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Molecular, Morphophysiological, Multidrug Resistance and Ultrastructural Analysis of Emerging Bacteria In Drinking Water

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Abstract

Tracking of drinking water excellent is essential for human health. The physicochemical consequences of the water samples had been below widespread degrees except for the HCO3- cation, which was high in any respect websites. The morphological results of fifty-eight pure bacterial isolates revealed two categories (Gram-positive and negative bacteria): biochemistry, PCR sequencing, and scanning electron microscopy (SEM) showed the distinction among the 14 selected bacterial isolates that they have been chosen based on morpho-chemical consequences of the same bacterial isolates. The antibiotics sensitivity outcomes discovered that all isolates were immune to nine to twelve antibiotics from fifteen examined exceptional antibiotics. The morphophysiological alignment of sequences and SEM records confirmed a rising sort of bacterial isolates from ingesting water sources. Phylogenetic trees confirmed all fourteen isolates, *Escherichia coli*, *Bacillus subtilis*, *Bacillus paralicheniformis*, *Bacillus licheniformis*, *Bacillus tequilensis*, *Bacillus velezensis*, *Pseudomonas fragi*, *Bacillus jeotgali*, *Klebsiella variicola* found in special clades primarily based on genetic distance. The combination of the cutting-edge microbial monitoring tools in consuming water-based totally on DNA sequencing and SEM analysis is essential. Additionally, the current research has a look at affords a record that distinguishes among extraordinary sorts of bacteria in ingesting water.

Keywords: Drinking water quality, water analysis, morpho-physiological analysis, multidrug resistance, *16S rRNA* gene, Sanger sequencing, Scanning electron microscopy

Introduction

The development of public consuming water resources became one of the maximum crucial technological advancements in developed countries throughout the 20th century, with a significant impact on public fitness (Ashour, El Attar, Rafaat, & Mohamed, 2009). Today, potable water is recognized as a human proper and is currently to be had to round eighty-three in line with cent of the sector's population, with a shortage of secure resources, particularly in rural regions of growing nations **(Water & Organization, 2006)**. The provision of potable water is a fundamental aspect of public health and improvement in any country. Egypt has each floor and groundwater source, such as lakes, streams, the Nile, and ponds (El Bedawy, 2014). The exception of a network's water resources is usually connected to its environmental hygiene or sanitation practices (Wagdy, 2008). Overpopulation, growing urbanization, agricultural sports, industrialization, and unsanitary behavior have affected the great of faucet water resources (Elarabawy, Tosswell, & Attia, 2000). Many water sources in Egypt have been observed now not to meet WHO standards, in keeping with research (Abdel-Dayem, 2011; Allam & Allam, 2007; Ayad, Khalifa, & ElFawy, 2021; El-Rawy, Abdalla, & El Alfy, 2020; El-Sadek, 2010; van Steenbergen & Dayem, 2007).

The chance associated with the pathogenicity of microbes is exacerbated using its capability to face up to destruction by antibiotics (León-Buitimea, Morones-Ramírez, Yang, & Peña-Miller, 2021). Biological treatment techniques inside the wastewater treatment flora can result in selective growth of antibiotic-resistant bacteria and consequently boom the incidence of multidrug-resistant organisms (Yahav, Tau, & Shepshelovich, 2021). Even though microorganisms in consuming water are decreased through chlorination, they could live on the remedy technique and enter the distribution community (Pichel, Vivar, & Fuentes, 2019).

The human fecal fabric is created from a significant portion of bacteria and carries an excessive level of coliform bacteria; these microbes are numerous with admire to their capability to travel through the soil matrix (Salam *et al*., 2021). Microorganisms, which include fecal coliform bacteria, *Escherichia coli*, and overall coliform microorganisms, serving as pathogenic

indicator species, do not always reason ailment, but they signal that the water is infected with ailment-inflicting pathogens (Aram, Saalidong, & Osei Lartey, 2021).

