



RECORDS OF PHARMACEUTICAL AND BIOMEDICAL SCIENCES



Monitoring Freshwater Quality in Egypt and the Suez Canal Region: The Critical Role of Metagenomics and Integrated Assessment

Noha Elkayal, Samira Zakeer, Marwa Azab, Ali Abdellah Ahmed, and Sarah Shabayek*

Microbiology & Immunology Department, Faculty of Pharmacy, Suez Canal University

Abstract

Freshwater quality plays a critical role in ensuring safe drinking water, highlighting the need for continuous and comprehensive monitoring. In Egypt, the Nile River serves as the main freshwater source, contributing over 90% of the nation's water supply. The Ismailia Canal and its branches, vital freshwater sources for the Suez Canal region, face significant degradation due to different sources of pollution. Despite its importance, limited information exists on the bacterial composition of these freshwater systems. Traditional culture-based methods are commonly employed to detect indicator bacteria, identify dominant species, and evaluate antibiotic resistance. While these methods are useful, they fail to capture the full microbial diversity. To address this limitation, metagenomic approaches, particularly high-throughput 16S rRNA gene sequencing, provide a more comprehensive characterization of the water microbiome. This integrated approach, combined with physico-chemical assessments, offers deeper insights into bacterial community dynamics and pollution impacts, supporting improved water resource management and drinking water safety.

Keywords: Freshwater, indicator bacteria, antibiotic resistance, microbiome, sewage.

Received: 16. 10. 2025

Revised: 26. 10. 2025

Accepted: 28. 10. 2025

*Correspondence Author:

E-mail:

noha.elkayal@gmail.com

1. Introduction

Water is a vital component of life and its availability and purity are crucial for human health. Clean drinking water remains fundamental to global health, as water-related diseases account for over 829,000 deaths annually. There is a major effect on human health, the environment, ecosystems, and economic security that is caused by the accessibility, storage, transportation, and utilization of water (**UN-Water Annual Report, 2014, UNICEF, 2023**). Effective risk assessment and comprehensive management strategies are essential to ensure a safe drinking water supply, as these build confidence in water quality (**Fewtrell & Bartram, 2001**). In evaluating water

quality, it is essential to consider physical, biological, and chemical parameters based on the intended use (**Sargaonkar & Deshpande, 2003**).

In Egypt, the Nile River serves as the main freshwater source, contributing over 90% of the nation's water supply (**Wahaab & Badawy, 2004**). The Ismailia Canal, a major freshwater source for multiple governorates, originates from the Nile River in Cairo and flows eastward toward the Ismailia governorate, traversing Cairo, Qalioubeya, and Sharqeya governorates (**Stahl & Ramadan, 2008**). However, the discharge of pollutants like sewage, industrial effluents, and agricultural runoff has significantly degraded the water quality in the Ismailia Canal. This has led to increased levels of heavy metals and fecal

contamination in the canal's waters (**Goher et al., 2014**).

To monitor water quality, indicator bacteria like total coliforms, fecal coliforms (FC), and fecal streptococci (FS) are routinely tested to detect fecal contamination and potential declines in freshwater quality (**Ezzat S. et al., 2012**). The predominant bacterial species found in Nile surface water consist of *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Escherichia coli*, and *Staphylococcus aureus* (**Rabeh, 2001; Rifaat, 2007; Azzam et al., 2017**). However, research shows that these bacteria have been becoming more resistant to antibiotics, primarily as a result of the extensive and improper use of antibiotics in a variety of fields, such as veterinary care, agriculture, and medicine (**Azzam et al., 2017**). When released into wastewater, these antibiotics create selective pressure, fostering the development of resistant strains (**Reinthal et al., 2003**).

Traditional culture-dependent methods for microbial analysis are limited in their capacity to capture the full diversity of microorganisms in environmental samples. Metagenomics, by contrast, enables the study of uncultured microbes within their native habitats, providing a comprehensive overview of microbial diversity (**Steele & Streit, 2005**). Advances in next-generation sequencing (NGS) have made metagenomic methods more widely available and cost-effective, allowing for detailed profiling of microbial communities, particularly in water ecosystems. This approach, utilizing bacterial microbiome analysis, provides insights into the sources of aquatic microbial contamination (**Nakatsu et al., 2019**) and may contribute to improved surface water quality assessments and future drinking water treatment strategies (**Fiedler et al., 2018**).

Microbiome profiling offers valuable insights into microbial interactions across different taxa. For example, network-based methods reveal complex microbial interaction patterns, helping elucidate the ecological factors that shape microbial community composition (**Matchado et al., 2021**). **Barberán et al. (2012)** suggested that varying network structures across habitats reflect unique ecological principles governing microbial communities. Additionally, understanding these microbial networks in the context of ecosystem processes is critical, as environmental microbes are often considered functionally redundant (**Chen et al., 2022**). **Gamfeldt et al. (2008)** proposed that evaluating microbial functions can yield deeper insights into how biodiversity sustains ecosystem functionality.

