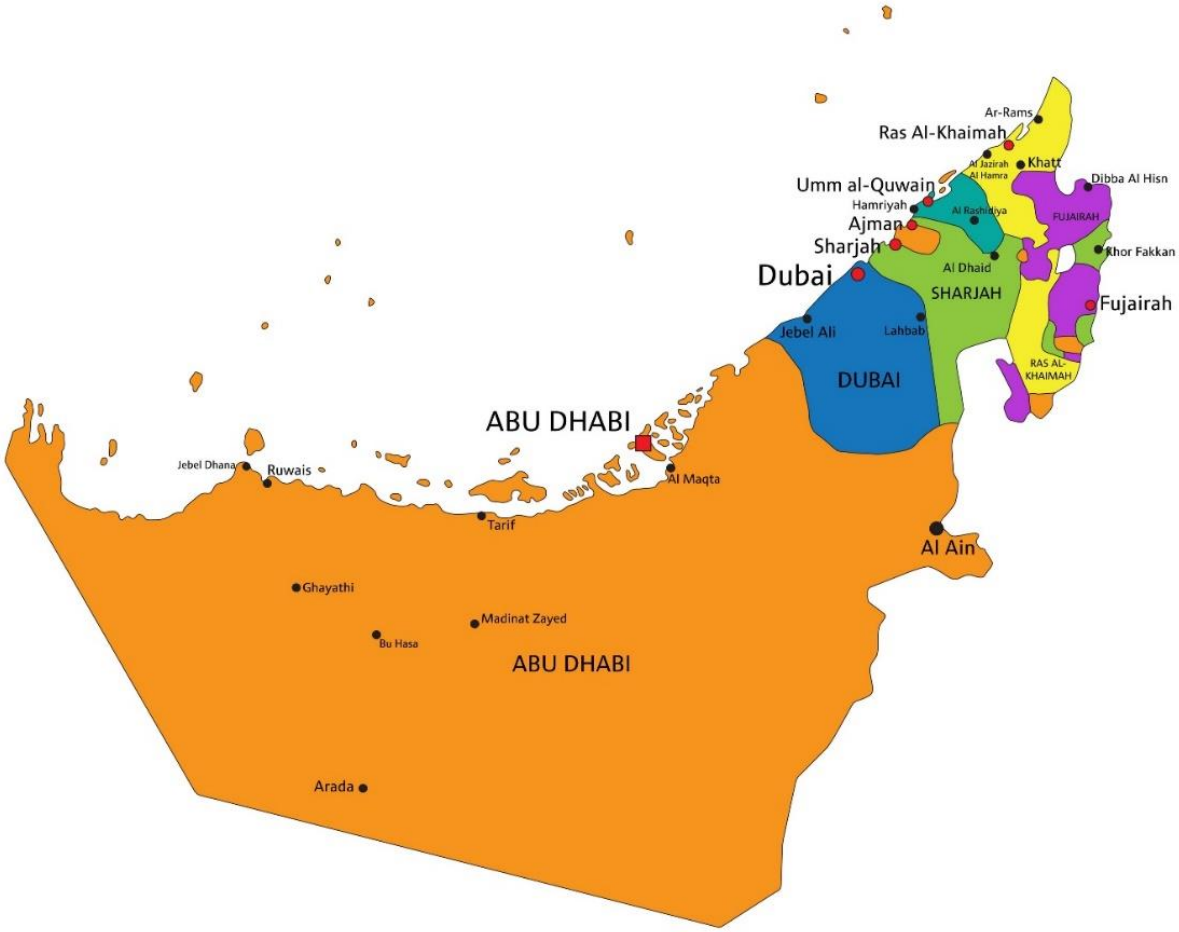



Figure 1.1 UAE map: outlining the United Arab Emirates in the Arabian Peninsula Location and presents the location of the Seven Emirates (Inc, 2022)

### 1.2.1 Natural and Conventional Water Resources in UAE

#### 1.2.1.1 Surface Water resources

Fresh surface water is very rare in UAE nature due to the low rainfall and high evaporation rates. The typical rainfall in the UAE is about 35 millimetres (1.4 in). It consists of seasonal floods, springs and falajes. (Falajes are human-made channels or streams that intercept the groundwater and bring it to the surface) (Irlinger, 2008).

Surface water is considered a source for feeding the aquifers in the country. About 114 dams of different sizes were built in the UAE to increase groundwater recharge and protect against damage caused by flash floods.

#### 1.2.1.2 Ground Water resources

Groundwater is an essential source of water supply in the UAE. Groundwater resources can be classified into renewable (occur primarily in shallow grainy aquifers) and Non-renewable water resources (encountered in deep aquifers). The water availability in shallow aquifers is small because it mainly depends on the rainfall, which differs from one year to another.

### 1.2.2 Non-Conventional Water Resources in UAE

#### 1.2.2.1 Desalination of seawater and salty groundwater

These resources are costly and used for drinking and irrigation purposes. In 1998 the Federal Electricity & Water Authority (FEWA) introduced multi-effect desalinated water (MED). (Abu-Hilal et al., 1994, Aburizaiza et al., 2013).The multiple-effect distillation is the low-temperature thermal process of finding freshwater by recovering the vapour of

boiling seawater in a sequence of vessels (called effects), each maintained at a lower temperature than the last. Treated wastewater is used for irrigation activities; around half of the annual water production from all sources is used for irrigation of green spaces, as well as in agriculture. The other half is used for domestic usage, such as washing streets and buildings. Each UAE resident uses an average of 550 litres of water per day against the international average of 170-300 litres per day (Alsalmi et al., 2013). In the UAE, better conservation practices can reduce water consumption per person by about 14 to 171 litres of water per day. The FEWA has adopted different strategies to minimise consumption, such as minimising the water usage for agricultural practices, controlling the leakage of the water distribution system, reducing personal consumption and raising awareness.

Moreover, it is essential to keep assessing recharge enhancement options such as recharge dams and salty water for agriculture and other activities. Periodic maintenance for falajes and springs in the country is another necessary action to conserve water resources; (Abu-Hilal et al., 1994, Banat et al., 1993, Hassan et al., 1995, Murad et al., 2007, Sommariva and Syambabu, 2001). Local UAE cities government have mandated periodical maintenance for all-natural water resources.

### 1.2.3 Drinking-Water

Abu Dhabi has nine desalination companies with gross production of about 280,000 MIG (Million Imperial Gallon) (1,245.42 million $m^3$). This represents an average of 750.58 MIGD (Per Day), equivalent to 3.41 million $m^3$/day, with the peak production reaching 821 MIGD (3.73 million $m^3$/day).

Around 1.30% of the annual gross production is consumed internally by the production plants (i.e. auxiliary consumption). About 267,406 MIG (1,215.63 million $m^3$) is dispatched into the distribution system. Thermal desalination accounted for 92.4% of the drinking water production in Abu Dhabi.

### 1.2.4 Water Quality

In 2014, the number of water samples tested for quality in the UAE were 204,462. These tests were performed by UAE sectors companies representing the annual average of water test required by authorities. Seventy-seven different quality determinants were examined in each test in all Emirates. These tests include -ve and +ve ions, minerals, total dissolved solids (TDS), accounting for the majority of test failures of the Water Quality Regulations (WQR). Microbial counts and Langelier Saturation Index (LSI, which is an approximate indicator of the degree of saturation of calcium carbonate in water) At the same time, residual fluctuation of chlorine and bromate levels were recorded and considered failures. Mitigation measures on bromate noncompliance levels were initiated, and significant improvements were made, which have reduced the average bromate limits in most of the areas in the Abu Dhabi region, meeting the regulation limits. Results of pilot studies conducted in 2014 have also demonstrated that bromate limits are considerably improved in the Al Ain region (Abu Dhabi). Overall compliance with the sampling frequency measure (SFM) was 99%, while Prescribed Concentration or Value (PCV) compliance increased slightly to 94% (Adqcc, 2014).

The maximum or minimum concentration or value specified in relation to that parameter are shown in the Tables below. Providing safe drinking water in distribution systems is a

top priority goal of health agencies in the UAE. The UAE standard mandates that the drinking water must be chemically safe and pathogen-free (Table 1.1 and 1.2).

Table 1. 1: Water quality standards for UAE: Physical Properties as set by the Department of Energy of Abu Dhabi (UAE) (DOE, 2021) .

| Item | Parameter | Unit of Measurement | Concentration or value (maximum unless otherwise stated) |
|---|---|---|---|
| 1 | Colour | mg/l pt/Co scale | 15.0 |
| 2 | turbidity | NTU (Nephelometric Turbidity Unit) | 4.0 |
| 3 | cdour (including hydrogen sulphide) | Dilution number | Unobjectionable |
| 4 | taste | Dilution number | Unobjectionable |
| 5 | total dissolved solids (TDS) | mg/l | 100 (minimum)<br>600 (maximum) |
| 6 | calcium hardness | mg/l as $CaCO_3$ | 200 at 25 ºC |
| 7 | total hardness | mg/l as $CaCO_3$ | 300 at 25 ºC |
| 8 | langelier saturation index | The value shall be slightly positive at all times | 0.0 (minimum)<br>0.5 (maximum) |
| 9 | hydrogen ion | pH value | 7.0 (minimum)<br>9.2 (maximum) |
| 10 | residual chlorine | mg/l $Cl_2$ | 0.2 (minimum)<br>0.5 (maximum) |
| 11 | chlorine Dioxide | mg/l $Cl_2$ | 0.1 (minimum)<br>0.4 (maximum) |

Table 1. 2:Water quality standards for UAE: Microbiological Properties as set by the Department of Energy of Abu Dhabi (UAE) (DOE, 2021) .

| Item | Parameter | Unit of measurement | Concentration or value (maximum unless otherwise stated) |
|---|---|---|---|
| 1 | total coliforms | number/100ml | 0 |
| 2 | *E. coli* or thermotolerent Faecal *coliform* bacteria | number/100ml | 0 |
| 3 | *Intestinal Enterococci* | number/100 ml | 0 |
| 4 | *Legionella* | CFU/l | <1.0 |
| | *Legionella* | GU/l | Negative |

Several standards have been established to provide high water quality, including sterilisation techniques to control and prevent microbial colonisation(Ashbolt, 2015). Microbial communities usually found in drinking water are made of both Eukaryotes & Prokaryotes and may include viruses, bacteria, parasitic protozoa, and fungi (Marciano-Cabral and Cabral, 2003). In the UAE, water testing does not include testing against *Acanthamoeba*; however, the UAE has a very strict standard for testing for bacteria in the water. In the UAE, no studies or outbreaks of *Acanthamoeba* have been recorded associated with water quality. Therefore, this study was undertaken to investigate UAE water through examining several samples of various types of water ranging from bottled water to beach water at different locations among UAE cities.

## 1.3 Water contaminants

Various types of bacteria/viruses are categorised as pathogens that are disease-causing microorganisms found in pretreated and/or inadequately treated water. The US Environmental Protection Agency (US-EPA) has assessed the presence of bacteria/viruses in drinking water and their health hazards. Their conclusions are as follow:

- Legionella is a bacterium found naturally in the environment — typically in water. It causes a health risk if *aerosolised* (e.g., in air conditioning systems or showerheads), resulting in a kind of pneumonia identified as *Legionnaires* illness. *Entero viruses, such as polio viruses, echo viruses and coxsackie viruses*, exist in the intestines of infected humans or animals, in addition to the three different polioviruses are 62-nonpolio *entero viruses* that can cause sickness in humans ranging from gastroenteritis to meningitis.According to US-EPA, bacteria and viruses can also be listed as “indicators" of water hygiene which, at a level outside of recognised limits, may reflect "a problem in the treatment process or the integrity of distribution system. The US-EPA has regulated water pollution indicators and their possible difficulties. Below are some of their related findings:

- Turbidity refers to the cloudiness of water and can hinder disinfection, providing an environment for microbial growth and can show the existence of bacteria and/or viruses as well as other disease-causing organisms such as *Escherichia coli* (*E.coli*) that can produce signs such as diarrhoea, cramps, nausea and headaches.

- Coliforms are bacteria naturally existing in the environment and used as indicators.
- Faecal contamination indicators, notably *Enterococcal* bacteria or coliphage, the viruses that infect *E. coli*, can create a short-term health effect, including camps, nausea, diarrhoea, or may pose a more significant risk for people with severely weakened immune systems such as the elderly, young children and infants.
- *Escherichia coli* and faecal coliform are indicators of water contaminated by human or animal wastes, causing short-term health issues or more serious illnesses, especially with elderly and young children.
- *Acanthamoeba* is a free-living amoeba widely distributed in the environment. In recent years, the incidence of infections due to *Acanthamoeba* spp. has shown a remarkable increase. It is the causative agent of a sight-threatening infection of the cornea known as *Acanthamoeba keratitis* (AK) and fatal disease of the central nervous system known as Granulomatous Amebic Encephalitis (GAE), mainly in immunocompromised patients.

## 1.4 *Acanthamoeba*

*Acanthamoeba* is considered a severe pathogen and potential water contaminant. It exists in two forms; trophozoites and cysts. For trophozoites the infectious stage diameter is about 12–35 μm with some variations depending on several factors, including specific genotype, surrounding environment, and nutrient availability. However, cyst size is approximately (13-23 μm). Under favourable physiological conditions, including abundant food supply, neutral pH, appropriate temperature (i.e. 30°C) and osmolarity between 50–80 osmole, cysts transform back into infectious trophozoites. *Acanthamoeba* spp. are usually classified based on the small nuclear subunit 18S ribosomal RNA whole gene

sequence, which permits the differentiation of *Acanthamoeba* spp. into 20 genotypes (T1-T20) (Corsaro et al., 2017b, Fuerst et al., 2015, Gast et al., 1996).

The genus *Acanthamoeba* contains around 20 genotypes (T1 – T20), some of them pathogenic such as *A. castellanii, A. culbertsoni, A. polyphaga, A. astronyxis*(Corsaro et al., 2017a),

*A*. *hatchetti, A. divionensis, A. rhysodes, A. lugdunensis*, and *A. lenticulata,* while others are not (Booton et al., 2005). Apart from the pathogenic risks, *Acanthamoeba* plays an essential role in the ecosystems. Environmental microbiology shows that *Acanthamoeba,* as a dominant microorganism, has two functions in nature; ecological constituent and pathogenic pollutant, making *Acanthamoeba* a suitable candidate for clinical microbiology research, public health and other related disciplines(Yang and Ji, 2014). The ability of *Acanthamoeba* to encyst itself enables it to survive severe environmental conditions such as extreme temperatures, pH and shortage of nutrients. These cysts can survive for long periods and under harsh conditions, including water where a chlorine concentration of 4 ppm or 4 mg/l is used as a disinfectant (Siddiqui et al., 2021).

### 1.4.1 Global Overview - Source and occurrence of *Acanthamoeba*

According to the World Health Organization (WHO), about 780 million individuals depend on water from risky sources lakes, rivers, and shallow wells with human or animal waste, chemicals, and other things that contaminate their water (Gadgil, 1998). Unclean hands or dirty containers have been shown to contaminate water during storage and transport. Drinking unsafe water can lead to several diseases and death.

According to the WHO, more than 2000 children under five years old die daily due to diarrhoea caused by contaminated water(Gadgil, 1998, WHO 2020). Nowadays this figure is around 820; Yet diarrhoea is largely preventable, and the deaths of 297 000 children aged under 5 years could be avoided each year (WHO 2022).

The worldwide distribution of *Acanthamoeba* in the natural environment makes soil, airborne dust, and water a potential source of illnesses. Depending on the species, *Acanthamoeba* can grow over a wide temperature range in water, with the optimum temperature for pathogenic species being 30°C. Trophozoites can exist and replicate in the water while feeding on bacteria, yeasts, and other organisms.

Giant viruses of amoebae such as Provirophages and transpovirons were listed among the *Acanthamoeba* resisting microorganisms. Their sympatric lifestyle within amoebae is suspected of promoting lateral nucleotide sequence transfers (Chelkha et al., 2018). Some *Acanthamoeba* species have shown differences in their susceptibility to giant viruses. Till recently, only the genome of a single *Acanthamoeba* castellanii Neff existed. (Chelkha et al., 2018)

### 1.4.2 Isolation and Identification

Laboratory identification of *Acanthamoeba* is based on different methods such as direct examination, culture, and molecular techniques. Immediate analysis and DNA-based assays can provide rapid detection of *Acanthamoeba*.

Molecular biology techniques such as Polymerase Chain Reaction (PCR), DNA sequencing, etc. have enhanced the identification of *Acanthamoeba* species. Therefore, sequence analysis of 18S ribosomal DNA (rDNA) enabled distinguishing 20 genotypes,

named T1 to T20, T4 genotype being the most common and linked to human infections. This method used 18S rDNA gene sequences larger than 2,000 base pairs (bp); however, *Acanthamoeba* isolates shorter than 500 bp have been isolated using 18S rDNA technique. (Chelkha et al., 2020)

Due to its low cost and simplicity, the culture method has been the main procedure used for *Acanthamoeba* detection. Published literature reveals that the culture method includes a long incubation time exceeding several weeks. Additionally, the culture of *Acanthamoeba* is genus-specific(Chelkha et al., 2020) . Since rapid diagnosis is crucial in treating cases caused by pathogenic *Acanthamoeba*, culture can be an obstacle for a quick diagnosis and recovery. A genetic characterisation is a valuable tool for identifying *Acanthamoeba*; it is quick, reliable, and species-specific (Chelkha et al., 2020) .

Previous studies showed that Polymerase Chain Reaction (PCR)-based methods using primers targeting different parts of 18S rDNA genes are useful for rapid detection of *Acanthamoeba.*

Identification at a genus level can be determined through the structural properties and movement of *Acanthamoeba*. *Acanthamoeba* moves fast with a locomotory rate of about 0.8 µm/second. The movement includes the development of a hyaline pseudopodium. The manner of *Acanthamoeba* movement is similar both at solid substrata and water-air interface. These features are inherited across the genus and can be used for identification(Preston et al., 2001). Some of the distinctive characteristic features of *Acanthamoeba* include the presence of spine-like acanthopodia. These spines are known as Acanthopodia. They also feature a distinguishing contractile vacuole in their cytoplasm. The other specific characteristics are the vesicular nucleus and big central

nucleolus. Amplification reactions and phylogenetic analysis are standard methods of *Acanthamoeba* identification. Remarkably, analysis of gene sequence and DNA sampling relies heavily on the species' unique characteristics. Health providers widely use *Acanthamoeba* culturing techniques to identify *Acanthamoeba* in water samples(Vijayakumar, 2018).

The abundance and wide spreading of *Acanthamoeba* in the environment make it relatively easy to identify. The isolation process might be a little more complex or inefficient in the whole process. Various methods are commonly used to identify the *Acanthamoeba* isolates(Diplock et al., 2009) ,such as the sequencing of DNA has enabled further classification of the species asT1, T2, T3 and T4. The PCR method is a definite way of identifying *Acanthamoeba*. In fact, this method is commonly used to diagnose *Acanthamoeba* keratitis (AK). The PCR technology works by amplifying copies of a specimen in several magnitudes, followed by sequencing and identifying the cell.

### 1.4.3 *Acanthamoeba* Worldwide Case Studies

*Acanthamoeba* is distributed worldwide. Reported cases of *Acanthamoeba* have been documented by several countries, particularly: Taiwan, Iran, Turkey, Jamaica, and the South of France. In table 1.3, it is clear that the primary method used to identify *Acanthamoeba* is culturing, DNA extraction of DNA, PCR amplification of DNA, and finally, Sequencing & Identification. The most prevalent genotype is T4.

Table 1. 3*:* Summary of some published global case studies documenting *Acanthamoeba* existence worldwide and can pose a serious health issue by providing safe shelter for pathogenic bacteria (References indicated in the table)

| Case Study | Location | Number of samples | Testing & analysis method | Results | Reference |
|---|---|---|---|---|---|
| 1 | Taiwan | 200 | Culturing, DNA extraction, PCR Amplification, Sequencing & Identification | 17% of samples contain T4 to T8, T11, T12, T15 Genotypes | (Kao et al., 2012) |
| 2 | Iran | 50 | Culturing, DNA extraction, PCR Amplification, Sequencing & Identification | 32% of samples T4 and T5 genotypes | (Nazar et al., 2011) |
| 3 | Turkey | 32 | Culturing, DNA extraction, PCR Amplification, Sequencing & Identification | 19.23% of samples were T4 genotype | (Kuk et al., 2013) |
| 4 | Turkey | 150 | Culturing, DNA extraction, PCR Amplification, Sequencing & Identification | 22% Thirty-three of samples were T4 genotype | (Coşkun et al., 2013) |
| 5 | Jamaica | 83 | Culturing, DNA extraction, PCR Amplification, Sequencing & Identification | 50.6% (42/83) Recreational water contains T3, T4, T5, T10 and T11, T4 genotype is the most frequent | (Todd et al., 2015) |
| 6 | South of France | 77 | Co-Culture growth of ARB with Amoeba | 244 isolates that corresponded to 89 different species of ARB | (Pagnier et al., 2008) |

| | | | | | |
|---|---|---|---|---|---|
| 7 | Thailand | 100 | Culturing, DNA extraction, PCR Amplification, Sequencing & Identification | genotype T4 found in 87 isolates | (Putaporntip et al., 2021) |
| 8 | Turkey | 148 | Culturing, DNA extraction, PCR Amplification, Sequencing & Identification | 71 (47.97%) positive for FLA. 18 (12.16%) positive for *Acanthamoeba* spp | (Aykur and Dagci, 2021) |

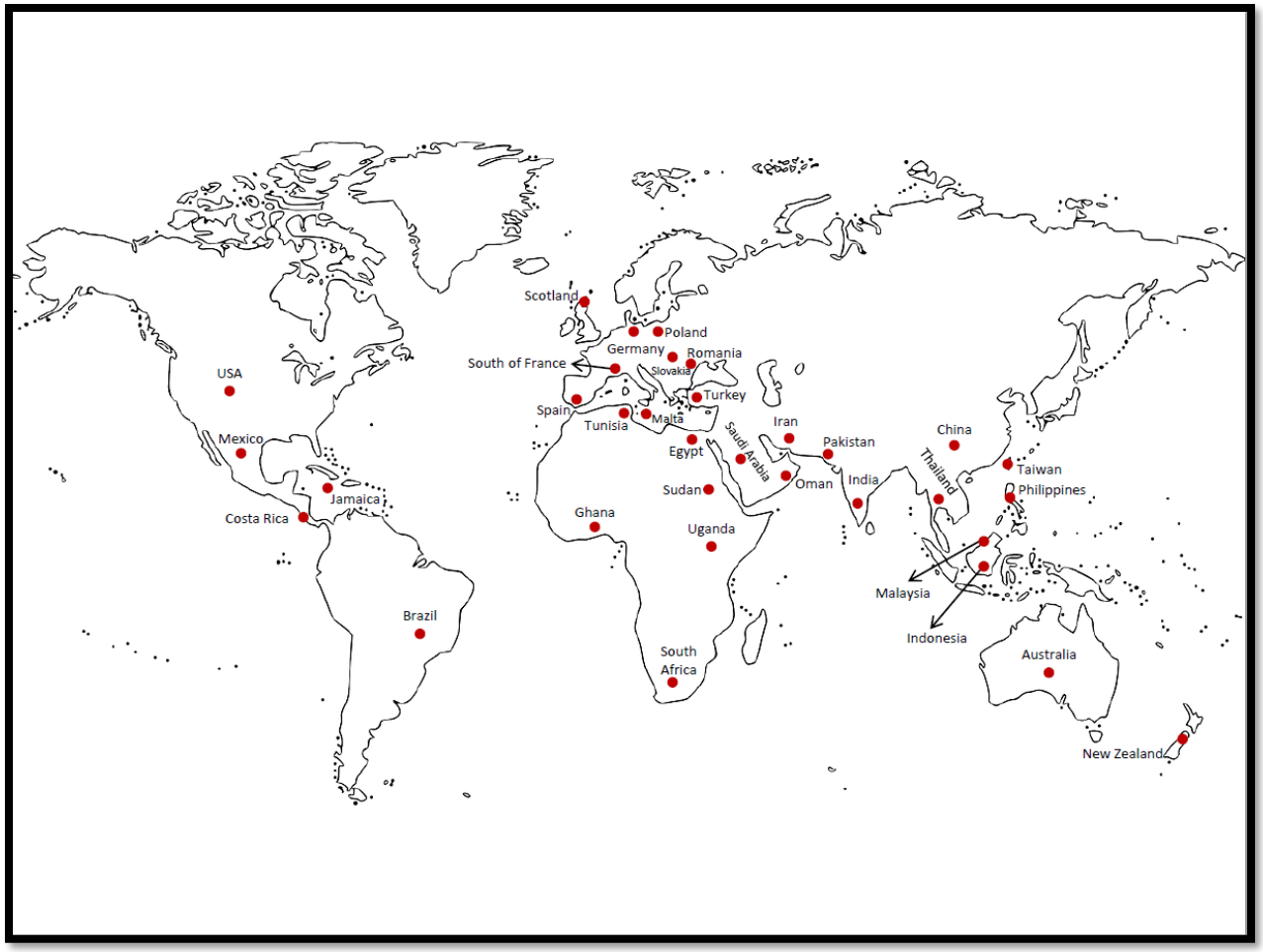
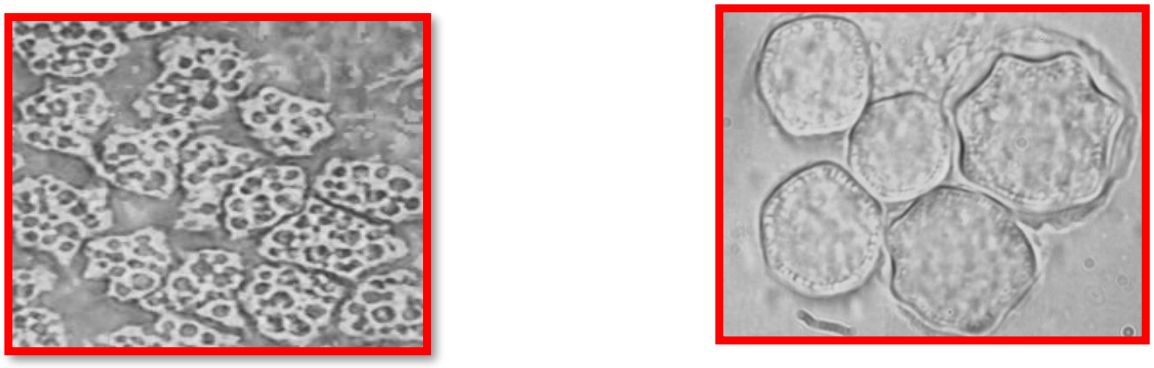



Figure 1.2: Global distribution of *Acanthamoeba* in different continent. *Acanthamoeba* can be found everywhere. Common habitats for *Acanthamoeba* include dirt, dust, freshwater sources (lakes, rivers, and hot springs), salt water (such as a marsh), and seawater. *Acanthamoeba* can also be found in humidifiers, heaters, ventilation system, and air conditioning (HVAC) systems, pools, bathtubs, drinking water systems (such as slime layers in pipelines and taps), and drinking water (Al-Malki, 2020, Bouten and Elsheikha, 2022)

### 1.4.4 Life Cycle

*Acanthamoeba* has two phases in its life cycle; Phase one is a mobile vegetative and infectious trophozoite form with characteristic acanthopodia (Figure: 1.2a). The second phase is a dormant cyst form (Figure: 1.2b). Both phases are readily visible under light microscopy. The ability of *Acanthamoeba* to encyst itself enables it to survive severe environmental conditions such as extreme temperatures, pH, shortage of nutrients and chlorination (Lakhundi et al., 2017).

The trophozoites reproduce by mitosis (nuclear membrane does not remain intact). Although both cysts and trophozoites can gain entry into the body through various means, however, trophozoites are the infective forms. Entry can happen through the eye, the nasal passages, to the lower respiratory tract. or ulcerated or broken skin as shown in Figure 1.3 (WHO 2019).



(a) (b)

Figure 1.3: Two stages of *Acanthamoeba* Life cycle, a: trophozoites stage with a diameter of 15-35 µm b: dormant cyst stage of 13-23 µm (Photos were taken from Dr Alsam Lab). magnify 400 X

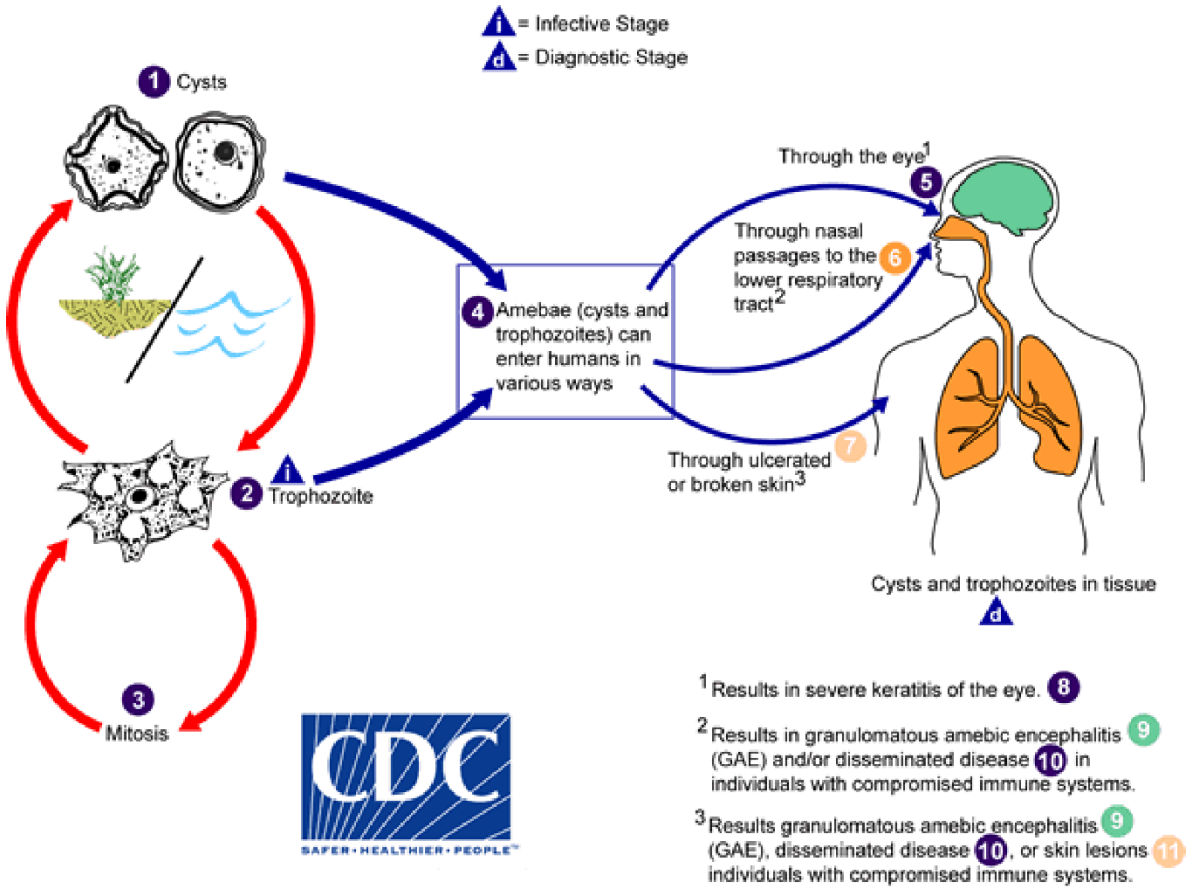



Figure 1.4: *Acanthamoeba* found in the environment can flip between trophozoite and cyst; when gaining entry to the human system, they can cause various diseases, A. keratitis, GAE, and skin lesions (WHO 2019).

When *Acanthamoeba* spp. infects the eye, it can cause severe keratitis in healthy persons, mainly contact lens users. When it enters the lungs, or through the skin, it can invade the central nervous system by haematogenous dissemination, causing GAE or skin lesions in individuals with compromised immune systems.

Tears Collected from patients with *Acanthamoeba* keratitis has shown that the presence of immunoglobulins in tears against *Acanthamoeba* did not protect the patient from infection (Alsam et al., 2008b, Gittleman, 2001)

During the trophozoites stage, *Acanthamoeba* lives on organic particles as a nutrient, as well as microbial species. It multiplies mitotically under optimum food supply conditions, neutral pH, and temperature of about 30°C. *Acanthamoeba* trophozoites have active metabolic machinery that furnishes energy in the form of Adenosine Triphosphate (ATP) by subjecting carbohydrates and lipids to undergo pathways including glycolysis and beta-oxidation of free fatty acids, respectively (Baig et al., 2021, Band and Mohrlok, 1973, Khan, 2006). Under harsh conditions such as nutrient deprivation and extremes in the environment, *Acanthamoeba* undergoes cellular differentiation resulting in a double-walled cyst (Anwar et al., 2019); instead, the outer wall consists of Polysaccharides and proteins, while the inner wall possesses cellulose (Dudley et al., 2005). Both walls are separated by a space, except at specific points. The cyst wall structure for *A. castellanii* belonging to the T4 genotype has been shown to contain 33% protein, 4 - 6% lipid, 35% carbohydrates (mainly cellulose), 8% ash, and 20% unidentified materials (Dudley et al., 2005) . With gas chromatography combined with mass spectrometry, the carbohydrate composition of cyst walls revealed a high percentage of galactose and glucose and small

amounts of mannose and xylose. Linkage analysis revealed several types of glycoside linkages, including the 1, 4-linked glycosylic conformation indicative of cellulose.

### 1.4.5 Role in the Ecosystem

In soil, *Acanthamoeba* has two ecological roles: (i) affecting the structure of the microbial community and (ii) increasing nutrient recycling. These actions are associated with soil *Acanthamoeba* feeding on bacteria, therefore, regulating bacterial populations in soil. In the environment, free-living amoebae are the dominant bacterial consumers and are responsible for up to 60% of the total reduction in the bacterial population (Kosoy, 2013, Sinclair et al., 1981). The bacteria are primary decomposers; it directly decomposes organic materials but is ineffective in liberating minerals from their mass. The secondary decomposers, such as *Acanthamoeba*, consume the primary decomposers and discharge mineral nutrients as waste products that are tied up in the primary decomposers biomass. In this way, *Acanthamoeba* makes nutrients available that would otherwise remain inaccessible for much longer. *Acanthamoeba* and bacteria in the soil showed significantly higher containment of mineralisation carbon, nitrogen, and phosphorous compared with the soil containing bacteria but without *Acanthamoeba* (Rosenberg et al., 2009a). As well as bacterial consumption, *Acanthamoeba* stimulates bacterial populations in the soil. The mineral regeneration by the secondary decomposers (such as *Acanthamoeba*) relieves nutrient limitation for the primary decomposers bacteria. (Rønn et al., 2002, Rosenberg et al., 2009a)

This was verified with the findings that when nitrogen is limited (but carbon present), nitrogen mineralisation by *Acanthamoeba* permitted continued growth of bacteria resulting in higher bacterial biomass (Rosenberg et al., 2009a). When carbon is present

in small quantities, *Acanthamoeba* is almost entirely responsible for nitrogen mineralisation. Overall, *Acanthamoeba* appears to play an essential role in regulating bacterial populations in the environment and nutrient cycling, thus contributing to the ecosystems' functioning.

### 1.4.6 Nutrition (Phagocytosis & Pinocytosis)

*Acanthamoeba* is selectively feeding on bacteria that affects the *Acanthamoeba's* growth (Bottone et al., 1994, Henriquez et al., 2021).

It is well documented that *Acanthamoeba* feeds on *E. coli.* Pathogenic bacteria survive and multiply inside *Acanthamoeba,* while the non-pathogenic ones are used as a food source (Alsam et al., 2006, Cardas et al., 2012, Visvesvara et al., 2007a).

*Acanthamoeba* receives food by consuming bacteria, algae, and yeast via phagocytosis; in addition to that, they ingest liquids through the process of pinocytosis. The nutrition of *Acanthamoeba* in the ecosystem plays an important role. As mentioned earlier in this article *Acanthamoeba* digests the bacteria and uses contractile vacuoles to release the environmentally useful bacterial nutrients from its food vacuole into the soil (Alsam et al., 2006).