Bacillus species cells are rod-shaped, gram-positive microorganisms that arise clearly in soil and vegetation (Shen *et al*., 2020). The microbiological mechanisms that influence the prevalence and pathophysiology of microbial infections in ingesting water are poorly understood (Rizzo *et al*., 2013). There are numerous reasons for this lack of awareness: first, the proper detection, identification, and quantification of microorganisms in water is difficult and calls for an aggregate of classical and molecular methods; second, the virulence of waterborne pathogens varies extensively, relying on environmental situations and the treatment of the water supply; third, the transmission of waterborne infections is difficult and only feasible with a combination of classical and molecular strategies; and fourth, the transmission of waterborne infections is difficult and most effectively viable with a mixture of different variables (Sanganyado & Gwenzi, 2019).

Microbial pathogens found in drinking water consist of viruses, bacteria, and protozoa, which are all three forms of microorganisms. In step with the maximum recent surveillance information, the microorganism has been diagnosed as a result of the causative agent of most waterborne outbreaks in Korea **(Lee, Yun, Lee, Jung, & Lee, 2021)**. Furthermore, owing to the way of life changes and the advent of various new bacterial diseases, the contribution of pathogenic bacteria to waterborne epidemics is increasing in different aspects of the advanced world (Cissé, 2019), hence, we can recognize pathogenic bacteria in drinking water assets and seek advice from many recent opinions for data on other kinds of waterborne pathogens. Notwithstanding those advances, numerous scientists have highlighted flaws inside the use of *16S rDNA* sequences to evaluate microbial variety and phylogenetic analysis (Roux, Enault, le Bronner, & Debroas, 2011). Besides the reality that *16S rDNA* best covers a small part of the genome, its loss of relevant characteristics and gradual price of evolution can make it difficult to differentiate closely related bacterial lines and to remedy evolutionary trees (Farhat, Kim, & Vrouwenvelder, 2020). Natural lakes and rivers, reservoirs, as well as groundwater provide the majority of ingesting water, relying on the freshwater assets to be had inside the location (Gleick & Palaniappan, 2010).

Depending on the supplied water quality, it is miles treated using diverse methods consisting of flocculation and sand filtration before being introduced into the supply system for human consumption after a disinfection step together with ozonation or chlorination. (Nakazawa *et al*., 2021). On account that no treatment is sufficient to make certain the microbiological protection of ingesting water, the "multi-barrier precept" is used to lessen the chance of waterborne diseases (Rebelo, Quadrado, Franco, Lacasta, & Machado, 2020). Consequently, one of the targets of the microbiology of consuming water is to assess the microbiological efficiency of the specific remedy approaches in phrases of reduction of pathogenic microorganisms and chemical inactivation. Pathogenic bacteria are already present within the water supply or are added later **(**El-Kowrany *et al*., 2016**)**. This work aimed to isolate and perceive bacterial isolates from tap water.

Materials and methods

Samples collection

The water samples had been accumulated from Moshtohor, Toukh, Shibin Alqanatir, and Albaradiea websites, as shown **in Figure 1.** From each region, 10 samples were accrued in a 1-L sterilized bottle each, then, they were filtered using a sterilized filtration machine with a 0.22-µm filter out on the same collection day. The received filters have been cultured on Nutrient agar, MacConkey (MAC) agar, Blood agar, and Chocolate agar plates using sterilized long forceps below sterilization conditions at 35°C–37°C for 16–24 h.