Continuous monitoring of freshwater quality is crucial to maintaining safe drinking water. In Egypt,

traditional cultural methods have frequently been used to study the bacterial quality of Nile water, with significant contributions from researchers such as **Rabeh (2001)**, **Sabae & Rabeh (2007)**, **Rifaat (2007)**, **Ezzat et al. (2012)**, and **Azzam et al. (2017)**. Metagenomic methods have been less commonly applied; however, one of the earliest studies on the Nile's microbiome was conducted by **Eraki et al. (2019)** at Cairo University. In this minireview will shed light on the water quality in Egypt and Suez Canal region with highlighting the importance of integrating metagenomics for water quality assessment.

2. Water Supplies and Demand in Egypt

It is thought that around 97% of Egypt's water supply is obtained from the Nile River, with the remaining water coming from groundwater and rain that falls during specific seasons. Although it is anticipated that Egypt's water supply will reach 74.2 billion m³ annually by the year 2025, there is a possibility that there will be a shortfall of 26 billion m³ if the policies that are currently in place are not altered (**Abdel-Shafy and Aly, 2002; Omar and Moussa, 2016**).

2.1 The Nile River's Influence

According to **Wahaab and Badawy (2004)**, the Nile has been important to Egypt in many different aspects, including its history, politics, health, society, and economy. The river and its main irrigation canals are the main sources of drinking water for the Nile delta and valley, according to research by **Shamrukh and Abdel-Wahab (2010)**.

2.2 Pollution Sources in the Nile

Rises in population, irrigation projects, and industrial activities have all contributed to an increase in pollution in the Nile system, including the River Nile, its canals, and drains (**Goher et al., 2015; Abdel-Dayem et al., 2007**). This pollution has been increasing over the past few decades. One of the most significant causes for concern is the increased pollution that is a consequence of decreased water levels. This problem is made worse by developments related to the Ethiopia Dam (**Radwan et al., 2019**).

3. The Ismailia Canal and Its Branches and sources of pollution

The Ismailia Canal serves as the main supply of freshwater for a number of cities, villages, and governorates. It was built in 1862 to provide drinking water to the towns around the Suez Canal as well as to the laborers who were digging the Suez Canal path (**Gerish et al., 2008**).

The canal branches from the Nile River at north Cairo, near Shubra, and heads straight to the east toward Ismailia governorate, Nefesha, passing Cairo, Qalioubeya, and Sharqeya governorates, and then by Tal-kebeer, Qassasin, and Abosoir districts in the west of Ismailia governorate (Stahl and Ramadan, 2008). The canal splits into two arms at Ismailia: the Port-Said canal, which runs north to supply the town of Port-Said via the Qantara-Gharb district, and the Suez canal, which runs south to supply the town of Suez via the Fayed district. The Ismailia Canal spans 125 km from the Nile to Lake Timsah, with a depth of roughly 1-3 meters and a width of roughly 30-70 meters. The length of the Port Said branch is estimated at about 90 km. As for the Suez Branch, its length is 87 km (Geriesh *et al.*, 2008). See Figure 1.

Manayef Canal is a small branch of the Ismailia Canal that runs parallel to it through Manayef district. The Sinai Canal is a linkage canal that connects both the Ismailia and Suez Canals.

A short canal is passing through Qantara-Sharq district and is named Al-Abtal Canal. Al-Abtal Canal receives water coming through the Serapeum siphon, located under the New Suez Canal, and then delivers it to farmers in the area east of the New Suez Canal and Sinai (Khairy & Kamal, 2021). It begins in the west on the eastern bank of the Suez Canal and ends in the east at Al-Huda Canal in Qantara Sharq .

The water quality in the Ismailia Canal and its branches is negatively impacted by a number of different waste streams, such as sewage and industrial effluents (Geriesh *et al.*, 2008). Facilities for petroleum, steel, fertilizers, aluminum sulfate, and detergents are all types of industrial zones that are located along the canal. These facilities all contribute to the contamination of the canal. Even further, seepage from rural areas, septic tanks, and agricultural runoff all contribute to the deterioration of water quality (Goher *et al.*, 2014; Youssef *et al.*, 2010).



Figure 1. Map for Ismailia governorate showing Ismailia Canal and its branches to the north (Port-Said Canal) and south (Suez Canal).

4. Freshwater and Wastewater Treatment in the Ismailia governorate

4.1 Freshwater Treatment

The main processes in water purification can be summarized as follows: screening, coagulation, flocculation, sedimentation, purification, and sterilization (Geriesh *et al.*, 2008). In the Ismailia Governorate, there are 37 water treatment plants that are dispersed across a variety of freshwater canals. Water is supplied to these plants by the canal's outlets, which are located on the canal stream channel.