### 1.4.7 Pathogenicity and Genotypes of *Acanthamoeba*

*Acanthamoeba* is widely distributed in the environment, including soil, air, and water. Some strains of *Acanthamoeba* are opportunistic pathogens, causing GAE and AK. Furthermore, the diagnosis of AK can pose a challenge. The disease can be misdiagnosed as bacterial or fungal keratitis, thus delaying therapy and potentially leading to the disease's aggravation, same as for GAE, which may be misdiagnosed with bacterial

or viral meningitis. The number of *Acanthamoeba* cases is increasing every year. Because of all the threats and their dangerous effects on human health, the early detection of pathogenic *Acanthamoeba* in environments is essential. Literature has documented 20 genotypes of *Acanthamoeba* T1 to T20 have been identified. Previous studies have shown that T3, T4, and T5 genotypes are highly pathogenic (Shokri et al., 2016). The T4 genotype is the most reported in the literature from clinical cases and is also the most prevalent in the environment

However, a clear relationship between the genotypes and their pathogenicity has not been established. Molecular methods based on the amplification of nucleic acids are optimal for sensitive, specific, and simultaneous detection and quantification of *Acanthamoeba* compared to conventional staining and microscopy assays. Moreover, sequencing data of the nuclear small subunit ribosomal DNA (18S rDNA) and genotyping systems provide distinct strain phylogeny and taxonomy (Corsaro et al., 2017a).

### 1.4.8 Pathogenesis and Treatment

*Acanthamoeba* adhesion to the host cell is a primary step of pathogenesis. A mannose-binding protein (MBP) mediates it, which is expressed on the surface of *Acanthamoeba* (Garate et al., 2004). The initial binding leads to secondary events like phagocytosis and toxin production resulting in the host cell death. (Alsam et al., 2003)

In the UAE, there have been no reports about cases of G*ranulomatous Amebic Encephalitis*; however, the local newspapers in UAE on July 1, 2007, has reported that; "Complete Moisture Plus multi-Purpose contact lens solution", made by Advanced Medical Optics Company, has been linked with 138 confirmed cases of AK in the United States".

*Acanthamoeba* keratitis is rare corneal infection that is increasing in frequency and is frequently transmitted by contact lens wearers, someone who experienced recent eye trauma, or someone exposed to contaminated waters. *Acanthamoeba* survive in air, soil, dust, and water. Therefore, eye trauma and poor contact lens hygiene practices lead to the entrapment of debris and thus infection.

This disease can successfully be treated with aromatic diamidines and biguanides in a combination therapy. As there is an increasing occurrence of contact lens usage in the developed world and presently no effective monotherapy for AK. However, one needs to remember that, phylogenetically speaking, *Acanthamoeba* is similar to humans and therefore make it challenging to find a drug that selectively harms the parasite without harming its host (Lorenzo-Morales et al., 2015) .

*Acanthamoeba keratitis* causes severe microbial infection (Fanselow et al., 2021b, Nina, 2007). Many studies worked on the photocatalytic utility of Titanium dioxide combined with Ultraviolet solar radiation (TiO2/UV) in a disinfection system, with efficacy on bacteria, fungi, and viruses. In the published study of (Gomart et al., 2018), results presented of a series *of* in vitro experiments evaluating the amoebicidal effects of TiO2 with UV-A on *Acanthamoeba* cysts and trophozoites in an effort to ultimately utilise the impact of antimicrobial agents for the treatment of amoebic keratitis. Given the *in vitro* synergistic effects of chlorhexidine association with TiO2 and UV-A against cysts, the treatment of *Acanthamoeba keratitis* could be improved by this new therapeutic approach (Gomart et al., 2018). *Acanthamoeba castellanii* was found to Induce Interleukin-12 (IL-12) and IL-6, which help control infection and mitigate aggressive and inflammatory responses against *Acanthamoeba.(*Cano et al., 2017*)*.

In many cases, initial interactions between the *Acanthamoeba* and the host are initiated by a carbohydrate-mediated recognition system. The cell surface of all eukaryotic cells includes glycoproteins in its structure, which are composed of oligosaccharides bound to a protein backbone. The oligosaccharide chains are composed of several sugars, including hexoses (e.g., mannose and galactose), pentoses (e.g., fucose), hexosamines (e.g., glucosamine and galactosamine) and sialic acids. The saccharide residues of the oligosaccharides are well known for their role in mediating host-parasite interactions leading to the development of infections (Bhat et al., 2007, Frederick and Petri Jr, 2005). Recently, significant progress has been made towards understanding the mechanism by which *Acanthamoeba* adhere to the host cells, a critical first step in its pathogenesis. These studies have concluded that the main virulence protein of *Acanthamoeba* is a mannose-binding protein that facilitates the adhesion of amoebae to the surface of the tissues and also plays a role in events that occur following the adhesion that leads to the development of damaging the host cell.

*Acanthamoeba* can be life-threatening if they infect a person with reduced immunity, such as those suffering from immunodeficiency disease (Alsam et al., 2008a). Various forms of these opportunistic pathogens have been known to cause mild to severe illnesses, most of which are spread by the use of contaminated water. The two most common variants of *Acanthamoeba* that are known to pose pathogenic risks to humans are *Acanthamoeba culbertsoni* and *Acanthamoeba castellanii.* The fact that *Acanthamoeba* is a pathogen raises the need to isolate and identify isolates from water (Gerba et al., 2003). Nutrient-rich environments such as surface water and soil are the most common habitat for these microorganisms. It has been postulated that the spread of most

waterborne diseases results from the proliferation of *Acanthamoeba* and its distribution by the water network via the municipal water system (Alsam et al., 2006). Some *Acanthamoeba* strains are more pathogenic than others, even though they all share the same set of inherent characteristics and movements (Khan and Siddiqui, 2009). Various forms of skin irritation and brain cell damage are associated with *Acanthamoeba*(Sissons et al., 2004).It is essential to mention that not all types of *Acanthamoeba* are pathogenic. This is particularly true for *Acanthamoeba,* whose habitat is air. In 2009, Santos and his group confirmed the presence of *Acanthamoeba* in urine samples collected from healthy individuals. Therefore, *Acanthamoeba* contaminated swimming pools is a dangerous source of urinary tract infections. Then, It has been confirmed that 10% of urine samples were positive for the presence of *Acanthamoeba* (Alsam, unpublished data).

Several studies suggest that *Acanthamoeba* might be a potential environmental host for *Mycobacterium avium,* which causes paratuberculosis in animals. In fact, this bacteria *(M. avium)* is found to survive the process of phagocytosis by *Acanthamoeba* and to be protected from the host immune system (Samba-Louaka et al., 2018). *Toxoplasma gondii* is a parasitic protozoan infecting humans and warm-blooded animals. *Acanthamoeba* T4 type was found to coexist in water with *Toxoplasma gondii;* thus, there is a possibility of mixed infection in humans after occasional contact with water. The effect of *T. gondii* may augment the effect of *Acanthamoeba.* Cases of Waterborne outbreaks of *Acanthamoeba* augmented toxoplasmosis have been described since 1979 in Panama, Canada and Brazil and other epidemics in some countries are supposed to be caused by consuming contaminated water (Adamska, 2018).

The genus Legionella comprises more than 60 species, and about half are associated with infection. *Legionella pneumophila* is the most commonly associated with these infections and by far the most studied, but *L. non-pneumophila* species, such as *L. feeleii, L. anisa,* etc., may also present clinical importance. Free-living amoebae are their preferred environmental host, where these bacteria not only survive but also succeed in multiplying, and this relationship can lead to an increase in bacterial virulence. (Gomes et al., 2018)

### 1.4.9 *Acanthamoeba* Resisting Bacteria (ARB)

According to Flemming, Amoeba Resisting Bacteria (ARB) are currently given recognition as possible human health pathogens that live in the environment(Flemming, 2011). To identify and detect the ARB such as Legionella pneumophila from humans as well as environmental samples, the co-culture with amoebae has proven effective for several years. The efficacy of this procedure have been explored mostly in hospital water and cooling towers water and found that it can be regarded as possible pools to enhance the growth of ARB (Muchesa et al., 2014). In another study, isolation and identification of ARB were improved by using a co-culture procedure (Evstigneeva et al., 2009).

Co-culture procedure is defined as a cell culture containing the growth of two distinct cell types. Amoeba Resisting Bacteria population was studied in seventy-seven ecological water samples comprising fountains, rivers, lakes and domestic wells in Southern France (Evstigneeva et al., 2009).The study identified 244 isolates that corresponded to 89 different species of ARB, excluding legionella species.

The above study and others have indicated that *Acanthamoeba* has a worldwide footprint; its occurrence features distribution that is not inclined towards any part of the globe. Its occurrence is international, not local or regional. Its pathogenic properties and the fact that it finds water a suitable natural habitat means that it is a significant health risk, even though it does not present any epidemic risks through moving from one individual to another by mere contact(Delafont et al., 2013, Flemming, 2011, Vaerewijck et al., 2014).

This study will be the first within the UAE to establish with evidence the existence of *Acanthamoeba* in the region.

### 1.4.10 Immunity against *Acanthamoeba*

Humans' serum naturally contains antibodies, mainly consisting of Immunoglobulin M (IgM) and Immunoglobulin G (IgG) to pathogenic species of *Acanthamoeba*. These antibodies and the capacity of the *Acanthamoeba* to activate complement within the human immune system make the first-line defence against *Acanthamoeba* infections. Both antibody and complement are essential in stimulating recognition of the *Acanthamoeba* by phagocytic cells such as neutrophils. These antibodies may also prevent tissue invasion by inhibiting its adherence, phagocytic activity, and migration and deactivating cytopathogenic agents within *Acanthamoeba* (Ferrante, 1991).

Alizadeh has attempted to examine the immunisation of lab animals against *Acanthamoeba;* their results showed that oral immunisation with aqueous extracts of *Acanthamoeba* induces strong immunity against the development of *Acanthamoeba* keratitis. Intramuscular immunisation with the same antigenic mixture stimulates the production of serum IgG antibodies and delayed-type hypersensitivity responsiveness to

*Acanthamoeba* antigens and fails to prevent *Acanthamoeba* keratitis. Therefore neither – IgG specific nor delayed-type hypersensitivity was able to protect the cornea against *Acanthamoeba* infection. The most apparent difference between orally immunised subjects and intramuscularly immunised subjects is the appearance of parasite-specific Immunoglobulin A (IgA) antibodies in mucosal secretions, including the tears (Alizadeh et al., 1995, Alsam et al., 2008a, Neelam and Niederkorn, 2017).

Immunological methodologies to test for *Acanthamoeba* cells utilising antibody-antigen reaction was useful in identifying *Acanthamoeba* cells in biological liquid samples such as body fluids. An Enzyme-linked Immunosorbent Assay (ELISA) method uses the whole fixed trophozoites rather than disrupted cell extracts as the antigen source was developed and shown to be an effective tool for identifying antibodies against *Acanthamoeba* in the clinical laboratory setting. An enzyme-linked immunosorbent assay for detecting *Acanthamoeba* in environmental samples was designed and optimised in which it was possible to see *Acanthamoeba* trophozoite in water samples at a low cell count of approximately ten cells per each well of *Acanthamoeba* (Reveiller et al., 2003). Furthermore, a detailed investigation has attempted the ELISA technic for the specific identification of *Naegleria fowleri* (the brain-eating Amoeba) in primary cultures of environmental water samples. Results of this study indicate that the ELISA method is reliable and can be considered a powerful tool for detecting *N. fowleri* and maybe other species such as *Acanthamoeba* in environmental water samples.

### 1.4.11 Seasonal and Geographical effects on *Acanthamoeba* Distribution

To study the effects of seasonal temperatures on the variety of *Acanthamoeba* species, Kao and colleagues had investigated the existence of *Acanthamoeba* in water samples (Kao et al., 2014)The investigators gathered every quarter at 19 reservoirs harbouring drinking water. The reservoirs were geographically distributed within the Northern, Southern as well as Central regions of Taiwan. Two hundred and eleven water samples were taken across the four Seasons: Winter 2012-Summer 2013. In brief, one litre of raw water was stored in a sterile polypropylene container and stored at temperatures of 4$^{o}$ C for subsequent analyses within 24hours. The study established that the species of *Acanthamoeba* differed from season to season due to temperature variation. To verify the identity of the isolated *Acanthamoeba* strains, they were compared with the *Acanthamoeba* reference once listed on the National Centre for Biotechnology Information (NCBI) GenBank. The study concluded that the *Acanthamoeba* detection rate in all reservoir samples collected was 39.5%. In terms of geographical distribution, the Northern region had a higher rate of *Acanthamoeba* isolates Compared with the Southern and Central areas across all four seasons. The study also concluded that *Acanthamoeba* detection rate was higher in the Fall and Winter samples than in the Spring and Summer. The study showed that *Acanthamoeba* genotype T4 was the most common among other genotypes isolated from this study. They have also concluded that dissolved oxygen (DO) is a favourable factor for the presence of *Acanthamoeba* in water.

The main aim of this project is to investigate whether the manmade water sources in the UAE will contain *Acanthamoeba*, their genotypes and whether they host or interact with other pathogenic microorganisms such as, bacteria.

Currently there has been no published studies locally, nationally or internationally, regarding the existence of *Acanthamoeba* in drinking water of the United Arab Emirates (UAE). The study was taken to investigate the *Acanthamoeba* occurrence in drinking water of the United Arab Emirates. Upon analysis, water contents of metal ions were very low, however microbial content was staggering. *Acanthamoeba* was isolated along with another bacterial cohabitant. *Acanthamoeba* was identified utilizing PCR, real-time PCR and nested PCR. Cytotoxicity and interaction between *Acanthamoeba* and bacterial cells were studied utilizing the human cell line KB cell. *Acanthamoeba* was found in close association with other bacterial species existed in cohabitation manner with local environmental bacterial species. Interaction between isolated *Acanthamoeba* and KB cell line was studied. This finding will be marked as the first evidence to show existence of *Acanthamoeba* in drinking water of UAE.

# Chapter 2

# Water Collection, Chemical and Bacterial Analysis

## 2.1 Introduction

Safe drinking water is anonymously accepted as an international agenda and priority, which is evident from the Millennium Development Goals (MDGs) and Sustainable Development Goals (SDGs) of the United Nations (UN) initiative and vision (MDGs 7 and SDGs 6); despite efforts by the MDGs, many people still lack access to safe drinking water, even lack access to basic water (Dinka, 2017). Nevertheless, providing safe drinking water in distribution systems is a top priority goal of health agencies in many countries.

Drinking water is derived from two basic sources: surface waters, such as rivers and reservoirs, and groundwater (Fawell and Nieuwenhuijsen, 2003) . In addition to having serious health implication for humans, contaminated drinking water also has adverse health implications for marine life that consume the impure water (Rather et al., 2017) . The Safe Drinking Water Act (established to protect the quality of drinking water in the USA) defines the term "contaminant" as meaning any physical, chemical, biological, or radiological substance or matter in water (broadly meaning anything other than water molecules). The sources of these contaminants are multiple including industrial and municipal discharges, natural geological formations, urban and rural run-off, drinking water treatment process, and water distribution materials (Rather et al., 2017). It is worth noting that all water contains natural contaminants, particularly inorganic contaminants that arise from the geological strata through which the water flows and, to a varying extent, anthropogenic pollution by both microorganisms and chemicals (Fawell and Nieuwenhuijsen, 2003).

Drinking water contaminants include several chemicals such as arsenic, fluoride, aluminium, lead, nitrates, cyanides, disinfection by-products, radon, and pesticides (Dinka, 2017, Rather et al., 2017). The health concerns associated with chemical constituents of drinking-water arise primarily from the ability of chemical constituents to cause adverse health effects after prolonged periods of exposure(WHO, 2017) . These adverse health effects range from numerous cancers, cardiovascular diseases, adverse reproductive outcomes, and neurological diseases (Rather et al., 2017). The great majority of evident water-related health problems are the result of microbial (bacterial, viral, protozoan or other biological) contamination (WHO, 2017) . Contamination of drinking water by pathogens of intestinal origin causing diarrhoeal disease is the most important aspect of drinking water quality (Fawell and Nieuwenhuijsen, 2003) . In addition to intestinal pathogens, other microbial hazards, such as toxic cyanobacteria and *Legionella*, may be of public health importance under specific circumstances (WHO, 2017).

Several standards have been established to provide high water quality, including sterilisation technics to control and prevent microbial colonisation. The standard of WHO is not exactly the same as that of USA, Canada, European Commission, Russia, India, South Africa, Ethiopia, etc. (Dinka, 2017). Although the WHO defines safe drinking water as water that does not represent any significant risk to health over a lifetime of consumption, including different sensitivities that may occur between life stages (WHO, 2017), the term “safe” depends on the particular resistance ability of an individual (Dinka, 2017) . Water that is safe for drinking in some African countries might not be safe in

European countries; some African countries already developed resistance to some of the water-related diseases (Dinka, 2017) .

Microbial communities that are normally found in drinking water are made of both Eukaryotes & Prokaryotes and may include viruses, bacteria, parasitic protozoa, and fungi (Nsanze et al., 1999). To name a few: *Acanthamoeba*, *E.coli*, *Legionella pneumophila*, and *Staphylococcus aureus* are examples of the coexistence of microbial communities (Marciano-Cabral and Cabral, 2003). In water, Free-living amoeba (FLA) or protists such as amoebae, flagellates and ciliates have two major ecological activities:

(1) Influencing the structure of the microbial community, and (2) Enhancing nutrient recycling. Both of these activities are associated with water FLA feeding on bacteria, thus regulating bacterial populations in the water. Among protists, free-living amoebae are the dominant bacterial consumers and are responsible for up to 60% of the total reduction in the bacterial population (Sinclair et al., 1981). The primary decomposers (bacteria) directly decompose organic materials but are inefficient in releasing minerals from their own mass. The secondary decomposers, such as free-living amoebae, consume the primary decomposers and release mineral nutrients as waste products that are tied up in the primary decomposer's biomass.

The government of the UAE has put tremendous effort into providing the consumer with high quality drinking water according to national and international standards; a major example is the establishment of Abu Dhabi Quality and Conformity Council (QCC) which is audited by International approved auditing committees such as ENAS, ILAC, UKAS and IAF. They examine all water sources (network water, bottled, unbottled, groundwater,

treated water and other types of water); they analyse water for chemicals, microbiology and radioactivity.

The aim of this chapter is to isolate and identify the microbial community from different water sources in the UAE, and to perform chemical and bacterial analysis from different water Sources.

## 2.2 Materials and Methods

A total of 171 water samples from 4 Emirates (table 2.1), (57 locations in triplicates) were collected (Figure 2.1). Water samples were collected in sterile 1000 ml bottles. When water was taken from taps, water was allowed to run for 3 minutes before collecting the sample to allow for the external deposits of dust or dirt inside the tap to be removed .

Bottled water was purchased from a nearby grocery store, and mist water was taken from the reservoir that supplies the mist pump before the water enters the pump. All samples were refrigerated in a cool box at a temperature less than 10°C during transit to the laboratory. All samples arrived at the lab within the same day of collection and were assigned a unique code to keep their identities. Sample information was recorded in a laboratory sample logbook for documentation. The maximum transport time to the laboratory was 3 hours, and samples were processed within 2 hours of receipt at the laboratory, following the procedure for microbial analysis as detailed in section 2.2.1.2 (Mahmoud et al., 2018).

Table 2. 1 : Showing the criteria of samples: locations , months, pH , and Tempreture

| Water Samples locations | Month | pH Winter / Summer | Temp. °C Winter / Summer |
|---|---|---|---|
| **Emirate 1** | | | |
| Residential Tank | February & August | 7.0/7.2 | 22/40 |
| Residential Tap | February & August | 7.1/7.2 | 21/38 |
| Residential Water cooler | February & August | 7.2/7.1 | 9/7 |
| Mosque tank | February & August | 7.4/7.8 | 20/38 |
| Mosque Tap | February & August | 7.2/7.8 | 21.4/38 |
| Mosque water cooler | February & August | 7.4/7.6 | 8.2/7 |
| Hospital tank | February & August | 6.9/7.2 | 19.8/41 |
| Hospital tap | February & August | 6.0/7.1 | 20/39 |
| Hospital Water cooler | February & August | 7.0/7.4 | 6/8.5 |
| **Emirate 2** | | | |
| Residential Tank | February & August | 6.6 /7.2 | 20/37.8 |
| Residential Tap | February & August | 6.4/7.2 | 20/38 |
| Residential Water cooler | February & August | 6.6/7.4 | 8/6.8 |
| Mosque tank | February & August | 6.8/7.8 | 18/36.8 |
| Mosque Tap | February & August | 6.6/7.6 | 19.2/37 |
| Mosque water cooler | February & August | 7.5/7.6 | 6.4/7.2 |
| Hospital tank | February & August | 7.2/7.9 | 18.4/40 |
| Hospital tap | February & August | 7.0/6.8 | 18.6/39.4 |
| Hospital Water cooler | February & August | 7.0/7.6 | 8.8/7.5 |
| **Emirate 3** | | | |
| Residential Tank | February & August | 6.8/7.4 | 22/39.8 |
| Residential Tap | February & August | 6.8/7.4 | 22.2/39.7 |
| Residential Water cooler | February & August | 6.2/7.6 | 5/6.2 |
| Mosque tank | February & August | 6.5/7.9 | 19.6/39.2 |
| Mosque Tap | February & August | 6.7/8.0 | 19.5/39.1 |
| Mosque water cooler | February & August | 7.5/7.9 | 8.4/7 |
| Hospital tank | February & August | 6.2/7.4 | 20.4/39.6 |
| Hospital tap | February & August | 6.0/6.4 | 20.0/39.6 |
| Hospital Water cooler | February & August | 7.9/7.6 | 7.8/7.0 |
| **Emirate 4** | | | |
| Residential Tank | February & August | 6.0/7.0 | 19/40.4 |
| Residential Tap | February & August | 6.1/7.2 | 22.2/40 |
| Residential Water cooler | February & August | 6.2/7.0 | 6.4/7.8 |
| Mosque tank | February & August | 7.4/8.0 | 19.0/40.2 |
| Mosque Tap | February & August | 7.3/8.0 | 19.0/40.0 |
| Mosque water cooler | February & August | 7.4/7.9 | 6.8/7.4 |
| Hospital tank | February & August | 6.8/7.4 | 18.6/39.9 |
| Hospital tap | February & August | 6.6/7.4 | 19.0/39.2 |
| Hospital Water cooler | February & August | 6.7/7.3 | 5.9/6.2 |

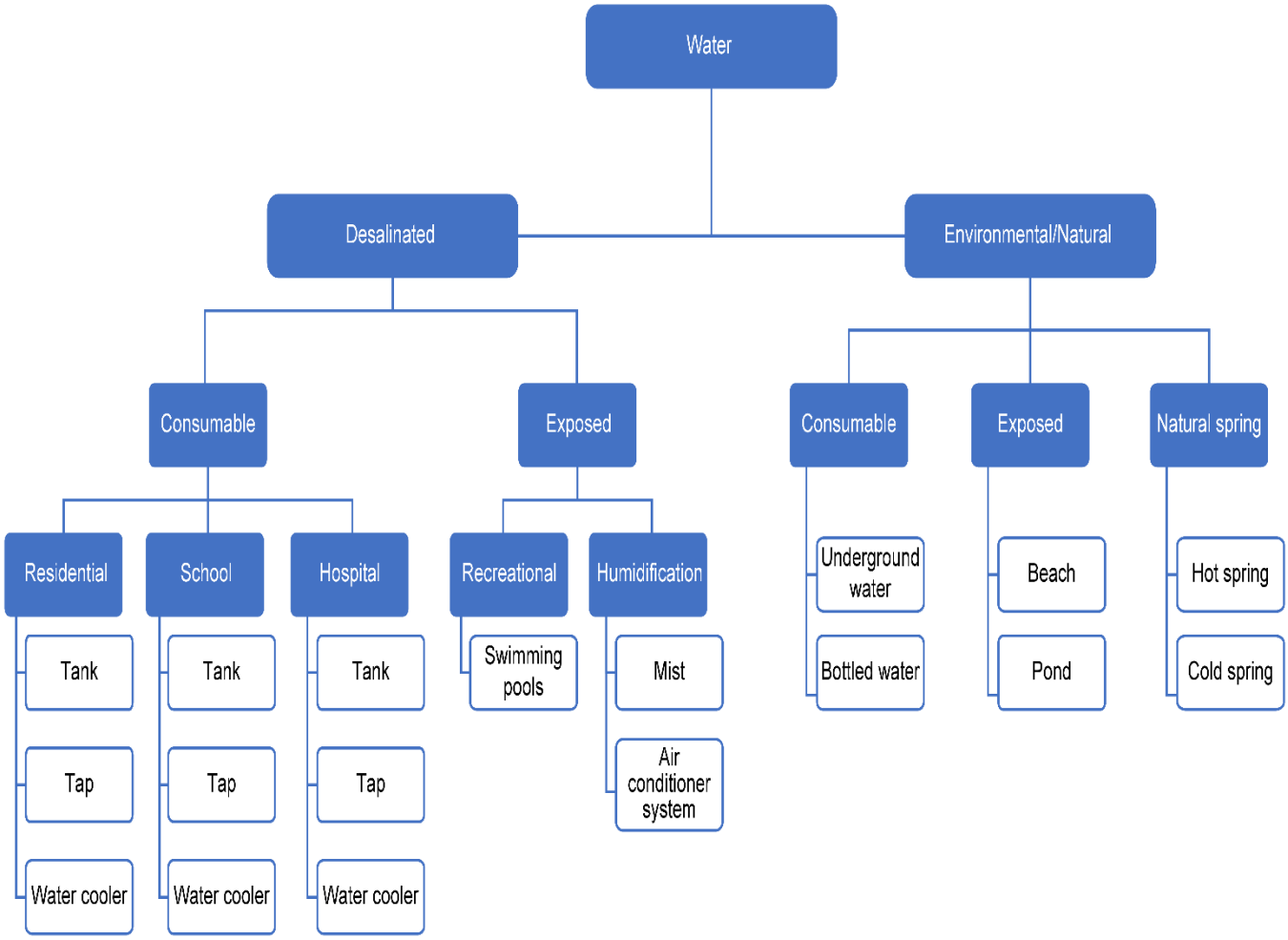



Figure 2.1: Water collected from 57residential locations in 4 Emirates. Water samples are collected from two major sources Desalinated plants and Natural water.

### 2.2.1 Water Analysis

#### 2.2.1.1 Chemical Analysis

Chemical analysis of water samples was conducted within 2 hours of arrival at the lab to determine the following parameters phosphate (PO4), Fluoride (F), Bromate ($BrO_3$), Chloride (Cl), Chlorate ($ClO_3$), Bromide (Br), Nitrate ($NO_3$), Sulphate ($SO_4$), Total dissolved solids (TDS), and Carbonate. Methods of testing were according to the standard in the Abu Dhabi Quality and Confirmity Council (QCC) lab (Tables 1.1 and 1.2) and US EPA, Test Method (300.1), EPA, 1993 utilizing the Dionex DX500 and the Columns: Dionex AG9-HC and AS9-HC, 2 mm:

A filtered aliquot of sample was injected into a stream of eluent and pumped through three different ion exchange columns and a conductivity detector. The first two columns, a precolumn and separator column were packed with a low capacity and strongly basic anion exchanger. The anions of interest were separated on the basis of their relative affinities for the exchange sites of the resin. The affinity of each anion is largely determined by its radius and valence. Because different anions have different migration rates, the sample anions elute from the column as discrete bands. Each anion is identified by its retention time within the exchange column. The sample anions were selectively eluted off the separator column and onto the last column, the suppresser column. The micromembrane suppressor which is bathed in a continuously flowing acid solution (regenerant solution) contains a cation exchange resin in the hydrogen form. In the micromembrane suppressor the separated anions are converted to their highly conductive acid forms which are detected in the conductance cell of the conductivity detector. The suppressor column decreases the background conductivity of the eluent to

a insignificant level by converting it to a weakly conductive form. The chromatograms formed are displayed on an integrator for measurement of peak height or zone which offers the basis for quantitation of anions by comparison to peaks of known values (Dasgupta and Maleki, 2019).

#### 2.2.1.2 Sample Filtration and Bacterial Analysis

Water samples were divided into two portions (500 ml each). Both portions were passed through the membrane sterile Nitrocellulose membrane filters (MicroFunnel-PALL) with a pore size of 0.45 µm, were placed on the filter base, grid side up, and a funnel was attached to the base so that the membrane filter was held between the funnel and the base. The water samples were inverted three times to distribute the microorganisms uniformly within the sample. Water samples were filtered using the filtration system with flasks in which nitrocellulose membrane filters were placed to collect suspended microorganisms. Samples were filtered, and membrane filters were collected. The two membrane filters were placed on plate count agar (PCA.) plates. One plate with the first filter membrane was incubated for 24 hours at 30°C, and the second plate with the second filter membrane was incubated at 37° C for 24 hours. After incubation single colonies were isolated, streaked on to PCA plates, and incubated for 24 hours at 30°C and 37°C. Colonies were characterised visually based on general morphology, colony edge, shape, colour, etc. and were tested for Gram staining reaction. Pure bacterial cultures were calorimetrically identified and fully diagnosed to the species levels by Vitek 2 compact system. This system uses advanced colourimetric technology to determine individual biochemical reactions contained in a variety of microorganism identification "cards"

(Pincus, 2006). Unknown bacteria were mixed with standardised suspensions, and each self-contained card was incubated and read by the instrument's internal optics. After identifying the microorganism by Gram staining, bacterial suspensions were prepared by taking sterile swabs with a sufficient number of colonies of pure fresh culture and were suspended in 3.0 ml of sterile saline (aqueous 0.45% to 0.50% NaCl, pH 4.5 to 7.0) in clear plastic (polystyrene) test tubes. The turbidity was adjusted to standardise the number of bacterial cells/ml according to the McFarland Turbidity table (Table 2.2) and measured using a DensiCHEK™ photometer to give an approximate number of bacterial cells per ml. ($5 \times 10^{5}$ CFU/ml).

Identification cards were inoculated with microorganism suspensions using an integrated vacuum apparatus within the testing machine. Test tubes containing the microorganism suspensions were placed into a special rack (cassette), and the identification cards were placed in a neighbouring slots while inserting the transfer tubes into the corresponding suspension tube. The filled cassette was placed into a vacuum chamber station for the purpose of introducing the microorganism suspension into micro-channels that fill all the test wells

Table 2. 2: Suspension Turbidity Used for Card Inoculation in Vitek 2 compact system

| **Product** | **McFarland Turbidity Range** |
|---|---|
| Gram-Negative (GN) | 0.50 -0.63 |
| Gram Positive (GP) | 0.50 -0.63 |

A transmittance optical system allows the interpretation of test reactions using different wavelengths in the visible spectrum. The system does the calculations on raw data, and test data from an unknown organism are compared to the respective database stored within the system to determine a quantitative value for proximity to each of the stored genus/species databases.

#### 2.2.1.2.1 Bacterial Colorimetric Identification

System cards come in two types: Gram-Negative (GN) cards to test for bacterial identity of GN bacteria and Gram-Positive (GP) cards to test for Gram-positive bacteria. The identification cards are based on established biochemical methods and developed identification procedures.

- GN Cards: the GN card system is based on established biochemical methods and newly developed substrates measuring carbon source utilisation, enzymatic activities, and resistance. The system has **47 biochemical** tests and one negative control well. Full bacterial identification could be obtained in approximately 10 hours or less.
- GP Cards have **43 biochemical tests** measuring carbon source utilisation, enzymatic activities and inhibition. Final identification results could be obtained in approximately 8 hours or less.

#### 2.2.1.2.2 Statistical Analysis

Experimental data were analysed using a one-tailed unpaired t-test (two-sample equal variance) for comparison between means. P values were considered statistically significant at a 95% confidence interval (i.e. $P < 0.05$).

## 2.3 Results

### 2.3.1 Chemical Analysis

Results of the chemical analysis of water samples obtained from sampling sites in the different Emirates during Winter were identical to the chemical analysis results obtained in summer; these findings are as presented in Tables 2.3 – 2.6. Levels of some detected chemical components, i.e. TDS and chlorate, from all the sampling sites were compared to the WHO standard for allowable limits of chemical ions in drinking water, as limits for these chemical components. In addition, There are no current UK and WHO standards for bromide as additive in drinking water or receiving water, because Bromine is primarily used as an alternative disinfectant for swimming pools, spas and cooling tower water, but not for municipal drinking-water, partly due to cost and partly to concerns about the formation of brominated DBPs (WHO 2018).

Chemical analysis of the water samples from Emirates -1- showed that the 'residential tank' sampling site had the highest amount of TDS i.e. 156.00 mg/l compared with sampling site 'Mosque water cooler' which had TDS of 134.67 mg/l (Table 2.3). The results also showed that the TDS for all the sample sites in Emirate -1- were with acceptable TDS limits stated in the UAE and WHO standards i.e. 100 – 600 mg/l and below 500 mg/l respectively (Table 2.3). Furthermore, the results also showed that the bromate levels in all the sampling sites are higher than the allowable limits of both the UAE and UK standards i.e. 0.01 mg/l in both cases (Table 2.3).

Table 2. 3 : Chemical Analysis of Water samples from Emirate 1.Water samples from Emirates -1- were physically cheracterized for chemical contents. Water samples were all checked for Phosphates(PO4) Detection limit(DL)* 0.009, Fluoride (F) (DL) 0.05 mg/l, Bromate (BrO3) (DL)0.0003 mg/l ,Chloride (Cl) (DL)* 0.0001 mg/l, Chlorate (ClO3) (DL) 0.0017 mg/l, Bromide (Br) (DL)* 1.0 mg/l, Nitrate (NO3) (DL) 1.00 mg/l, Sulphate (SO4) (DL) 15 to 321 mg/l, Total dissolved solids (T.D.S.), and Carbonate . Results indicated that Bromate, levels are higher than the allowable limits of the UAE and UK standards, but lower than WHO standard

| Water Samples locations Emirate -1- | Phosphates mg/l | Fluoride(F) mg/l | Bromate ($BrO_3$) mg/l | Chloride (Cl) mg/l | Chlorate ($ClO_3$) mg/l | Bromide (Br) mg/l | Nitrate ($NO_3$) mg/l | Sulphate ($SO_4$) mg/l | TDS mg/l | Carbonate -hardness mg/l |
|---|---|---|---|---|---|---|---|---|---|---|
| Tank Residential | 0.01 | BDL | 0.06 | 64.31 | 0.08 | 0.17 | 0.02 | 4.24 | 156.00 | 0.68 |
| Tap Residential | 0.01 | 0.03 | 0.06 | 65.88 | 0.08 | 0.21 | 0.02 | 4.28 | 160.00 | 1.02 |
| Water cooler Residential | 0.01 | BDL | 0.06 | 65.59 | 0.08 | 0.22 | 0.02 | 4.30 | 154.67 | 0.36 |
| Mosque tank | BDL | BDL | 0.09 | 59.13 | 0.12 | 0.18 | 0.03 | 3.48 | 142.33 | 0.48 |
| Mosque Tap | BDL | BDL | 0.09 | 62.19 | 0.13 | 0.24 | 0.03 | 3.65 | 136.00 | 0.28 |
| Mosque water cooler | 0.01 | BDL | 0.08 | 56.52 | 0.10 | 0.17 | 0.03 | 3.45 | 134.67 | 0 .36 |
| Hospital tank | BDL | BDL | 0.09 | 63.38 | 0.36 | 0.21 | 0.01 | 3.67 | 145.67 | 0.44 |
| Hospital tap | BDL | BDL | 0.09 | 58.06 | 0.37 | 0.12 | 0.02 | 3.36 | 137.00 | 0.52 |
| Hospital Water cooler | BDL | BDL | 0.09 | 59.95 | 0.11 | 0.18 | 0.02 | 3.54 | 139.33 | 0.12 |
| UAE Standard | 2.20 | 1.50 | 0.01 | 250.00 | 0.70 | 0.01 | 50.00 | 250.00 | 100 - 600 | 200.00 |
| UK Standard | 0.7 -1.9 | 1.00 | 0.01 | 250.00 | *0.70 | - | 50.00 | 250.00 | *Below 500 | 108.00 |

* indicates WHO standard (WHO 2011) , UK Standard (The Network and Information Systems Regulations, 2018)
UAE Standard (Department of Energy of Abu Dhabi, 2021) *DL: Detection limits

Similar to Emirates 1, results of the chemical analysis of water samples from Emirate 2 (Table 2.4) indicated that the bromide levels in all the sampling sites is higher than the allowable limits of the UAE and UK standards i.e. 0.01 mg/l, however they are within tha allowable limits of WHO (0.2 -0.6 mg/l). Results from Emirate 2 also showed that sampling site ‘residential tank’ had the highest TDS of 1430.00 mg/l while sampling site ‘Mosque tank’ had the least TDS of 169.67 mg/l. Only sample site ‘residential tank’ had a TDS value that was higher than the acceptable TDS limits of the UAE and WHO standards i.e. 100 – 600 mg/l and below 500 mg/l respectively; the TDS values of other sampling sites were within the acceptable limits put forward in the UAE and UK guidelines (Table 2.4).