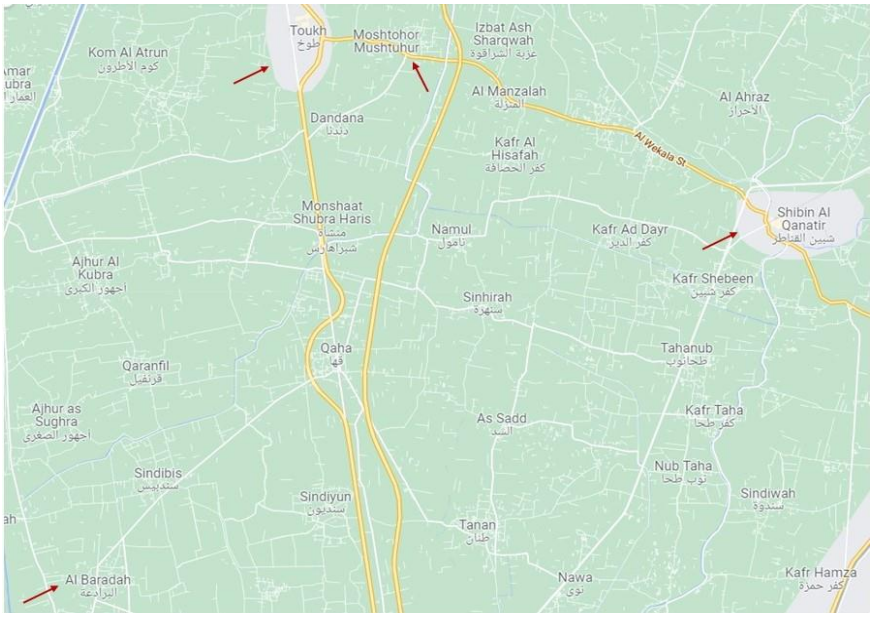

Fig.1. Map of the collection sites

Water analysis

The pH and EC were measured using a multi-pH-EC Bench Meter device (AD8000 professional multi-Parameter pH-ORP-Conductivity-TDS-TEMP Bench Meter, Hungary). The EC was finished using a digital conductivity probe in units of micro siemens per centimetre. The evaluation of water samples was conducted within the laboratory using a method established according to the Water and Wastewater standards exam guide (Federation, W. E., & APH Association, 2005). Sulfate was measured in water samples collected using a UV-Vis spectrophotometer, primarily based on the turbidimetric method (Federation, W. E., & APH Association, 2005; Hanrahan, Gardolinski, Gledhill, & Worsfold, 2002; Nagarkatti, 1991). Ions such as Cl^{-}, $HCO3^{-}$, $CO3^{2-}$, Ca^{2+} and Mg^{2+} have been measured using well-set up titrimetric techniques (Nagarkatti, 1991). Na+ and k+ were measured by the photometric flame emission technique using a flame photometer. These include the standard running techniques, widespread calibrations, blanks, and triplicate analyses of water samples.

Bacterial culture

The one-of-a-kind obtained bacterial colonies were subcultured on nutrient agar and MAC agar plates using the streaking method. Pure isolates were checked for purity through a simple stain (safranin stain). Approximately fifty-eight pure isolates were subjected to gram stain. Seven gram-positive and seven gram-negative bacterial isolates were taken into consideration for performing biochemical tests and *16S rDNA* approach. Furthermore, seven gram-positive and gram-negative organisms were decided on for conducting a scanning electron microscope.

Morphological and biochemical assays

For taxonomic identity, the isolates were subjected to a sequence of biochemical checks. Each isolates become checked for cell shapes, motility, Endospore forming, and Gram stain via phase-contrast Leica microscope. The physiological exams were conducted for indole production, aesculin (Esculin) hydrolysis, citrate usage, acetate utilization, Voges Proskauer, nitrate reduction, methyl pink, casein hydrolysis, increase in KCN, histamine-forming, lysine hydrolysis, tyrosine hydrolysis, gelatin hydrolysis, growth at 5% NaCl and 13% NaCl, tween 40 hydrolysis and carbohydrates fermentation (adonitol, arabinose, glycogen, malonate, dextrin, D-maltose, D-trehalose, D-cellobiose, xylose, gluconate, sorbose, sucrose, erythritol, dulcitol, D-melezitose, D-raffinose, α-D-lactose, D-melibiose, xylitol, ribose, D-salicin, N-acetyl-D glucosamine, α -D-glucose, D-mannose, D-fructose, D-galactose, ribose, L-rhamnose, inosine, D-sorbitol, D-mannitol, D-arabitol, Myo-inositol, glycerol, and inulin). Furthermore, the enzymatic activities examined have been Arginine dihydrolase, lipase production, pectinolytic activity, starch hydrolysis, lecithin's production, and proteolytic enzyme production **(**El-Gayar, 2017; Feng, Feng, & Shu, 2018; Javid, Shahabadi, Amirkhani, Amrollahi, & Ranjbar,