4.2 Wastewater treatment

In Egypt, the primary wastewater treatment methods include technologies such as trickling filters, activated sludge systems, oxidation ditches, stabilization ponds, constructed wetlands, rotating biological contactors (RBC), sequencing batch reactors (SBR), up-flow anaerobic sludge blankets, and modified septic tanks (Abdallah, 2014).

4.3 Al Mahsama waste water treatment plant

The construction of Al-Mahsama (waste water

treatment plant) WWTP, which is a large-scale wastewater treatment plant, was finished in the year 2020 in the Sinai Peninsula, which is found in the eastern region of the Suez Canal (Khairy & Kamal, 2021). The purpose of its establishment was to gather agricultural drainage water that was previously discharged into Timsah Lake (Abd El Samie *et al.*, 2008). in the Suez Canal with the intention of treating and transferring it to Al-Abtal Canal . That plant's function is to handle a blend of household waste and agricultural runoff from smaller agricultural drains, such as El-Mahsama Drain, with the intention of treating and transferring it to Al-Abtal Canal (Khairy & Ghany, 2021). The Al-Mahsama drain water treatment plant and the Serapeum siphon are located near the village of Serapeum, affiliated with the center and city of Fayed, south of Ismailia. The cultivation of 70,000 acres east of the Suez Canal in the Sinai Peninsula was made possible by the construction of water cranes that could reach the tertiary treatment plant through wells underneath it (Khairy & Kamal, 2021). See Figure 2.



Figure 2. Al-Mahsama (waste water treatment plant) WWTP in the eastern region of the Suez Canal.

5. Monitoring of Freshwater Quality

The simplest and most widely used description of water quality is "it is the physical, chemical, and biological characteristics of water." (Spellman, 2013). In addition to specific physical properties, the concentration and state of any or all of the organic and inorganic material found in the water can be used to describe the quality of the water. Therefore, water quality, biological life, microbiological traits, chemical analyses, particulate matter, and the physical characteristics of the water body must all be examined and assessed in order to fully evaluate the quality of the aquatic environment (Bartram & Ballance, 1996). The measured physical, biological, and chemical characteristics as well as the uses of the water, such as drinking, irrigation, or industrial use, are what determine the classification of water quality, according to Sargaonkar and Deshpande (2003).

6. Physicochemical Parameters

Numerous studies on altering the chemical and physical properties of Nile water have been conducted, and it has been confirmed that pollution sources can alter these properties. Among these are studies recorded by Abd El-Salam et al. (2017), Sabae and Rabeh (2006), Ezzat S. et al. (2012), Goher et al. (2014), Abdel-Shafy & Aly (2002), and Shamrukh & Abdel-Wahab (2010). These changes have the potential to drastically alter the environmental characteristics of the Nile River water (Abdel-Satar et al., 2017; Badr et al., 2013).

Physical and chemical parameters are usually measured as per Standard Methods for Examination of Water and Wastewater by the American Public Health Association (APHA, 2017) and mainly include: PH, total dissolved solids (TDS), biochemical oxygen demand (BOD), dissolved oxygen (DO), ammonia (NH₃), nitrate (NO₃-), nitrite (NO₂-), chloride (Cl-), calcium hardness (CaH), magnesium hardness (MgH), calcium (Ca), magnesium (Mg), total hardness (TH), and total alkalinity (T.Alk). Egypt Law 48/1982 for the Protection of the Nile and Waterways from Pollution (Egyptian Law 48/1982, 2013) and the WHO recommendation in Guidelines for Drinking Water Quality (WHO, 2022) serve as the reference range for the parameters.

7. Bacterial Indicators

Coliform bacteria are common in the environment. They are generally not harmful but indicate contamination of water with disease-causing organisms. They have long been used as an indicator

that the water is potentially harmful due to feces contamination (Rawway et al., 2016). Enterococci, *Escherichia coli*, and other aerobic bacteria are common markers of contamination that are present when pathogens are present (Sabae & Rabeh, 2007). Typical indicator bacteria, like total coliforms, fecal coliform bacteria (FC), and fecal streptococci (FS), are frequently used to assess fecal pollution and the risk of deterioration in water quality in freshwater sources (Ezzat S. et al., 2012). The main four genera of the family *Enterobacteriaceae*—*Escherichia*, *Klebsiella*, *Enterobacter*, and *Citrobacter*—make up the majority of the total coliforms found in surface water (Stevens et al., 2003). In the case of fecal coliforms, *E. coli*, *K. oxytoca*, and *K. pneumoniae* are the main species (Cabral and Marques, 2006). *Enterococci faecium* and *Enterococci faecalis* are among the dominant fecal streptococci in surface water (Sinton et al., 1993). Coliforms are rod-shaped, facultative anaerobic, gram-negative, non-spore-forming bacteria that ferment lactose with the formation of gas and acid at 35°C in 48 hours when the fermentation technique is used (APHA, 2017).