Table 2. 4: Results of chemical analysis of water samples from Emirate -2- characterized for Phosphates($PO_4$), Fluoride (F), Bromate ($BrO_3$), Chloride (Cl), Chlorate ($ClO_3$), Bromide (Br), Nitrate ($NO_3$), Sulphate ($SO_4$), Total dissolved solids (T.D.S.), and Carbonate . Results indicated that Bromide is higher than the allowable limits of the UAE standard.

| Water Samples locations Emirate -2- | Phosphates mg/l | Fluoride mg/l | Bromate ($BrO_3$) mg/l | Chloride (Cl) mg/l | Chlorate ($ClO_3$) mg/l | Bromide | Nitrate ($NO_3$) mg/l | Sulphate ($SO_4$) mg/l | TDS mg/l | Carbonate -hardness mg/l |
|---|---|---|---|---|---|---|---|---|---|---|
| Residential tank | 0.002 | 4.21 | BDL | 304.50 | 0.202 | 0.63 | 15.66 | 925.69 | 1430.00 | 0.24 |
| Residential tap | BDL | 0.15 | BDL | 91.29 | 0.20 | 0.37 | 0.18 | 9.27 | 191.33 | 1.60 |
| Residential water cooler | BDL | 0.11 | BDL | 85.72 | 0.21 | 0.33 | 0.02 | 8.95 | 186.33 | 0.48 |
| Mosque tank | BDL | 0.02 | BDL | 77.38 | 0.23 | 0.29 | 0.10 | 8.11 | 169.67 | 0.16 |
| Mosque tap | BDL | 0.01 | BDL | 92.63 | 0.20 | 0.37 | 0.01 | 9.50 | 200.00 | 0.08 |
| Mosque Water cooler | BDL | 0.03 | BDL | 81.00 | 0.21 | 0.31 | 1.05 | 11.26 | 180.67 | 0.24 |
| Hospital tank | 0.023 | 0.01 | BDL | 85.14 | 0.26 | 0.32 | 0.22 | 8.80 | 184.33 | 0.20 |
| Hospital tap | BDL | BDL | BDL | 85.15 | 0.27 | 0.33 | 0.04 | 8.56 | 184.33 | 0.16 |
| Hospital Water cooler | BDL | 0.01 | BDL | 48.42 | 0.25 | 0.32 | 0.03 | 8.41 | 184.67 | 0.12 |
| **UAE Standard** | 2.2 | 1.50 | 0.01 | 250.00 | 0.70 | 0.01 | 50.00 | 250.00 | 100 - 600 | 200.00 |
| **UK Standard** | 0.7 -1.9 | 1.00 | 0.01 | 250.00 | *0.70 | - | 50.00 | 250.00 | *Below 500 | 108.00 |

* indicates WHO standard (WHO 2011) , UK Standard (The Network and Information Systems Regulations, 2018)
UAE Standard (Department of Energy of Abu Dhabi, 2021)

Chemical analysis of water samples from sampling sites in Emirate 3 showed that only sampling sites hospital tap and hospital water cooler had TDS values higher (2790.00 mg/l, 3080.00 mg/l and 2913.33 mg/l respectively) than the acceptable limits set in the UAE and WHO guidelines i.e. 100 – 600 mg/l and below 500 mg/l respectively (Table 2.5). The results also showed that while sampling site ‘hospital tap’ had the highest TDS (3080.00 mg/l), sampling site ‘mosque tank’ had the least TDS (1.62 mg/l) (Table 2.5).

Similar to Emirates 1, results of the chemical analysis of water samples from sampling sites from Emirate 3 showed bromate levels to be higher than the allowable limits of the UAE standard i.e. 0.01 mg/l in both cases (Table 2.5).

Table 2. 5 : Results of chemical analysis of water samples from Emirate -3- characterized for Phosphates(PO4), Fluoride (F), Bromate (BrO3), Chloride (Cl), Chlorate (ClO3), Bromide (Br), Nitrate (NO3), Sulphate (SO4), Total dissolved solids (T.D.S.), and Carbonate . Results indicated that Bromide is higher than the allowable limits of the UAE standard. BDL :Below Detection Limit

| Water Samples locations Emirate-3- | Phosphates mg/l | Fluoride mg/l | Bromate ($BrO_3$) mg/l | Chloride (Cl) mg/l | Chlorate ($ClO_3$) mg/l | Bromide (Br) mg/l | Nitrate ($NO_3$) mg/l | Sulphate ($SO_4$) mg/l | TDS mg/l | Carbonate -hardness mg/l |
|---|---|---|---|---|---|---|---|---|---|---|
| Residential tank | 0.01 | 0.03 | BDL | 114.36 | 0.29 | 0.38 | 0.89 | 50.01 | 270.00 | 1.20 |
| Residential tap | 0.01 | 0.03 | BDL | 98.64 | 0.30 | 0.38 | 1.04 | 33.36 | 235.00 | 1.20 |
| Residential water cooler | 0.01 | 0.01 | BDL | 98.70 | 0.30 | 0.37 | 0.66 | 33.65 | 233.33 | 0.96 |
| Mosque tank | 0.01 | 0.01 | BDL | 74.38 | 0.31 | 0.35 | 1.24 | 4.52 | 1.62 | 0.16 |
| Mosque tap | 0.01 | 0.02 | BDL | 74.14 | 0.31 | 0.36 | 1.33 | 4.48 | 163.00 | 1.20 |
| Mosque water cooler | 0.01 | 0.02 | 0.02 | 73.83 | 0.17 | 0.31 | 1.33 | 3.51 | 162.33 | 1.20 |
| Hospital tank | 0.02 | 0.14 | BDL | 1325.60 | BDL | 1.40 | 6.87 | 621.14 | 2790.00 | 0.28 |
| Hospital tap | 0.02 | 0.13 | BDL | 1350.50 | BDL | 1.46 | 7.55 | 640.47 | 3080.00 | 0.16 |
| Hospital Water cooler | 0.13 | 0.27 | BDL | 1324.30 | BDL | 1.40 | 6.91 | 621.76 | 2913.33 | 0.12 |
| **UAE Standard** | 2.20 | 1.50 | 0.01 | 250.00 | 0.70 | 0.01 | 50.00 | 250.00 | 100 - 600 | 200.00 |
| **UK Standard** | 0.7 -1.9 | 1.00 | 0.01 | 250.00 | *0.70 | - | 50.00 | 250.00 | *Below 500 | 108.00 |

* indicates WHO standard (WHO 2011) , UK Standard (The Network and Information Systems Regulations, 2018)UAE Standard (Department of Energy of Abu Dhabi, 2021)

All sampling sites within Emirate 4 were seen to have TDS values within the acceptable limits by the UAE standard and WHO guidelines i.e. 100 – 600 mg/l and below 500 mg/l respectively (Table 2.6). While sampling site 'residential tap' had the highest TDS of 279.33 mg/l, sampling site 'residential water cooler' had the least TDS of 257.33 mg/l (Table 2.6).

Table 2. 6 *:* Table 2.6: Results of chemical analysis of water samples from Emirate -4- characterized for Phosphates($PO_4$), Fluoride (F), Bromate ($BrO_3$), Chloride (Cl), Chlorate ($ClO_3$), Bromide (Br), Nitrate ($NO_3$), Sulphate ($SO_4$), Total dissolved solids (T.D.S.), and Carbonate . Results indicated that Bromide and Bromate are higher than the allowable limits of the UAE standards. BDL :Below Detection Limit

| Water Samples locations Emirate-4- | Phosphates mg/l | Fluoride mg/l | Bromate ($BrO_3$) mg/l | Chloride (Cl) mg/l | Chlorate ($ClO_3$) mg/l | Bromide (Br) mg/l | Nitrate ($NO_3$) mg/l | Sulphate ($SO_4$) mg/l | TDS mg/l | Carbonate - hardness mg/l |
|---|---|---|---|---|---|---|---|---|---|---|
| Residential Tank | BDL | BDL | BDL | 157.85 | 0.10 | 0.72 | 0.01 | 2.86 | 277.67 | 0.24 |
| Residential Tap | BDL | 0.02 | BDL | 158.78 | 0.09 | 0.71 | 0.02 | 3.21 | 279.33 | 0.24 |
| Residential Water cooler | BDL | BDL | BDL | 145.38 | 0.09 | 0.62 | 0.04 | 3.26 | 257.33 | 0.24 |
| Mosque tank | BDL | 0.01 | 0.01 | 142.67 | 0.05 | 0.56 | 0.04 | 4.68 | 260.33 | 0.72 |
| Mosque Tap | BDL | 0.01 | 0.01 | 145.24 | 0.05 | 0.58 | 0.04 | 4.71 | 265.67 | 0.44 |
| Mosque Water cooler | BDL | 0.02 | 0.01 | 144.94 | 0.05 | 0.57 | 0.05 | 4.76 | 263.33 | 0.24 |
| Hospital Tank | BDL | BDL | 0.01 | 151.20 | 0.02 | 0.57 | 0.10 | 4.45 | 273.00 | 0.68 |
| Hospital Tap | BDL | BDL | 0.01 | 152.25 | 0.02 | 0.61 | 0.04 | 4.61 | 274.00 | 0.48 |
| Hospital Water cooler | BDL | 0.02 | 0.05 | 152.40 | 0.02 | 0.60 | 0.04 | 4.58 | 274.33 | 0.48 |
| **UAE Standard** | 2.20 | 1.50 | 0.01 | 250.00 | 0.70 | 0.01 | 50.00 | 250.00 | 100 - 600 | 200.00 |
| **UK Standard** | 0.7 -1.9 | 1.00 | 0.01 | 250.00 | *0.70 | - | 50.00 | 250.00 | *Below 500 | 108.00 |

2.3.2 * indicates WHO standard (WHO 2011) , UK Standard (The Network and Information Systems Regulations, 2018)
2.3.2 UAE Standard (Department of Energy of Abu Dhabi, 2021)

### 2.3.2 Bacterial Species in Water Samples

Results show that the count of microbial species in Emirate-1-residential water samples is 30% to 50% higher than similar samples of the other three Emirates (Tables 2.7 – 2.14) .Emirate -4- Residential water samples show a lower level of microbial pollution. Water samples from mosques facilities within the 4 Emirates have shown that the least microbial contents were detected in Emirate -1-mosque facilities while the highest bacterial counts were detected in Emirate -3- and Emirate -4-mosque facilities. Hospital water samples were collected from hospitals in the 4 Emirates. Results showed that a higher bacterial count 44 CFU/ml was detected in Emirates -4-Hospital facilities compared to Emirates 1, 2, and 3 . The lowest microbial count was detected in Emirate -1- hospital facilities 33 CFU/ml .

The total numbers of bacterial species in the 12 residential water samples collected from each Emirate were; 48, 40, 39 and 32 in Emirates 1, 2, 3 and 4 respectively. Emirate-1- is 8 bacterial species content higher than Emirate-2 (16.7%) and 9 bacterial species content higher than Emirate-3 (18.8%) and 16 bacterial species content higher than Emirate-4 (33.3%).

In the Mosque water samples are 10/18 sampls , 36/18 sampls, 53/18 sampls and 42/18 sampls for emirates 1, 2, 3 and 4 respectively. Emirate-3- is 43 bacterial species content higher than Emirate-1 (81%) and Emirate-4 is 32 bacterial species content higher than Emirate-1 (60%) and Emirate-2 is 26 bacterial species content higher than Emirate-1 (49%).

In the Hospital water samples are 33/18 sampls , 39/18 sampls, 38/18 sampls and 44/18 sampls for emirates 1, 2, 3 and 4 respectively. Emirate-4- is 11 bacterial species content higher than Emirate-1 and Emirate-2 is 6 bacterial species content higher than Emirate-1 and Emirate-3 is 5 bacterial species content higher than Emirate-1.

Chemical analysis of the same water samples from the 4 Emirates was performed to measure the levels of the following ions: Phosphates, Floride, bromate, chloride, chlorate, Bromide, nitrate, sulphate, total dissolved solids, and carbonates. All parameters of water samples from Emirate -1-, Emirate -2-, Emirate -3- ,and Emirate -4- fall within the U.A.E. standard and or the U.K. standards. except Bromide in all Emirates are higher than the allowable limits of the UAE & UK standards

#### 2.3.2.1 Emirate 1

The microbial contents of all samples are summarised in (Table 2.7 and Table 2.8). All samples were filtered, and membranes were applied onto the surface of Plate Count Agar (PCA.). Plates were incubated for 24 hours. All samples appeared to be mixed with various bacterial species of bacteria. Results of testing samples from Emirate -1- for microbial contents at 30/37˚C indicated that 100% of water samples were contaminated with bacteria except for mosque water cooler samples which show 0% contamination .

The sampling site ‘mosque water cooler’ which had the lowest TDS of 134.67 mg/l (Table 2.3) was seen to have the least number of identified bacterial species (0) compared to other sampling sites (Table 2.7). On the other hand, sampling site ‘residential tap’ which had the highest TDS in Emirate -1- (Table 2.3) was seen to have > 10 identified bacterial species (Table 2.7).

Table 2. 7 : Numbers of Bacteria species in water samples of Emirates -1- in winter and summer

| Locations in Emirate -1- | Number of species in winter | Number of species in Summer | Total number of species | Mean number of species | SD | SE |
|---|---|---|---|---|---|---|
| Residential tank | 10 | 13 | 23 | 11.5 | 2.1 | 1.5 |
| Residential tap | 3 | 8 | 11 | 5.5 | 3.5 | 2.5 |
| Residential water cooler | 7 | 7 | 14 | 7 | 0.0 | 0.0 |
| Mosque tank | 4 | 4 | 8 | 4 | 0.0 | 0.0 |
| Mosque tap | 2 | 2 | 4 | 2 | 0.0 | 0.0 |
| Mosque water cooler | 0 | 0 | 0 | 0 | 0.0 | 0.0 |
| Hospital tank | 3 | 6 | 9 | 4.5 | 2.1 | 1.5 |
| Hospital tap | 6 | 6 | 12 | 6 | 0 | 0 |
| Hospital water cooler | 8 | 4 | 12 | 6 | 2.8 | 2 |
| **Total** | **45** | **50** | | **5.2** | | |

able 2. 8 : Bacterial species in water samples of Emirates -1- in Winter and Summer

| Locations in Emirates -1- | Species | |
|---|---|---|
| | Winter | Summer |
| Residential tank | *Kocuria kristinae* | *Enterococcus columbae* |
| | *Aeromonas sobria* | *Pantoea spp* |
| | *Gemella bergeri* | *Pasteureeka testudinis* |
| | *Pantoea spp* | *Pseudomonas aeruginosa* |
| | *Pasteurella pneumotropica* | *Rhizobium radiobacter* |
| | *Rhizobium radiobacter* | *Serratia marcescens* |
| | *Sphimgobacterium thalpophilum* | *Serratia plymuthica* |
| | *Sphingomonas paucimobilis* | *Sphingomonas aeruginosa* |
| | *Staphylococcus lentus* | *Sphingomonas paucimobilis* |
| | *Streptococcus parasanguinis* | *Staphylococcus haemolyticus* |
| | | *Staphylococcus lentus* |
| | | *Staphylococcus vitulinus* |
| | | *Streptococcus thoraltensis* |
| Total | 10 | 13 |

| | | |
|---|---|---|
| Residential tap | *Sphingomonas paucimobilis* | *Staphylococcus haemolyticus* |
| | *Staphylococcus haemolyticus* | *Aeromonas salmonicida* |
| | *Staphylococcus lentus* | *Enterococcus casseliflavus* |
| | | *Gemella sanguinis* |
| | | *Serratia plymuthica* |
| | | *Sphingomonas paucimobilis* |
| | | *Staphylococcu sciuri* |
| | | *Staphylococcus lentus* |
| Total | 3 | 8 |
| Residential Water cooler | *Aerococcus viridans* | *Aerococcus viridans* |
| | *Aeromonas salmonicida* | *Pantoea spp* |
| | *Kocuria kristinae* | *Pseudomonas aeruginosa* |
| | *Pantoea spp* | *Sphingomonas aeruginos* |
| | *Staphylococcus haemolyticus* | *Staphylococcus haemolyticus* |
| | *Staphylococcus lentus* | *Staphylococcus lugdunensis* |
| | *Staphylococcus vitulinus* | *Staphylococcus vitulinus* |
| Total | 7 | 7 |
| Mosque tank | *Rhizobium radiobacter* | *Entercoccus casseliflavus* |
| | *Sphingomonas paucimobilis* | *Rhizobium radiobacter* |
| | *Staphylococcus haemolyticus* | *Sphingomonas paucimobilis* |
| | *Staphylococcus lentus* | *Staphylococcus haemolyticus* |
| Total | 4 | 4 |
| Mosque tap | *Staphylococcus thoraltensis* | *Staphylococcus thoraltensis* |
| | *Leuconostos mesenteroides ssp cremoris* | *Leuconostos mesenteroides ssp cremoris* |
| Total | 2 | 2 |
| Mosque Water cooler | 0 | 0 |
| Total | 0 | 0 |
| Hospital tank | *Staphylococcus lentus* | *Aeromonas sobria* |
| | *Kiebsiella pneumoniae ssp pneumoniae* | *Sphingomonas paucimobilis* |
| | *Enterobacter aerogeness* | *Staphylococcus aureus* |
| | | *Staphylococcus lentus* |
| | | *Staphylococcus sciuri* |
| | | *Staphylococcus vitulinus* |
| Total | 3 | 6 |

| | | |
|---|---|---|
| Hospital tap | *Staphylococcus lentus* | *Staphylococcus lentus* |
| | *Aerococcus viridans* | *Aeromonas salmonicida* |
| | *Aeromonas sobria* | *Leuconostos mesenteroides ssp cermoris* |
| | *Sphingomonas paucimobilis* | *Proteus vulgaris* |
| | *Staphylococcus aureus* | *Rhizobium radiobacter* |
| | *Staphylococcus lugdunensis* | *Sphingomonas paucimobilis* |
| Total | *6* | 6 |
| Hospital Water cooler | *Staphylococcus lentus* | *Staphylococcus lentus* |
| | *Aeromonas sobria* | *Staphylococcus vitulinus* |
| | *Pantoea spp* | *Granulicatella adiacens* |
| | *Serratia ficaria* | *Sphingomonas paucimobilis* |
| | *Sphingomonas paucimobilis* | |
| | *Staphylococcus aureus* | |
| | *Staphylococcus gallinarum* | |
| | *Staphylococcus vitulinus* | |
| Total | 8 | 4 |

The common bacteria in Emirate 1 are *Pantoea spp, Aerococcus viridans Leuconostos mesenteroides ssp cremoris , Rhizobium radiobacter ,Sphingomonas paucimobilis ,Staphylococcus haemolyticus ,Staphylococcus lentus ,Staphylococcus thoraltensis , Staphylococcus vitulinus*

#### 2.3.2.2 Emirate 2

The microbial contents of all samples were summarised in (Table 2.9 and Table 2.10). All samples were filtered, and membranes were applied onto the surfaceof plate count Agar PCA. Plates were incubated for 24 hours. All samples appeared to be mixed with various bacterial species . Results of tested samples from Emirates -2- for microbial contents indicated that 100% of water samples were contaminated with bacteria; Microbial contents of drinking water are higher in most samples in summertime than in

wintertime.Mosque tap and Hospital Tap are the highest number of bacteria in summer, Mosque tank and Hospital Water cooler are the lowest number of bacteria in summer. Similar to what was observed in Emirate -1-, sampling site ‘mosque tank’ which had the lowest TDS in Emirate -2- i.e.169.67 mg/l (Table 2.4) was seen to have the lowest number of identified bacterial species (Table 2.9). Although sampling site ‘residential tank’ had the highest TDS of 1430.00 mg/l, (Table 2.4) the number of bacterial species identified (12) there was not the highest; the highest was 18 (Table 2.9).

Table 2. 9 : Numbers of Bacteria species in water samples of Emirate-2- in winter and summer

| Locations in Emirate -2- | Number of species in winter | Number of species in Summer | Total number of species | Mean number of species | SD | SE |
|---|---|---|---|---|---|---|
| Residential tank | 7 | 5 | 12 | 6 | 1.4 | 1.0 |
| Residential tap | 7 | 8 | 15 | 7.5 | 0.7 | 0.5 |
| Residential water cooler | 7 | 6 | 13 | 6.5 | 0.7 | 0.5 |
| Mosque tank | 3 | 3 | 6 | 3 | 0.0 | 0.0 |
| Mosque tap | 9 | 9 | 18 | 9 | 0.0 | 0.0 |
| Mosque water cooler | 5 | 7 | 12 | 6 | 1.4 | 1.0 |
| Hospital tank | 6 | 8 | 14 | 7 | 1.4 | 1.0 |
| Hospital tap | 8 | 9 | 17 | 8.5 | 0.7 | 0.5 |
| Hospital water cooler | 3 | 5 | 8 | 4 | 1.4 | 1.0 |
| **Total** | **55** | **60** | | **6.4** | | |

Table 2. 10 *:* Bacteria species in water samples of Emirates -2- in winter and summer

| Location in Emirate-2- | Species | |
|---|---|---|
| | Winter | Summer |
| Residential tank | *Kocuria kristinae* | *Staphylococcus aureus* |
| | *Staphyloccus pseudintermedius* | *Aeromonas sobria* |
| | *Streptococcus thoraltensis* | *Pantoea spp* |
| | *Pseudomonas aeruginosa* | *Photobacterium damselae* |
| | *Pseudomonas oryzihabitans* | *Staphylococcus haemolyticus* |
| | *Pseudomonas luteola* | |
| | *Cedecea lapagei* | |
| Total | *7* | *5* |
| Residential tap | *Staphylococcus lugdunensis* | *Staphylococcus sciuri* |
| | *Staphylococcus aureus* | *Aeromonas sobria* |
| | *Staphylococcus pseudintermedius* | *Chromobacterium violaceum* |
| | *Staphylococcus haemolyticus* | *Pantoea spp* |
| | *Aeromonas sobria* | *Photobacterium damselae* |
| | *Sphingomonas paucimobilis* | *Sphingomonas paucimobilis* |
| | *Escherichia coli* | *Staphylococcus aureus* |
| | | *Staphylococcus lugdunensis* |
| Total | 7 | 8 |
| Residential water cooler | *Staphylococcus lugdunensis* | *Staphylococcus haemolyticus* |
| | *Granulicatella elegnas* | *Aerococcus viridans* |
| | *Kocuria kristinae* | *Aeromonas sobria* |
| | *Aeromonas sobria* | *Pantoea spp* |
| | *Aeromonas salomicida* | *Staphylococcus aureus* |
| | *Sphingomonas paucimobilis* | *Staphylococcus sciuri* |
| | *Staphylococcus aureus* | |
| Total | 7 | 6 |

| | | |
|---|---|---|
| Mosque tank | *Staphylococcus haemolyticus* | *Staphylococcus lentus* |
| | *Granulicatella adiacens* | *Aerococcus viridans* |
| | *Sphingomonas paucimobilis* | *Serratia plymuthica* |
| Total | 3 | 3 |
| Mosque tap | *Staphylcoccus vitulinus* | *Staphylococcus lentus* |
| | *Staphylcoccus sciuri* | *Enterococcus columbae* |
| | *Staphylcoccus lugdunensis* | *Leuconostoc mesenteroides ssp cermoris* |
| | *Staphylococcus pseudintermedius* | *Pantoea spp* |
| | *Staphylcoccus aureus* | *Sphingomonas paucimobilis* |
| | *Staphylococcous haemoyticus* | *Staphylococcus aureus* |
| | *Aeromonas sobria* | *Staphylococcus pseudintermedius* |
| | *Sphingomonas paucimobilis* | *Staphylococcus sciuri* |
| | *Pseudomonas luteola* | *Staphylococcus vitulinus* |
| Total | *9* | 9 |
| Mosque Water cooler | *Granulicatella elegnas* | *Streptococcus iniae* |
| | *Staphylococcus sciuri* | *Pantoea spp* |
| | *Pseudomonas alcaligenes* | *Serratia marcescens* |
| | *Sphingomonas paucimobilis* | *Sphingomonas paucimobilis* |
| | *Rhizobium radiobacter* | *Staphylococcus aureus* |
| | | *Staphylococcus lentus* |
| | | *Staphylococcus vitulinus* |
| Total | *5* | *7* |
| Hospital tank | *Staphylococcus vitulinus* | *Staphylococcus vitulinus* |
| | *Staphylococcus lentus* | *Aeromonas salmonicida* |
| | *Pantoea spp* | *Enterococcus columbae* |
| | *Klebsiella pneumoniae ssp ozaenae* | *Pantoea spp* |
| | *Sphingomonas paucimobilis* | *Pasteureeka testudinis* |
| | *Aeromonas salmoicida* | *Rhizobium radiobacter* |
| | | *Sphingomonas paucimobilis* |

| | | |
|---|---|---|
| | | *Staphylococcus sciuri* |
| | | |
| Total | 6 | 8 |
| Hospital tap | *Granulicatella elegnas* | *Staphylococcus vitulinus* |
| | *Staphylococcus lentus* | *Aeromonas sobria* |
| | *Aerococcus viridans* | *Pantoea spp* |
| | *Kocuria rhizophila* | *Rhizobium radiobacter* |
| | *Kocuria kristinae* | *Serratia plymuthica* |
| | *Sphingomonas paucimobilis* | *Sphingomonas paucimobilis* |
| | *Pasteurella testudinis* | *Staphylococcus lentus* |
| | *Chromobacterium indologenes* | *Staphylococcus sciuri* |
| | | *Staphyococcus lugdunensis* |
| Total | 8 | 9 |
| Hospital Water cooler | *Staphylococcus vitulinus* | *Staphylococcus vitulinus* |
| | *Kocuria kristinae* | *Aeromonas sobria* |
| | *Pseudomonas aeruginosa* | *Enterococcus columbae* |
| | | *Sphingomonas paucimobilis* |
| | | *Staphylococcus aureus* |
| Total | 3 | 5 |

The common bacteria in Emirate 2 are : *Aeromonas sobria,Pantoea spp, Sphingomonas paucimobilis, Staphylcoccus aureus, Staphylcoccus sciuri, Staphylcoccus vitulinus, Staphylococcus aureus, Staphylococcus lentus,Staphylococcus lugdunensis, Staphylococcus pseudintermedius and Staphylococcus vitulinus .*

### 2.3.2.3 Emirate 3

The microbial contents of all samples were summarised in (Table 2.11 and Table 2.12). Results of testing samples from Emirate – 3 for microbial contents indicated that all water samples are contaminated with bacteria at winter and summer .Microbial contents of drinking water are higher in most of samples in winter time than in the summer time, Mosque Water cooler has the highest number of bacteria and Hospital tank has the lowest number of bacteria in winter, additionally , Mosque tank has the highest number

of bacteria in Summer time and Residential Tank, tap ,and Hospital tap, had the lowest number of bacteria in summer.

The results further showed that in Emirate -3-, sampling site ‘mosque tank’ which had the least TDS of 1.62 mg/l (2.5) had the highest number of identified bacterial species (21) compared to other sampling sites (Table 2.11). Also, it was seen that a total of 12 bacterial species were identified in sampling site ‘hospital tap’ (Table 2.11) which had the highest TDS of 3080.00 mg/l.

Table 2. 11 *:* Numbers of Bacteria species in water samples of Emirate-3- winter and summer

| Locations in Emirate -3- | Number of species in winter | Number of species in Summer | Total number of species | Mean number of species | SD | SE |
|---|---|---|---|---|---|---|
| Residential tank | 7 | 4 | 11 | 5.5 | 2.1 | 1.5 |
| Residential tap | 9 | 4 | 13 | 6.5 | 3.5 | 2.5 |
| Residential water cooler | 10 | 5 | 15 | 7.5 | 3.5 | 2.5 |
| Mosque tank | 9 | 12 | 21 | 10.5 | 2.1 | 1.5 |
| Mosque tap | 6 | 10 | 16 | 8 | 2.8 | 2.0 |
| Mosque water cooler | 11 | 5 | 16 | 8 | 4.2 | 3.0 |
| Hospital tank | 5 | 9 | 14 | 7 | 2.8 | 2.0 |
| Hospital tap | 8 | 4 | 12 | 6 | 2.8 | 2.0 |
| Hospital water cooler | 6 | 6 | 12 | 6 | 0.0 | 0.0 |
| **Total** | **71** | **59** | | **7.2** | | |

Table 2. 12 : Bacteria species in water samples of Emirates -3- in winter and summer

| Locations in Emirate-3- | Species | |
|---|---|---|
| | Winter | Summer |
| Residential tank | *Cedecea lapagei* | *Enterococcus faecium* |
| | *Chromobacterium violaceum* | *Pantoea spp* |
| | *Psedomonas luteola* | *Serratia rubidaea* |
| | *Sphingomonas paucimobilis* | *Streptococcus thoraltensis* |
| | *Staphylococcus aureus* | |
| | *Staphylococcus lugdunensis* | |
| | *Staphylococcus pseudintermedius* | |
| Total | 7 | 4 |
| Residential tap | *Chromobacterium violaceum* | *Streptococcus thoraltensis* |
| | *Escherichia coli* | *Entrobacter aerogenes* |
| | *Francisella tularensis* | *Klebsiella pneumoniae ssp pneumoniae* |
| | *Psedomonas aeruginosa* | *Serratia rubidaea* |
| | *Pseudomonas oryzihabitans* | |
| | *Staphylococcus aureus* | |
| | *staphylococcus haemolyticus* | |
| | *Staphylococcus lugdunensis* | |
| | *Staphylococcus pseudintermedius* | |
| Total | 9 | 4 |
| Residential Water cooler | *Escherichia coli* | *Alloiococcus otitis* |
| | *Kocuria kristinae* | *Pantoea spp* |
| | *Photobacterium damselae* | *Pseudomonas aeruginosa* |
| | *Psedomonas luteola* | *Serratia rubidaea* |
| | *Pseudomonas aeruginosa* | *Streptococcus thoraltensis* |
| | *Pseudomonas oryzihabitans* | |
| | *Pseudomonas putida* | |
| | *Sphingomonas paucimobilis* | |
| | *Staphylococcus lugdunensis* | |
| | *Staphylococcus pseudintermedius* | |
| Total | 10 | 5 |
| Mosque tank | *Enterococcus casseliflavus* | *Aeromonas salmonicida* |

| | | |
|---|---|---|
| | *Klebsiella oxytoca* | *Burkholderia mallei* |
| | *Klebsiella pneumoniae ssp pneumoniae* | *Helcococcus kunzii* |
| | *Kocuria varians* | *Leuconostoc mesenteroides ssp cermoris* |
| | *Pasteurella pneumotropica* | *Leuconostoc pseudomesnteroides* |
| | *Psedomonas stutzeri* | *Pantoea spp* |
| | *Rhizobium radiobacter* | *Pasteureeka testudinis* |
| | *Sphingomonas paucimobilis* | *Pseudomonas mendocina* |
| | *Staphylococcus hominis ssp hominis* | *Rhizobium radiobacter* |
| | | *Sphingomonas paucimobilis* |
| | | *Staphylococcus lentus* |
| | | *Staphylococcus vitulinus* |
| | 9 | 12 |
| Mosque tap | *Enterococcus casseliflavus* | *Aerococcus viridans* |
| | *Kkiebsiella pneumoniae ssp ozaenae* | *Helcococcus kunzii* |
| | *Leuconostos pseduomesenteroides* | *Leuconostoc mesenteroides ssp cermoris* |
| | *Pantoea ssp* | *Leuconostoc mesenteroides ssp dextranicum* |
| | *Sphingomonas paucimobilis* | *Pantoea spp* |
| | *Staphylococcus lentus* | *Pseudomonas luteola* |
| | | *Rhizobium radiobacter* |
| | | *Sphingomonas paucimobilis* |
| | | *Staphylococcus lentus* |
| | | *Staphylococcus pseudintermedius* |
| Total | 6 | 10 |
| Mosque water cooler | *Aeromonas salmonicida* | *Leuconostoc mesenteroides ssp cermoris* |
| | *Burkholderia cepacia group* | *Leuconostoc pseudomesnteroides* |
| | *Erysipelothrix rhusiopathiae* | *Pseudomonas oryzihabitans* |
| | *Gardnerella vaginalis* | *Sphingomonas paucimobilis* |
| | *Ochrobacturm anthropi* | *Staphylococcus pseudintermedius* |
| | *Pseudomonas aeruginosa* | |
| | *Pseudomonas fluorescens* | |
| | *Pseudomonas oryzihabitans* | |

| | | |
|---|---|---|
| | *Sphingomonas paucimobilis* | |
| | *Staphylococcus aureus* | |
| | *Staphylococcus vitulinus* | |
| Total | 11 | *5* |
| Hospital tank | *Enterobacter aerogenes* | *Aeromonas salmonicida* |
| | *Klebsiella oxytoca* | *Enterococcus columbae* |
| | *Serratia fonticola* | *Gardnerella vaginalis* |
| | *Staphyloccus lentus* | *Kocuria kristinae* |
| | *Staphyloccus pseudintermedius* | *Pseudomonas oryzihabitans* |
| | | *Sphingomonas paucimobilis* |
| | | *Staphylococcus aureus* |
| | | *Staphylococcus pseudintermedius* |
| | | *Staphylococcus sciuri* |
| | 5 | 9 |
| Hospital tap | *Enterobacter aerogenes* | *Staphylococcus haemolyticus* |
| | *Enterobacter cloacoae complex* | *Enterococcus durans* |
| | *Kiebsiella pneumoniae ssp pneumoniae* | *Pseudomonas luteola* |
| | *Kocuria kristinae* | *Sphingomonas paucimobilis* |
| | *Pseudomonas aeruginosa* | |
| | *Serratia marcescens* | |
| | *Streptococcus parasanguinis* | |
| | *Streptococcus thoraltensis* | |
| Total | 8 | 4 |
| Hospital water cooler | *Burkholderia pseudomallei* | *Lactococcus garvieae* |
| | *Enterobacter aerogenes* | *Pediococcus pantosaceus* |
| | *Rhizobium radiobacter* | *Psedomonas luteola* |
| | *Staphyloccus lentus* | *Sphingomonas paucimobilis* |
| | *Staphyloccus pseudintermedius* | *Staphylococcus haemolyticus* |
| | *Vibrio fluvialis* | *Staphylococcus pseudintermedius* |
| Total | 6 | 6 |

Common bacteria in Emirate 3 are : *Pantoea ssp, Pseudomonas aeruginosa, Pseudomonas oryzihabitans, Rhizobium radiobacter, Sphingomonas paucimobilis Staphylococcus lentus and Staphylococcus pseudintermedius*

#### 2.3.2.4 Emirate 4

The microbial contents of all samples were summarised in (Table 2.13 and Table 2.14). Results of testing samples from Emirates -4- for microbial contents indicated that all water samples were contaminated with bacteria. Microbial contents of drinking water are higher in the wintertime than in the summertime.

Twelve bacterial species were identified in sampling site 'residential water cooler' (Table 2.13) which had the lowest TDS (257.33 mg/l) among the sampling sites in Emirate -4- (Table 2.6). Sampling site 'residential tap' which had the highest TDS of 279.33 mg/l (Table 2.6) was also seen to have 12 bacterial species (Table 2.13).