2020; Li *et al*., 2018; Nayarisseri, Singh, & Singh, 2019; Wang, Li, *et al*., 2017; Yoon *et al*., 2001).

Antibiotics sensitivity

An antibiotic sensitivity test was conducted using the Kirby-Bauer disk diffusion approach (**Bauer *et al*. 1966**). The following antibiotic discs (HIMEDIA, India) at the final concentrations, which can be indicated were used: Chloramphenicol (30-µg), Ampicillin (10-µg), Erythromycin (15-µg), Tetracycline (30-µg), Cefixime (5-µg), Nalidixic acid (30 µg), Amoxiclav (Amoxicillin/Clavulanic acid) (20/10-µg), Penicillin 10 units, Ciprofloxacin (5-µg), Amikacin (30-µg), Gentamicin (1-µg), Kanamycin (30-µg), Tobramycin (10-µg), Levofloxacin (5-µg) and Doripenem (10-µg). These antibiotics were selected because they are used in each human medication and animal veterinary practice or because previous studies have reported microbial resistance to them (Ateba & Bezuidenhout, 2008). Three colonies were picked from each sample, and each colony changed into transferred to 3-ml sterile distilled water to prepare a bacterial suspension. A 100-µL aliquots of each suspension were plated onto Mueller-Hinton agar plates. Antibiotic discs had been applied to the plates using sterile needles and the plates have been incubated at 37°C for 24 hours (National Committee for Medical Laboratory Standards, 1999). After incubation, the diameters of the antibiotic inhibition zone were measured as indicated in **Table 1**. The results received have been used to categorize the isolates as resistant, intermediate resistant, or sensitive to a selected antibiotic using well-known reference values in line with the National Committee for Clinical Laboratory Standards (National Committee for Clinical Laboratory Standards, 1999), now scientific and Laboratory Institute for Standardization. Numerous antibiotic resistance phenotypes were produced for isolates that exhibited resistance to three or extra antibiotics.

Table1. Sensitivity breakpoints (mm diameter) for the used antibiotics

Antibiotic (µg/disk)	Susceptible	Intermediate	Resistant
Chloramphenicol (30 µg)	≥18	13–17	≤12
Ampicillin (10 µg)	≥17	14–16	≤13
Erythromycin (15 µg)	≥23	14–22	≤13
Tetracycline (30 µg)	≥15	12–14	≤11
Cefixime (5 µg)	≤19	16–18	≤15
Nalidixic acid (30 µg)	≤19	14–18	≤13
Amoxyclav (Amoxicillin/Clavulanic acid) (20/10 µg)	≤18	14–17	≤13
Penicillin 10 units	≤21	18–20	≤17
Ciprofloxacin (5 µg)	≤21	16–20	≤15
Amikacin (30 µg)	≥17	15 to 16	≤14
Gentamicin (10 µg)	≥15	13 to 14	≤12
Kanamycin (30 µg)	≥18	14–17	≤13
Tobramycin (10 µg)	≥15	13–14	≤12
Levofloxacin (5 µg)	≥17	14–16	≤13
Doripenem (10 µg)	≥23	20–22	≤19