8. Pathogenic Species

Microbiological studies on the Nile over its different parts from north to south revealed the presence of many pathogenic bacterial strains. A study by Rifaat (2007) near Cairo revealed the presence of *Alcaligenes*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Rahnella*, *Rhodococcus*, *Xanthobacter*, *Pseudomonas*, *Streptomyces*, *Aeromonas*, and *Arthrobacter*. Osman et al. (2011) also found similar results beside *Salmonella* and *staphylococci* in Helwan, Giza, Embaba, and Shubbra El-Khema in Greater Cairo. A study by Sabae and Rabeh (2007) on Nile water at the Damietta branch found *Klebsiella pneumoniae*, *Pseudomonas flourcense*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella colerasuis*, *Shigella sp.*, *Proteus vulgaris*, and *Serratia liquefaciens*. However, *Citrobacter freundii*, *Proteus vulgaris*, *Salmonella typhi*, and *Escherichia coli* were isolated by Abo-State et al. (2012) from fifteen sites within the Rosetta branch. AbdelRahim et al. (2013) found that *Escherichia coli*, *Salmonella sp.*, *Pseudomonas aeruginosa*, and *Shigella sp.* were the dominant species in the Nile water in the Upper Egypt region, specifically in the Sohag governorate. The previously mentioned studies are corroborated by a collective meta-analysis conducted by Rabeh (2009) regarding the microbial conditions of the Nile River water between Aswan and Cairo. According to him, most domestic wastewater, both treated and untreated, is dumped into agricultural

drains, which leads to fast degradation in the delta (Damietta and Rosetta Branches) and in front of Cairo, especially when flow is low. **El-Taweel and Shaban (2001)** conducted a study that identified the classical bacterial indicators at two points on the Ismailia Canal; however, there is a dearth of information regarding pathogenic strains in the eastern part of the canal, specifically around the Ismailia Governorate. **Diab et al.** evaluated the Ismailia governorate's water treatment plants' capacities in a study they carried out in 2000. They compared the dominant species in the freshwater before and after treatment; they found 21 species, the majority of which are pathogenic and resistant to chlorine treatment. Among these species were *Staphylococcus* sp., *Escherichia coli*, *Bacillus* sp., *Corynebacterium* sp., *Klebsiella pneumoniae*, *Salmonella* sp., *Enterobacter cloacae*, *Moraxella* sp., *Shigella* sp., and *Proteus* sp.

9. Antimicrobial Resistance (AMR)

Water resources contaminated with pathogens can affect human health when consumed, applied to agricultural products, or used in any other way by humans (**Ramírez-Castillo et al., 2015**). When combined with the rise in antibiotic resistance, the issue of microbial water pollution could become even more complex (**Kraemer et al., 2019**). Since antibiotics are introduced into aquatic environments through medical treatment, agriculture, and animal farming, antibiotic resistance has been discovered in a variety of aquatic habitats (**Economou & Gousia, 2015**). A large proportion of consumed antibiotics end up in wastewater, where they can exert selective pressure on microorganisms or make them resistant (**Reinthal et al., 2003**). Previous studies on Nile water show an increase in antibiotic resistance because of the extensive use and misuse of antibiotics in medicine, veterinary medicine, and agriculture (**Azzam et al., 2017**).

More than 40% of the coliform and fecal coliform bacteria isolated from drinking water supplies were antibiotic-resistant, according to **Cooke (1976)**. Multiple antibiotic resistance were found to be prominently present in coliforms, including *Enterobacter aerogens*, *Citrobacter freundii*, *Citrobacter diversus*, *E. coli*, and *Klebsiella* species, according to a study conducted at the Godavari River in India (**Chitanand et al., 2010**). Enzymes known as extended-spectrum beta-lactamases (ESBLs) are capable of hydrolyzing the majority of penicillins and cephalosporins, such as cefuroxime, third- and fourth-generation cephalosporins, and aztreonam, but not cephamycins or carbapenems (**EUCAST, 2017**). *Klebsiella pneumoniae* and *Escherichia coli* are the