Table 2. 13 : Numbers of Bacteria species in water samples of Emirate-4- winter and summer

| Locations in Emirate -4- | Number of species in winter | Number of species in Summer | Total number of species | Mean number of species | SD | SE |
|---|---|---|---|---|---|---|
| Residential tank | 5 | 3 | 8 | 4 | 1.4 | 1.0 |
| Residential tap | 5 | 7 | 12 | 6 | 1.4 | 1.0 |
| Residential water cooler | 8 | 4 | 12 | 6 | 2.8 | 2.0 |
| Mosque tank | 6 | 6 | 12 | 6 | 0.0 | 0.0 |
| Mosque tap | 8 | 7 | 15 | 7.5 | 0.7 | 0.5 |
| Mosque water cooler | 8 | 10 | 18 | 9 | 1.4 | 1.0 |
| Hospital tank | 10 | 4 | 14 | 7 | 4.2 | 3.0 |
| Hospital tap | 9 | 7 | 16 | 8 | 1.4 | 1.0 |
| Hospital water cooler | 10 | 4 | 14 | 7 | 4.2 | 3.0 |
| **Total** | **69** | **52** | | **6.7** | | |

Table 2. 14 : Bacteria species in water samples of Emirates -4- in Winter and Summer

| Emirate-4- | Species | |
|---|---|---|
| | Winter | Summer |
| Residential tank | *Kocuria kristinae* | *Staphylococcus aureus* |
| | *Kocuria rhizophila* | *Staphylococcus lugdunensis* |
| | *Pseudomonas aeruginosa* | *Pseudomonas oryzihabitans* |
| | *Sphingomonas paucimobilis* | |
| | *Staphylococcus pseudintermedius* | |
| Total | 5 | 3 |
| Residential tap | *Cedecea lapagei* | *Aeromonas sobria* |
| | *Enterobacter aerogenes* | *Lactococcus garvieae* |
| | *Kocuria kristinae* | *Pantoea spp* |
| | *Serratia marcescens* | *Sphingomonas paucimobilis* |
| | *Staphylococcus chromogenes* | *Staphylococcus pseudintermedius* |
| | *Streptococcus thoraltensis* | *Staphylococcus sciuri* |
| | | *Staphyococcus lugdunensis* |
| Total | 5 | 7 |
| Residential Water cooler | *Burkholderia cepacia group* | *Aeromonas sobria* |
| | *Chromobacterium violaceum* | *Enterococcus faecium* |
| | *Enterobacter cloacae complex* | *Escherichia coli* |
| | *Psedomonas oryzihabitans* | *Staphylococcus lugdunensis* |
| | *Sserratia marcescens* | |
| | *Staphylococcus aureus* | |
| | *Staphylococcus pseudintermedius* | |
| | *Staphylococcus sciuri* | |
| Total | 8 | 4 |
| Mosque tank | *Staphylococcus sciuri* | *Aeromonas sobria* |
| | *Klebsiella pneumoniae ssp rhinoscleromatis* | *Leuconostoc mesenteroides ssp dextranicum* |
| | *Pantoea ssp* | *Pseudomonas luteola* |

| | | |
|---|---|---|
| | *Rhizobium radiobacter* | *Sphingomonas paucimobilis* |
| | *Sphingomonas paucimobilis* | *Staphylococcus aureus* |
| | *Staphylococcus lentus* | *Staphylococcus lugdunensis* |
| Total | 6 | 6 |
| Mosque tap | *Aeromonas sobria* | *Aeromonas sobria* |
| | *Chromobacterium violaceum* | *Kocuria kristinae* |
| | *Gemella sanguinis* | *Pseudomonas luteola* |
| | *Myroides ssp* | *Sphingomonas paucimobilis* |
| | *Rhizobium radiobacter* | *Staphylococcus aureus* |
| | *Sphingomonas paucimobilis* | *Staphylococcus lugdunensis* |
| | *Staphylococcus aureus* | *Staphylococcus sciuri* |
| | *Staphylococcus lentus* | |
| Total | 8 | 7 |
| Mosque Water cooler | *Aeromonas salmoicida* | *Aeromonas sobria* |
| | *Gardnerella vaginalis* | *Enterococcus columbae* |
| | *Gemella sanguinis* | *Enterococcus columbae* |
| | *Globicatella sanguinis* | *Pantoea spp* |
| | *Kocuria kristinae* | *Photobacterium damselae* |
| | *Pseudimonas fluorescens* | *Pseudomonas luteola* |
| | *Sphingomonas paucimobilis* | *Serratia odorifera* |
| | *Staphylococcus pseudintermedius* | *Sphingomonas paucimobilis* |
| | | *Staphylococcus aureus* |
| | | *Staphylococcus lentus* |
| Total | 8 | 10 |
| Hospital tank | *Aeromonas salmoicida* | *Enterococcus columbae* |
| | *Chromobacterium violaceum* | *Kocuria rosea* |
| | *Gemella morbillorum* | *Sphingomonas paucimobilis* |
| | *Lactococcus garvieae* | *Staphylococcus aureus* |
| | *Photobacterium damselae* | |
| | *Sphingobacterium thalpophilum* | |
| | *Sphingomonas paucimobilis* | |
| | *Staphyloccus aureus* | |
| | *Staphylococcus pseudintermedius* | |
| | *Staphylococcus vitulinus* | |
| Total | 10 | 4 |
| Hospital tap | *Chromobacterium violaceum* | *Enterococcus faecium* |

| | | |
|---|---|---|
| | *Delftia acidovorans* | *Pantoea spp* |
| | *Granulicatella elegnas* | *Pseudomonas oryzihabitans* |
| | *Kocuria rhizophila* | *Serratia ficaria* |
| | *Micrococcus luteus aylae* | *Staphylococcus aureus* |
| | *Sphingomonas paucimobilis* | *Staphylococcus lugdunensis* |
| | *Staphylococcus aureus* | *Staphylococcus sciuri* |
| | *Staphylococcus lentus* | |
| | *Staphylococcus pseudintermedius* | |
| Total | 9 | 7 |
| Hospital Water cooler | *Aerococcus viridnas* | *Enterococcus cassliflavus* |
| | *Aeromonas salmonicida* | *Klebsiella pneumoniae ssp ozaenea* |
| | *Neisseria aninaloris/zoodegmatis* | *Lactococcus gravieae* |
| | *Pantoea ssp* | *Sphingomonas paucimobilis* |
| | *Sphingomonas paucimobilis* | |
| | *Staphylococccus pseudintermedius* | |
| | *Staphylococcus aureus* | |
| | *Staphylococcus lentus* | |
| | *Staphylococcus lugdunensis* | |
| | *Staphylococcus vitulinus* | |
| Total | 10 | 4 |

Common bacteria in Emirate 4 are: *Sphingomonas paucimobilis and Staphylococcus aureus*

#### 2.3.2.5 Other locations

Isolated bacterial species from water samples are listed below (Tables 2.15 – 2.23). Samples were collected during - winter and summer. Results shows similarities between bacterial counts in water samples at winter and summer bearing in mind that winter time is very mild which might be more convinient for microbial growth than summer time.

Table 2. 15 : Playground pool- Emirate number 2- , Swimming pool number 1 – Emirate number 2, Swimming pool number 2 -Emirates number 2, Swimming pool number 3- Emirate number 2 ,Swimming pool number 1 Emirates number 1- , Swimming pool number 2 Emirates number 1, Playground pool number 3 Emirates number 1).

| Locations | Number of species | | Total |
|---|---|---|---|
| | Winter | Summer | |
| Hot Spring 2 | 7 | 3 | 10 |
| Beach water | 9 | 4 | 13 |
| Water well 1 | 1 | 6 | 7 |
| Water well 2 | 6 | 0 | 6 |
| District cooling | 10 | 6 | 16 |
| Desalination plant 1 | 0 | 0 | 0 |
| Desalination plant -2 | 0 | 0 | 0 |
| Desalination plant -3 | 0 | 0 | 0 |
| Mist Tank -1 | 4 | 5 | 9 |
| Mist Tank -2 | 4 | 10 | 14 |
| Mist Tank -3 | 2 | 9 | 11 |
| Pond-1 | 1 | 7 | 8 |
| Pond-2 | 24 | 5 | 29 |
| Playground pool - Emirates -2 | 7 | 8 | 15 |
| Swimming pool -1 - Emirates -2 | 7 | 8 | 15 |
| Swimming pool - 2 -Emirates -2 | 9 | 9 | 18 |
| Swimming pool- 3 Emirates -2 | 4 | 5 | 9 |
| Swimming pool -1 Emirates -1 | 4 | 4 | 8 |
| Swimming pool -2 Emirates -1 | 2 | 2 | 4 |
| Playground pool - 3 Emirates -1- | 10 | 11 | 21 |

Table 2. 16 *:* Table 2.16: Bacteria species in water samples in winter and summer from Hot Spring 1 and Hot Spring 2 .

| Locations | Species | |
|---|---|---|
| | Winter | Summer |
| Hot Spring 1 | *Enterococcus casseliflavus* | *Staphylococcus pseudintermedius* |
| | *Escherichia coli O157* | *Pediococcus pentosaceus* |
| | *Rhizobium radiobacter* | |
| | *Sphingobacterium thalpophilum* | |
| | *Staphylococcus lentus* | |
| | *Streptococcus iniae* | |
| | *Vibrio fluvialis* | |
| Total | 7 | 2 |
| Hot Spring 2 | *Aeromonas sobria* | *Staphylococcus lentus* |
| | *Enterobacter aerogenes* | *Rhizoblum raduobacter* |
| | *Pseudomonas aeruginosa* | *Serratia plymuthic* |
| | *Sphingomonas paucimobilis* | |
| | *Staphylococcus lentus* | |
| | *Staphylococcus vitulinus* | |
| | *streptococcus thoraltensis* | |
| Total | 7 | 3 |

Table 2. 17: Bacteria species in water samples in winter and summer from Beach water

| Location | Species | |
|---|---|---|
| | Winter | Summer |
| Beach water | *Staphyococcus lugdunensis* | *Alloiococcus otitis* |
| | *Enterobacter gergoviae* | *Francisella tularensis* |
| | *Gemella sanguinis* | *Sphingomonas paucimobilis* |
| | *Kocuria kristinae* | *Staphylococcus pseudintermedius* |
| | *Psedomonas oryzihabitans* | |
| | *Sphingomonas paucimobilis* | |
| | *Sphingomonas paucimobilis* | |
| | *Staphylococcus pseudintermedius* | |
| | *Stenotrophomonas maltophilia* | |
| Total | 9 | 4 |

Table 2. 18: Bacteria species in water samples in winter and summer from Water well 1, Water well 2.

| Location | Species | |
|---|---|---|
| | Winter | Summer |
| Water well 1 | *Streptococcus thoraltensis* | *Aerococcus viridans* |
| | | *Citrobacter sedlakii* |
| | | *Cronobacter sakazakii* |
| | | *Entrobacter aerogenes* |
| | | *Serratia marcescens* |
| | | *Streptococcus thoraltensis* |
| Total | 1 | 6 |
| Water well 2 | *Citrobacter sedlakii* | |
| | *Enterobacter aerogenes* | |
| | *Klebsiella oxytoca* | |
| | *Serratia fonticola* | |
| | *Serratia marcescens* | |
| | *Staphylococcus lentus* | |
| Total | 6 | 0 |

Table 2. 19 Bacteria species in water samples in winter and summer from District cooling

| Location | Species | |
|---|---|---|
| | Winter | Summer |
| District cooling | *Aeromonas salmonicida* | *Aeromonas sobria* |
| | *Aeromonas veronii* | *Pseudomonas luteola* |
| | *Kocuria kristinae* | *Serratia fonticola* |
| | *Pseudomonas aeruginosa* | *Staphulococcus pseudintermedius* |
| | *Pseudomonas luteoia* | *Staphylococcus aureus* |
| | *Pseudomonas oryzihabitans* | *Staphylococcus lugdunensis* |
| | *Serratia odorifera* | |
| | *Staphylococcus pseudintermedius* | |
| | *Staphylococcus schleiferi* | |
| | *Streptococcus thoraltensis* | |
| Total | 10 | 6 |

Table 2. 20: Bacteria species in water samples in winter and summer from Desalination plant 1- ,Desalination plant -2 and Desalination plant-3 .

| Location | Species | |
|---|---|---|
| | Winter | Summer |
| Desalination plant 1- | 0 | 0 |
| Desalination plant -2 | 0 | 0 |
| Desalination plant -3 | 0 | 0 |

Table 2. 21: Bacteria species in water samples in winter and summer from Mist Tank -1- , Mist Tank -2-and Mist Tank -3- .

| Location | Species | |
|---|---|---|
| | Winter | Summer |
| Mist Tank -1- | *Bacillus cereus* | *Aeromonas salmonicide* |
| | *Serratia fonticola* | *Aeromonas sobria* |
| | *Serratia grimesii* | *Pseudomonas luteola* |
| | *Staphylococcus pseudintermedius* | *Staphelococcus paucimobillis* |
| | | *Stenotrophomonas maktophilia* |
| Total | *4* | 5 |
| Mist Tank -2- | *Aeromonas sobria* | *Aeromonas salmonicida* |
| | *Enterobacter aerogenes* | *Aeromonas sobria* |
| | *Staphylococcus hominis ssp hominis* | *Gemella sanguinis* |
| | *Staphylococcus pseudintermedius* | *Kocuria kristinae* |
| | | *Photobacterium damselae* |
| | | *Sphingomonas paucimobilis* |
| | | *Staphelococcus pseudintermedius* |
| | | *Staphylococcus aureus* |
| | | *Staphylococcus lentus* |
| | | *Staphylococcus lugdunensis* |
| Total | 4 | 10 |
| Mist Tank -3- | *Sphingomonas paucimobilis* | *Aeromonas salmonicida* |
| | *Aeromonas salmonicida* | *Leuconostoc mesenteroides ssp cermoris* |
| | | *Pantoea spp* |
| | | *Pseudomonas fluorescens* |
| | | *Pseudomonas luteola* |
| | | *Serratia plymuthica* |
| | | *Sphingomonas paucimobilis* |
| | | *Staphylococcus agalactiae* |
| | | *Staphylococcus aureus* |
| Total | 2 | 9 |

Table 2. 22 : Bacteria species in water samples in winter and summer from Pond-1- , Pond-2-.

| Location | Species | |
|---|---|---|
| | Winter | Summer |
| Pond-1 - | *Aeromonas sobria* | *Staphylococcus lentus* |
| | | *Aeromonas veronii* |
| | | *Kocuria kristinae* |
| | | *Pantoea spp* |
| | | *Pseudomonas aeruginosa* |
| | | *Staphyococcus pseudintermedius* |
| | | *Staphylococcus lentus* |
| Total | 1 | 7 |
| Pond-2 - | *Aeromonas salmoncida* | *Staphylococcus lentus* |
| | *Cronobacter sakazakii* | *Aeromonas salmonicida* |
| | *Enterococcus faecalis* | *Enterococcus casseliflavus* |
| | *Entrococcus casseliflavus* | *Klebsiella pneumoniae spp pneumoniae* |
| | *Gemella sanguinis* | *Leuconostoc mesenteroides ssp cermoris* |
| | *Granulicatella elegans* | |
| | *Kocuria kristinae* | |
| | *Lactococcus gravieae* | |
| | *Pantoea spp* | |
| | *Providencia rettgeri* | |
| | *Pseudomonas luteola* | |
| | *Pseudomonas oryzihabitans* | |
| | *Serratia fonticola* | |
| | *Serratia marcescens* | |
| | *Serratia plymuthica* | |
| | *Sphingomonas paucimobils* | |
| | *Staphylococcus aureus* | |
| | *Staphylococcus haemolyticus* | |
| | *Staphylococcus lentus* | |
| | *Staphylococcus pseudintermedius* | |
| | *Staphylococcus sciuri* | |
| | *Stenotrophomonas maltophilia* | |
| | *Streptococcus agalactiae* | |
| | *Streptococcus thoraltensis* | |
| Total | 24 | 5 |

Table 2. 23: Bacteria species in water samples in winter and summer from Playground pool- Emirates -2-, Swimming pools -1- Emirates -2-, Swimming pools -2- Emirates -2-, Swimming pools-3- Emirates -2-, Swimming pools -1 Emirates -1-, Swimming pools -2 Emirates -1-, Playground pool -3 Emirates -1-

| Location | Number of species | |
|---|---|---|
| | Winter | Summer |
| Playground pool - Emirates -2- | *Aeromonas salmonicida* | *Aeromonas salmonicida* |
| | *Pantoea spp* | *Pantoea spp* |
| | *Rhizobium radiobacter* | *Pseudomonas fluorescens* |
| | *Serratia plymuthica* | *Rhizobium radiobacter* |
| | *Sphingomonas paucimobilis* | *Serratia plymuthica* |
| | *Staphylococcus lentus* | *Sphingomonas paucimobilis* |
| | *Staphylococcus sciuri* | *Staphylococcus lentus* |
| | | *Staphylococcus sciuri* |
| Total | 7 | 8 |
| Swimming pools -1 - Emirates -2- | *Aerococcus viridans* | *Streptococcus uberis* |
| | *Granulicatella elegans* | *Staphylococcus lentus* |
| | *Kocuria kristinae* | *Kocuria kristinae* |
| | *Staphylococcus lentus* | *Granulicatella elegans* |
| | *Streptococcus uberis* | *Aerococcus viridans* |
| | *Aeromonas sobria* | *Sphingomonas paucimobilis* |
| | *Chryseobacterium gleum* | *Chryseobacterium gleum* |
| | | *Aeromonas sobria* |
| Total | 7 | 8 |
| Swimming pools - 2 - Emirates -2- | *Staphylococcus lentus* | *Streptococcus sanguinis* |
| | *Granulicatella elegans* | *Staphylococcus vitulinus* |
| | *Kocuria kristinae* | *Staphylococcus sciuri* |
| | *Staphylococcus sciuri* | *Staphylococcus lentus* |
| | *Staphylococcus vitulinus* | *Kocuria kristinae* |
| | *Streptococcus sanguinis* | *Granulicatella elegans* |

| | | |
|---|---|---|
| | *Serratia marcescens* | *Cedecea davisae* |
| | *Sphingomonas paucimobilis* | *Serratia marcescens* |
| | *Cedecea davisae* | *Sphingomonas paucimobilis* |
| Total | 9 | 9 |
| Swimming pools- 3 Emirates -2- | *Staphylococcus lentus* | *Staphylococcus lentus* |
| | *Aerococcus viridans* | *Aerococcus viridans* |
| | *Sphingomonas paucimobilis* | *Staphylococcus vitulinus* |
| | *Pantoea spp* | *Pantoea spp* |
| | | *Sphingomonas paucimobilis* |
| Total | 4 | 5 |
| Swimming pools -1 Emirates -1- | *Enterococcus faecalis* | *Enterococcus faecalis* |
| | *Staphylococcus lentus* | *Staphylococcus lentus* |
| | *Aeromonas salmonicida* | *Pseudomonas putida* |
| | *Pseudomonas putida* | *Pantoea spp* |
| Total | 4 | 4 |
| Swimming pools -2 Emirates -1- | *Aeromonas sobria* | *Aeromonas sobria* |
| | *Rhizoblum radiobacter* | *Rhizoblum radiobacter* |
| Total | 2 | 2 |
| Playground pool - 3 Emirates -1- | *Sphingomonas paucimobilis* | *Staphulococcus pseudintermedius* |
| | *Staphylococcus aureus* | *Staphylococcus aureus* |
| | *Staphylococcus lentus* | *Staphylococcus haemolyticus* |
| | *Staphylococcus sciuri* | *Staphylococcus lentus* |
| | *Aeromonas sobria* | *Staphylococcus sciuri* |
| | *Chromobacterium violaceum* | *Photobacterium damselae* |
| | *Photobacterium damselae* | *Aeromonas sobria* |
| | *Sphingomonas paucimobilis* | *Chromobacterium violaceum* |
| | *Staphulococcus pseudintermedius* | *Pantoea spp* |
| | | *Photobacterium damselae* |
| | | *Sphingomonas paucimobilis* |
| Total | 10 | 11 |

Results above (Tables 2.7 – 2.23) indicate that all water samples appeared to be contaminated with bacterial species at various levels between 0 CFU/ml to 13 CFU/ml. Table 2.24 shows that Emirate 1 has the highest level of bacterial count in water during summer and winter and Emirate 4 has the lowest bacterial count in winter however Emirate 3 appeared to be having the least amount of bacterial count in summer time.

Table 2. 24 *:* Summary of samples that contains highest number of species in winter season and summer season

| Sample Description | Highest in microbial number of species -Winter | Highest in microbial number of species - Summer |
|---|---|---|
| Residential Tank | Emirate 1 | Emirate 1 |
| Residential Tap | Emirate 3 | Emirate 1 and Emirate 2 |
| Residential Water cooler | Emirate 3 | Emirate 1 |
| Mosque tank | Emirate 3 | Emirate 3 |
| Mosque tap | Emirate 2 | Emirate 3 |
| Mosque Water cooler | Emirate 3 | Emirate 4 |
| Hospital tank | Emirate 4 | Emirate 3 |
| Hospital tap | Emirate 4 | Emirate 2 |
| Hospital Water cooler | Emirate 4 | Emirate 3 |

### 2.3.3. Comparison of means

A comparison of the mean of the numbers of bacterial species identified in the water samples from different sampling sites in Emirate 1 (both winter and summer i.e. 5.2 – Table 2.7) with the mean of the numbers of bacterial species identified in the water samples from different sampling sites in Emirate 2 (both winter and summer i.e. 6.4 – Table 2.9) showed that there was no significant difference in the number of bacterial species identified in both Emirates as the calculated P value was > 0.05 i.e. 0.17 (Table 2.25). Similarly, no significant difference was observed when the mean of the numbers of bacterial species identified in the water samples from different sampling sites in Emirate 1 (both winter and summer i.e., 5.2 – Table 2.7) with the mean of the numbers of bacterial species identified in the water samples from different sampling sites in Emirate 4 (both winter and summer i.e. 6.7 – Table 2.13) as the calculated P value was > 0.05 i.e. 0.11 (Table 2.25). A significant difference was also not observed when the mean of the numbers of bacterial species identified in the water samples from different sampling sites in Emirate 1 (both winter and summer i.e. 5.2 – Table 2.7) was compared with the mean of the numbers of bacterial species identified in the water samples from different sampling sites in Emirate 3 (both winter and summer i.e. 7.2 – Table 2.11) as the calculated P value was > 0.05 i.e. 0.06 (Table 2.25) . When the mean of the numbers of bacterial species identified in the water samples from different sampling sites in Emirate 2 (both winter and summer i.e. 6.4 – Table 2.9) was compared with the mean of the numbers of bacterial species identified in the water samples from different sampling sites in Emirate 3 (both winter and summer i.e. 7.2 – Table 2.11) and that from Emirate 4, no significant difference

was observed as the calculated P value was > 0.05 i.e. 0.16 and 0.34 respectively (Table 2.25) . No significant difference was also seen when the mean number of bacterial species identified in the water samples from different sampling sites Emirate 3 (both winter and summer i.e. 7.2 – Table 2.11) was compared with the mean of the numbers of bacterial species identified in the water samples from different sampling sites in Emirate 4 (both winter and summer i.e. 6.7 – Table 2.13); the calculated P values was > 0.05 i.e. 0.24 (Table 2.25).

Table 2. 25 : Summary of P values calculated from the means of the numbers of bacterial species from different sampling sites in the different Emirates (in winter and summer). P values < 0.05 are considered significant.

| | **P values** | | | |
|---|---|---|---|---|
| | **Emirate -1-** | **Emirate -2-** | **Emirate -3-** | **Emirate -4-** |
| **Emirate -1-** | - | 0.17 | 0.06 | 0.11 |
| **Emirate -2-** | 0.17 | - | 0.16 | 0.34 |
| **Emirate -3-** | 0.06 | 0.16 | - | 0.24 |
| **Emirate -4-** | 0.11 | 0.34 | 0.24 | - |

Further to these, it was seen that the total numbers of isolates identified in Winter from all the sampling sites in Emirates 2 and 3 (45 and 55 respectively) were less than the numbers identified in summer (50 and 60 respectively) (Tables 2.7, 2.9 and 2.26). The reverse was the case with Emirates 3 and 4, as the total numbers of isolates identified in Winter from all the sampling sites in Emirates 3 and 4 (71 and 69 respectively) were more than the numbers identified in summer (59 and 52 respectively) (Tables 2.11, 2.13 and

2.26). Results however showed that there was no significant difference between the total number of bacterial species identified during winter (240) and the total number of bacterial species identified in summer from all the sampling sites (221), in all the Emirates combined (calculated P value = 0.25). Nevertheless, there was considerable variation in the types/composition of bacterial species identified in winter and summer from the different sampling sites in the different Emirates (Tables 2.8, 2.10, 2.12 and 2.14).

Table 2. 26: Summary of the total numbers of bacterial species isolated from the different Emirates. P values < 0.05 are considered significant.

| | Total number of species in winter | Total number of species in summer | **P value** |
|---|---|---|---|
| Emirate -1- | 45 | 50 | **0.25** |
| Emirate -2- | 55 | 60 | |
| Emirate -3- | 71 | 59 | |
| Emirate -4- | 69 | 52 | |
| **Total** | **240** | **221** | |

## 2.4 Discussion

Pathogenic contamination of drinking water results in the most significant health risk to humans, and there have been an incalculable number of disease outbreaks and poisonings throughout the history resulting from exposure to untreated or poorly treated drinking water. United Arab Emirates (UAE) is in no better position than other countries when it comes to waterborne pathogens and diseases. The UAE is relying heavily on the desalination of seawater for human consumption. The desalinated water often results in clean, purified water with acceptable limits of microbial contents as specified by the Regulation & Supervision Bureau (RSB) of Abu Dhabi-UAE. The RSB has specified the allowable biological water contents as follows:

Table 2. 27 *:* Department of Energy of Abu Dhabi (UAE) (2021) -Drinking water microbial parameters showing the allowable limits of microbial contamination. Drinking water should always be free of Algae, Mold, Parasites, Insects, Ovules, Larvae, and Protozoa including Amoeba

| Item | Parameter | Unit of Measurement | Concentration orValue (maximum unless otherwise stated) |
|---|---|---|---|
| 1 | Total coliforms | CFU//100 ml | 0 |
| 2 | *E. coli* or Thermo tolerant Fecal coliform bacteria | CFU/100 ml | 0 |
| 3 | Intestinal *Enterococci* | CFU/100 ml | 0 |
| 4 | *Legionella* | CFU/liter | <1.0 |

A comparison of the UK and UAE standards for drinking water (Archives, 2000), show comparable requirements for bacterial contents in water.

Both components of the Standard Drinking Water Test, i.e. microbiological and chemical quality tests, were conducted in this study. The microbiological test conducted in this study was to identify total number of bacterial species in water. The chemical quality test checks for a range of chemical components; some of them are heavy metals that may pose a risk to human health while others may only affect the taste, odour and appearance of the water (i.e. 'aesthetic' characteristics). The bacterial cultures were only made to test for number of species in each sample regardless of the number of colonies forming units per unit volume. Furthermore, chemical analysis was done to ascertain if detected levels of chemical ions had any influence on microbial diversity.

A clean and treated water supply to each house may be the norm in Europe and North America, but in developing countries, access to both clean water and sanitation are not the rule, and waterborne infections are common. Two and a half billion people have no access to improved sanitation, and more than 1.5 million children die each year from diarrheal diseases (Fenwick, 2006). According to the WHO, the mortality of water associated diseases exceeds 5 million people per year. From these, more that 50% are microbial intestinal infections, with cholera standing out in the first place. In general terms, the greatest microbial risks are associated with ingestion of water that is contaminated with human or animal intestinal waste. Wastewater discharges in fresh waters and costal seawaters are the major source of intestinal microorganisms, including pathogens (Fenwick, 2006, Grabow, 1996, WHO, 2004, WHO 2004) In this study, results showed that all the sampling sites were contaminated with a variety of pathogenic bacterial

species (as detailed in section 2.3.2), an indication that water from these sources are potentially hazardous to health. Unfortunately, insufficient monitoring of contamination in water could be the reason for this finding.

Findings from their study, to assess the effect of TDS in reverse osmosis concerntrate on microbial activity and diversity, led (Choi et al., 2014) to suggest that presence of TDS (i.e. TDS injection into effluent) may have led to diversification of microbial species. However, that kind of suggestion cannot be made from findings from this study as no clear cut relationship was seen between the presence and levels of TDS and bacterial diversity in the sampling sites (sections 2.3.2.1 – 2.3.2.4).

The higher than acceptable levels of TDS in the sampling sites 'residential tank' in Emirate 2 (i.e. 1430.00 mg/l – Table 2.4), 'hospital tank' and 'hospital water cooler' (both in Emirate 3 with TDS of 2790.00 mg/l and 2913.33 mg/l respectively) could be a function of sediment/impurities accumulating over time as a result of irregular/poor maintenance practice. On the other hand, the higher than acceptable TDS level in sampling site 'hospital tap' in Emirate 3 (3080.00 mg/l) may be due to the source of the water flowing through the tap or even the pipes through which the water flows.

Seasonal variation was observed in the bacterial composition/species of the sampling sites in this study (section 2.3.3). This finding is similar to the findings in the study by they studied the microbiomes of a subarctic thermokarst lake and found the microbial communities to be strikingly different in summer and winter. Findings from the study by Wilheim et al., 2014, are also in agreement with the finding from this study; they tested hypotheses concerning seasonal changes in microbial community diversity/production

and found that distinct communities were present in winter and summer samples collected.

Therefore, this finding points to the possibility that the weather might have an effect on the physicochemical properties of the sampling sites used/involved in this study, which in turn determined the types of bacteria that could survive under the prevailing conditions per time. Findings from this study also showed that there are no significant differences between the numbers of species identified in all the sampling sites from the different Emirates (in both winter and summer) (Section 2.3.3; Table 2.26).

Bromate is a disinfection by-product mainly generated from the oxidation of bromide during the ozonation and disinfection process in order to remove pathogenic microorganism of drinking water (Kim et al., 2020). The International Agency for Research of Cancer (IARC) specifies that the concentration of bromate in drinking water and mineral water must be monitored as it is a potential human carcinogen(Kim et al., 2020). Results from this study showed that the levels of Bromate detected in all water samples in Emirate 1 were higher (in the range of 0.06 – 0.09 mg/l) that the acceptable limits set in both the UAE and UK standards (0.01 mg/l in both cases) (Table 2.3).

The bromate levels in the sampling sites in Emirate 2 were below detection limits, moreover a few sampling sites in both Emirates 3 (1 sampling site) and 4 (3 sampling sites) had bromate limits above the limit set in both the UAE and UK standards (0.01 mg/l in both cases) (Tables 2.4 – 2.6)(Alomirah et al., 2020), in their study to determine the occurrence of bromate in desalinated drinking water and groundwater from Kuwait, also detected bromate in higher than acceptable standard limits in the water samples (i.e. tap water samples).

They were able to conclude that desalinated water was a source of bromate exposure. In all four (4) Emirates involved in this study, water is also sourced from desalination plants; so it is possible that the desalination plant(s) that supplies water in Emirate 1 is/are responsible for the higher than acceptable limits of bromate in the water samples from this Emirate. Thus, factors unique to the different Emirates (the desalination plants) may have played a role in the differences observed in the levels of bromate from the sampling sites (Hautman and Munch, 1997).

In conclusion, the presence of bacterial, as well as chemical contaminants, in the water samples from different sampling sites in different locations in this study shows the potentially hazardous nature of water from these sites. Water authorities in the UAE need to therefore implement improved surveillence and other precautionary measures.

# Chapter 3

# *Acanthamoeba* Identification in UAE water

## 3.1 Introduction

*Acanthamoeba* is an anaerobic, containing mitochondria, eukaryotic protist that is ubiquitous in the environment. It was given its name because of the spine-like structures on its surface, "Acantha" is the Greek word for spiky, spiny, thorny, etc. Originally observed in the 1600s by Antonio van Leeuwenhoek, it was not until the late 1900s that *Acanthamoeba* was conclusively linked to diseases in humans, including *Acanthamoeba keratitis* (AK) and Granulomatous Amebic Encephalitis (GAE) (Jager and Stamm, 1972, Nagington et al., 1974). The confusion between different free-living amoeba resulted from both organisms having similar life cycles and plastic morphology. A bacterivore, *Acanthamoeba,* contributes to the microbial community by releasing bacterial biomass back into the environment. In the rhizosphere (the zone of soil surrounding a plant root where the biology and chemistry of the soil are influenced by the root), it is thought to play a role in enhancing the bacterial community composition through feeding (Kreuzer et al., 2006). This enhancement improves the root structure of various plants. Additionally, *Acanthamoeba* increases phosphorus uptake in plants as well as liberates nitrogen, which had been previously sequestered in its several preys.

*Acanthamoeba* is found in environments beyond just soil. It has been found in air, sand, fresh and saltwater, glaciers and sandstorm dust. In manmade environments, it has been isolated from swimming pools, tap water, bottled water (Aghajani et al., 2016, Balczun and Scheid, 2017, Delafont et al., 2013, Magnet et al., 2012) showerheads, faucets, and domestic water systems(Al-Malki, 2020, Eftekhari-Kenzerki et al., 2021).

Rarely, *Acanthamoeba* is an opportunistic pathogen of more complex organisms, including birds, fish, dogs, reptiles, and of course, humans (Dykova et al., 1999, Frank and Bosch, 1972, Kent et al., 2011, Visvesvara et al., 2007a). *Acanthamoeba* is able to inhabit all these environments and organisms because of its ability to differentiate reversibly. *Acanthamoeba* has been largely investigated because of its capability to become an opportunistic pathogen of humans. However, the role of *Acanthamoeba* in the environment is often understated. *Acanthamoeba* is accountable for discharging bacterial biomass back into the microbial community. Sampling *Acanthamoeba* in the environment is an excellent way to begin to estimate the diversity of the genus *Acanthamoeba*. An environmental sample (soil, water, etc.) will contain numerous single-celled organisms, which may prevent the isolation of *Acanthamoeba* DNA. In order to end up with a pure culture of *Acanthamoeba* DNA, a single *Acanthamoeba* is removed from plates and allowed to grow clonally. The serial culturing of environmental samples results in a pure culture of *Acanthamoeba*.

As *Acanthamoeba* can live and survive in most environments, it is difficult to identify exactly where *Acanthamoeba*-human interaction occurs. It was believed a long time ago that tap water was an important factor in the aetiology of *Acanthamoeba* keratitis infections (Kilvington et al., 2004). Previous studies have shown the presence of *Acanthamoeba* in domestic water supplies in Japan, Korea, Jamaica, Mexico and the United Kingdom (Bonilla-Lemus et al., 2010, Edagawa et al., 2009, Jeong and Yu, 2005, Kilvington et al., 2004, Lorenzo-Morales et al., 2005).

*Acanthamoeba* is a free-living non-parasitic amoeboid organism that is found in surface and sub-surface waters, soil, dust and air. It can be present in low numbers in healthy

environments but it also can form large populations when certain environmental conditions are met. *Acanthamoeba* is a major concern as it is not only capable to survive under extreme conditions, but also can cause severe immunopathological reactions in the human host. *Acanthamoeba* can cause serious infections, such as encephalitis, and blindness in humans. In addition, they can damage environmentally sensitive areas due to their production of toxic compounds and mainly due to their capacity to act as vectors, enabling the transmission of several known human pathogens, such as viruses of poliovirus and measles. *Acanthamoeba* has also been found to degrade wastewater and pollutants, often leading to the spread of substances through the environment and making them available for human consumption. *Acanthamoeba*, a single-celled organism, is widely studied and has been the subject of numerous research projects over the years. Recent studies have discovered a plethora of information that goes far beyond its basic characteristics. In 2020, a study conducted by researchers at University Libre de Bruxelles revealed that the *Acanthamoeba* species ATCC 30010 could cause irreversible changes in the epithelial cells of the human respiratory tract. This species is common in nature and can be found in soil and water, as well as air samples. In addition, this study adds to the increasing evidence that *Acanthamoeba* is an important pathogen in human respiratory disorders.

Additional studies have been conducted in recent years, investigating the *Acanthamoeba*-host interaction. In 2021, researchers at the University of Barcelona demonstrated the same species' ability to adhere to and consume epithelial cells in a respiratory environment. Furthermore, this study also suggested that molecular mechanisms are involved in *Acanthamoeba*'s ability to cause respiratory infections. This research is

invaluable for understanding how the organism can colonize and cause diseases in humans.

A recent study published by researchers at the University of Manchester has yielded information about the *Acanthamoeba*'s effects on the immune system of humans. The findings suggest that exposure to the organism can reduce the immune response that would normally protect against other pathogens or agents of disease. This would make it easier for the person to suffer from an infection. In addition to this, this study suggests that exposure to *Acanthamoeba* may be a key factor in cases of “immune suppression” that have been reported in a variety of contexts.

These findings provide a greater understanding of *Acanthamoeba*'s capabilities and implications, demonstrating how even a single-celled organism can have great effects on human health. Overall, the studies provide invaluable knowledge and should spark further investigations into the organism’s complex interactions and mechanisms.

(Borecka et al., 2020, Chidambaram, Hendiger et al., 2021, Shi et al., 2021, Ting, 2022)

### 3.1.1 *Acanthamoeba* life cycle

*Acanthamoeba* has a biphasic life cycle: mobile trophozoites and dormant cysts. As trophozoites, they are actively feeding by pinocytosis and phagocytosis and prefer to live at interfaces such as soil-water, water-air, etc. (De Moraes and Alfieri, 2008). Under optimal growth conditions, trophozoites will grow and replicate by binary fission. *Acanthamoeba* has no documented sexual reproduction. *Acanthamoeba* attaches to its prey through the Mannose-Binding protein (MBP), which binds to mannosylated glycoproteins on the surface of bacteria (Garate et al., 2004). MBP is thought to be

responsible for all of *Acanthamoeba*'s receptor-mediated endocytosis regardless of its prey. However, the unique features of *Acanthamoeba* are most noted in its alternate form, the cyst (Al-Malki, 2020, Eftekhari-Kenzerki et al., 2021).