Genomic DNA extraction

The acquired bacterial isolates were sub-cultivated in LB Broth in a single day at 37°C. Five milliliter of bacterial boom turned into harvested through a spin at 8000 rpm for five minutes. The supernatant turned into discarded, and the pellet changed into stored for genomic DNA extraction. The genomic DNA changed into extracted the use of Wizard® Genomic DNA Purification kit (Cat# A1620, Promega, USA) consistent with manufacture commands. Moreover, the concentration and genomic purity of DNA were evaluated in every sample using a Nanodrop spectrophotometer set to 260-nm and 280-nm. Also, the integrity of genomic DNA was visualized on 1% agarose gel that stained with EtBr beneath Gel doc XR+ machine (Bio-Rad, USA). Then the purified DNA samples were saved at −20ºC for further research.

Molecular identification analysis

Oligonucleotide primers, widespread 27 forward, and 1429 reverse (Mao, Zhou, Chen, & Quan, 2012) were used to enlarge the *16S rRNA* gene. The *16S rDNA* was amplified from the received DNA isolates in response to PCR, and the situations were as follows: 25-µl Premix Taq™ DNA Polymerase (TaKaRa Taq™ model 2.0, cat# R004A), 0.5-µM from each ahead and reverse primers, 0.6-µg DNA, sterilized double-distilled water as much as 50-µl. this system for PCR turned into 30 cycles as follows: 98ºC for 10 s., 55ºC for 30 s., and 72ºC for one minutes. Amplification has accomplished the usage of the SensoQuest PCR system. Amplicons have been visualized using electrophoresis on 1.2% agarose gel after staining with EtBr underneath Gel

document XR+ machine (Bio-Rad, USA). The PCR product becomes purified through MinElute PCR purification kit (Cat. No. / id: 28004, Qiagen) as proven **in Fig. 2**, then, the purified PCR product turned into dispatched to MICROGEN company (South Korea) for sanger sequencing service.

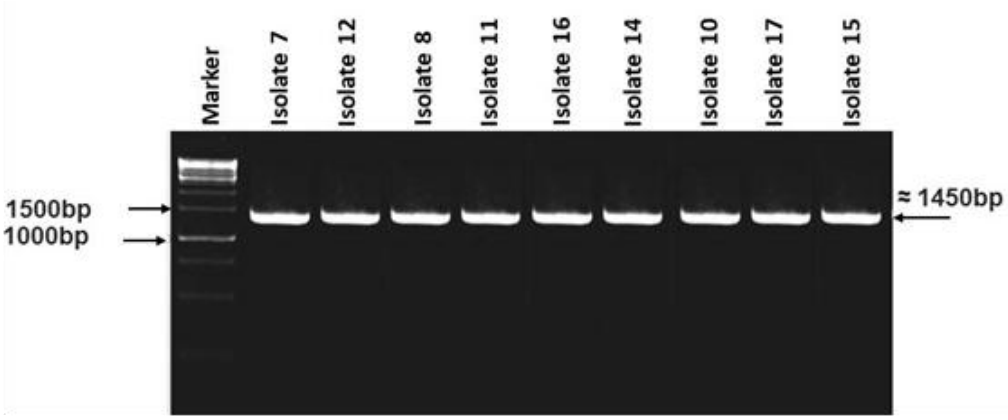

Fig.2. the amplicons of the 16S rRNA gene of the different bacterial isolates were visualized with Gel Doc System on 1.5% agarose gel that stained with EtBr. The detected amplified band was almost 1450bp.

Phylogenetic analysis

The alignment changed into completed the use of Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/) and was included in MEGA X software. The maximum appropriate nucleotide substitution model for the *16S rDNA* sequences was identified using the algorithm of the MEGA X software program. The nucleotide substitution and transition-transversion deviation have been assessed with the most chance method and Tamura-Nei version. The Phylogenetic tree changed into conducted the use of the maximum likelihood method with the most suitable version. The bootstrap consensus timber deduced from 500 replicates were purchased to represent the evolutionary history of the taxa and the use of MEGA X software was analyzed (Kumar, Stecher, Li, Knyaz, & Tamura, 2018; Tamura & Nei, 1993).