most common Enterobacteriaceae ESBL-producing species, and the CDC states in its 2019 report on antibiotic resistance that they pose a serious threat. *Acinetobacter*, *Aeromonas*, and *Pseudomonas* species are notable because they can survive and multiply in the environment, which gives them opportunities to promote horizontal gene transfer with other environmental bacteria (**Milligan et al., 2023**). *P. aeruginosa* is one of the many different types of *Pseudomonas* sp. that is well-known for being an agent of infections that are resistant to multiple antibiotics (**Horcajada et al., 2019**). Methicillin-resistant *Staphylococcus aureus* (MRSA) is a popular example of a bacteria that possesses resistance to several antibiotics, including aminoglycosides, macrolides, tetracycline, chloramphenicol, and lincosamides. These strains exhibit resistance to disinfectants, and MRSA serves as a significant source of hospital-associated infections (**de Lencastre et al., 2007**). Even though *Staphylococcus aureus* does not usually show microbiological resistance to vancomycin, it does happen a lot during clinical treatment. MRSA strains found in patients after long-term vancomycin treatment failure are still susceptible; in this case, they can be defined as vancomycin-resistant *Staphylococcus aureus* (VRSA) (**Machado et al., 2021**). When it comes to enterococci, previous studies have provided evidence that suggests vancomycin-resistant enterococcus (VRE) can still be found in wastewater effluent even after it has been treated through the treatment process. This finding indicates that VRE might contribute to the transmission of the infection to the environment (**Araújo et al., 2010**) and can cause highly epidemic infections (**Nnadozie & Odume, 2019**).

Culture-based antimicrobial susceptibility testing (AST) is considered the gold standard for assessing antibiotic resistance (**Poupard et al., 1994**). The Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) are two organizations that have standardised AST methods and test result interpretation, respectively (**CLSI, 2020; EUCAST, 2017**). The most often used techniques among these are the agar dilution method, the disc diffusion/Kirby-Bauer method, the broth microdilution method, Etest, the microdilution method, and the microcalorimetry method. (**Schumacher et al., 2017**).

According to recent research, a large number of clinically significant antimicrobial resistance genes (ARGs) were initially obtained from environmental microbes (**Milligan et al., 2023**). Techniques for determining ARGs in freshwater environments

include culture-based single strain approaches and molecular methods such as quantitative polymerase chain reaction (qPCR), metagenomics, and polymerase chain reaction (PCR) (**Nnadozie & Odume, 2019**). Although molecular methods can measure target pathogens and ARGs at a lower threshold for detection, they cannot link ARGs to host organisms (**Keenum et al., 2022**).

Developments in high-throughput DNA sequencing have facilitated and increased the accessibility of antibiotic resistance research (**Pillay et al., 2022**). According to **Schloss and Handelsman (2003)**, there are two types of metagenomic approaches: sequence- or function-based. Sequence-based methodologies rely on established sequences from the target gene families, whereas function-based methodologies exclude metagenomic sequencing data and may consequently result in the identification of novel gene sequences with specific functions (**Ngara & Zhang, 2018**). Bacterial resistance to antibiotics is an inherent trait that has developed across generations, resulting in a multitude of phenotypic and genotypic resistance mechanisms (**Bello-López et al., 2019**). These mechanisms, which include the antibiotic's inactivation, modification, degradation, and elimination, give the bacteria the ability to resist antimicrobial drugs (**Blair et al., 2014**).

Kyoto Encyclopaedia of Genes and Genomes (KEGG) metabolic pathways can be used for mapping genes linked to drug resistance (**Cloete et al., 2016**).

10. Metagenomic Analysis

Current advancements in molecular microbiology techniques have shown that over 99.8% of the microbes in some environments cannot be cultured (**Steele and Streit, 2005**). The most popular method for examining environmental prokaryotic diversity is the sequencing of 16S ribosomal RNA genes (**Logares et al., 2013**). The highly conserved 16S rRNA gene has nine rapidly evolving hypervariable regions that help identify different species (**Cheng et al., 2015**). According to **López-Aladid et al. (2023)**, microbiome studies increase the resolution capability for identifying bacterial taxa by combining two or more region sequences, typically V3–V4. 16S rRNA gene sequencing enables the taxonomic classification of individual organisms, the identification of metabolic and regulatory pathways, and the investigation of functional relationships among microbial community members (**Eraki et al., 2019**). The metagenomic analysis workflow is described in Figure 3.

10.1 Raw Data Manipulation

Many strategies have been put forth for the analysis of 16S rRNA (gene) sequencing data, and a growing body of scientific literature recommends software applications. Tools like AmpliconNoise, Quantitative Insights Into Microbial Ecology 2 (QIIME2), Mothur, SILVAngs, and MEGAN are frequently used for denoising and analyzing 16S data. Although there are many different programs and algorithms available for analyzing 16S metagenomics datasets, QIIME appears to be the "gold standard." (**Oulas et al., 2015**) Raw data is usually received as FASTQ files. Trimming low-quality reads is important to prepare the reads for downstream analysis. This can be done using platform-specific tools such as the FASTX-Toolkit (**Su et al., 2012**) and FastQC (**Huson et al., 2007**), which can provide summary statistics for FASTQ files.