### 3.1.2 Encystment

Under strain conditions, the amoeba encloses itself in a double-layered cyst from which it will not appear until conditions are again optimal for reproduction. Encystment is induced by desiccation, starvation, anoxia, osmolarity, extreme pH, temperature, or other environmental stresses (Aksozek et al., 2002, Lloyd et al., 2001) . There are three stages of encystment: pre-encystment, cyst initiation and cyst wall synthesis (Khunkitti et al., 1998). During early stages of encystment, the trophozoites round up and withdraw their pseudopodia (Murti and Shukla, 1984). Trophozoites store glucose in the form of glycogen, which is used to synthesise cellulose (Lorenzo-Morales et al., 2008). Glycogen is the most rapidly-degraded macromolecule during the initial phase of *Acanthamoeba* encystment (Lorenzo-Morales et al., 2008). This glucose is then used for the construction of the cyst walls. There is an 80% decrease in cell volume, and autolysosomes appear to remove mitochondria, excess food and other materials (Tomlinson, 1967). During encystment, there is a loss of DNA, RNA and protein, though encystment is repressed if protein and RNA synthesis are inhibited (Murti and Shukla, 1984). The outer ectocyst, containing an acid-insoluble protein, is synthesised first and is continuous over the entire cell. The endocyst, made of cellulose, is created after the exocyst and becomes attached to the ectocyst at the ostioles, which are closed by an operculum plug(Mattar and Byers, 1971). The mechanism of encystment is not entirely understood but recent studies have found that encystment is dependent on genes responsible for glycogen breakdown,

cellulose synthesis, autophagy proteins and cysteine proteases (Bouyer et al., 2009, Byers et al., 1991, Chambers and Thompson, 1974, Lorenzo-Morales et al., 2008). Little research has been done on the cyst once fully formed, so little is known about how it is able to remain dormant for years or at what level it is still functioning metabolically.

There appears to be an unknown maturation process that occurs over time as newly made cysts are not capable of immediately excysting (Chambers and Thompson, 1974). Several studies have found that the age of the cyst corresponds with its ability to exocyst, which suggests that further mechanisms occur following visible encystment that lends to complete cyst maturation. Cysts can synchronously excyst when kept in cyst form for more than 800 hours (Chambers and Thompson, 1974). When kept in cyst form for less than 800 hours, not all cysts are capable of excystment. As a cyst, *Acanthamoeba* can remain dormant for more than twenty years and retain its ability to excyst and reproduce(Sriram et al., 2008). Cysts are capable of surviving in low pH, UV and gamma radiation, antibiotics and heavy metals (Aksozek et al., 2002). Cysts play a larger role in mammalian infections because they are largely invisible to the immune system.

### 3.1.3 Excystment

Very little is understood about what triggers excystment. While the presence of prey can stimulate excystment, *Acanthamoeba* will not always excyst even under apparent optimal conditions. The process of excystment has been divided into four stages: mature cyst, activated cyst, pre-emergence stage, and emergence(Khan, 2007, Mattar and Byers, 1971). Excystation is dependent on protein and RNA synthesis (Chambers and Thompson, 1974, Murti and Shukla, 1984). However, only very low levels of RNA synthesis occur during excystment, suggesting that the cyst is preloaded mainly with the

necessary RNA needed during excystment(Murti and Shukla, 1984). The first clear sign of excystment is the movement of the trophozoite within the cyst walls. From there, the trophozoite emerges from an ostiole (a small hole or opening through which algae or fungi release their mature spores) from which the operculum (a specialised type of covering that usually protects spores) has been removed. Beyond the operculum, no other parts of the two walls are visually degraded. However, the endo- and ectocyst at the operculum is significantly thinner than elsewhere in the cyst. Following excystment, the empty cyst walls persist in culture and eventually break down (Chambers and Thompson, 1974, Sriram et al., 2008).

### 3.1.4 *Acanthamoeba* and human health (GAE)

In 1972, *Acanthamoeba* was conclusively linked to the Granulomatous Amebic Encephalitis (GAE) (Jager and Stamm, 1972). GAE is a brain infection with near 100% mortality. Its symptoms include headache, fever, aphasia, ataxia, vomiting, intracranial pressures, seizures and eventually death (Jung et al., 2007). GAE results when *Acanthamoeba* crosses the blood-brain barrier and begins to destroy the brain's cells, resulting in brain lesions and ultimately death. GAE appears to occur only in chronically ill or immunocompromised individuals (Khan, 2007) . It is believed that *Acanthamoeba* accesses the brain by either entering the bloodstream through the skin or the lung, then trans versing the Blood-Brain Barrier or by migrating up the nasal route and invading through the olfactory bulb. GAE usually leads to a disseminated infection throughout the body with *Acanthamoeba* pneumonitis in the pulmonary parenchyma, and skin lesions can also be observed (Aichelburg et al., 2008, Martinez and Visvesvara, 1997). While GAE is the most severe disease caused by *Acanthamoeba*, it is also the rarest.

Once the amoebas enter the human body, these organisms can damage organs and tissues, causing a wide range of symptoms (eye infection, skin infection, abscesses, and encephalitis). *Acanthamoeba* infections are difficult to diagnose due to the wide range of symptoms, the difficulty in identifying the organism from clinical samples, and the lack of reliable testing methods.

*Acanthamoeba* infections in humans have been increasingly recognized over the past several decades, as the organism has been found in a wide variety of clinical specimens in patients with a range of diseases. In addition, *Acanthamoeba* species have been associated with cases of fatal meningoencephalitis and keratitis, caused by contact with contaminated material or contact lenses.

In order to prevent *Acanthamoeba* infections in humans, it is important to practice good hygiene. This includes washing the hands frequently, avoiding contact with contaminated water, and wearing contact lenses properly. In addition, patients should not share contact lens care supplies, as this may lead to the transmission of *Acanthamoeba*. Other preventive measures include wearing protective eyewear when swimming, avoiding contact lens wear in bath water, and avoiding use of bath sponges.

*Acanthamoeba* is a potentially dangerous organism which can cause serious health issues in humans if contact is made with contaminated material or contact lenses. It is important to take the necessary precautions to avoid contact with this organism, as prompt diagnosis and treatment is necessary to prevent serious injury or death in some cases.

### 3.1.5 *Acanthamoeba* in water and human health

In 1973, *Acanthamoeba* was linked to an eye infection, *Acanthamoeba* keratitis (AK) (Fredenslund et al., 1975, Nagington et al., 1974). The most prominent infection caused by this organism, AK, is characterised by inflammation of the cornea. *Acanthamoeba* causes cytotoxicity to corneal cells by releasing various cysteine and serine proteases. This results in an intense inflammatory response followed by the necrosis of the stromal cells of the eye. The large number of cysts seen late in *Acanthamoeba* infection suggests that cell-mediated immune response to *Acanthamoeba* is primarily directed towards trophozoites as opposed to cysts, which results in a high incidence of persistent infections (Aksozek et al., 2002). AK is classically identified by a ring in the stroma, which results from neutrophils infiltrating the stroma in an attempt to clear the *Acanthamoeba*. *Acanthamoeba* spp normal habitat is the surface of water source, sand is frequently found in different environments where human can be exposed to amoeba and other protists. The presence of *Acanthamoeba* in water channels/rivers and places such as hospitals, pools, hot springs has a great effect on human health. Contact with contaminated water, wearing of contact lens during evening time, insufficient cleaning of the contact lens, swimming with lens and other kinds of poor lens hygiene are main reasons for AK. The incidence in people using rigid contact lens is 9.5 times lower than for soft contact lens wearers because they have to be removed at night and be cleaned daily(Haddad et al., 2019). In a relevant study *Acanthamoeba* occurrence and its rising tide of *Acanthamoeba* keratitis was studied in New Zealand. 52 patients diagnosed with AK were selected for this study. Demographic and clinical data were collected. Result showed that contact lens (CL) use was noted in 96% of unilateral and 100% of bilateral cases. The mean duration

of symptoms at presentation was 21 days and the mean duration from presentation to definitive diagnosis was 14 days. Diagnosis is often challenging and when delayed is associated with worse outcomes.

In Australia, AK occurs predominantly in contact lens wearers with typical clinical features including epitheliopathy and perineural infiltrates. The patient demographics, frequency of possible inclining factors and clinical presentation of AK were similar to case series from the UK and New Zealand (Höllhumer et al., 2020).

AK is a rare disease in developing countries in comparison with bacterial and fungal keratitis. In this study 43 eyes of 129 patients were examined and found that the left eyes of 55 patients (38.4%) and the right eyes of 60 patients (41.9%) and both eyes of 14 cases (9.7%) were suffering from keratitis.

The outbreak of UK does not appear to be due to any one of the identified risk factors in isolation. Improving contact lenses (CLs) and hand hygiene, avoiding CLs contamination with water and use of effective CL disinfection solutions, or daily disposable CLs, will reduce the incidence of AK. In the longer-term, water avoidance publicity for CL users can be expected to reduce the incidence further (Carnt et al., 2018).

AK can result in blindness if not rigorously treated. *Acanthamoeba* is resistant to most antimicrobials and under certain drug conditions, *Acanthamoeba* will encyst and become dormant within the cornea. This can result in a persistent infection that can last or re-emerge years after the original infection. It is also a major concern in cornea transplants where infected donor corneas can be lost following transplant. Treatment typically of AK includes hourly topical applications of chlorhexidine or polyhexamethylene biguanide, but

a relapse may occur(Padzik et al., 2018). Treatment can last from weeks to months. While AK is exceptionally rare, cases of AK are steadily increasing, the diagnosis of AK is frequently missed and/or delayed. In fact, a delayed diagnosis often allows for deeper corneal involvement with severe sequelae requiring more intensive treatment, including surgery.

AK is a vision-threatening condition that can be challenging to both diagnose and treat. Importantly, a prompt diagnosis reduces the risk of prolonged medical treatment and the need for surgical interventions. Disease severity and the time from symptom onset to diagnosis can predict the duration of treatment, final visual outcomes, and the eventual need for surgery.

In Australia, contact lens users tended to develop AK, which had usual clinical signs like epitheliopathy and perineural infiltrates. In case series from the UK and New Zealand, the patient demographics, occurrence of possible predisposing factors, and clinical presentation of AK were comparable, at University of Iowa, cases of *Acanthamoeba keratitis* have risen over the past 20 years. AK was frequently misdiagnosed in an extensive retrospective analysis, with late diagnosis and high rates of unsuccessful medical therapy. Poorer results were caused by corticosteroid use prior to an AK diagnosis. results highlight the necessity for ophthalmologists to rule out *Acanthamoeba* as a possible cause of contact lens-associated keratitis prior to actually starting topical steroids (Rayamajhee et al., 2022, Scruggs et al., 2022).

AK is most prevalent among contact lens wearers, especially when contact lenses are washed or stored in tap water, The diagnosis of AK is quite often delayed or missed, which has an adverse impact on patient health and living standards while also producing

less favourable final visual results. (Stehr-Green et al., 1989, Varacalli et al., 2021). Bacteria found in tap water create a biofilm on the contact lens and their cases that *Acanthamoeba* (also from tap water) can feed upon. AK is also associated with cornea trauma because injured corneas have increased expression of mannose-containing glycoproteins, which are the primary receptors for *Acanthamoeba* (Jaison et al., 1998) .It is thought that another risk factor is an immune deficiency that may protect most people from AK. Mannosylated glycoproteins that associate with IgA in tears ordinarily may protect against AK. People without these specific antibodies may be at higher risk for AK infection.

Humans are exposed to *Acanthamoeba* consistently. Up to 80% of unaffected individuals have been shown to harbour anti-*Acanthamoeba* antibodies, indicating widespread exposure to these organisms(Alizadeh et al., 2001, Chappell et al., 2001). However, the diagnosis of AK is generally very low. An estimated 700 cases were reported in the United States between 1973 and 1996 (Martinez and Visvesvara, 1997). In histological preparations, the amoebae look very similar to keratoplasty, neutrophils, or monocytes, which can often lead to false negative and/or wrong positive results. It has been assessed that 70% of AK cases are wrongly diagnosed clinically as viral keratitis (Bacon et al., 1993, Goodall et al., 1996, Thebpatiphat et al., 2007).Thus, many patients are initially treated with inappropriate drug therapies. This lag time hampers disease resolution as several studies have found that earlier diagnosis and treatment results in lesser morbidity and healthier visual outcome (Bacon et al., 1993).

*Acanthamoeba* has gained public attention in recent years as a result of an outbreak of *Acanthamoeba* keratitis (AK) in Chicago, Illinois, USA and a subsequent increase in AK

in several other locations (Thebpatiphat et al., 2007). Prior to 2002, Chicago clinicians would document 2-3 cases of AK per year. Since 2003, they have been averaging 30 cases a year. These increases in AK incidences corresponded with EPA-mandated water treatment changes that required US cities to reduce the amount of chlorine by-products in the water. This has potentially allowed the biofilm which *Acanthamoeba* feeds upon to proliferate in the water system. In 2009, the AK outbreak resulted in the voluntary recall of Advanced Medical Optics Complete Moisture Plus lens care solution. However, no solution was found to be contaminate (Verani et al., 2009).

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### 3.1.6 Testing for *Acanthamoeba* by PCR.

Confirmation of *Acanthamoeba* existence by laboratory diagnosis is an important step to study *Acanthamoeba*. Polymerase Chain Reaction (PCR) protocol assays were developed for the detection of genus-specific *Acanthamoeba* DNA. Polymerase Chain Reaction (PCR) has proven itself to be an invaluable tool in identifying *Acanthamoeba*. *Acanthamoeba* in is an ideal candidate for routine PCR testing because it can be a slow growing organism or not grow at all on the non-nutrient agar. PCR technology has become a much broader field since its discovery in 1986 which amplifies a specific region of a DNA strand (the DNA target). Most PCR methods amplify DNA fragments of between 0.1 and 10 kilo base pairs (kbp) in length (Holmgaard et al., 2021, Yera et al., 2021) .

PCR is now popular for detecting genus-specific *Acanthamoeba* DNA.

The assay targeted a portion of the 18S rDNA and was shown to have good amplification efficiency against extracted trophozoites and cysts, showed no non-specific PCR amplification against other free-living amoebae and has sensitivity that could detect as

low as 10 cysts or trophozoites per reaction (Qvarnstrom et al., 2006). They found the amplification efficiency to be appropriate there was no cross-reactivity between the other free-living amoebae and the assay was sensitive, having a limit of detection of less than one trophozoite per total amount of sample processed.

The aim of this study was to estimate the occurrence of the *Acanthamoeba* spp. in chosen water sources in the UAE using molecular techniques. The investigations were also carried out to determine genotypes of the obtained environmental isolates.

## 3.2 Materials and Methods

### 3.2.1 *Acanthamoeba* Isolation

#### 3.2.1.1 Sampling

A total of 171 water samples from 4 Emirates (57 locations in triplicates) were collected from different resources (Figure 2-1) in sterile 1000ml bottles. All samples were then refrigerated in a cool box at a temperature less than 10°C during transportation to the laboratory. All samples arrived at the lab within the same day of collection and were assigned a unique code to keep their identities. The maximum transport time to the laboratory was 3 hours, and samples were processed within 2 hours of receipt at the laboratory. Samples were analysed for physicochemical properties (as described in section 2.2.1.1) as per the standard procedure set in the Standard Methods for the Examination of Water and Wastewater (Rice et al., 2012).

#### 3.2.1.2 Culture

Samples were cultured as described by(Ghaderifar et al., 2018). Briefly, 1000 ml of each water sample was filtered using nitrocellulose membranes, following which the filters were placed directly on non-nutrient agar (NNA) plates. Non-nutrient agar (1.5g Bacto agar powder [Canada Quelab Company] dissolved in 99ml distilled water) was prepared with 1 ml Amoeba Page Saline; each NNA plate was enriched with a 5ml suspension of heat killed Escherichia coli (and allowed to dry before use) which served as food for *Acanthamoeba*. Amoeba Page Saline consists of 2.5 mM NaCl, 1 mM $KH_2PO_4$, 0.5 mM $Na_2HPO_4$, 40 µm $CaCl_2$-$6H_2O$, and 20 µm $MgSO_2$. 7H2O (pH 6.9). Cultured NNA plates were then incubated aerobically at 30°C for one week.

Afterward, plates with filter paper were examined under inverted microscope (200 X) for positive existence of *Acanthamoeba* cells. To isolate pure Cysts/ trophozoites from a mixed culture, cultures were refreshed weekly by removing a one $cm^2$ section of agar from the surface of the plate, containing Cysts/ trophozoites, and placing it face down onto the fresh NNA-*E. coli* plate and incubated.

Sub culturing to maintain the *Acanthamoeba* cell culture was performed by using Peptone Yeast Glucose (PYG) medium in T75 plastic culturing flasks and incubated for at 30˚C, all plates were observed for the occurrence of amoebae.

*Acanthamoeba* was also isolated from water samples through filtering water samples and collecting *Acanthamoeba* directly from the filter paper and then Identification using PCR**.**

### 3.2.2 *Acanthamoeba* Identification

#### 3.2.2.1 Testing for *Acanthamoeba* Morphologically

T75 flasks were examined daily under inverted microscope for developing a confluent single layer growth of *Acanthamoeba* cells.

#### 3.2.2.2 Identification of *Acanthamoeba* using Conventional PCR

##### 3.2.2.2.1 DNA extraction

From all *Acanthamoeba* positive samples, the growing *Acanthamoeba* cells were harvested from culture plates, described in section 3.2.1, and from the filtration membranes. To harvest *Acanthamoeba* from culture plates, 2000 µl of PBS was added to the medium, after which the surface and bottom of agar was scratched with sharp edge to isolate *Acanthamoeba*. The distilled water with a suspension of trophozoites and cysts of *Acanthamoeba* were collected in 1.5 ml micro centrifuge tubes and centrifuged for 10

min at 10000 rpm, to remove additional agar. *Acanthamoeba* cells were also directly harvested from filter papers; and to do this, water samples (1000 ml) were first filtered through nitrocellulose membrane, as previously described in section 3.2.1, following which cells were scratched from the filter paper and collected in Eppendorf tubes. DNA was then extracted from *Acanthamoeba* cells collected in Eppendorf tubes using Chelex resin DNA extraction method described by Iovieno and Miller, 2011. 200 µl Chelex solution (10% [wt/vol]) in 0.1% Triton X-100 and 10 mM Tris buffer [pH 8.0]) was added to a suspension of *Acanthamoeba* cells in PYG broth. This was then vortexed for 10 secs, centrifuged at 10,000 rpm for 10 secs, heated at 95°C for 20 mins in a water bath and then centrifuged at 10,000 rpm for 20 s. The supernatant was the substrate used for PCR.

#### 3.2.2.2.2 Conventional PCR

Conventional PCR was used to confirm the identity of the cells harvested in section 3.2.1 as *Acanthamoeba*. The PCR was done to amplify the *Acanthamoeba*-specific amplimer of the 18S rDNA-gene defined as ASA.S1. Genus-specific primers, i.e. forward primer JDP1 (5'-GGC CCA GAT CGT TTA CCG TGA A-3') and reverse primer JDP2 (5'-TCT CAC AAG CTG CTA GGG AGT CA-3') (Schroeder et al., 2001), were used to amplify the hyper variable diagnostic fragment (≈ 423-551 bp) from the extracted *Acanthamoeba* DNA (section 3.2.2.2.1). All PCR amplification reaction mixtures consisted of 12.5µL Dr. MAX DNA Polymerase (Doctor Protein) PCR master mix (composed of Dr. MAX DNA Polymerase, 10X Dr. MAX buffer 2 mM each dNTPs and GC Solution), 5µL template DNA, and 0.6 mM of each primer; each mix was made up to 25µL with distilled water. All PCR reactions were carried out in a Basic Thermocycler (DNA Engine Tetrad 2 Peltier

Thermal Cycler (BIO-RAD). Amplification conditions were: 5 min pre-heating at 94°C, followed by 35 cycles at 94°C for 30 sec, 52°C for 30 sec, 72°C for 1min, and a final extension for 10 min at 72° C. A cloned ASA.S1 amplicon of a T4 genotype strain was used as a positive control. Amplified DNA was detected by electrophoresis utilizing 1% gels. PCR products were purified using Millipore plate MSNU030. The purified PCR products were then Sanger-sequenced with the BigDye terminator v3.1 sequencing kit and a 3730xl automated sequencer (Applied Biosystems, Foster City, CA). Nucleotide sequences were determined on both strands of PCR amplification products

#### 3.2.2.3 Testing for *Acanthamoeba* using Nested PCR

As a proof of concept, additional PCR protocols, highly sensitive nested PCR, was used (Walochnik et al., 2004). The first PCR step used the primers JDP1 and P3rev 5'-CTA AGG GCA TCA CAG ACC TG-3' (Walochnik et al., 2004). In this PCR about 800 bp fragment was identified, under reaction conditions of 15 min at 95˚C and 30 cycles at 95˚C for 1 min, 52˚C for 2 min, 72˚C for 3 min and final extension for 7 min at 72˚C. The second PCR step was performed with 3µl of the first PCR product respectively, using the primer pair P2fw 5'-GAT CAG ATA CCG TCG TAG TC-3'(Walochnik et al., 2004) and JDP2, amplifying an amplicon of about 180 bp. Reaction conditions were: 15 min at 95˚C, followed by 30 cycles at 95˚C for 1 min, 54˚C for 2 min, 72˚C for 3 min and final extension for 7 min at 72°C. The PCR products were visualized by electrophoresis on 1% agarose gel. PCR amplicons were directly sequenced on both strands. Sequences were edited and aligned using Blast (http://www.ncbi.nlm.nih.gov). To assign the isolates to the

species/genotype level, phylogenetic analysis was performed by comparison of the obtained sequences with those of reference strains.

## 3.3 Results

Polymerase Chain Reaction results has shown the existence of *Acanthamoeba* in water samples taken from different location as shown in (tables 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 3.10). *Acanthamoeba* cells were isolated successfully and identified morphologically, DNA from *Acanthamoeba* was amplified and copied several times by the PCR, and through the electrophoresis it was obvious to isolate the DNA of the *Acanthamoeba* which was sequenced.

*Acanthamoeba* was isolated from water samples of the four Emirates utilizing PCR testing to reveal identity and gene sequence. *Acanthamoeba* was identified by traditional PCR and nested PCR. *Acanthamoeba* was also isolated from environmental water samples through filtering water samples and collecting *Acanthamoeba* directly from the filter paper. *Acanthamoeba* was identified by regular PCR and by nested PCR and it was observed that nested PCR was more effective at amplifying DNA as evidenced in results presented in Tables 3.1 – 3.8.

Table 3. 1: *Acanthamoeba* isolated from Emirates -1- in Winter (1. *Acanthamoeba* was grown on NNA and then Isolated using Conventional PCR, 2. *Acanthamoeba* was grown on NNA and then Isolated using nested PCR,3. *Acanthamoeba* was collected directly from filter paper and then Isolated using Conventional PCR, 4. *Acanthamoeba* was collected directly from filter paper and then Isolated using nested PCR)

| Emirates -1- Winter | 1 *Acanthamoeba* was grown on NNA, | 2 *Acanthamoeba* was grown on NNA, | 3 *Acanthamoeba* result-direct from filter paper, | 4 *Acanthamoeba* result-direct from filter paper | Genotypes |
|---|---|---|---|---|---|
| Residential tank | - | + | - | + | T4 |
| Residential tap | - | + | - | + | T4 |
| Residential water cooler | + | + | + | + | T4 |
| Mosque tank | + | + | - | + | T4 |
| Mosque tap | - | + | - | + | T4 |
| Mosque water cooler | + | + | + | + | T4 |
| Hospital tank | + | + | + | + | T4 |
| Hospital tap | - | + | - | + | T4 |
| Hospital water cooler | - | + | - | + | T4 |

Table 3. 2: *Acanthamoeba* isolated from Emirates -1- in Summer(1. *Acanthamoeba* was grown on NNA and then Isolated using Conventional PCR, 2. *Acanthamoeba* was grown on NNA and then Isolated using nested PCR,3. *Acanthamoeba* was collected directly from filter paper and then Isolated using Conventional PCR, 4. *Acanthamoeba* was collected directly from filter paper and then Isolated using nested PCR)

| Emirates -1- Summer | 1 *Acanthamoeba* was grown on NNA, | 2 *Acanthamoeba* was grown on NNA, | 3 *Acanthamoeba* result-direct from filter paper, | 4 *Acanthamoeba* result-direct from filter paper | Genotypes |
|---|---|---|---|---|---|
| Residential tank | - | + | - | + | T4 |
| Residential tap | - | + | - | + | T4 |
| Residential water cooler | + | + | + | + | T4 |
| Mosque tank | + | + | - | + | T4 |
| Mosque tap | - | + | - | + | T4 |
| Mosque water cooler | + | + | + | + | T4 |
| Hospital tank | + | + | + | + | T4 |
| Hospital tap | - | + | - | + | T4 |
| Hospital water cooler | - | + | - | + | T4 |

Table 3. 3: *Acanthamoeba* isolated from Emirates -2- in Winter (1. *Acanthamoeba* was grown on NNA and then Isolated using Conventional PCR, 2. *Acanthamoeba* was grown on NNA and then Isolated using nested PCR,3. *Acanthamoeba* was collected directly from filter paper and then Isolated using Conventional PCR, 4. *Acanthamoeba* was collected directly from filter paper and then Isolated using nested PCR)

| Emirates -2- Winter | 1 *Acanthamoeba* was grown on NNA, | 2 *Acanthamoeba* was grown on NNA, | 3 *Acanthamoeba* result-direct from filter paper, | 4 *Acanthamoeba* result-direct from filter paper | Genotypes |
|---|---|---|---|---|---|
| Residential tank | - | + | - | + | T4 |
| Residential tap | - | + | - | + | T4 |
| Residential water cooler | + | + | + | + | T4 |
| Mosque tank | + | + | - | + | T4 |
| Mosque tap | - | - | - | - | - |
| Mosque water cooler | + | + | + | + | T4 |
| Hospital tank | - | - | - | - | - |
| Hospital tap | - | + | - | + | T4 |
| Hospital water cooler | - | + | - | + | T4 |

Table 3. 4: *Acanthamoeba* isolated from Emirates -2- in Summer (1. *Acanthamoeba* was grown on NNA and then Isolated using Conventional PCR, 2. *Acanthamoeba* was grown on NNA and then Isolated using nested PCR,3. *Acanthamoeba* was collected directly from filter paper and then Isolated using Conventional PCR, 4. *Acanthamoeba* was collected directly from filter paper and then Isolated using nested PCR)

| Emirates -2- Summer | 1 *Acanthamoeba* was grown on NNA, | 2 *Acanthamoeba* was grown on NNA, | 3 *Acanthamoeba* result-direct from filter paper, | 4 *Acanthamoeba* result-direct from filter paper | Genotypes |
|---|---|---|---|---|---|
| Residential tank | - | + | - | + | T4 |
| Residential tap | - | + | - | + | T4 |
| Residential water cooler | + | + | + | + | T4 |
| Mosque tank | + | + | - | + | T4 |
| Mosque tap | - | - | - | - | - |
| Mosque water cooler | + | + | + | + | T4 |
| Hospital tank | - | - | - | - | - |
| Hospital tap | - | + | - | + | T4 |
| Hospital water cooler | - | + | - | + | T4 |

Table 3. 5: *Acanthamoeba* isolated from Emirates -3 - in Winter (1. *Acanthamoeba* was grown on NNA and then Isolated using Conventional PCR, 2. *Acanthamoeba* was grown on NNA and then Isolated using nested PCR,3. *Acanthamoeba* was collected directly from filter paper and then Isolated using Conventional PCR, 4. *Acanthamoeba* was collected directly from filter paper and then Isolated using nested PCR)

| Emirates -3- Winter | **1** *Acanthamoeba* was grown on NNA, | **2** *Acanthamoeba* was grown on NNA, | **3** *Acanthamoeba* result-direct from filter paper | **4** *Acanthamoeba* result-direct from filter paper | Genotypes |
|---|---|---|---|---|---|
| Residential tank | **-** | **+** | **-** | **+** | T4 |
| Residential tap | **-** | **+** | **-** | **+** | T4 |
| Residential water cooler | **-** | **+** | **+** | **+** | T15 |
| Mosque tank | **-** | **-** | **-** | **-** | - |
| Mosque tap | **-** | **-** | **-** | **-** | - |
| Mosque water cooler | **-** | **+** | **+** | **+** | T4 |
| Hospital tank | **-** | **+** | **-** | **+** | T4 |
| Hospital tap | **-** | **-** | **-** | **-** | - |
| Hospital water cooler | **+** | **+** | **-** | **+** | T4 |

Table 3. 6: *Acanthamoeba* isolated from Emirates -3 -in Summer (A*Acanthamoeba* isolated from Emirates -3 - in Summer (1. *Acanthamoeba* was grown on NNA and then Isolated using Conventional PCR, 2. *Acanthamoeba* was grown on NNA and then Isolated using nested PCR,3. *Acanthamoeba* was collected directly from filter paper and then Isolated using Conventional PCR, 4. *Acanthamoeba* was collected directly from filter paper and then Isolated using nested PCR)

| Emirates -3- Summer | 1 *Acanthamoeba* was grown on NNA, | **2** *Acanthamoeba* was grown on NNA, | **3** *Acanthamoeba* result-direct from filter paper | **4** *Acanthamoeba* result-direct from filter paper | Genotypes |
|---|---|---|---|---|---|
| Residential tank | **-** | **+** | **-** | **+** | T4 |
| Residential tap | **-** | **+** | **-** | **+** | T4 |
| Residential water cooler | **-** | **+** | **+** | **+** | T15 |
| Mosque tank | **-** | **-** | **-** | **-** | - |
| Mosque tap | **-** | **-** | **-** | **-** | - |
| Mosque water cooler | **-** | **+** | **+** | **+** | T4 |
| Hospital tank | **-** | **+** | **-** | **+** | T4 |
| Hospital tap | **-** | **-** | **-** | **-** | - |
| Hospital water cooler | **-** | **+** | **-** | **+** | T4 |

Table 3. 7: *Acanthamoeba* isolated from Emirates -4 - in Winter ( *Acanthamoeba* isolated from Emirates -3 - in Winter (1. *Acanthamoeba* was grown on NNA and then Isolated using Conventional PCR, 2. *Acanthamoeba* was grown on NNA and then Isolated using nested PCR,3. *Acanthamoeba* was collected directly from filter paper and then Isolated using Conventional PCR, 4. *Acanthamoeba* was collected directly from filter paper and then Isolated using nested PCR)

| Emirates -4- Winter | 1 *Acanthamoeba* was grown on NNA, | **2** *Acanthamoeba* was grown on NNA, | **3** *Acanthamoeba* result-direct from filter paper | **4** *Acanthamoeba* result-direct from filter paper | Genotypes |
|---|---|---|---|---|---|
| Residential tank | **-** | **-** | **-** | **-** | - |
| Residential tap | **-** | **-** | **-** | **-** | - |
| Residential water cooler | **-** | **-** | **-** | **-** | - |
| Mosque tank | **-** | **+** | **+** | **+** | T4 |
| Mosque tap | **-** | **-** | **-** | **-** | - |
| Mosque water cooler | **-** | **+** | **-** | **+** | T4 |
| Hospital tank | **-** | **+** | **-** | **+** | T4 |
| Hospital tap | **-** | **-** | **-** | **-** | - |
| Hospital water cooler | **-** | **-** | **-** | **-** | - |

Table 3. 8: *Acanthamoeba* isolated from Emirates -4 -in Summer (*Acanthamoeba* isolated from Emirates -3 - in Winter (1. *Acanthamoeba* was grown on NNA and then Isolated using Conventional PCR, 2. *Acanthamoeba* was grown on NNA and then Isolated using nested PCR,3. *Acanthamoeba* was collected directly from filter paper and then Isolated using Conventional PCR, 4. *Acanthamoeba* was collected directly from filter paper and then Isolated using nested PCR)

| Emirates -4- Summer | 1 *Acanthamoeba* was grown on NNA, | 2 *Acanthamoeba* was grown on NNA, | 3 *Acanthamoeba* result-direct from filter paper | **4** *Acanthamoeba* result-direct from filter paper | Genotypes |
|---|---|---|---|---|---|
| Residential tank | - | - | - | - | - |
| Residential tap | - | - | - | - | - |
| Residential water cooler | - | - | - | - | - |
| Mosque tank | - | + | + | + | T4 |
| Mosque tap | - | - | - | - | - |
| Mosque water cooler | - | + | - | + | T4 |
| Hospital tank | - | + | - | + | T4 |
| Hospital tap | - | - | - | - | - |
| Hospital water cooler | - | - | - | - | - |

Table 3. 9 : Summary of the number of samples collected from different Emirates

| Locations | Number of collected samples | Number of positive samples (Conventional PCR) | Percentage (%) | Number of positive samples (Nested PCR) | Percentage (%) |
|---|---|---|---|---|---|
| Emirates -1 | 18 | 8 | 44 | 18 | 100 |
| Emirates -2 | 18 | 6 | 33 | 14 | 77 |
| Emirates -3 | 18 | 5 | 28 | 12 | 66 |
| Emirates -4 | 18 | 2 | 11 | 6 | 33 |
| Total | 72 | 21 | | 52 | |

Table 3. 10: *Acanthamoeba* isolated from (Hot Spring 1 Hot Spring 2, Beach water ,Water well 1, Water well 2, District cooling ,Desalination plant 1- ,Desalination plant -2 , Desalination plant-3 , Mist Tank -1- , Mist Tank -2- , Mist Tank -3- , Pond-1- , Pond-2- , Playground pool- Emirates -2- , Swimming pools -1 -Emirates -2- , Swimming pools -2 - Emirates -2-, Swimming pools-3- Emirates -2- ,Swimming pools -1 Emirates -1- , Swimming pools -2 Emirates -1- , Playground pool -3 Emirates -1--) in - Winter ( 1. *Acanthamoeba* was grown on NNA and then Isolated using nested PCR, 2. *Acanthamoeba* was collected directly from filter paper and then Isolated using nested PCR, 3. *Acanthamoeba* was collected directly from filter paper and then Isolated using Conventional PCR, 4. *Acanthamoeba* was collected directly from filter paper and then Isolated using nested PCR)

| Locations - Winter | **1** *Acanthamoeba* was grown on NNA, | **2** *Acanthamoeba* was grown on NNA, | **3** *Acanthamoeba* result-direct from filter paper | **4** *Acanthamoeba* result-direct from filter paper | Genotypes |
|---|---|---|---|---|---|
| Hot Spring 1 | **-** | **+** | **-** | **+** | **T4** |
| Hot Spring 2 | **-** | **-** | **-** | **-** | **-** |
| Beach water | **-** | **-** | **-** | **-** | **-** |
| Water well 1 | **-** | **+** | **-** | **+** | **T4** |
| Water well 2 | **-** | **+** | **+** | **+** | **T4** |
| District cooling | **-** | **+** | **+** | **+** | **T4** |
| Desalination plant 1- | **-** | **-** | **-** | **-** | **-** |
| Desalination plant -2 | **-** | **-** | **-** | **-** | **-** |
| Desalination plant -3 | **-** | **-** | **-** | **-** | **-** |
| Mist Tank -1- | **-** | **+** | **-** | **+** | **T4** |
| Mist Tank -2- | **+** | **+** | **+** | **+** | **T4** |
| Mist Tank -3- | **+** | **+** | **-** | **+** | **T4** |
| Pond-1- | **+** | **+** | **+** | **+** | **T4** |
| Pond-2- | **+** | **+** | **+** | **+** | **T4** |
| Playground pool - Emirates -2- | **+** | **+** | **-** | **+** | **T4** |
| Swimming pools -1 - Emirates -2- | **-** | **-** | **-** | **-** | **-** |
| Swimming pools - 2 -Emirates -2- | **-** | **+** | **-** | **+** | **T4** |
| Swimming pools- 3 Emirates -2- | **-** | **+** | **-** | **+** | **T4** |
| Swimming pools -1 Emirates -1- | **+** | **+** | **-** | **+** | **T4** |
| Swimming pools -2 Emirates -1- | | **-** | **-** | **-** | **-** |
| Playground pool - 3 Emirates -1- | **-** | **+** | **-** | **+** | **T4** |

Table 3. 11 : *Acanthamoeba* isolated from (Hot Spring 1 Hot Spring 2, Beach water ,Water well 1, Water well 2, District cooling ,Desalination plant 1- ,Desalination plant -2 , Desalination plant-3 , Mist Tank -1- , Mist Tank -2- , Mist Tank -3- , Pond-1- , Pond-2- , Playground pool- Emirates -2- , Swimming pools -1 -Emirates -2- , Swimming pools -2 - Emirates -2-, Swimming pools-3- Emirates -2- ,Swimming pools -1 Emirates -1- , Swimming pools -2 Emirates -1- , Playground pool -3 Emirates -1--) in Summer (1. *Acanthamoeba* was grown on NNA and then Isolated using Conventional PCR, 2. *Acanthamoeba* was grown on NNA and then Isolated using nested PCR,3. *Acanthamoeba* was collected directly from filter paper and then Isolated using Conventional PCR, 4. *Acanthamoeba* was collected directly from filter paper and then Isolated using nested PCR)

| Locations - Summer | **1** *Acanthamoeba* was grown on NNA, | **2** *Acanthamoeba* was grown on NNA, | **3** *Acanthamoeba* result-direct from filter paper | **4** *Acanthamoeba* result-direct from filter paper | Genotypes |
|---|---|---|---|---|---|
| Hot Spring 1 | **-** | **+** | **-** | **+** | **T4** |
| Hot Spring 2 | **-** | **-** | **-** | **-** | **-** |
| Beach water | **-** | **-** | **-** | **-** | **-** |
| Water well 1 | **-** | **+** | **-** | **+** | **T4** |
| Water well 2 | **-** | **+** | **+** | **+** | **T4** |
| District cooling | **-** | **+** | **+** | **+** | **T4** |
| Desalination plant 1- | **-** | **-** | **-** | **-** | **-** |
| Desalination plant -2 | **-** | **-** | **-** | **-** | **-** |
| Desalination plant -3 | **-** | **-** | **-** | **-** | **-** |
| Mist Tank -1- | **-** | **+** | **-** | **+** | **T4** |
| Mist Tank -2- | **+** | **+** | **+** | **+** | **T4** |
| Mist Tank -3- | **+** | **+** | **-** | **+** | **T4** |
| Pond-1- | **+** | **+** | **+** | **+** | **T4** |
| Pond-2- | **+** | **+** | **+** | **+** | **T4** |
| Playground pool - Emirates -2- | **+** | **+** | **-** | **+** | **T4** |
| Swimming pools -1 - Emirates -2- | **-** | **-** | **-** | **-** | **-** |
| Swimming pools - 2 -Emirates -2- | **-** | **+** | **-** | **+** | **T4** |
| Swimming pools- 3 Emirates -2- | **-** | **+** | **-** | **+** | **T4** |
| Swimming pools -1 Emirates -1- | **+** | **+** | **-** | **+** | **T4** |
| Swimming pools -2 Emirates -1- | | **-** | **-** | **-** | **-** |
| Playground pool - 3 Emirates -1- | **-** | **+** | **-** | **+** | **T4** |

Table 3. 12: Occurrence rate of AC in each water source, 68% of the collected samples were positive to AC.

| Locations | Number of collected samples | Number of positive samples | (%) |
|---|---|---|---|
| Emirates -1 | 18 | 18 | 100 |
| Emirates -2 | 18 | 14 | 77 |
| Emirates -3 | 18 | 12 | 66 |
| Emirates -4 | 18 | 8 | 44 |
| Hot Spring 1 | 2 | 2 | 100 |
| Hot Spring 2 | 2 | 0 | 0 |
| Beach water | 2 | 0 | 0 |
| Water well 1 | 2 | 2 | 100 |
| Water well 2 | 2 | 2 | 100 |
| District cooling | 2 | 2 | 100 |
| Desalination plant 1- | 2 | 0 | 0 |
| Desalination plant -2 | 2 | 0 | 0 |
| Desalination plant -3 | 2 | 0 | 0 |
| Mist Tank -1- | 2 | 2 | 100 |
| Mist Tank -2- | 2 | 2 | 100 |
| Mist Tank -3- | 2 | 2 | 100 |
| Pond-1- | 2 | 2 | 100 |
| Pond-2- | 2 | 2 | 100 |
| Playground pool - Emirates -2- | 2 | 2 | 100 |
| Swimming pools -1 - Emirates -2- | 2 | 0 | 0 |
| Swimming pools - 2 -Emirates -2- | 2 | 2 | 100 |
| Swimming pools- 3 Emirates -2- | 2 | 2 | 100 |
| Swimming pools -1 Emirates -1- | 2 | 2 | 100 |
| Swimming pools -2 Emirates -1- | 2 | 0 | 0 |
| Playground pool - 3 Emirates -1- | 2 | 2 | 100 |
| Total | 117 | 80 | 68 |

## 3.4 Discussion

This is the first investigation in the UAE describing *Acanthamoeba* detection/occurrence in water resources. Morphological characteristics were used to presumptively identify *Acanthamoeba* in this study. Both conventional and nested PCR (as described in sections 3.2.2.2 and 3.2.2.3) were then used for the conclusive identification of the morphologically detected *Acanthamoeba*-like isolates; this grouping based on specific genome sequences is more sensitive and specific than morphological techniques. Amplification was successful in 52 (72%) of the 72 water samples tested using nested PCR compared to 21 positive water samples (29%) based on conventional PCR; this is an indication of the higher sensitivity of nested PCR in amplifying DNA from samples with low number of *Acanthamoeba* cells.