Scanning electron microscopy

Bacterial growth after 12 h was centrifuged (8000 × g) and prefixed in 2.5% glutaraldehyde (Sigma−Aldrich) in a single day at room temperature, washed thrice in 0.1-mM phosphate-buffered saline (PBS) for 15 minutes, and centrifuged again (8000 × g). The received bacterial cells had been fixed in 1% osmium tetroxide (Sigma-Aldrich) for 90 minutes and washed thrice in 0.1-mM PBS for 15 minutes, and finally dried with alcohol collection (50%, 70%, 80%, 90%, and 100%). The dried bacterial cell samples were stored at −20°C for 20 minutes and uncovered to important point drying. The bacterial cell samples were sputter-coated in about 12-nm gold and below vacuum for the '40s and subsequently tested using scanning electron microscopy. Four captures of regular structures at 1500 magnification confirmed the usage of a scanning electron microscope (FEI Company, Netherlands) model quanta 250 FEG (subject emission gun) concerned with EDX unit (strength dispersive x-ray analyses). The captures had been taken at an excitation voltage of 20-K.V., at distinctive magnifications varying from 400 to 6000 and working distance various from 13.7–14.2-mm. Handiest 1500 magnification was identified for the contemporary studies work. **(Wang, Li, *et al*., 2017).**

Results and Discussion

The quality of drinking water is an extremely vital factor in public health (Exum *et al*., 2018). Analysis of water samples had pH, EC, anions (Cl^-, $SO4^{2-}$ and $CO3^{2-}$), and cations (Na^+, K^+, Ca^{2+}, and Mg^{2+}) intensities within the acceptable limits set for drinking water except for $HCO3^-$ (Cotruvo, 2017). In this study, the EC records among different sites were ranged from 0.46–1.14-ds m^{-1}. pH records ranged from 6.64–7.11. The cations and anions records were under standard levels except for $HCO3^-$, which was higher than the standard level in different locations as shown in Table 2.

Table2. pH, EC, cations, and anions records in drinking waters

Property	Result				Permissible limit
	Shibin Alqanatir	Toukh	Moshtohor	Albaradiea	
EC (ds m^{-1})	0.46	1.14	0.8	0.9	0.75
pH	6.89	6.64	6.88	7.11	---
					Soluble cations (mmolec L^{-1})
Ca^{2+}	3.2	7.2	5.1	6.9	---
Mg^{2+}	0.4	2.0	0.9	1.56	---
Na^{+}	0.86	2.06	1.82	1.89	3.0
K^{+}	0.14	0.0	0.0	0.16	---
					Soluble Anions (mmolec L^{-1})
Cl^{-}	1.0	2.8	2.1	2.45	4.0
CO_3^{2-}	0.0	0.0	0.0	0.01	---
HCO_3^{-}	3.6	8.3	5.7	8.13	1.25
SO_4^{2-}	0.0	0.16	0.02	0.9	---
SSP %	18.7	20.07	23.3	17.98	60
SAR	0.64	0.96	1.05	0.75	13
Mg ratio	11.1	21.7	15	14.8	50
RSC	0.0	---	---	---	1.25

SAR: sodium adsorption ratio; SSP: Soluble Sodium Percent; RSC: residual sodium carbonate