The dataset is then demultiplexed, or reads are assigned to samples based on barcode information. Usually, demultiplexing is done when fastq files are created from the raw read data (**Wilkins et al., 2021**). For metagenomic data analysis, denoising is crucial (**Fischer-Hwang et al., 2019**), but it varies depending on the platform; for example, Illumina requires less denoising than others (**Oulas et al., 2015**).

There are three major categories of methods that can be identified: Three methods are available for the analysis of sequencing raw reads: grouping sequencing reads together to create operational taxonomic units (OTUs); creating amplicon sequence variants (ASVs) using corrected reads; and directly taxonomizing raw reads (**Straub et al., 2020**). Traditional methods involve assembling all 16S rRNA sequences that are similar by 97% and then assigning these to OTUs using reference trees (**Caporaso et al., 2010**). These methods do not include all the data; in particular, the assignments did not include sequence quality information or statistical data on the reads (**Callahan et al., 2016**).

Nevertheless, ASV techniques detect sequence variations that differ by as little as one nucleotide and identify the biological sequences in the sample prior to the introduction of amplification and sequencing errors. According to **Callahan et al. (2017)**, ASV methods offer superior ecological pattern discrimination and have shown sensitivity and specificity compared to OTU methods.

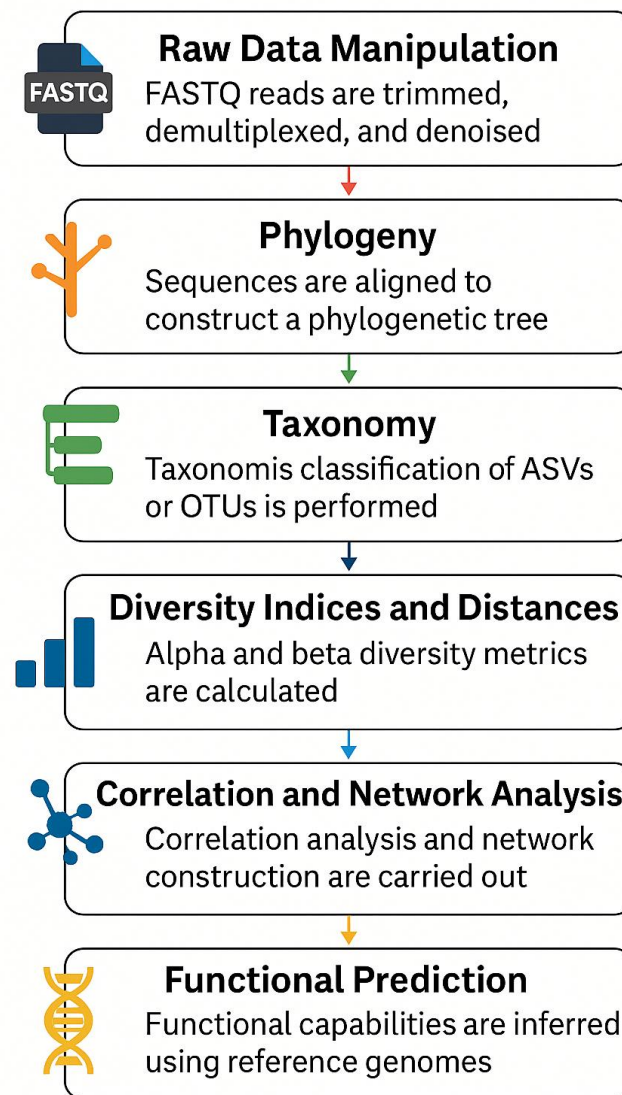


Figure 3. Workflow of 16S rRNA microbiome analysis showing the main analytical steps, beginning with raw data processing (quality control, trimming, and denoising), followed by phylogenetic reconstruction and taxonomic classification of microbial communities. Diversity analyses assess within- and between-sample variation, while correlation and network analyses reveal microbial interactions. The workflow concludes with functional prediction, identifying potential metabolic pathways and resistance genes to interpret ecological and health-related implications.

10.2 Phylogeny

A phylogenetic tree, or phylogeny, is a visual depiction that illustrates the evolutionary connections between different species, organisms, or genes that have descended from a common ancestor. It serves as a valuable tool for organizing information about biological diversity, creating classifications, and gaining understanding about evolutionary events (Baum, 2009). Phylogeny and microbial taxonomy may resemble each other in that each branch in the phylogeny has a taxonomic name. Although the

phylogeny is a more detailed framework for classifying microbes, it is not enough because only a small number of the branches in the phylogeny are currently labeled with taxonomic names (Washburne *et al.*, 2018). The most popular approaches for sequence analysis problems are most likely alignment-based ones (Geetika *et al.*, 2016). Sequence alignment is a technique used to find homologous sequences. It can be classified into two categories: Pairwise alignment, which involves only two sequences compared at a time, and multiple

sequence alignment, which compares more than two sequences. Alignment-based can be local or global (Munjal *et al.*, 2019). Multiple sequence alignments improve phylogenetic tree construction from sequence data (Ashkenazy *et al.*, 2018).