In Emirates 1, nested PCR confirmed the presence of *Acanthamoeba* in all the samples taken from all the sampling sites during winter; it was seen that all the isolates were of the T4 genotype (Table 3.1). These findings were identical for summer (as presented in Table 3.2). Overall, it was seen that all 18 samples collected from the nine sampling sites, were positive for the presence of *Acanthamoeba* (100% occurrence - Table 3.9).

In Emirates 2, nested PCR showed the presence of *Acanthamoeba*, of the T4 lineage, in seven of the nine sampling sites during winter (Table 3.3) and summer (Table 3.4); findings were also identical for samples taken during winter and summer. Altogether, nested PCR showed the presence of *Acanthamoeba* in 14 of the 18 samples collected from the nine sampling sites (77% occurrence - Table 3.9).

For emirates 3, findings were also similar for winter and summer and it was seen that *Acanthamoeba* was present in six of the nine sampling sites. However, unlike Emirates 1

and 3, *Acanthamoeba* belonging to the T15 lineage (one sampling site i.e., residential water cooler) was also isolated in addition to *Acanthamoeba* of the T4 lineage (Tables 3.5 and 3.6). The results showed 66% occurrence of *Acanthamoeba* in the samples collected from the different sampling sites in Emirates 3 i.e., 12 positive samples of the 18 samples collected (Table 3.9).

Similar to all other Emirates, the findings from Emirate 4 are identical during winter and summer (Tables 3.7 and 3.8). However, *Acanthamoeba* was only found to be present in three of the nine sampling sites and they all belonged to the T4 lineage. The lowest level of occurrence of *Acanthamoeba* was seen in this Emirate as only six of the 18 samples (33%) collected were positive for *Acanthamoeba* (Table 3.9).

These findings did not show any seasonal variability in the occurrence of *Acanthamoeba* as occurrence in the winter was similar to occurrence in the summer in all sample's sites for all Emirates (Tables 3.1 – 3.8). This is in agreement with the study by (Carnt et al., 2020) also did not see any significant seasonal variation (in winter compared to summer) in the prevalence of *Acanthamoeba* in domestic tap water in the greater Sydney region of Australia. However unlike findings from this study, studies like that by Kao et al., (2013) have previously reported significant seasonal variation in the prevalence of *Acanthamoeba* in outdoor water bodies; they further reported that seasonal effects influenced the diversity of the isolated *Acanthamoeba* species. Kao et al., (2013) suggested, based on their findings, that seasonal characteristics affected parameters in the studied river. Thus, the absence of seasonal variation in the findings of this study is an indication that physicochemical characteristics, which could influence variation in occurrence, were not affected by the changing seasons.

Of the total 72 samples collected from the four Emirates, 52 (72%) were seen to be positive for *Acanthamoeba*. Only one (≈2%) was of the T15 lineage while the other ≈98% were of the T4 lineage; the T4 lineage is the *Acanthamoeba* genotype most commonly associated with *Acanthamoeba* keratitis (Carnt et al., 2020, Lass et al., 2014). This finding is similar to results from the study by (Koyun et al., 2020) who investigated the occurrence of free living amoeba in water resources (rivers and tap water) in the Black Sea. They found that 98 of 192 water samples collected were positive for *Acanthamoeba* and that the T4 lineage was the predominant genotype among the *Acanthamoeba* (96.94%). Furthermore, in their study to evaluate the seasonal impact of *Acanthamoeba* species in a subtropical watershed, Kao et al., (2013) also found *Acanthamoeba* of the T4 lineage to be the predominant genotype among the identified *Acanthamoeba* species. The high prevalence of the T4 genotype relative to others in these studies as well as findings from this study may be due to the greater virulence and greater transmissibility of this T4 genotype.

The T15 *Acanthamoeba* lineage is also known to be associated with human infections (Castro-Artavia et al., 2017) . Although not the most common cause, T15 *Acanthamoeba* have also been reported to cause *Acanthamoeba* keratitis (Di Cave et al., 2009, Fanselow et al., 2021a) .

Results from this study further showed that no *Acanthamoeba* positive samples were obtained from some sampling sites i.e. Hot Spring 2, Beach water, Desalination Plants 1, 2 and 3, as well as Swimming pool -1- Emirate -2- and Swimming pool -2- Emirates -1- (Table 3.12). Being a protozoan usually found in environmental sources, it is quite unusual that *Acanthamoeba* was not identified from the Hot Spring 2 and Beach water

samples. This absence may be due to the fact that only a few samples were assessed in the course of this study; this also applies to the Desalination plants. Despite being bodies of water liable to contamination, the absence of *Acanthamoeba* in the swimming pool samples, i.e., Swimming pool -1- Emirate -2- and Swimming pool -2- Emirates -1-, may be due to the fact that these particular pools were better treated than the other pools involved in this study.

In conclusion, presence of potentially pathogenic T4 and T15 *Acanthamoeba* in water resources is an indication that more stringent measures are needed for the treatment of the water resources that supplies water for everyday/regular use.

# Chapter 4

# Cytotoxic Effects of Bacteria from Various Water Sources On Human KB Epithelial Cells

## 4.1 Introduction

Worldwide, access to safe drinking water is becoming a major issue and factors such as human contamination, misuse, and overuse of water resources have been identified as the main contributing factors (Shawish et al., 2019). According to the WHO, about 600 million cases of diarrhoea and dysentery and 4600 infant deaths were stated per year because of insufficient sanitation and polluted drinking water (Some et al., 2021). Added to the public health aspects, the safety and quality of coastal and recreational inland waters are of utmost importance to bathers and other users, with a significant impact on the economy, particularly in tourist zones (Rodrigues and Cunha, 2017). In the UAE, where the isolates used in this study originated, water is scarce and contamination of the few existing water resources has been documented; this contamination has been known to cause serious problems (Shawish et al., 2019).

The microbial contamination of water is often of faecal nature related to humans, domesticated animals or wildlife(Jung et al., 2014). Wherever it comes in contact with sewage or human waste from the surrounding, surface water becomes potentially dangerous as a carrier of pathogenic microorganisms; this microbial contamination makes the water unsafe for bathing, swimming, drinking, etc.(Some et al., 2021). The main origins of microbial contamination of natural aquatic resources are discharges of hospitals, water treatment plants, industries considered as point sources, etc. (Jung et al., 2014).

In drinking water, coliform bacteria, such as *E. coli*, can be found commonly, which reach the water from point sources such as sewage discharges and runoff from manure-

fertilized agricultural land. Other bacteria most commonly found in drinking water are *Shigella spp*., *Salmonella spp*., *Vibrio parahaemolyticus*, and *Vibrio Cholerae* (Jung et al., 2014). The presence of bacteria in drinking water poses a significant danger to human health; although many bacteria found in drinking water cause only mild illnesses, bacteria that can cause potentially life-threatening conditions may also be present in the drinking water (Pandey et al., 2014). Enumeration of faecal bacteria in water is important for the development of parameter-based technologies for drinking water quality assessment because existence of these faecal coliform bacteria in a water body specifies that the water has been polluted with faecal substance of warm-blooded animals (Pandey et al., 2014).

The diseases most commonly acquired through contaminated drinking water with bacteria are cholera, gastroenteritis, diarrhoea, *shigellosis*, and *salmonellosis* (Cabral, 2010). Research conducted by Hikal et al. (2013) on drinking water in Egypt showed the presence of bacteria such as *Escherichia coli (E. coli)*, *Klebsiella pneumoniae*, *Kluyvera ascorbata*, *Staphylococcus aureus*, *Salmonella arizonae*, *Proteus mirabilis*, *Enterobacter cloacae*, and *Providencea spp*. alone and in symbiosis with *Acanthamoeba*.

In order to curb/minimise the dangers associated with the use and consumption of contaminated waters, some countries/governments have predefined standards that govern quality of water to be used for different purposes. For example, within the European Union, bathing water quality is regulated under the Bathing Water Directive; the current Bathing Water Directive (2006/7/EC) monitors water quality at designated bathing waters and focuses on the enumeration of faecal indicator organisms (FIO); *Escherichia coli* and Intestinal *Enterococci* (Farrell et al., 2021) .

In the UAE- Abudhabi, the Regulatory and Supervisory Bureau (RSB) for the Electricity and Water sector mandates that the presence of *E. coli* and other coliform organisms, as well as *Clostridium perfringens* (when water originates from or is influenced by surface water) be check monitored in order to determine whether or not water intended for human consumption complies with the parametric values laid down in their regulations (DOE, 2021) .

Bacterial infections are the leading cause of morbidity and mortality worldwide, and bacteria can cause infections in nearly all host tissues (Los et al., 2013). Many diverse bacterial pathogens use common strategies to cause infection and disease; they share common mechanisms in terms of their abilities to adhere, invade and cause damage to host cells and tissues, as well as to survive host defences and establish infection (Wilson et al., 2002). That is to say that bacterial pathogens employ a number of special factors or so-called ‘virulence factors’ during host-pathogen interactions (Niu et al., 2013). Microbial virulence factors encompass a wide range of molecules produced by pathogenic microorganisms (Leitão, 2020) ; conventional virulence factors include secreted proteins, such as protein toxins and enzymes, and cell surface structures such as capsular polysaccharides, lipopolysaccharides and outer membrane proteins, which directly contribute to disease processes (Chen et al., 2005).

Water hygiene is critical and has a major effect on human health; therefore, the toxicity of a group of bacteria, isolated from different water sources, on Human KB Epithelial Cells will be assessed in this chapter.

In addition to the above, this study was conducted to determine the cytotoxic effects of bacterial isolates from various water sources on the ATCC Epithelial Cell Line KB (human

epithelial carcinoma cells) as a model of epithelial cells. Cytotoxicity experiment were performed. The ATCC Epithelial Cell Line KB was used as an in vitro model to assess the cytotoxic effect of the bacterial isolates on the human skin. The toxic effect of the different bacterial strains on KB cells was determined in a colorimetric assay which measures the activity of lactate dehydrogenase-LDH in the supernatants using the Cytotoxicity Detection Kit plus; Roche. As cells get damaged or die off, the amount of LDH in the supernatants increase proportionally, therefore, the number of damaged or lysed cells directly correlates with the amount/colour of formazan formed in the assay. Percentage cytotoxicity was calculated as follows:

$$\left(\%\ Cytotoxicity = \frac{[Sample\ value - \ Low\ control\ value]}{[High\ control\ value - \ Low\ control\ value]} \times 100\right)$$

Results showed that 6 hrs post infection at Multiplicity of Infection (MOI 10:1), the cytotoxic effects of the bacterial isolates on KB cells ranged from 3.84% - 11.33%. *Streptococcus* was seen to have been the most toxic on KB cells at 11.33% toxicity while isolate STAHA was the least toxic at 3.84% toxicity (Table 4.3 and Figure 4.1).

## 4.2 Materials and Methods

All chemicals were purchased from Sigma, unless otherwise stated.

### 4.2.1 Bacterial Isolates

All bacterial isolates used in the course of this study were obtained from various water sources as indicated in Table 4.1. All isolates were grown overnight at 37$^{o}$C on Cysteine Lactose Electrolyte Deficient (CLED) agar under aerobic conditions and refreshed every week. One or two single colonies of bacteria were grown under aerobic conditions in Luria-Bertani (LB) broth for 6 hrs or overnight at 37$^{o}$C as necessary. In addition, the Optical Density (OD) of each broth culture was measured in a spectrophotometer (Cecil CE 2041) at a wavelength of 595 nm and optical density 0.22, 0.3 or 0.35, as well as at wavelength of 600 nm and optical density of 0.8 as appropriate, List of bacteria used in this Chapter is shown in Table 4.1.

Table 4. 1: Information on bacterial isolates used in this study

| **Isolates** | | **Water sources** | **Number of CFU/ml present** | | | |
|---|---|---|---|---|---|---|
| **Abbreviation used in the study** | **Full name of isolate** | | **At $OD_{595}$ 0.22** | **At $OD_{595}$ 0.35** | **At $OD_{595}$ 0.3** | **At $OD_{600}$ 0.8** |
| *E. coli* | *Escherichia coli (-)* | Residential tap, hot springs, residential water cooler, bottled water (gn) | ~$10^8$ | | | |
| *E.coli* ESBL | *Escherichia coli* ESBL | | ~$10^8$ | | | |
| *Klebsiella* | *Klebsiella* sp | Residential tap, pond, bottled water, mosque tank and water cooler, | ~$10^8$ | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | hospital tank and water cooler, water well | | | | |
| *Kleb* ESBL | *Klebsiella* ESBL extended spectrum beta-lactamase | | $\sim10^8$ | | | |
| MSSA | Methicillin susceptible *Staphylococcus aureus* | Bottled water, pond | $\sim10^8$ | | | |
| SERRU | *Serratia rubidaea* | Residential tank, tap and water cooler | $\sim10^8$ | | | |
| ENTAE | *Enterobacter aerogenes* | Pond, water well, hospital tank, tap and water cooler, residential tank, tap | $\sim10^8$ | | | |
| *S. epidermidis* | *Staphylococcus epidermidis* | *Control (gp)* | | $\sim10^8$ | | |
| *Enterococcus* | *Enterococcus* sp. | Residential tank, tap and water cooler, mosque tap, and tank, hospital tank tap and water cooler, recreation, hot spring, pond, bottled water | $\sim10^8$ | | | |
| *B. subtilis* | *Bacillus subtilis* | | | | $\sim10^7$ | |
| *B. cereus* | *Bacillus cereus* | | | | $\sim10^7$ | |
| STRAG | *Streptococcus agalactiae* | Mist, pond | $\sim10^7$ | | | |
| STAHA | *Staphylococcus haemolyticus* | Mist tank, residential tap water cooler and tank, mosque tank, hospital tank tap and water cooler, recreation | $\sim10^7$ | | | |
| *Streptococcus* | *Streptococcus* sp | Residential tap and tank, | | | $\sim10^7$ | |
| STAHO | *Staphylococcus hominis* | Mist, mosque tank, hospital tap | | | | $\sim10^8$ |

### 4.2.2 Cell lines and cell culture

The ATCC Epithelial Cell Line KB (carcinoma cells) was used in the course of this study as an in vitro model to study the cytotoxic effect of the isolates described in Table 4.1 - above with the human skin. The cells were cultured and maintained in Minimum Essential Medium Eagle with L-Glutamine (EMEM). EMEM (catalogue number 12-611Q from BioWhittaker™-Lonza) was supplemented with 10% v/v fetal bovine serum and 1% minimum essential amino acids. Cells were placed into T25 flasks and incubated at 37$^{o}$C in a $CO_2$ incubator (95% humidity, 5% $CO_2$).

### 4.2.3 Cell transferred into a T25 flask

Growth medium was aspirated from confluent flasks and the cells washed once with phosphate-buffered saline (PBS) and 0.5 ml accutase was added into the flask. The flask was rocked for a few seconds and then warmed 1 -2 min at 37$^{o}$C. Once the cells were totally detached from the tissue culture flask, 5 ml of growth medium were added. The content of the flask was subsequently transferred into a 15 ml centrifuge tube which was then centrifuged for 4 min at 1000 rpm. The supernatant was discarded, and the pellet was re-suspended in warm growth medium. Finally, the suspended cells were transferred into other flasks and incubated at 37$^{o}$C in a $CO_2$ incubator (95% humidity, 5% $CO_2$) for 24 hours or till flask is confluent.

### 4.2.4 Seeding cells in 24 well plates

Cells were detached and re-suspended as described in the section above and seeded in 24 well plates. After this, plates were incubated at 37$^{o}$C in a $CO_2$ incubator (95% humidity, 5% $CO_2$) for 24 hours or till flask is confluent.

### 4.2.5 Cytotoxicity assays

The cytotoxic effects of two infectious doses (IDs) i.e. ~$10^6$ and ~$10^7$ CFU of different bacterial strains on KB Epithelial cells were evaluated in this study. Bacteria were added to KB cells, prepared as described in section 4.2.3, at the rate of ~$10^6$ or ~$10^7$ CFU per well which correspond to Multiplicity of Infection (MOIs), which is the number of bacteria per cell at the onset of infection, 10:1 and 100:1 respectively. Control wells free of bacteria, were also set up for this experiment.

### 4.2.6 Infection of KB cells with bacteria

Epithelial Cell Line KB cell line was cultured and grown in 24-well plates in EMEM medium and incubated until 80-100% confluence was attained (there are ~$10^5$ cells in each 80-100% confluent well of a 24-well plate for KB cell line). Growth medium from each confluent well was refreshed, after which bacteria from a 6-hr broth culture was added to each well at the rate of ~$10^6$ or ~$10^7$ CFU per well. This corresponds to MOIs (number of bacteria per cell at the onset of infection) 10:1 and 100:1 respectively. Plates were then incubated at 37$^o$C (in humidified 95% air, 5% $CO_2$) for 40 minutes; after this, supernatants were discarded and each well washed with PBS to remove non-adherent bacteria. After this removal of non-adherent bacteria, the antibiotic Meropenem was added to each well at a final concentration of either 200 µg/ml or 150 µg/ml, as appropriate (Table 4.2), and incubated at 37°C (in humidified 95% air, 5% $CO_2$) for 45 min to kill extracellular bacteria. Supernatant from each well was then discarded and the wells washed again with PBS. Thereafter, 500 µl of medium appropriate for each cell line were added to each well, and plates were then incubated for 24 hrs at 37$^o$C (in humidified 95% air, 5% $CO_2$).

Table 4. 2: Concentration of antibiotic used to kill extracellular bacteria

| Isolates | Concentration of Meropenem (µg/ml) |
|---|---|
| *E. coli* | 150 |
| *E.coli* ESBL | 200 |
| *Klebsiella* | 150 |
| *Kleb* ESBL | 200 |
| MSSA | 150 |
| SERRU | 150 |
| ENTAE | 150 |
| *S. epidermidis* | 150 |
| *Enterococcus* | 150 |
| *B. subtilis* | 150 |
| *B. cereus* | 150 |
| STRAG | 150 |
| STAHA | 200 |
| *Streptococcus* | 150 |
| STAHO | 150 |

After the elimination of un-internalized bacteria (with appropriate antibiotic) and the subsequent addition of medium to each well, plates were incubated for both 6 and 24 hrs at 37$^{o}$C (in humidified 95% air, 5% $CO_2$).

### 4.2.7 Measurement of lactate dehydrogenase

Following the incubations described in the section above, the toxic effect of the different bacterial strains on KB cells was determined in a colorimetric assay which measures the activity of lactate dehydrogenase-LDH in the supernatants using the Cytotoxicity

Detection Kit plus; Roche. The LDH activity in the supernatants is determined by a coupled enzymatic reaction whereby the yellow Iodo-Nitro-Tetrazolium (INT) salt in the dye solution (solution 2) is reduced to red formazan (dye). As cells get damaged or die off, the amount of LDH in the supernatants increase proportionally, therefore, the number of damaged or lysed cells directly correlates with the amount/colour of formazan formed in the assay.

Lysis buffer (5 μl) was added to some control wells to bring about release of a high amount of LDH from the cells; these were the high controls, while the low controls were just cells alone in growth medium. Fifty μl of supernatant were collected from each well and loaded in a fresh 96-well plate. Fifty μl of a reaction mixture (solution 1 + solution 2) were then added to all wells (sample and control) and incubated for 15 min at room temperature. Next, the stop solution was added to all wells to stop all reactions. Lastly, absorbance in each well was read at a wavelength of 595 nm in a FLUO star Omega Multiplate Reader (BMG Labtech). Percentage cytotoxicity was calculated as follows:

$$\left(\%\ Cytotoxicity = \frac{[Sample\ value - \ Low\ control\ value]}{[High\ control\ value - \ Low\ control\ value]} \times 100\right)$$

### 4.2.8 Statistical analysis

Unless otherwise stated every experiment was repeated 3 times. Experimental data were analysed using a one-tailed unpaired t-test (two-sample equal variance) for comparison between means. *P* values (calculated by Microsoft Excel) were considered statistically significant at a 95% confidence interval (i.e., $P < 0.05$).

## 4.3 Results

### 4.3.1 Cytotoxic effects of bacteria on KB cells

#### 4.3.1.1 Cytotoxic effects of bacteria on KB cells 6 hrs post-infection

##### 4.3.1.1.1. Cytotoxic effects at Multiplicity of Infection 10:1

Results from this study showed that 6 hrs post infection at MOI 10:1 the cytotoxic effects of the bacterial isolates on KB cells ranged from 3.84% - 11.33%. *Streptococcus* was seen to have been the most toxic on KB cells at 11.33% toxicity while isolate STAHA was the least toxic at 3.84% toxicity (Table 4.3 and Figure 4.1).

Table 4. 3 : Cytotoxic effects of bacterial isolates on KB cells 6 hrs post-infection at MOI 10:1. The highest and lowest percentages are highlighted yellow and turquoise respectively

| Isolates | Cytotoxic effects (%) |
|---|---|
| *Escherichia coli* | 9.08 |
| *E.coli* ESBL | 4.32 |
| *Klebsiella* | 4.77 |
| *Kleb* ESBL | 4.98 |
| Methicillin susceptible *Staphylococcus aureus* | 5.07 |
| *Serratia rubidaea* | 9.60 |
| *Enterobacter aerogenes* | 8.42 |
| *Staphylococcus epidermidis* | 9.87 |
| *Enterococcus* | 8.46 |
| *Bacillus subtilis* | 6.71 |
| *Bacillus cereus* | 8.76 |
| *Streptococcus agalactiae* | 9.19 |
| *Staphylococcus haemolyticus* | 3.84 |
| *Streptococcus* | 11.33 |

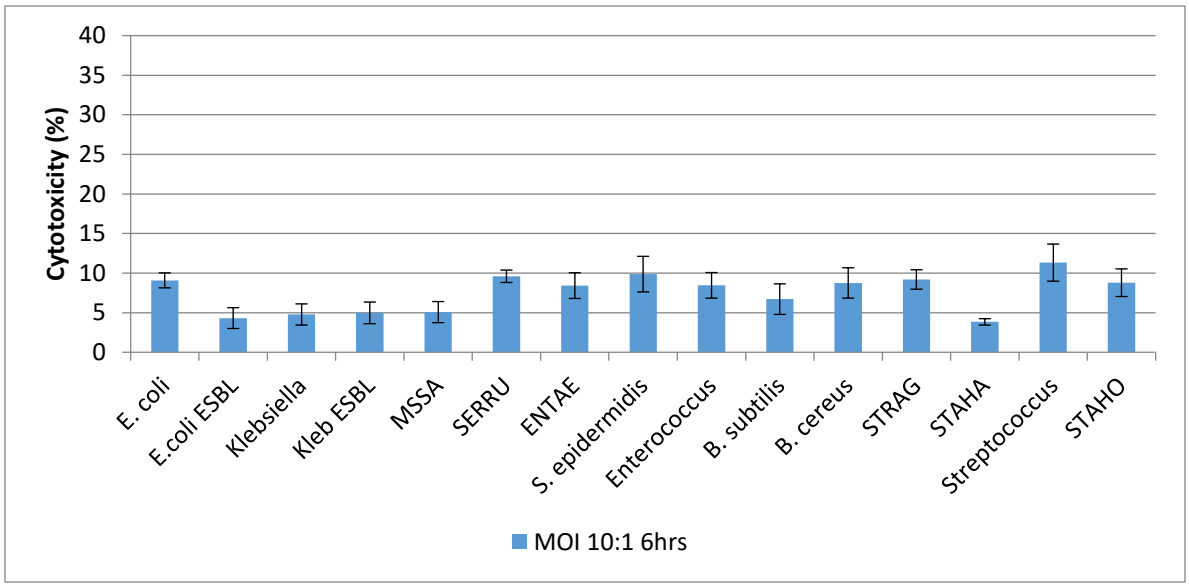


Figure 4.1: Cytotoxic effect of different bacteria, at Multiplicity of Infection 10:1, on KB Epithelial Cell Line 6 hours post-infection. Results are the average of six independent experiments; error bars represent standard error.

#### 4.3.1.1.2. Cytotoxic effects at Multiplicity of Infection 100:1

Findings from this study showed that 6 hrs post-infection at MOI 100:1, the toxic effects of the bacterial isolates on KB cells ranged from 3.49% - 15.89%. Unlike what was seen 6 hrs post-infection at MOI 10:1, it was isolate STAHA and not isolate *Streptococcus* that was most toxic on the KB cells at 15.89% toxicity. Also, isolate *Enterococcus* was the least toxic on KB cells at MOI 100:1, 6 hrs post-infection at 3.49% toxicity unlike the case at MOI 10:1, 6 hrs post-infection, where it was seen that isolate STAHA was the least toxic on KB cells (Table 4.4 and Figure 4.2).

Table 4. 4 : Cytotoxic effects of bacterial isolates on KB cells 6 hrs post-infection at MOI 100:1. The highest and lowest percentages are highlighted yellow and turquoise respectively.

| **Isolates** | **Cytotoxic effects (%)** |
|---|---|
| *E. coli* | 8.83 |
| *E.coli* ESBL | 3.72 |
| *Klebsiella* | 4.79 |
| *Kleb* ESBL | 5.36 |
| MSSA | 4.40 |
| SERRU | 5.03 |
| ENTAE | 5.23 |
| *S. epidermidis* | 5.20 |
| *Enterococcus* | 3.49 |
| *B. subtilis* | 10.04 |
| *B. cereus* | 6.23 |
| STRAG | 14.03 |
| STAHA | 15.89 |
| *Streptococcus* | 10.96 |
| STAHO | 10.15 |

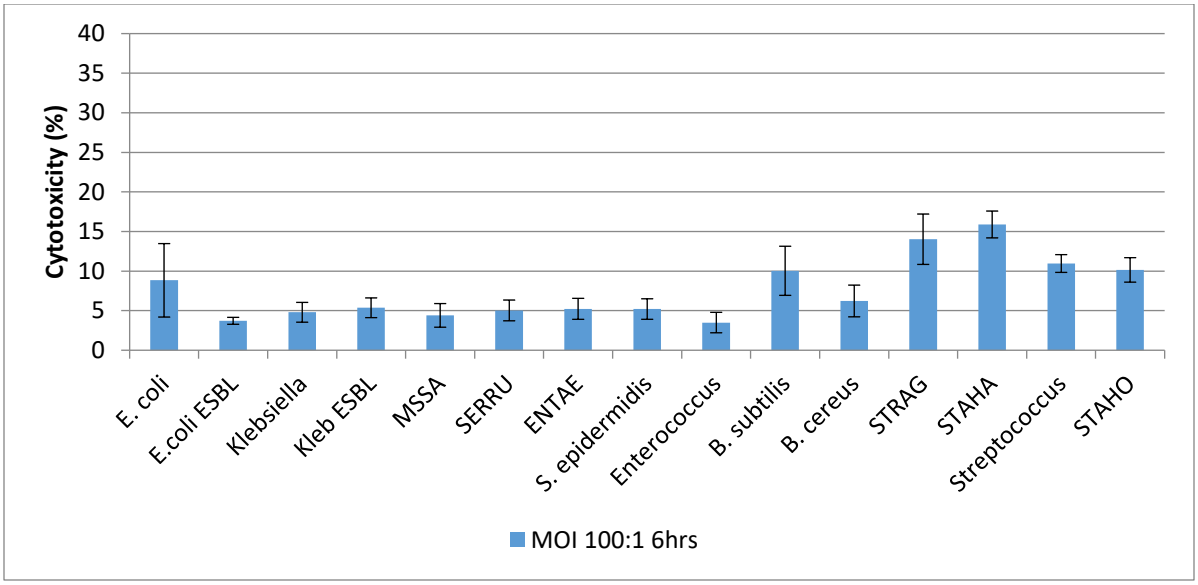


Figure 4.2: Cytotoxic effect of different bacteria, at MOI 100:1, on KB Epithelial Cell Line 6 hours post-infection. Results are the average of six independent experiments; error bars represent standard error.

### 4.3.1.2 Cytotoxic effects of bacteria on KB cells 24 hrs post-infection

#### 4.3.1.2.1. Cytotoxic effects at Multiplicity of Infection 10:1

Results from this study showed that at MOI 10:1, 24 hrs post-infection, the toxic effects of the bacterial isolates on KB cells ranged from 5.21% - 33.73% (Table 4.5 and Figure 4.3). The *Enterococcus* isolate was seen to be the least toxic under this condition 5.21% toxicity unlike the case at MOI 10:1, 6 hrs post-infection where isolate STAHA was seen to be the least toxic on KB cells. Rather isolate STAHA was seen to be the most toxic on KB cells at MOI 10:1, 24 hrs post-infection at 33.73% toxicity which is the opposite of what was observed at MOI 10:1, 6 hrs post-infection.

Table 4. 5 : Cytotoxic effects of bacterial isolates on KB cells 24 hrs post-infection at MOI 10:1. The highest and lowest percentages are highlighted yellow and turquoise respectively.

| **Isolates** | **Cytotoxic effects (%)** |
|---|---|
| *E. coli* | 20.65 |
| *E.coli* ESBL | 9.56 |
| *Klebsiella* | 9.16 |
| *Kleb* ESBL | 8.95 |
| MSSA | 17.39 |
| SERRU | 11.07 |
| ENTAE | 14.11 |
| *S. epidermidis* | 9.27 |
| *Enterococcus* | 5.21 |
| *B. subtilis* | 9.87 |
| *B. cereus* | 13.44 |
| STRAG | 12.41 |
| STAHA | 33.73 |
| *Streptococcus* | 10.45 |
| STAHO | 10.13 |

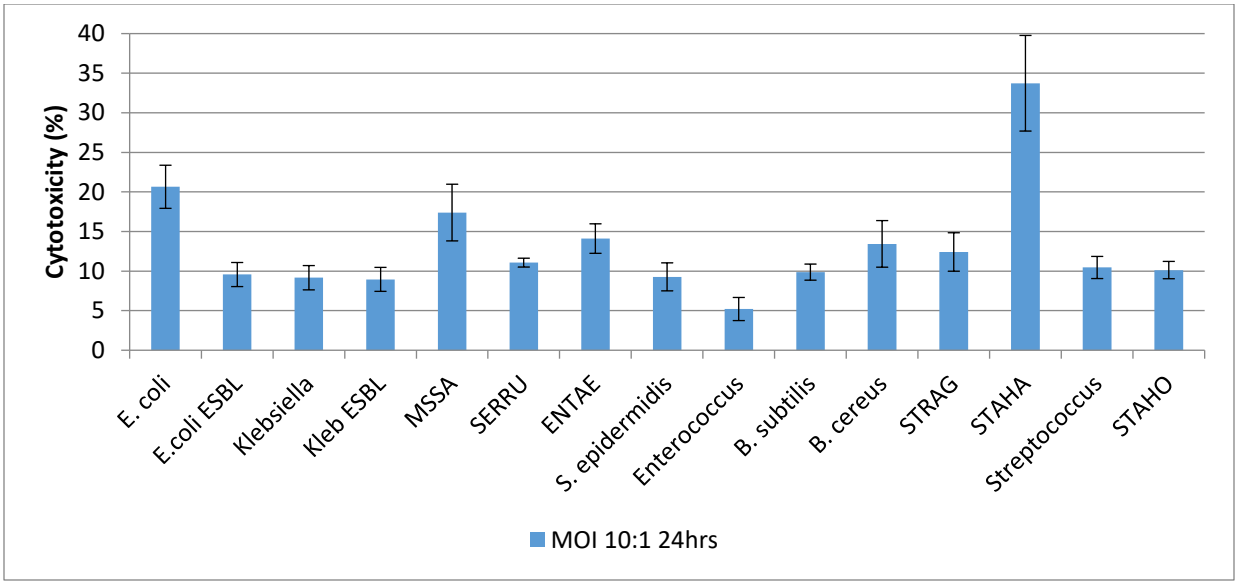


Figure 4.3: Cytotoxic effect of different bacteria, at MOI 10:1, on KB Epithelial Cell Line 24 hours post-infection. Results are the average of six independent experiments; error bars represent standard error.