The traditional method of identifying microorganisms based totally on phenotypic and biochemical traits is less specific than extra cutting-edge genotypic and SEM methods (Franco-Duarte *et al*., 2019). Within the current have a look at, the gram stain results divided the obtained bacterial isolates into two categories: gram-positive and negative microorganisms. Also, the easy stain outcomes displayed the prevailing isolates cell form in long rods and brief rods as proven in **Table 3 and 4**. The floating water drop research effects are prominent between the motile and nonmotile cells, as displayed in **Table 3 and 4.** The biochemical results of contemporary bacterial isolates like indole manufacturing, aesculin (Esculin) hydrolysis, citrate usage, acetate usage, Voges Proskauer, nitrate discount, methyl crimson, casein hydrolysis, increase in KCN, histamine-forming, lysine hydrolysis, tyrosine hydrolysis, gelatin hydrolysis, tween forty hydrolysis, boom at 5% NaCl, and 13% NaCl were recorded as proven in **Table 3 and 4.** Furthermore, the distinctive fermented sugars information noted of the received bacterial isolates that the examined sugars had been adonitol, arabinose, glycogen, malonate, dextrin, D-maltose, D-trehalose, D-cellobiose, xylose, gluconate, sorbose, sucrose, erythritol, dulcitol, D-melezitose, D-raffinose, α-D-lactose, D-melibiose, xylitol, ribose, D-salicin, N-acetyl-D glucosamine, α -D-glucose, D-mannose, D-fructose, D-galactose, ribose, L-rhamnose, inosine, D-sorbitol, D-mannitol, D-arabitol, Myo-inositol, glycerol, and inulin as represented in Table 3 and 4, moreover, the enzymatic activity records identified a diffusion of responses of bacterial isolates for arginine dihydrolase, lipase manufacturing, pectinolytic hobby, starch hydrolysis, lecithin's production, and proteolytic enzyme manufacturing as noted in Table 3 and 4. Many previous styles of literature completed the traditional biochemical strategies for identifying bacteria to manipulate organic infection (Al Moosa, Khan, Alalami, & Hussain, 2015; Havelaar & Melse, 2003).

Table3. Morphological and biochemical characteristics of gram-negative bacterial isolates

Isolates No. **Performed testes**	**Isolate 19**	**Isolate 23**	**Isolate 35**	**Isolate 39**	**Isolate 42**	**Isolate 10**	**Isolate 15**
Simple stain	**Short Rods**	**Short Rods**	**Short Rods**	**Short Rods**	**Short Rods**	**Short Rods**	**Short Rods**
Gram stain	-	-	-	-	-	-	-
Motility	+	+	+	+	+	-	
Growth at 35-37ºC	+	+	+	+	+	+	+
Oxidase	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+
Indole production	+	+	+	+	+	-	-
Citrate	-	-	-	-	-	**ND**	+
Voges Proskauer	-	-	-	-	-	**ND**	+
Methyl Red	-	-	**ND**	-	-	**ND**	**V**
Maltose	+	+	+	+	+	-	**ND**
Xylose	+	+	+	+	+	**ND**	**ND**
Sorbose	+	+	+	+	+	**ND**	+
Sucrose	+	**V**	**V**	+	+	**ND**	+
Raffinose	+	-	**V**	+	+	**ND**	**ND**
Lactose	+	+	+	+	+	**ND**	+
Glucose	+	+	+	+	+	+	**ND**
Mannose	+	+	+	+	+	+	**ND**
Rhamnose	**V**	+	+	**V**	+	**ND**	**ND**
Scientific Name	***Escherichia coli***	***Escherichia coli***	***Escherichia coli***	***Escherichia coli***	***Escherichia coli***	***Pseudomonas fragi***	***Klebsiella variicola***

ND: not detected; +: positive; -: negative; V: variable

Table4. morphological and biochemical characteristics of gram-positive bacterial isolates