10.3 Taxonomy

The taxonomic classification of metagenomic sequences aims to identify the precise species from which they originated. This data is primarily used to classify different microbial groups that inhabit a specific environment (Rosen *et al.*, 2010). The taxonomic assignment of ASV/OTUs requires marker gene reference databases; some often used databases are Greengenes (16S), Silva (16S + 18S), Ribosomal Database Project (16S), and Greengenes (16S) (Oulas *et al.*, 2015).

Many approaches are developed for the taxonomic assignment of ASV/OTUs, mainly: direct comparison of local alignments, e.g. basic local alignment and search tool (BLAST) (Altschul *et al.*, 1997), post-processing of local alignments, e.g. MEGAN-like Last Common Ancestor LCA algorithms (Kahlke & Ralph, 2019), which is then developed to give higher classification accuracy under the software called Bayesian-based LCA method (BLCA) (Gao *et al.*, 2017), global alignment between query and reference reads, e.g., vsearch plugin in QIIME2 (Rognes *et al.*, 2016), approaches for machine learning based on k-mers (Orozco-Arias *et al.*, 2021), phylogenetic placement techniques, like building a de novo tree from the short sequences (Ewers *et al.*, 2023), probabilistic methods, e.g., PROTAX (Somervuo *et al.*, 2016), and the Rasbolasomal Database Project's (RDP) naïve Bayesian classifier (Lan *et al.*, 2012). Using QIIME, BLAST, and LCA techniques, along with suitable reference database curation and building a mock community that replicates system variation, seem to be the most efficient ways to generate accurate taxonomic assignments (Hleap *et al.*, 2021).

10.4 Diversity Indices and Distances

An important area of study in environmental microbiology is the evaluation of microbial diversity and distribution. It has been shown that the diversity and abundance of bacteria significantly influence how different ecosystems function in terrestrial settings (Delgado-Baquerizo *et al.*, 2017). Our knowledge of the diversity of bacteria in the environment is still lacking on several scales, despite the fact that they are the most diverse organisms on Earth. In order to be able to answer this big question, three interrelated components of biodiversity should be considered: alpha-, beta-, and gamma-diversity (Walters & Martiny, 2020). Alpha diversity refers to diversity on

a local scale, which describes the species richness in a single sample (Andermann *et al.*, 2022). Meanwhile, beta diversity describes variation in community composition among geographic locations (Lozupone *et al.*, 2007). The total species diversity among communities spread over a broader geographic area is referred to as gamma diversity (Walters & Martiny, 2020; Andermann *et al.*, 2022).

The statistical analysis of microbial diversity indices and distances can be done with a variety of tools and software programs. Alpha diversity measures the variety within a sample and is usually described by the number of variants present; this is determined with the Chao1 (Chao, 1984), observed number of OTUs/ASVs, and Abundance Coverage-based Estimator (ACE) (Chao *et al.*, 2000). Additional measures include evenness and species richness, such as the Simpson or Shannon indices (Simpson, 1949; Shannon, 1948). Faith's phylogenetic diversity, on the other hand, takes into account the phylogenetic relationship when it comes to predicting the diversity of microbes (Faith, 1992).

To describe variation in community composition (beta diversity), two sets of varying distances can be used: "phylogenetic" distances like unweighted and weighted UniFrac (Lozupone *et al.*, 2007) and "non-phylogenetic" ones like Bray-Curtis (Bray & Curtis, 1957) or Jaccard (Real & Vargas, 1996). Bray-Curtis and weighted Unifrac are quantitative metrics, whereas Jaccard and unweighted Unifrac are qualitative metrics.

10.5 Correlation and network analysis

Ecosystem function is a complicated result of the ecological and metabolic interactions of microbial communities that are always changing and growing in the environment (Falkowski *et al.*, 2008). These interactions take place on a very small scale and involve many different types of organisms with a wide range of abilities. There are numerous ways in which individual cells, strains, or functional groups that constitute a community can affect one another (Goldford *et al.*, 2018). When integrated with supplementary data on rate processes and environmental variables, network analyses can create reproducible hypotheses about potential ecological interactions that support ecosystem function (Wright *et al.*, 2012).

Finding microbial components that might have mutualistic relationships is done through network analysis, which primarily consists of calculating correlations, visualizing networks, and calculating network properties (Wen *et al.*, 2023). According to Matchado *et al.* (2021), network-based techniques

have been shown to be useful in understanding complex patterns of microbial interaction. They are employed in the assessment of co-occurrences between individual taxa and the identification of core community members (**Jameson et al., 2023**). These methods usually involve the pairwise approach, whereby a metric like Pearson or Spearman correlation is used to compare the co-occurrence or mutual exclusion pattern of two taxa (**Barberán et al., 2012**). Selecting an appropriate correlation cut-off to regulate the resulting network's density in correlation-based networks is a challenging task. Although the user is typically in charge of selecting the cut-off, certain approaches can automatically determine the best correlation threshold using a method based on random matrix theory (**Luo et al., 2007**).