#### 4.3.1.2.2. Cytotoxic effects at Multiplicity of Infection 100:1

Results from this study showed that the toxic effects of the bacterial isolates on KB cells at MOI 100:1, 24 hrs post-infection ranged from 4.04% - 25.15% as seen in (Table 4.6 and Figure 4.4). As was the case at MOI 10:1, 24 hrs post-infection, isolate STAHA was seen to be the most toxic on KB cells at 25.15% toxicity; however, this is still lower than what was observed at MOI 100:1, 24 hrs post-infection i.e., 33.73% toxicity. This finding is also similar to what was observed at MOI 100:1, 6 hrs post-infection where it was also seen that isolate STAHA was the most toxic on KB cells at 15.89% toxicity. The *B. cereus* isolate was seen to be the least toxic on KB cells at MOI 100:1, 24 hrs post-infection at 4.04% toxicity unlike the case at MOI 10:1, 24 hrs post-infection where isolate *Enterococcus* was seen to be the least toxic on KB cells at 5.21% toxicity.

Table 4. 6 : Cytotoxic effects of bacterial isolates on KB cells 24 hrs post-infection at MOI 100:1. The highest and lowest percentages are highlighted yellow and turquoise respectively.

| **Isolates** | **Cytotoxic effects (%)** |
|---|---|
| *E. coli* | 21.92 |
| *E.coli* ESBL | 9.61 |
| *Klebsiella* | 11.19 |
| *Kleb* ESBL | 13.11 |
| MSSA | 24.15 |
| SERRU | 11.75 |
| ENTAE | 14.95 |
| *S. epidermidis* | 11.23 |
| *Enterococcus* | 6.89 |
| *B. subtilis* | 15.42 |
| *B. cereus* | 4.04 |
| STRAG | 20.03 |
| STAHA | 25.15 |
| *Streptococcus* | 13.77 |
| STAHO | 11.73 |

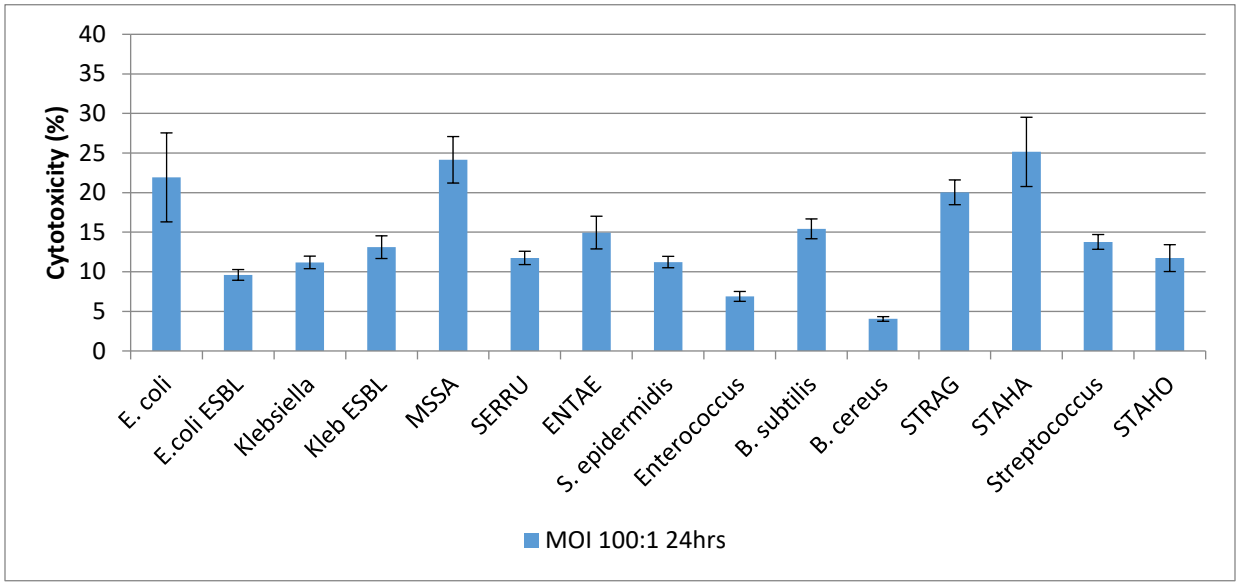


Figure 4.4: Cytotoxic effect of different bacteria, at MOI 100:1, on KB Epithelial Cell Line 24 hours post-infection. Results are the average of six independent experiments; error bars represent standard error.

#### 4.3.1.3 Comparison of means

A comparison of the mean toxic effect of the bacterial isolates on KB cells at MOI 10:1, 6 hrs post-infection with the mean toxic effect at MOI 100:1, 6 hrs post-infection showed that although the mean toxic effect at MOI 100:1 6 hrs post-infection was higher at 7.557%, it was not significantly higher than the mean toxic effect at MOI 10:1 6 hrs post-infection (7.546%) as the calculated *P* value was $\geq$ 0.05 (Table 4.7). Further comparison of the mean cytotoxic effect at MOI 10:1, 24 hrs post-infection with that at MOI 100:1, 24 hrs post-infection showed that there was no significant differences between the means (calculated *P* value was $\geq$ 0.05 (Table 4.7)) even though the mean at MOI 100:1, 24 hrs post-infection was higher compared to that at MOI 10:1, 24 hrs post infection (Table 4.7).

Table 4. 7 : Comparison of the cytotoxic effects of bacterial isolates on KB cells at MOIs 10:1 and 100:1 and at 6 hrs and 24 hrs post-infection. Values of $P < 0.05$ were accepted as significantly different based on the 1-tailed unpaired t-test (two sample equal variance)

| **Isolates** | **Cytotoxicity (%)** | | | | | |
|---|---|---|---|---|---|---|
| | **6hrs** | | | **24hrs** | | |
| | **MOI 10:1** | **MOI 100:1** | | **MOI 10:1** | **MOI 100:1** | |
| *E. coli* | 9.08 | 8.83 | | 20.65 | 21.92 | |
| *E.coli* ESBL | 4.32 | 3.72 | | 9.56 | 9.61 | |
| *Klebsiella* | 4.77 | 4.79 | | 9.16 | 11.19 | |
| *Kleb* ESBL | 4.98 | 5.36 | | 8.95 | 13.11 | |
| MSSA | 5.07 | 4.40 | | 17.39 | 24.15 | |
| SERRU | 9.60 | 5.03 | | 11.07 | 11.75 | |
| ENTAE | 8.42 | 5.23 | ***P* value = 0.496** | 14.11 | 14.95 | ***P* value = 0.293** |
| *S. epidermidis* | 9.87 | 5.20 | | 9.27 | 11.23 | |
| *Enterococcus* | 8.46 | 3.49 | | 5.21 | 6.89 | |
| *B. subtilis* | 6.71 | 10.04 | | 9.87 | 15.42 | |
| *B. cereus* | 8.76 | 6.23 | | 13.44 | 4.04 | |
| STRAG | 9.19 | 14.03 | | 12.41 | 20.03 | |
| STAHA | 3.84 | 15.89 | | 33.73 | 25.15 | |
| *Streptococcus* | 11.33 | 10.96 | | 10.45 | 13.77 | |
| STAHO | 8.79 | 10.15 | | 10.13 | 11.73 | |
| **Mean** | 7.546 | 7.557 | | 13.027 | 14.329 | |

Upon comparison of the means cytotoxic effects of the bacterial isolates at MOI 10:1 6 hrs post-infection with that at MOI 10:1, 24 hrs post-infection, it was seen that the mean cytotoxic effect at MOI 10:1, 24 hrs post-infection (13.027%) was significantly higher than the mean cytotoxic effect at MOI 10:1, 6 hrs post-infection (7.546%); calculated *P* value

< 0.05 (Table 4.8). Similarly, the mean cytotoxic effect of the isolates on KB cells at MOI 100:1 24 hrs post-infection (14.329%) was seen to be significantly higher than the mean cytotoxic effect of the isolates on KB cells at MOI 100:1, 6hrs post-infection (7.557%); calculated *P* value < 0.05 (Table 4.8).

Table 4. 8 : Comparison of the cytotoxic effects of bacterial isolates on KB cells at MOIs 10:1 and 100:1 and at 6 hrs and 24 hrs post-infection. Values of *P* < 0.05 were accepted as significantly different based on the 1-tailed unpaired t-test (two sample equal variance)

| **Isolates** | **Cytotoxicity (%)** | | | | | |
|---|---|---|---|---|---|---|
| | **6 hrs** | **24 hrs** | | **6 hrs** | **24 hrs** | |
| | **MOI 10:1** | **MOI 10:1** | | **MOI 100:1** | **MOI 100:1** | |
| *E. coli* | 9.08 | 20.65 | | 8.83 | 21.92 | |
| *E.coli* ESBL | 4.32 | 9.56 | | 3.72 | 9.61 | |
| *Klebsiella* | 4.77 | 9.16 | | 4.79 | 11.19 | |
| *Kleb* ESBL | 4.98 | 8.95 | | 5.36 | 13.11 | |
| MSSA | 5.07 | 17.39 | | 4.4 | 24.15 | |
| SERRU | 9.6 | 11.07 | ***P* value = 0.003** | 5.03 | 11.75 | ***P* value = 0.001** |
| ENTAE | 8.42 | 14.11 | | 5.23 | 14.95 | |
| *S. epidermidis* | 9.87 | 9.27 | | 5.2 | 11.23 | |
| *Enterococcus* | 8.46 | 5.21 | | 3.49 | 6.89 | |
| *B. subtilis* | 6.71 | 9.87 | | 10.04 | 15.42 | |
| *B. cereus* | 8.76 | 13.44 | | 6.23 | 4.04 | |
| STRAG | 9.19 | 12.41 | | 14.03 | 20.03 | |
| STAHA | 3.84 | 33.73 | | 15.89 | 25.15 | |
| *Streptococcus* | 11.33 | 10.45 | | 10.96 | 13.77 | |
| STAHO | 8.79 | 10.13 | | 10.15 | 11.73 | |
| **Mean** | 7.546 | 13.027 | | 7.557 | 14.329 | |

## 4.4 Discussion

Results obtained from this study showed isolate STAHA (*Staphylococcus haemolyticus*) to be the most toxic on KB epithelial cells as it was seen to exert the highest toxicities at MOI 100:1, 6 hrs post infection (4.3.1.1.2, Table 4.4). at MOI 10:1 24 hrs post infection (4.3.1.2.1, Table 4.5), as well as at MOI 100:1 24 hrs post infection (4.3.1.2.2, Table 4.6).

*Staphylococci* are ubiquitous Gram-positive and non-motile bacteria that colonize mucous membranes of warm-blooded animals and humans (Kloos and Bannerman, 1994, Lowy, 1998) , and can be divided into two major groups according to their ability to coagulate rabbit plasma: coagulase-positive *Staphylococci* (CoPS) and coagulase-negative *Staphylococci* (CoNS). The cell structure of *Staphylococci* makes them highly tolerant to drying, dehydration and low water activity, which explains, in part, their widespread distribution and persistence in the environment Pfennig, (1992)

In addition to being present in soil, air, humans, a variety of animals, as well as in processed food products (such as cheeses or fermented sausages), the presence of *Staphylococci* has also been documented in water (Blaiotta et al., 2004, Iacumin et al., 2006, Irlinger, 2008, Norton and Lechevallier, 2000).

In the past, CoNS were regarded to be non-pathogenic as most of these species can establish a commensal relationship with humans and animals(Lindsay, 2008) (Lindsay, 2008, Victoria et al., 2010) , but this opinion is changing. The few reports of infections published by clinicians and microbiologists before the 1970s considered CoNS to be contaminants of clinical samples (Murray et al., 2007). However, there is now increasing

evidence that some species of CoNS (e.g. *S. capitis, S. cohnii, S.epidermidis, S. haemolyticus*, *S. hyicus* ssp. *hyicus* and *S.xylosus) (*Breckinridge and Bergdoll, 1971*)* can cause human diseases and nosocomial infections and/or may endanger human health through enterotoxin production (Balaban and Rasooly, 2001).

Results of this study appears to agree with results from the study by (Eltwisy et al., 2020) in which pathogenesis of *Staphylococcus haemolyticus* on primary human skin fibroblast cells was assessed. Results of this study showed that all *Staphylococcus haemolyticus* isolates induced lysis of the RBCs, but with different extents. The hemolytic activities ranged between 0.1% and 53.7%. The highest activity was observed for strain SH2 (53.7%), which induced hemolysis levels comparable to that of the *Staphylococcus aureus* strains. Generally, most of the clinical SH isolates had higher hemolytic activities compared to the control *Staphylococcus haemolyticus* strain used in their study (ATCC 29970). Those findings highlighted the significant development of *S. haemolyticus*, which was previously considered a contaminant when detected in cultures of clinical samples. Their high ability to adhere, invade and kill the host cells illustrate the severe damage associated with ulceration infections caused by *Staphylococcus haemolyticus*.

Findings from this study further showed that isolate *Streptococcus* (*Streptococcus* sp.) was the most toxic on KB cells at MOI 10:1, 6 hrs post infection (section 4.3.1.1.1, Table 4.3). Although this isolate was not characterised to species level, *Streptococc*i (including *Enterococci* and faecal *Streptococci*) are known to be indicators water contamination.

There were no significant differences between the mean toxic effects of the bacteria on KB cells at MOI 10:1 and MOI 100:1 6 hrs post infection (7.546% and 7.557%

respectively) as well as between the mean toxic effects of bacteria on KB cells at MOI 10:1 and MOI 100:1 24 hrs post infection (13.027% and 14.329% respectively) (calculated *P* values were <0.05 in both instances as presented in Table 4.7). Although the mean cytotoxic effects exerted by bacteria at MOI 100:1 6 hrs and 24 hrs post infection were higher than the mean toxic effects exerted by bacteria at MOI 10:1 6 hrs post infection, the differences were insignificant (Table 4.7). This showed that the bacteria used in the course of this study exerted similar effect on KB cells regardless of the number of bacteria present at the start of infection (MOIs) in this study, an indication that these bacteria exerted optimal toxicity on KB cells within the MOI 10:1 and MOI 100:1 range.

When the mean toxic effects of bacteria on KB cells at MOI 10:1 6 hrs post infection (7.546%) was compared to the mean toxic at MOI 10:1 24 hrs post infection (13.027%), the calculated *P* value was seen to be 0.003 (Table 4.8). The mean toxic effects of bacteria on KB cells at MOI 100:1 6 hrs post infection (7.557%) was also seen to be significantly higher than the mean toxic effects of bacteria on KB cells at MOI 100:1 24 hrs post infection (14.329%) as the calculated *P* value was 0.001 (Table 4.8). These findings indicate the effect of an increase in time on the toxic effect exerted by bacteria on the KB cells i.e., toxicity increased with time. This could be due to the fact that the bacteria were multiplying and producing a higher amount of virulence factors as time went by. Toxins are proteinaceous or non-proteinaceous molecules produced by bacteria to destroy or damage the host cell (Wilson et al., 2002). Pore forming toxins (PFTs) are common among bacteria, and about 25 to 30% of cytotoxic bacterial proteins are PFTs, making them the single largest category of virulence factors(Los et al., 2013) . Several well-known pathogenic bacteria including *E. coli, S. aureus, Streptococcus pneumoniae*

and *Mycobacterium tuberculosis* and many other pathogenic bacteria employ PFTs as virulence factors (Los et al., 2013). Therefore, it is highly possible that the increase in the cytotoxic effect of the bacteria, involved in this study, on KB cells with time resulted from the increased production of PFTs. This possible increase in production of the PFTs most likely came about due to the fact that the bacterial population got larger due to proliferation with time.

The presence of these bacterial pathogens in water indicate contamination of some sort and findings from this study shows that they are harmful/toxic to cells. Therefore, it is recommended that the appropriate government body/bodies, responsible for the monitoring of water quality, include more organisms to the list of organisms monitored when assessing the quality of water to be used for different purposes. In particular, it is recommended that the UAE government body/bodies, responsible for the monitoring of water quality, include *Staphylococcus haemolyticus* (the organism seen to be most toxic on KB cells in this study) to the list of organisms to be check monitored while assessing water quality.

# Chapter 5

# Effects of Chlorine on *Acanthamoeba* Survival and UAE Drinking Water Guidelines

## 5.1 Introduction

*Acanthamoeba*, a ubiquitously spread free-living amoeba, is known to cause a unique, but potentially sight-threatening, painful, often misdiagnosed, and difficult to treat corneal infection, keratitis, and meningoencephalitis, a fatal infection of the central nervous system (CNS) (Dunand et al., 1997, Goldschmidt et al., 2008, Neelam and Niederkorn, 2017, Rayamajhee et al., 2020, Torno Jr et al., 2000) . *Acanthamoeba* spp. can also cause sinusitis and skin lesions in immunocompromised individuals, such as AIDS patients (Dunand et al., 1997, Friedland et al., 1992, Torno Jr et al., 2000) . It has two discrete stages in its life cycle, an active phagotrophic trophozoite and an inactive double walled cyst stage, with the cyst stage allowing the amoeba to remain viable for many years, even in severe conditions, including chlorine treated water (Schuster, 2002, Visvesvara et al., 2007b). The infective form is the trophozoite stage, although both trophozoites and cysts can gain entry into the human body via different routes, such as debrided skin, cornea, and nasal passages (CDC, 2021) . Based on their morphology, *Acanthamoeba* species have been broadly classified into three groups (I, II, and III) (Siddiqui and Khan, 2012) and pathogenic strains are common of group II(Xuan et al., 2017) . *Acanthamoeba* species are the most important and most predominant protozoa found in the environment. They can inhabit water systems and have been isolated from drinking water plants (Hoffmann and Michel, 2001, Thomas et al., 2008), hospital water networks(Rohr et al., 1998), domestic water networks (Kilvington et al., 2004), and cooling towers (Barbaree et al., 1986). *Acanthamoeba* species are, so far, the most commonly encountered in human infections. The development of *Acanthamoeba* involves two stages. In the first stage, the vegetative trophozoite form is feeding and replicating. The

second stage develops under unfavourable environmental conditions, such as nutrient starvation, heat, cold, and desiccation, and also in the presence of high concentrations of various chemical compounds, with the trophozoites differentiating into cysts (Byers et al., 1991, Neff et al., 1964). During the encystment phase, *Acanthamoeba* become spherical and forms two distinct layers around itself: the endocyst, containing cellulose, and the ectocyst, containing various polysaccharides and proteins (Khunkitti et al., 1998, Lloyd et al., 2001). Cysts are metabolically inactive and can remain alive for more than 20 years under dry conditions (Lloyd et al., 2001) and 24 years at 4°C in water (Mazur et al., 1995). The most common infection associated with *Acanthamoeba* spp. is amoebic keratitis (AK), first described in 1974 (Nagington et al., 1974). This is a particularly difficult to treat ocular infection that leads to trauma and corneal necrosis.

In a 2009 study, *Acanthamoeba* spp. was also encountered in the urine of seriously ill patients in intensive care units, signifying that FLA might be a factor in hospital-acquired infections by enabling the transmission of bacteria to the patients (Santos et al., 2009). In fact, in addition to their naturally inherited pathogenicity, *Acanthamoeba* can possibly harbour various bacterial, viral, and eukaryotic species that are pathogenic to humans and animals (Greub and Raoult, 2004). Thus, among 539 bacterial species listed as being pathogenic for humans and/or animals, 102 bacterial species (including *Acinetobacter*, *Enterobacter*, *Legionella*, various *Mycobacteria, Pseudomonas*, and *Serratia* spp.) were labelled in the literature as being able to survive and potentially multiply after amoebal ingestion (Thomas et al., 2010). Survival of bacteria within amoeba cysts has been demonstrated for several of these species, mainly *mycobacteria* (Adékambi et al., 2006), and cysts are thus considered potential reservoirs of pathogens in water systems.

From a public health perspective, *Acanthamoeba*, and particularly *Acanthamoeba* cysts, are highly resistant to various physical and chemical stresses and can thus protect any intracellular microorganism from harmful environmental conditions that would normally kill them (Barker et al., 1992, Kilvington and Price, 1990, King et al., 1988). This protective effect is of increasing concern because they may even provide favourable conditions directly knowing that the biocide treatment itself is found to be stimulating FLA growth (Srikanth and Berk, 1993) or indirectly due to the biocidal treatment of killing extracellular bacteria that are then used as a food source by *Legionella pneumophila* as demonstrated by (Berjeaud et al., 2016, Temmerman et al., 2006) .

In spite of increasing health concerns, there is still a lack of information concerning the *Acanthamoeba* resistance to various biocides and disinfectants. Trophozoites are considered relatively sensitive to most chemicals, but cysts have been shown to be more resistant (Thomas et al., 2010). *Acanthamoeba* cysts have been reported in several studies to have high levels of resistance to radiation, such as UV, X-ray, and gamma irradiation, but limited resistance to high temperatures(Thomas et al., 2010). There is no official standard available to test the effectiveness of disinfectants against *Acanthamoeba*. The strains, the media used, exposure and neutralization conditions, as well as methods to evaluate residual viability after treatments, vary from one study to another and might be responsible for the observed discrepancies (Anger and Lally, 2008, Buck et al., 2000, Coulon et al., 2010).

Furthermore, there are few data available on the effectiveness of various chemicals used for surface and medical device disinfection in health care settings, such as alcohols, aldehyde-based products, and hydrogen peroxide-based products official standard to

teste the activities of disinfectants against *Acanthamoeba* is not available. The association established between the different microorganisms in the water systems are directly related to the effectiveness of the disinfection treatments applied (Lau and Ashbolt, 2009, Lechevallier et al., 1988) . Free-living amoebae (FLA) are eukaryotic microorganisms, commonly found in drinking water systems, and phagocyte bacteria, their nutritional source. However, some bacterial genera have developed strategies to survive the grazer effect of amoebae (Greub and Raoult, 2004).Some authors have reported that stress conditions such as exposure to disinfectant including free chlorine or heat (Alleron et al., 2008, Dusserre et al., 2008) can induce bacterial cells to enter a viable but non-cultivable state. Because of that, the effect of disinfection treatments on bacterial cells could be even lower, as the viability quantification method used was based on the number of cultivable cells, which is still considered the gold standard today.

As an overall, *Acanthamoeba* spp. is a natural inhabitant of drinking water systems that is able to survive the free chlorine concentration and temperatures used to ensure the microbial quality of the water system and to control and prevent bacteria colonization.

Globally, drinking water is not routinely monitored for the presence of *Acanthamoeba* species, and there is limited information on the occurrence or concentrations of *Acanthamoeba* in drinking water systems. Internationally, no standards or guideline values have been established for *Acanthamoeba* in drinking water systems. Studies have shown that conventional water treatment using coagulation and filtration greatly reduces the levels of amoebae in water, but some trophozoites and cysts are able to pass through the filter beds. *Acanthamoeba* trophozoites are killed by chlorination but the cysts are able to survive disinfection processes and subsequently pass into the water distribution

system where they may grow if conditions are favourable. Intrusion of soil contamination during pipe breaks and repairs may also be a source of *Acanthamoeba*. Good management practices such as maintaining disinfectant residuals, flushing and disinfection after repairs, and regular cleaning of sediment from pipes and storage tanks may reduce opportunities for regrowth of these organisms in the distribution system. However, it is likely that *Acanthamoeba* are present intermittently in water supplies and in building plumbing (Carnt and Stapleton, 2016, WHO 2011).

*Acanthamoebal* strains, the media used to grow and encyst trophozoites, and the exposure and neutralization conditions, as well as methods to evaluate residual viability after treatments, vary from one study to another and might thus be responsible for the observed discrepancies (Anger and Lally, 2008, Buck et al., 2000). Furthermore, there are few data available on the activities of the various chemicals used for surface and medical device disinfection in health care settings, such as alcohols, aldehyde-based products, peracetic acid-based products, and hydrogen peroxide-based products (Coulon et al., 2010).

As mentioned earlier in chapter 1 (Table 1.1 and Table 1.2), the current guidelines for physical/microbiological parameters for water authority in the UAE calls for implementing RSP standards (Department of Energy) , However residual chlorine ideal concentration shall be between 0.2 to 0.5 mg/l. It may increase to 1.00 mg/l or even above in situations where the licensee or the Transmission System Operator request that, in accordance with the Water Transmission Code or for controlling possible bacteriological contamination.

The concentration for either *E.coli*, standard method Total coliforms or *Enterococci* is zero; i.e. must not be detectable in any 100 ml sample. If the presence of either *E.coli*,

Total *coliforms* or Intestinal *Enterococci is* indicated, a repeat sample should be collected from the original sample location within 24 hours of the result of the original sample being known. If the repeat sample is non-compliant, the water operators should implement their immediate corrective action.

The aim of the existing work, is to test the effectiveness of various disinfection procedures of chlorine used in municipalities and in health care settings, including widely used high-level disinfectants, against environmental isolate strains of *Acanthamoeba*.

## 5.2 Materials and Methods

### 5.2.1 Preparation of *Acanthamoeba*

*Acanthamoeba* used in this chapter were isolated from environmental water in the UAE. They were grown in [Peptone 0.75% (w/v), - yeast 0.75% (w/v),- glucose 1.5% (w/v)] medium (PYG) and gravity filtered to remove culture broth. *Acanthamoeba* were suspended in PYG culture broth and were incubated at 25°C for 24 hrs to reduce the effects of medium changes and subsequent osmotic shock. This procedure also prevents cyst formation should *Acanthamoeba* be transferred into another culture suspension. After 24 hrs, *Acanthamoeba* were again filtered as described above, washed three times in phosphate-buffered saline (PBS), and resuspended in fresh PYG. *Acanthamoeba* were enumerated by using a haemocytometer after addition of cell suspension and were adjusted to an experimental concentration of $5x10^{5}$ cells per ml.

### 5.2.2 Preparation of chlorine viability assay

Stock chlorine solutions were prepared from a 5.25% solution of laboratory-grade sodium hypochlorite (7C's Safety and Environmental, Inc.). All chlorine test solutions were made

by dilution of chlorine stock solutions into PBS. Total chlorine residuals in all stock solutions were determined with a model Extech Chlorine meter (Anaum International Electronics LLC UAE).

Axenic culture of *Acanthamoeba* isolates were treated with free chlorine residuals to test for survivability in chlorine solutions. The isolates were prepared and enumerated in PBS as described above. Assays consisted of inoculating 1.0 ml portions of $1x10^{5}$ *Acanthamoeba* per ml into 19 ml of chlorine solution; 5.00, 6.00, 12.00, 25.00, 50.00 mg/litre of free available chlorine . The 5 ppm was chosen to resemble the dose for UAE disinfection of drinking water, the 6ppm was used because we have been informed by the water authority that some time, they go bit higher. other concentrations were chosen in order to determine at which dose we achieve complete disinfection, want to determine at which dose of chlorination we achieve 100% disinfection of *Acanthamoeba*. Disinfectant contact times for each dilution were 1 min, 5 min, 10 min, 15 min, 30 min, 60 min, 90 min, 120 min, and 24 hours for each chlorine concentration. *Acanthamoeba* cysts in water served as a control for the survival of *Acanthamoeba* in experimental procedures. *Acanthamoeba* viability was monitored using 0.2% trypan blue exclusion staining method (Nih ).

### 5.2.3 Determining of number of live *Acanthamoeba* using Cell Viability Testing with Trypan Blue Exclusion Method

To determine the number of viable *Acanthamoeba* after treatment with a chlorine solution, by pipetting 100 µl of growth suspension into a 1.5 ml Eppendorf tube, then one hundred µl of 0.4% Trypan blue was added. The mixture was incubated for 2-5

min. The counting was achieved by taking 10 µl of cell suspension and using haemocytometer cleaned with 70% ethanol before and after each useage. The number of viable *Acanthamoeba* and dead cells were counted in the middle square of both chambers of the haemocytometer, and the average was obtained (dead cells stained blue, viable cells were unstained). The number of cells counted were ranged between 40 to 70 cells. *Acanthamoeba* with chlorin were incubated at 30$^{o}$ C for 6 and 24 hrs. The number of viable and dead *Acanthamoeba* in each well was determined by adding 150µl of 0.4% Trypan blue in each well and incubating for 15 minutes. The numbers of viable and dead *Acanthamoeba*, as judged by dye exclusion or staining, were counted by a haemocytometer.

**To count cells per ml**

Average number of cells in one large square x dilution factor* x $10^4$

*dilution factor was 2 (1:1 dilution with trypan blue), $10^4$ = conversion factor to convert $10^4$ ml to 1 ml

Calculation of Cell Viability:

No. of Viable Cells Counted / Total Cells Counted (viable and dead) x 100 = % viable cells

## 5.3 Results

Trophozoites and cyst disinfection results (Tables 5.1 to 5.12) indicated that chlorination has a significant impact on the viability of *Acanthamoeba* in water. The concentration of chlorine of 5 ppm at a minimum contact time of 1 minute has deleted about 68% of the microorganism. As the contact time between the cells and the disinfectant time increased, the survivability diminished accordingly. Increasing the disinfectant concentration has shown more effect and killed almost instantly all cells of the experiment. Images of the treated and untreated amoebae add in Appendix 3 .

Table 5. 1: Chlorine concentration of 5.00 ppm (mg/l) tested for *Acanthamoeba* trophozoites survivability. Results indicated that a contact time of 1 minute between *Acanthamoeba* (5 X 105) and chlorine resulted in disinfecting of 68% of AC. Contact time of 5 minutes has achieved about 72% efficiency of AC disinfection. Contact time beyond 60 minutes between AC and Chlorine is 100% lethal to all AC cells.

| chlorine concentration | AC cell/ml | Contact time(min) | viable AC cells /ml | Dead AC cells /ml | % viable cells/ml |
|---|---|---|---|---|---|
| 5 ppm | $5 \times 10^5$ | 1 | 8 | 17 | 32% |
| | | 5 | 7 | 18 | 28% |
| | | 10 | 6 | 19 | 25% |
| | | 30 | 5 | 20 | 20% |
| | | 60 | 1 | 24 | 4% |
| | | 90 | 0 | 21 | 0.00 |
| | | 120 | 0 | 19 | 0.00 |
| | | 24 hours | 0 | 17 | 0.00 |

Table 5. 2 : Chlorine concentration of 5.00 ppm (mg/l) tested for *Acanthamoeba* trophozoites survivability. Results show Standard deviation (SD) and standard error (SE) of the values reported in table 5.2

| chlorine concentration | | Contact time(min) | reading 1 | standard deviation (SD) | standard error(SE) |
|---|---|---|---|---|---|
| 5 ppm | viable AC cells /ml | 1 | 8 | 1.00 | 0.58 |
| | | 5 | 7 | 0.58 | 0.33 |
| | | 10 | 6 | 0.58 | 0.33 |
| | | 30 | 5 | 1.53 | 0.88 |
| | | 60 | 1 | 0.00 | 0.00 |
| | | 90 | 0 | 0.00 | 0.00 |
| | | 120 | 0 | 0.00 | 0.00 |
| | | 24 hours | 0 | 0.00 | 0.00 |
| | Dead AC cells /ml | 1 | 17 | 3.00 | 1.73 |
| | | 5 | 18 | 1.00 | 0.58 |
| | | 10 | 19 | 3.00 | 1.73 |
| | | 30 | 20 | 2.00 | 1.15 |
| | | 60 | 24 | 2.00 | 1.15 |
| | | 90 | 21 | 1.00 | 0.58 |
| | | 120 | 19 | 1.53 | 0.88 |
| | | 24 hours | 17 | 2.00 | 1.15 |

Table 5. 3 : Chlorine concentration of 6.00 ppm (mg/l) tested for *Acanthamoeba* trophozoites survivability. Results indicated that at a contact time of 1 minute between AC ($5 X 10^{5)}$ and chlorine resulted in disinfecting of 84% of AC. Contact time of 5 minutes has achieved about 91.7% efficiency of AC disinfection. Contact time beyond 5 minutes between AC and Chlorine is 100% lethal to all AC cells.

| chlorine concentration | AC Trophozoite/ml | Contact time/min | viable AC cells /ml | Dead AC cells /ml | % viable cells/ml |
|---|---|---|---|---|---|
| 6 ppm | $5 X 10^{5}$ | 1 | 4 | 21 | 16% |
| | | 5 | 2 | 22 | 8.3% |
| | | 10 | 0.00 | 25 | 0.00 |
| | | 30 | 0.00 | 23 | 0.00 |
| | | 60 | 0.00 | 21 | 0.00 |
| | | 90 | 0.00 | 22 | 0.00 |
| | | 120 | 0.00 | 20 | 0.00 |
| | | 24 hours | 0.00 | 18 | 0.00 |

Table 5. 4 : Chlorine concentration of 6.00 ppm (mg/l) tested for *Acanthamoeba* trophozoites survivability. Results show Standard deviation (SD) and standard error (SE) of the values reported in table 5.3

| chlorine concentration | | Contact time(min) | reading 1 | standard deviation (SD) | standard error(SE) |
|---|---|---|---|---|---|
| 6 ppm | viable AC cells /ml | 1 | 4 | 1.00 | 0.58 |
| | | 5 | 2 | 1.00 | 0.58 |
| | | 10 | 0 | 0.00 | 0.00 |
| | | 30 | 0 | 0.00 | 0.00 |
| | | 60 | 0 | 0.00 | 0.00 |
| | | 90 | 0 | 0.00 | 0.00 |
| | | 120 | 0 | 0.00 | 0.00 |
| | | 24 hours | 0 | 0.00 | 0.00 |
| | Dead AC cells /ml | 1 | 21 | 3.06 | 1.76 |
| | | 5 | 22 | 1.00 | 0.58 |
| | | 10 | 25 | 1.00 | 0.58 |
| | | 30 | 23 | 1.00 | 0.58 |
| | | 60 | 21 | 1.53 | 0.88 |
| | | 90 | 22 | 2.52 | 1.45 |
| | | 120 | 20 | 2.00 | 1.15 |
| | | 24 hours | 18 | 2.00 | 1.15 |

Table 5. 5 : Chlorine concentration of 12.00 ppm (mg/l) tested for *Acanthamoeba* trophozoites survivability. Results indicated that at contact time of 1 minute between AC (5 X $10^{5)}$ and chlorine resulted in disinfecting of 100% of *Acanthamoeba*.

| chlorine concentration | AC cell/ml | Contact time/min | viable AC cells /ml | Dead AC cells /ml | % viable cells/ml |
|---|---|---|---|---|---|
| 12 ppm | 5 X$10^{5}$ | 1 | 0.00 | 23 | 0.00 |
| | | 5 | 0.00 | 25 | 0.00 |
| | | 10 | 0.00 | 21 | 0.00 |
| | | 30 | 0.00 | 23 | 0.00 |
| | | 60 | 0.00 | 22 | 0.00 |
| | | 60 | 0.00 | 21 | 0.00 |
| | | 90 | 0.00 | 22 | 0.00 |
| | | 24 hours | 0.00 | 25 | 0.00 |

Table 5. 6 : Chlorine concentration of 12.00 ppm (mg/l) tested for *Acanthamoeba* trophozoites survivability. Results show Standard deviation (SD) and standard error (SE) of the values reported in table 5.5

| chlorine concentration | | Contact time(min) | reading 1 | standard deviation (SD) | standard error(SE) |
|---|---|---|---|---|---|
| 12 ppm | viable AC cells /ml | 1 | 0 | 0.00 | 0.00 |
| | | 5 | 0 | 0.00 | 0.00 |
| | | 10 | 0 | 0.00 | 0.00 |
| | | 30 | 0 | 0.00 | 0.00 |
| | | 60 | 0 | 0.00 | 0.00 |
| | | 90 | 0 | 0.00 | 0.00 |
| | | 120 | 0 | 0.00 | 0.00 |
| | | 24 hours | 0 | 0.00 | 0.00 |
| | | | | | |
| | Dead AC cells /ml | 1 | 23 | 1.00 | 0.58 |
| | | 5 | 25 | 0.58 | 0.33 |
| | | 10 | 21 | 1.00 | 0.58 |
| | | 30 | 23 | 1.00 | 0.58 |
| | | 60 | 22 | 0.58 | 0.33 |
| | | 90 | 21 | 1.53 | 0.88 |
| | | 120 | 22 | 1.00 | 0.58 |
| | | 24 hours | 25 | 1.00 | 0.58 |

Table 5. 7 : Chlorine concentration of 25.00 ppm (mg/l) tested for *Acanthamoeba* trophozoites survivability. Results indicated that at contact time of 1 minute between AC ($5 X 10^{5)}$ and chlorine resulted in disinfecting of 100% of *Acanthamoeba*.

| chlorine concentration | AC cell/ml | Contact time/min | viable AC cells /ml | Dead cells AC/ml | % viable cells/ml |
|---|---|---|---|---|---|
| 25 ppm | $5X10^{5}$ | 1 | 0.00 | 21 | 0.00 |
| | | 5 | 0.00 | 24 | 0.00 |
| | | 10 | 0.00 | 23 | 0.00 |
| | | 30 | 0.00 | 22 | 0.00 |
| | | 60 | 0.00 | 25 | 0.00 |
| | | 60 | 0.00 | 21 | 0.00 |
| | | 90 | 0.00 | 22 | 0.00 |
| | | 24 hours | 0.00 | 17 | 0.00 |

Table 5. 8 : Chlorine concentration of 25.00 ppm (mg/l) tested for *Acanthamoeba* trophozoites survivability. Results show Standard deviation (SD) and standard error (SE) of the values reported in table 5.7

| chlorine concentration | | Contact time(min) | reading 1 | standard deviation (SD) | standard error(SE) |
|---|---|---|---|---|---|
| 25 ppm | viable AC cells /ml | 1 | 0 | 0.00 | 0.00 |
| | | 5 | 0 | 0.00 | 0.00 |
| | | 10 | 0 | 0.00 | 0.00 |
| | | 30 | 0 | 0.00 | 0.00 |
| | | 60 | 0 | 0.00 | 0.00 |
| | | 90 | 0 | 0.00 | 0.00 |
| | | 120 | 0 | 0.00 | 0.00 |
| | | 24 hours | 0 | 0.00 | 0.00 |
| | | | | | |
| | Dead AC cells /ml | 1 | 21 | 1.00 | 0.58 |
| | | 5 | 24 | 2.00 | 1.15 |
| | | 10 | 23 | 0.58 | 0.33 |
| | | 30 | 22 | 1.00 | 0.58 |
| | | 60 | 25 | 1.00 | 0.58 |
| | | 90 | 21 | 2.00 | 1.15 |
| | | 120 | 22 | 0.58 | 0.33 |
| | | 24 hours | 17 | 1.00 | 0.58 |

Table 5. 9 : Chlorine concentration of 50.00 ppm (mg/l) tested for *Acanthamoeba* trophozoites survivability. Results indicated that at contact time of 1 minute between AC cysts (5 X $10^{5)}$ and chlorine resulted in disinfecting of 100% of *Acanthamoeba*.

| chlorine concentration | AC cell/ml | Contact time/min | viable AC cells /ml | Dead AC cells /ml | % viable cells/ml |
|---|---|---|---|---|---|
| 50 ppm | $5X10^5$ | 1 | 0.00 | 25 | 0.00 |
| | | 5 | 0.00 | 24 | 0.00 |
| | | 10 | 0.00 | 21 | 0.00 |
| | | 30 | 0.00 | 18 | 0.00 |
| | | 60 | 0.00 | 24 | 0.00 |
| | | 60 | 0.00 | 21 | 0.00 |
| | | 90 | 0.00 | 22 | 0.00 |
| | | 24 hours | 0 | 17 | 0.00 |

Table 5. 10 : Chlorine concentration of 50.00 ppm (mg/l) tested for *Acanthamoeba* trophozoites survivability. Results show Standard deviation (SD) and standard error (SE) of the values reported in table 5.9

| chlorine concentration | | Contact time(min) | reading 1 | standard deviation (SD) | standard error(SE) |
|---|---|---|---|---|---|
| 50 ppm | viable AC cells /ml | 1 | 0 | 0.00 | 0.00 |
| | | 5 | 0 | 0.00 | 0.00 |
| | | 10 | 0 | 0.00 | 0.00 |
| | | 30 | 0 | 0.00 | 0.00 |
| | | 60 | 0 | 0.00 | 0.00 |
| | | 90 | 0 | 0.00 | 0.00 |
| | | 120 | 0 | 0.00 | 0.00 |
| | | 24 hours | 0 | 0.00 | 0.00 |
| | | | | | |
| | Dead AC cells /ml | 1 | 25 | 1.00 | 0.58 |
| | | 5 | 24 | 1.00 | 0.58 |
| | | 10 | 21 | 0.58 | 0.33 |
| | | 30 | 18 | 1.00 | 0.58 |
| | | 60 | 24 | 2.00 | 1.15 |
| | | 90 | 21 | 1.53 | 0.88 |
| | | 120 | 22 | 2.00 | 1.15 |
| | | 24 hours | 17 | 2.00 | 1.15 |

Table 5. 11 : Chlorine concentration of 5.00 ppm (mg/l) tested for *Acanthamoeba* cysts survivability, results indicated that at contact time of 1 minute between AC (5 X $10^{5)}$ and chlorine resulted in disinfecting of 60% of AC. Contact time of 5 minutes has achieved about 64% efficiency of AC disinfection. Contact time beyond 60 minutes between AC and Chlorine is 100% lethal to all *Acanthamoeba*.

| chlorine concentration | AC cell/ml | Contact time(min) | viable AC cells /ml | Dead AC cells /ml | % viable cells/ml |
|---|---|---|---|---|---|
| 5 ppm | 5 x $10^{5}$ | 1 | 10 | 15 | 40% |
| | | 5 | 9 | 16 | 36% |
| | | 10 | 6 | 19 | 25% |
| | | 30 | 5 | 20 | 20% |
| | | 60 | 1 | 24 | 4% |
| | | 90 | 0 | 21 | 0.00 |
| | | 120 | 0 | 19 | 0.00 |
| | | 24 hours | 0 | 17 | 0.00 |

Table 5. 12 : Chlorine concentration of 5.00 ppm (mg/l) tested for *Acanthamoeba* cysts survivability. Results show Standard deviation (SD) and standard error (SE) of the values reported in table 5.11

| chlorine concentration | | Contact time(min) | reading 1 | standard deviation (SD) | standard error(SE) |
|---|---|---|---|---|---|
| 5 ppm | viable AC cells /ml | 1 | 10 | 0.58 | 0.33 |
| | | 5 | 9 | 1.00 | 0.58 |
| | | 10 | 6 | 0.58 | 0.33 |
| | | 30 | 5 | 1.00 | 0.58 |
| | | 60 | 1 | 0.00 | 0.00 |
| | | 90 | 0 | 0.00 | 0.00 |
| | | 120 | 0 | 0.00 | 0.00 |
| | | 24 hours | 0 | 0.00 | 0.00 |
| | | | | | |
| | Dead AC cells /ml | 1 | 15 | 0.58 | 0.33 |
| | | 5 | 16 | 1.00 | 0.58 |
| | | 10 | 19 | 2.00 | 1.15 |
| | | 30 | 20 | 2.00 | 1.15 |
| | | 60 | 24 | 1.00 | 0.58 |
| | | 90 | 21 | 2.00 | 1.15 |
| | | 120 | 19 | 1.00 | 0.58 |
| | | 24 hours | 17 | 2.00 | 1.15 |

**Statistical analysis:** The data reported in this study were an average of triplicate reading. The results are reported as means.

## 5.4 Discussion

Free-living amoebae that belong to the *Acanthamoeba* genus represent a potential threat to human health due to not only their intrinsic pathogenicity (Marciano-Cabral and Cabral, 2003) but also their role as protective and disseminating hosts for other pathogenic microorganisms (Thomas et al., 2010). Due to their capacity to resist chemical and physical treatments used for drinking water production and distribution (Loret et al., 2008, Thomas et al., 2004, Thomas et al., 2008), they can colonize virtually any water system. They are also widely distributed in moist areas, air, and dust (Carlesso et al., 2010, Rivera et al., 1994, Rohr et al., 1998). Despite increasing health concerns caused by these organisms, there is still a scarcity of information concerning their inactivation by various physical and chemical biocides (Thomas et al., 2010).

Chlorine at 10 ppm (mg/l) was reported to be effective against *Hartmannella vermiformis* cysts, which is another species of *Amoeba,* after 30 min exposure (Kuchta et al., 1993) , but it was reported that it is ineffective against *Acanthamoeba* cysts because they can resist exposure to 100 mg/l chlorine for 10 min (Storey et al., 2004) or 50 mg/l for 18 hour (Kilvington and Price, 1990). Limited activity of chlorine was also reported against *Balamuthia mandrillaris* cysts and trophozoites (Siddiqui et al., 2008) , whereas (Chang, 1978) reported that exposure to 1–7 mg/l free chlorine (for 5–30 min) was cysticidal against *Naegleria* spp. Differences in cysticidal activity to chlorine and also to chlorine dioxide and ozone have been reported *with Acanthamoeba* spp. being less susceptible than *Naegleria* spp. (Cursons et al., 1980) . Monochloramine at 3.9 mg/l killed *Nigeria lovanensis* cysts within 1 hour (Ercken et al., 2003) .

Chlorine dioxide, a potent oxidant and antimicrobial, is a disinfectant widely used in surface disinfection, biofilm removal in water distribution systems, and, increasingly, in water treatment to produce potable water (CDC, 2022) . Several authors have reported similar efficacy of chlorine dioxide and chlorine to eliminate free living amoeba cells (Ma et al., 2017). Mogoa et al. (2010) reported that a concentration of free chlorine lower than 3 mg/l was not able to inhibit *Acanthamoeba* castellanii Neff. On the other hand, the CDC recommends a concentration of 1–3 mg/l (CDC, 2022) of free chlorine to avoid the growth of Naegleria fowleri in swimming pools.

When tested against *Acanthamoeba* and *Naegleria* spp., trophozoites, chlorine dioxide in water was effective after treatment for 30 min at concentrations of approximately 2 mg/l (*Naegleria* spp.) or 3 mg/l (*Acanthamoeba* spp.) (Cursons et al., 1980) .

The amoebicidal capacity of chlorine on the trophozoite stage of *Acanthamoeba* spp. and Naegleria fowleri was studied over 48 hur using an inverted light-microscope. In *Acanthamoeba castellanii* (ATCC 30234), Mogoa et al. (2010) reported that chlorine treatment led to the loss of *pseudopodia* and shrinking in the cell size and membrane permeabilization (Mogoa et al., 2011) . The same authors proved in a second paper that chlorine dioxide was as effective as chlorine in eliminating *Acanthamoeba* castellanii Neff by reducing the cell size more and inducing membrane permeabilization, as shown by propidium iodide staining (Coulon et al., 2010) .

In our study, Chlorine was shown to have a stronger effect on the environmental *Acanthamoeba* species. The chlorine was highly effective at eliminating all environmental *Acanthamoeba* strains used in this study. The chlorine dioxide greatly affected the morphology shape and increased the cell size shrinkage. The chlorine was highly

effective at eliminating environmental *Acanthamoeba* used in this study. Further experiments are needed to better understand the mode of action of chlorine and to evaluate its in situ efficacy in a scale up process.

The rationale of this study was to test the trophocidal and cysticidal concentration of chlorine doses used in municipalities to treat drinking water. Our results has indicated that chlorination at a concentration of 5 ppm (mg/l) is cysticidal for *Acanthamoeba* in both forms cyst and trophozoite in a duration of 30 minutes. Doses of chlorine higher than 5 ppm can produce more effect on killing a higher number of cysts and trophozoites up to a complete 100% kill of all *Acanthamoeba* instantly. Our results is in agreement with(Loret et al., 2008) results in which they reported that a clear effect on *Acanthamoeba polyphaga* cysts was observed when *Acanthamoeba* cysts or trophozoites were exposed to 5 mg/ L for 60 min.

Microbial testing of UAE water samples needs to be conducted at relevant locations in the distribution system following any consumer complaints or observations associated with taste, odour and colour. Target should be zero organism. Under normal conditions cultural methods are followed. However, in cases where suspected detections are identified and immediate confirmations are required, PCR methods or any other appropriate accredited methods shall be used. Drinking water shall be free at all times from algae, mould, parasites, insects and their eggs, larvae, and protozoa. Microbial water quality is being monitored in UAE by the Q & C central government laboratories. Water samples are being collected for testing on daily frequencies.

# Chapter 6

# Conclusion

## 6.1 Overview

*Acanthamoeba spp*. are common amoebae found in a wide range of natural habitats, living freely and can be pathogenic to humans (Al-Herrawy et al., 2015). A high percentage of *Acanthamoeba* in various environmental sources represents a sanitary risk to public health (M Manesh et al., 2016). *Acanthamoeba* can be found in water, soil, and air. It is isolated from purified water, seawater, adulterated water, swimming pool, sewage water, aquarium, lens wash solution, soil, dust in the air, food products, digestive organs, air conditioners, dialysis machines(Bagheri et al., 2010), distilled water, lake water, surgical instruments, sediments, etc. (Al-Herrawy et al., 2015). *Acanthamoeba* is also isolated from skin lesions, nasal cavities, pharynx, cerebral fluid, lung tissue, cornea, and human brain necrosis (Al-Herrawy et al., 2015). *Acanthamoeba* posed a risk to patients with compromised immune systems and people wearing contact lenses. In individuals with compromised immune systems, *Acanthamoeba* can cause otitis, granulomatous amoebic encephalitis, lung lesions, skin infections (M Manesh et al., 2016) and amoebic keratitis (Al-Herrawy et al., 2015). But not all strains of *Acanthamoeba* are pathogenic, just *Acanthamoeba culbertsoni, Acanthamoeba astronyxis, Acanthamoeba castellanii, Acanthamoeba divionensis, Acanthamoeba rhysodes, Acanthamoeba healyi, Acanthamoeba hatchetti,* and *Acanthamoeba polyphaga* can cause infections in humans (Al-Herrawy et al., 2015). Pathogenic isolates of *Acanthamoeba* are essential in medicine and represent potential causes of dangerous disease granulomatous amebic encephalitis that can be potentially fatal brain infection (Booton et al., 2005, Hewett et al., 2003).

Studies using the DNA sequence of mitochondrial and nucleic subunits of rRNA genes (SSU rRNA genes) determined the genotypic diversity of *Acanthamoeba*. Such research

has revealed many named species of *Acanthamoeba* associated with specific genotypes. The study conducted by Booton et al. (2005) showed that almost all *Acanthamoeba* keratitis conditions are caused by a single molecular genotype T4. Also, the T4 genotype is dominant in other infections such as infections of the brain, cerebrospinal fluid, skin, lungs, and nasal passages. In brain infections, rare genotypes T12, T10, and T1 were also captured. *Acanthamoeba spp*. comprises more than 24 species (Jercic et al., 2019). Molecular analyzes indicate that there are 22 or more *Acanthamoeba* genotypes designated as T1, T2, T3, ..., T22 (R Moreira et al., 2020).

*Acanthamoeba spp*. is used to isolate and cultivate microbes that resist after their phagocytosis, such as *Legionella* bacteria. *Acanthamoeba spp.* is also used to giant viruses in which there is a difference in permissiveness. However, there are problems with *Acanthamoeba* identification at the species level (Chelkha et al., 2020). Chelkha et al. (2020) conducted a study where they used a specific PCR system to detect and identify *Acanthamoeba* species. The study (Chelkha et al., 2020) included 33 *Acanthamoeba* isolates, 20 reference layers, and 13 isolates from clinical samples, soil, and water. Based on the blueprint of the genome sequences of 14 *Acanthamoeba*, the core genome is mapped for 826 genes, which enabled the design of a PCR system for one of the essential genes encoding alanine-tRNA ligase. These primers provided specific and compelling screening to detect the presence of *Acanthamoeba*. They identified 20 reference strains, while complete and partial sequences encoding 18S ribosomal RNA are identified in only 11 strains. Chelkha et al. (2020) found that four

isolates of *Acanthamoeba* could be a new species and that some isolates are assigned to the wrong species (Chelkha et al., 2020).

*Acanthamoeba* has two phases in its life cycle. One phase is the vegetative stage of the trophozoite when it is 13 µm to 23 µm in diameter, and the other phase is the dormant cyst in diameter of 5 µm to 20 µm. When *Acanthamoeba* is in the trophozoite stage, it feeds on organic particles and other microbes such as algae, bacteria, and yeasts (Khan, 2006). Cell division occurs by binary fission, and it is asexual. Cell division mainly occurs through the G2 phase (90%), G1 phase is negligible, M phase 2% to 3%, and S phase 2% to 3%. *Acanthamoeba* divides under osmolarity between 50 and 80 mOsmol, at a temperature of about 30°C, neutral pH values, and supplied with food (Khan, 2006). When exposed to harsh conditions, cellular differentiation occurs in the form of double cysts. The inner wall is made of cellulose, and the outer walls consist of polysaccharides and proteins. Both walls are usually separated by a space, except at specific points where they form opercula in the center of the ostiole, which is the exit point for the rejection of trophozoites(Khan, 2012) (Siddiqui and Khan, 2012). Cysts can live up to several years and can be transmitted by air (Khan, 2006) .

Cysts have pores called ostiole and are used to monitor changes in the environment. When the conditions are favorable, the trophozoites come out of the cyst and leave the outer shell. After that, they actively reproduce and thus complete the cycle. Excision and enzyme processes require the active synthesis of macromolecules. Excision and enzyme processes can be blocked by the protein synthesis inhibitor cyclohexane (Khan, 2006).

*A. castellanii* (T4 genotype) has a cell wall consisting of 35% carbohydrates (primary cellulose), 33% protein, 20% unidentified materials, 8% ash, and 4-6% lipids (Khan,

2012). Dudley et al. (2009) analyzed *A. castellanii* cysts and walls using gas chromatography in combination with mass spectrometry. They found various sugar residues in the intact cysts and cyst walls. Research has found that *Acanthamoeba* cysts and walls consist of a high percentage of glucose and galactose, as well as small amounts of xylose and mannose Dudley et al., 2009 .

The two prominent ecological roles of amoebae in the soil are to influence the structure of the microbial community and to improve the recycling of nutrients in the soil. Amoebae feed on bacteria in the soil and thus regulate their population in the soil. Free-living amoebae are the dominant consumers of bacteria, and they are responsible for about 60% of the total reduction in the bacterial population in the soil. Bacteria, which are the primary decomposers, directly decompose organic matter but are not effective in releasing minerals from their mass. Amoebae that are secondary decomposers consume primary decomposers and thus release mineral nutrients as waste products. Therefore, with the help of *Acanthamoeba*, nutrients become available. Otherwise, they would be unavailable for a more extended period. Soil containing bacteria and *Acanthamoeba* has significantly higher mineralization of nitrogen, phosphorus, and carbon than soil containing only bacteria. Also, in addition to consuming bacteria, amoebae promote the population of bacteria in the soil. Mineral regeneration by secondary degraders (amoebae) mitigates nutrient restriction for primary degrades (bacteria). When nitrogen in the soil is limited, and carbon is available, *Acanthamoeba's* mineralization has allowed continuous growth of bacteria leading to higher bacterial mass. However, when carbon is limited, *Acanthamoeba* is entirely responsible for mineralization, and bacteria contributed very little (Khan, 2012).

(Rosenberg et al., 2009b)constructed an experimental model system to study the effect of the common earth amoeba (*Acanthamoeba castellanii*) on the composition of the bacterial community in the rhizosphere of *Arabidopsis thaliana* (Thale cress). According to specific species of bacteria, the amoeba has shown marked tendencies to feed on bacteria (grazing). However, *Acanthamoeba,* when bacteria are replaced by species of tolerant grazing, bacteria comes an increase in the relative proportion of active bacteria, but amoebae still significantly reduce the number of bacteria. Two days after amoeba inoculation, specific bacterial taxa disappeared. The most pronounced decrease in the number of bacteria was in *Firmicutes*, while the increase was in *Nitrospira, Actinobacteria, Planctomycetes,* and *Verrucomicrobia*.

Research by (Rosenberg et al., 2009b) *Acanthamoeba* plays an important role in regulating bacterial populations and the circulation of nutrients in the environment, which contributes to the functioning of ecosystems (Khan, 2012) .

*Acanthamoeba* feeds on microorganisms that are present in different environments on surfaces and the water-air interface. *Acanthamoeba* uses acanthopods or sensitive structures that form on its surface to capture food particles that are primarily bacteria. *Acanthamoeba* also feeds on other protists, yeast and algae. Food intake in *Acanthamoeba* takes place by pinocytosis and phagocytosis. Pinocytosis is a nonspecific process through membrane intussusception, and the amoeba is used to carry large amounts of food particles/solutes. Phagocytosis is a receptor-dependent process. *Acanthamoeba* uses pinocytosis and phagocytosis to ingest large amounts of solutes and food particles. In *Acanthamoeba* at a similar rate (2 µl/h per $10^6$ cells) enters dissolved substances of different molecular weights, which include leucine (MW 131),

glucose (MW 180), inulin (MW 5000), and albumin (MW 65000). However, it is still unclear why *Acanthamoeba spp.* use phagocytosis or pinocytosis, how *Acanthamoeba* distinguishes pinocytosis and phagocytosis, whether there are differences between pathogenic and nonpathogenic *Acanthamoeba* in this regard. After the particles in the vacuole are adopted, *Acanthamoeba* distinguishes vacuoles that contain indigestible and digestible particles. The fate of the particles eaten by *Acanthamoeba* depends on the latex grains, particles' nature, and the food particles. Latex granules are exocytosed upon presentation of new particles, and vacuoles containing food particles are retained and digested. Particle uptake into *Acanthamoeba* is a complex process and can play a significant role in food intake and its pathogenesis (Khan, 2006).

## 6.2 Conclusion

The test for the quality of the drinking water has two components: Microbiological test and chemical test. The microbiological test conducted in this study was to identify total bacteria in water. The chemical quality test is conducted to assess the chemical cations and anions in the water. The test is made up of a range of chemical components. Some of the elements are heavy metals that may pose a great risk to human health while others may only affect the taste, odour and appearance of the water (These are called 'aesthetic' characteristics).UAE water was sampled from four Emirates and were found to be contaminated (100%) with pathogenic bacterial species.Municipality water samples, obtained from UAE, were tested for *Acanthamoeba.* Majority of samples (68%) were

found to harbour *Acanthamoeba* cells despite of the chlorination level involved during treatment. *Acanthamoeba* cells were genotyped and identified to the species level utilizing the PCR and nested PCR technology. The risk of having AC in potable water is magnified several folds by the possibility of having bacterial species engulfed internally by the AC cells. In this situation the *Acanthamoeba* may play a source of continuous contamination. It may act as a pathogen, parasite, carrier, and as a vehicle of pathogenic microorganism's transportation such as bacteria or viruses.

The cytotoxicity test was conducted to assess the virulence of the associated environmental bacteria on AC. Results obtained showed isolate STAHA (*Staphylococcus haemolyticus*) is the most toxic on KB epithelial cells as it was seen to exert the highest toxicities at MOI 100:1, 6 hrs post infection, as well as at MOI 100:1 24 hrs post infection. Results of this study appears to agree with the results from the study by (Eltwisy et al., 2020) in which pathogenesis of *Staphylococcus haemolyticus* on primary human skin fibroblast cells was assessed. The haemolytic activities ranged between 0.1% and 53.7%. The highest activity was observed for strain SH2 (53.7%), which induced haemolysis levels comparable to that of the *Staphylococcus aureus* strains. Their high ability to adhere, invade and kill the host cells illustrate the severe damage associated with ulceration infections caused by *Staphylococcus haemolyticus*. Findings from this study further showed that isolate *Streptococcus* (*Streptococcus* sp.) was the most toxic on KB cells at MOI 10:1, 6 hrs post infection. There were no significant differences between the mean toxic effects of the bacteria on KB cells at MOI 10:1 and MOI 100:1 6 hrs post infection (7.546% and 7.557% respectively) as well as between the mean toxic effects of

bacteria on KB cells at MOI 10:1 and MOI 100:1 24 hrs post infection (13.027% and 14.329% respectively).

Water treatment with the disinfection indicated that chlorination has a significant impact on the viability of *Acanthamoeba* in water. The concentration of chlorine of 5 ppm at a minimum contact time of 1 minute has deleted about 68% of the microorganism. As the contact time between the cells and the disinfectant time increased, the survivability chances diminish accordingly. Increasing the disinfectant concentration has shown more effect and killed almost instantly all cells of the experiment.

## 6.3 Limitations

Numerous limitations have been faced such as:

1. Difficulty in obtaining the types and numbers of water samples from the emirates with an average distance of about 200 km between the cities chosen for sampling. The process required an average of 12 hours to obtain the samples from each location and bringing them back to the laboratory on the same day and driving time of about 2.5 hours each way, plus the time consumed in between.
2. In most of the time, the lab work on samples continued after arriving at the lab to avoid changing the conditions of the samples. Experimental follow up takes a whole night until morning time to conclude.
3. Difficulties in getting any reagents for work on time from Sigma Aldrich representatives in the region. Finally, the entire company has closed and could not get anything locally.

4. Due to corona pandemics, I had to undergo some quarantine periods, which extended to weeks each time, due to contacting friends and relatives who tested positive for corona virus infection.

## 6.4 Recommendations for future work

The current work is the first and is the only work available so far to examine the quality of water at the UAE. The outcome of this study indicated that almost all water samples taken were infected with *Acanthamoeba*. Through my personal contact with water authorities, I found that *Acanthamoeba*, Ameoba or protozoa testing is not mandated in quality testing of potable or surface water. The following recommendations need to be taken into consideration:

1. Further research to explore water quality available regionally in terms of bacteria and *Acanthamoeba* is recommended.
2. Specifying diseases associated with environmental species of bacteria and *Acanthamoeba.*
3. Training technical individuals to carry out the lab work.
4. Re writing of the local guidelines to ensure protozoa protists species are enumerated and accounted.
5. Microbial testing of UAE water samples needs to be conducted at relevant locations in the distribution system following any consumer complaints or observations associated with taste, odour and Colour. Target should be zero organism. Under normal conditions cultural methods are followed. However, in cases where suspected detections are identified and immediate confirmations are required, PCR methods or any other appropriate accredited methods shall

be used. Drinking water shall be free at all times from algae, mould, parasites, insects and their eggs, larvae, and protozoa. Microbial water quality is being monitored in UAE by the Q & C central government laboratories. Water samples are being collected for testing on daily frequencies.

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# Appendices

## Appendix 1

Chemicals and Kits List

Chemicals:

| | |
|---|---|
| Minimum Essential Medium Eagle (EMEM) | (BioWhittaker™-Lonza 12-611Q) |
| Dulbecco's Modified Eagle's Medium (DMEM) | (Gibco™ 61965026) |
| MEM Non-Essential Amino Acid Solution (100×) | (Sigma 7145) |
| Fetal Bovine Serum | (Fisher Scientific10117272) |
| Phosphate buffered saline (PBS) | (Sigma P4417) |
| Nutrient agar | (Sigma N9405) |
| CLED Agar | (Sigma 55420) |
| Ringers solution | (Sigma 96724) |
| Peptone Water | (Sigma 70179) |
| Yeast Extract | (Sigma 92144) |
| D-Glucose | (RPI G32040-1000) |
| Plate Count agar | (Meerck 105463) |
| LB Broth | (LIF10855021) |
| Saline Solution | (BMX01204V) |
| Chelex 100 Resin | |
| Accutase® solution | (Sigma A6964) |
| Trypan Blue | |
| Vancomycin | (Sigma V2002) |
| Meropenem antibiotic 50mg | |
| Fetal bovine serum | |
| Minimum essential amino acids | |
| Triton X-100 | |
| Tris buffer | |
| Cytotoxicity Detection Kitplus; Roche | |
| GN Test Kit VTK2 | BMX021341 |

| | |
|---|---|
| GP Test Kit VTK2 | BMX021342 |
| CelLytic™ M | (Sigma C2978) |
| Virkon | (Fisher Scientific 12358667) |
| Ethanol | (Fisher Scientific BP2818) |

1 % virkon (w/v): 1 g in 100 ml water

70 % ethanol (v/v): 70 ml in 100 ml $H_2O$

**Cell lines and microorganisms; and storage and thawing methods :**

ATCC Epithelial Cell Line KB (carcinoma cells) The cells were cultured and maintained in Minimum Essential Medium Eagle with L-Glutamine (EMEM). The EMEM was supplemented with 10% v/v fetal bovine serum and 1% minimum essential amino acids. Cells were placed into T25 flasks and incubated at 37°C in a $CO_2$ incubator (95% humidity, 5% CO2).

# Appendix 2

## 1. Ion Chromatography:

This method covers the determination of **disinfectant by-products (**Bromate, Bromide, Chlorite and Chlorate) and **inorganic anions (**fluorides, chlorides, nitrites, nitrates, and sulfates) in drinking water

Chromatographic separation of ionic species and detection by electrical conductivity. A filtered aliquot of sample is injected into a stream of eluent and pumped through three different ion exchange columns and into a conductivity detector. The first two columns, a pre-column and separator column are packed with a low-capacity, strongly basic anion exchanger. The anions of interest are separated on the basis of their relative affinities for the exchange sites of the resin. The affinity of each anion is largely determined by its radius and valence. Because different anions have different migration rates, the sample anions elute from the column as discrete bands. Each anion is identified by its retention time within the exchange column. The sample anions are selectively eluted off the separator column and onto the last column, the suppresser column. The micromembrane suppressor which is bathed in a continuously flowing acid solution (regenerant solution) contains a cation exchange resin in the hydrogen form. In the micromembrane suppressor the separated anions are converted to their highly-conductive acid forms which are detected in the conductance cell of the conductivity detector. The suppressor column reduces the background conductivity of the eluent to a negligible level by converting it to a weakly conductive form. The chromatograms produced are displayed on an integrator or other data acquisition device for measurement of peak height or area which provides the basis for quantitation of anions by comparison to peaks of known standards

**Equipment**

Ion Chromatograph System Dionex ICS-3000 ( Dionex Auto sampler, Conductivity detector,

**Reference**

United States Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Test Method (300.1), EPA, 1993.

**2. Alkalinity (Carbonate)**

This method is suitable for the titration of waters containing hydroxide, carbonate, or *bicarbonate* alkalinity. The water should be free of color or turbidity which might obscure and affect the indicator response. The residual chlorine content of the water should not exceed 1.8 mg/l.

**Principle:**

Hydroxyl ions present in a sample as a result of dissociation or hydrolysis of solutes react with addition of standard acid (HCl 0.02 N).

**Equipment:**

Digital Burette *(Range 0-50 ml).*
Graduated cylinder 50 ml

- To Calculate $CO_3^{-2}$ and Bicarbonate $HCO_3^{-}$ as CaCO3 , We have to multiplying by conversion factor as following:

- Conversion Factor of $CO_3^{-2}$ = Equivalent weight of $CO_3^{-2}$/Equivalent weight of CaCO3 = 30/50 = 0.6
- Final result for Carbonate ($CO_3^{-2}$) = P-Alkalinity * 0.6
- Conversion Factor of $HCO_3^{-}$ = Equivalent weight of $CO_3^{-2}$/Equivalent weight of CaCO3 = 61/50 = 1.22
- Final result for Bicarbonate ($HCO_3^{-}$) = M-alkalinity (Total Alkalinity) * 1.22

**References:**

Standard Methods for the Examination of Water and Wastewater.22nd Ed. American Public Health Association, American Water Works Association, Water Environment Federation 2320 B. Titration Method.

**3. ICP**

Using ICP Varian720-ES (Inductively coupled plasma –720 Emission Spectrometry) to quantify trace elements and water matrix elements in

drinking water (bottled and unbottled samples). (Aluminum, Barium, Boron, Cadmium, Chromium, Phosphorus, Copper, Iron, Lead, Magnesium, Manganese, Potassium, Sodium, Zinc, Antimony, Arsenic, Nickel, Selenium, Calcium

The procedures follow EPA 200.5 method and guidelines provided by the manufacturer. The analytical determinative step described in this method involves multi-elemental determinations by ICP-720ES using simultaneous instrument. The instrument measure characteristic atomic-line emission spectra by optical spectrometry. Standard and sample solutions are nebulized by pneumatic nebulization and the resulting aerosol is transported by argon carrier-gas to the plasma torch. Element specific emission spectra are produced by radio-frequency inductively coupled plasma. The spectra are dispersed by a grating spectrometer, and the intensities of the line spectra are monitored at specific wavelengths by a photosensitive device. Photo currents from the photosensitive device are processed and controlled by a computer system. A background correction technique is required to compensate for variable background contribution to the determination of the analyte.

**Equipment**

Axially viewed inductively coupled plasma emission spectrometer. Computer- conrolled emission spectrometer with background-correction capability. The spectrometer must be capable of meeting and complying with the requirements.

## Appendix 3

Images of the treated and untreated amoebae:

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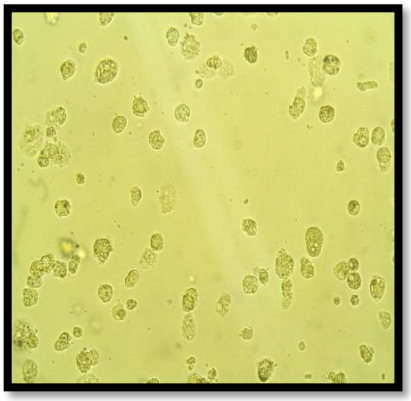
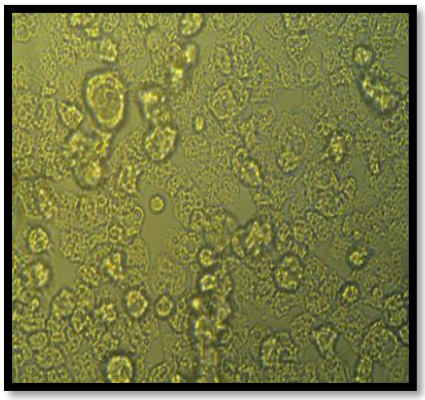
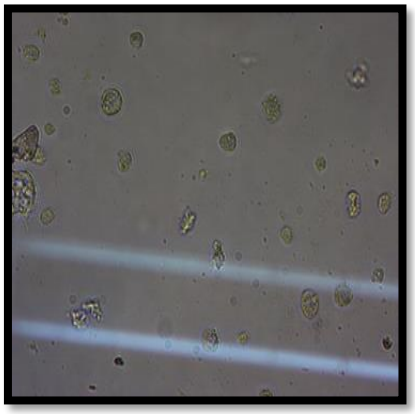
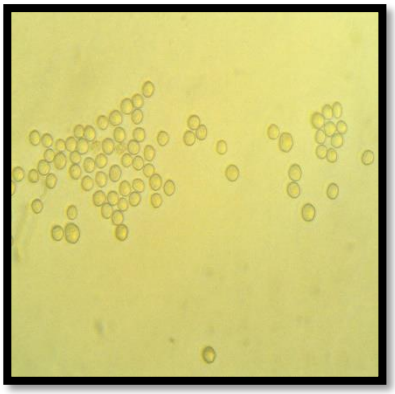
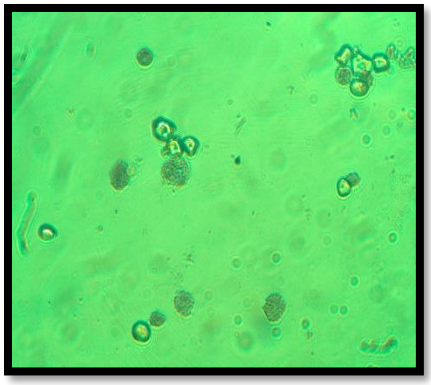
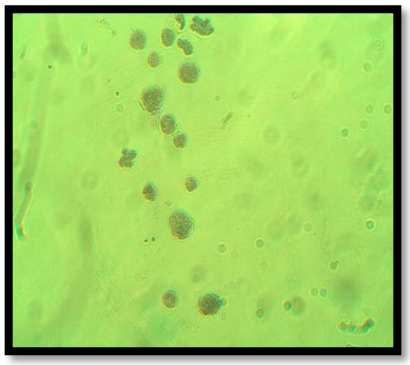
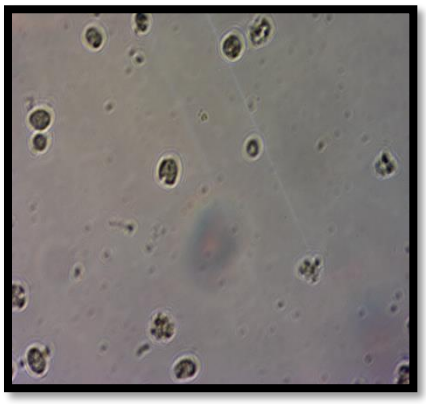
3.1 *Acanthamoeba* trophozoite:

| | |
|---|---|
|  |  |
| *Acanthamoeba* trophozoite 400x | *Acanthamoeba* trophozoite 400x |
|  |  |
| *Acanthamoeba* trophozoite 200X ( 5ppm Cl ) | *Acanthamoeba* trophozoite 200X (6 ppm Cl) |

3.2 *Acanthamoeba* cysts:

| | |
|---|---|
|  |  |
| *Acanthamoeba* cysts 400X | *Acanthamoeba* cysts 400X (5 ppm Cl) |
|  |  |
| *Acanthamoeba* cysts 400X (6 ppm Cl) | *Acanthamoeba* cysts 400X (12 ppm Cl) |