10.6 Functional Prediction

Although it is an essential method for studying microbial communities, profiling phylogenetic marker genes, such as the 16S rRNA gene, does not directly demonstrate functional abilities of the community. Thus, computational methods were developed to predict a metagenome's functional composition using reference genome databases and marker gene data (**Langille et al., 2013**). The predominant approach to function prediction makes use of software for sequence homology. The fundamental idea behind this strategy is that proteins are far more frequently modified or reused for use in related functional systems across species than when they are introduced completely new (**National Research Council, 2010**). Tools like Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt2), Tax4Fun2, Pangenome-based Functional Profiles (PanFP), and metagenome-scale models (MetGEM) are used to find functional profiles from 16S rRNA gene sequencing data using various algorithms (**Matchado et al., 2024**). Using the KEGG database's recorded genomic data, these methods seek to forecast the abundances of functional genes (**Kanehisa & Goto, 2000**) or based on genomic models (**Ortiz-Estrada et al., 2018**). Currently, Tax4Fun2 and PICRUSt2 are the most commonly used methods. Functional capabilities and phylogeny have varying degrees of correlation, depending on how complex the traits are. However, **Langille et al. (2013)** found strong correlations between them. Several investigations have demonstrated the close relationship between the two. For example, **Yosef et al. (2021)** found a significant fraction of the functions involved in the metabolism and biodegradation of xenobiotics, as well as a high frequency of halotolerant Proteobacteria members, which are

important for biological reactor systems and the bioremediation of anthropogenic compounds. Many studies also found a strong relationship between functions and environmental conditions. **Azaroual et al. (2022)** conducted a study on nitrogen fixation proteins and nitrogenase cofactors in the rhizosphere of wheat plants. These functions exhibited lower levels of enrichment in plants from nitrogen-rich environments resulting from fertilizers, whereas they observed an increase in nitrogenase functions in plants from environments that lack fertilizers. Research conducted on lake water revealed a high concentration of metabolic functions linked to adaptation, the possibility of bioremediation, and the capacity of different types of microorganisms to endure in extremely salinized water (**Oyewusi et al., 2020**).

Functional prediction can give strong evidence about many important parts of an ecosystem's microbiome that can have an impact on public health, including pathogenicity and antibiotic resistance. It is important to understand that pathogenic microorganisms are not defined by "taxonomy"; rather, it is common for a particular microbial species to include both pathogenic and non-pathogenic members. For example, by analyzing the protein structure or the decoded catalytic site from a nucleic acid sequence, we can make predictions or at least have suspicions about the sequence of many significant microbial toxins (**National Research Council, 2010**). Several studies have suggested the hypothesis that antibiotic-resistant bacteria (ARB) play a role in pollutant metabolism processes, and their dominance is a significant factor in the increase of ARGs. **Li et al.** found that the most prevalent genera, like *Acinetobacter* spp., *Elizabethkingia* spp., *Aeromonas* spp., and *Bacillus* spp., were the main hosts for ARGs. These genera made the ARGs resistant to sulfamethoxazole, tetracycline, and β -lactams (**Li et al., 2024**).

The presence and degree of functional redundancy in microbial communities should be understood, as this could contribute to the stability of ecosystem processes (**Allison & Martiny, 2008**). There is a positive correlation between the Relative Functional Redundancy Index (FRI) and ecological stability and resilience, as stated by **Biggs et al. (2020)**. The Tax4Fun2 tool accurately predicted functional gene redundancies, which closely matched those observed in simulated microbial communities (**Wemheuer et al., 2020**).

11. Conclusion

This article provides a comprehensive analysis of freshwater quality in the Suez Canal region, focusing on the Ismailia Canal and its branches, which serve as

a critical water source for multiple Egyptian governorates. The study highlights the significant degradation of water quality due to pollution from sewage, industrial effluents, agricultural runoff, and residential wastewater. Traditional culture-based methods for monitoring bacterial contamination are limited, prompting the adoption of metagenomic approaches, such as high-throughput 16S rRNA gene sequencing, to explore the microbial diversity and antibiotic resistance in these water systems. The findings emphasize the importance of integrating physico-chemical measurements with advanced microbiome analysis to assess pollution impacts on water quality. Metagenomic methods offer a detailed understanding of microbial communities and their functional roles, providing valuable insights for improving water treatment strategies, addressing antibiotic resistance, and ensuring safe drinking water. This study also highlights the need for continuous monitoring and sustainable management of freshwater resources in the region.

12. References

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