Isolates No. / Performed testes	Isolate 7	Isolate 12	Isolate 8	Isolate 11	Isolate 16	Isolate 14	Isolate 17
Simple stain	Rods	Rods	Rods	Rods	Rods	Rods	Rods
Gram stain	+	+	+	+	+	+	V
Endospore forming	+	+	+	+	+	+	+
Motility	+	+	+	+	+	+	+
Indole production	-	-	ND	-	-	-	ND
Aesculin (Esculin)	-	+	+	-	-	-	+
Citrate	+	+	+	+	+	+	ND
Voges Proskauer	+	+	+	+	+	+	ND
Nitrate Reduction	+	V	ND	+	+	+	+
Methyl Red	-	-	ND	-	-	-	ND
Adonitol	-	-	ND	-	-	-	
Arabinose	+	-	+	+	+	+	-
Glycogen	+	+	ND	+	+	+	ND
Casein Hydrolysis	+	+	ND	+	+	+	ND
D-Maltose	+	-	+	+	+	+	-
D-Trehalose	+	+	+	+	+	+	ND
D-Cellobiose	+	V	+	+	+	+	ND
Xylose	+	-	ND	+	+	+	ND
Sucrose	+	V	V	+	+	+	+
Erythritol	-	ND	ND	-	-	-	ND
Dulcitol	-	ND	ND	-	-	-	ND
D-Raffinose	+	-	V	+	+	+	ND
α-D-Lactose	V	-	+	V	V	V	ND
D-Melibiose	V	-		V	V	V	ND
Xylitol	ND	ND	-	ND	ND	ND	ND
Ribose	+		+	+	+	+	ND
D-Salicin	+	V	+	+	+	+	ND
Lysine	-	-	ND	-	-	-	ND
Tyrosine Hydrolysis	-	+	ND	-	-	-	ND
N-Acetyl-D Glucosamine	ND	ND	ND	ND	ND	ND	-
5% NaCl	ND	ND	ND	ND	+	ND	+
13% NaCl	ND	ND	ND	ND	+	ND	+
Starch	+	+	+	+	+	+	ND
α-D-Glucose	+	-	+	+	+	+	+
D-Mannose	+	-	+	+	+	+	ND
D-Fructose	+	+	+	+	+	+	ND
D-Galactose	V	-	ND	V	V	V	ND
Ribose	ND	+	ND	ND	ND	ND	ND
L-Rhamnose	-	-	+	-	-	-	ND
D-Sorbitol	+	-	+	+	+	+	ND
D-Mannitol	+	-	+	+	+	+	-
D-Arabitol	-	-	ND	-	-	-	ND
myo-Inositol	+	-	ND	+	+	+	ND
Glycerol	+	+	+	+	+	+	ND
Inulin	V	-	V	V	V	V	ND
Gelatin	+	+	ND	+	+	+	+
L-Arginine	-	V	ND	-	-	-	ND
Oxidase	V	-	V	V	V	V	-

Catalase	+	+	+	+	+	+	+
Urease	-	**ND**	**V**	-	-	-	**ND**
Lecithinase	-	+	**ND**	-	-	-	**ND**
Ornithine Decarboxylase	-	-	**ND**	-	-	-	**ND**
Phenylalanine Deaminase	-	-	**ND**	-	-	-	**ND**
Scientific Name	***Bacillus subtilis***	***Bacillus paralichenifor mis***	***Bacillus licheniformis***	***Bacillus tequilensis***	***Bacillus velezensis***	***Bacillus subtilis***	***Bacillus jeotgali***

ND: not detected; +: positive; -: negative; V: variable

The wide distribution of antibiotic-resistant microorganisms in floor and groundwater has been said in subsequent studies (Amarasiri, Sano, & Suzuki, 2020). The multiplied prevalence of MAR in microorganisms from numerous water resources (Jeena, Deepa, Rahiman, Shanthi, & Hatha, 2006). Resistance can be assigned to heavy pollutants from exceptional wastewater kinds from the Nile River and groundwater (Ewida, Hegab, Zaghloul, Salem, & Abdel-Rahman, 2021). They have an impact on these results and cannot be overemphasized. Bacterial resistance reports are reliable with preceding investigations in other floor and consuming water systems (Zhang, Ying, Pan, Liu, & Zhao, 2015). Within the modern research, the bacterial isolates were exposed to an antibiotic sensitivity test using fifteen one-of-a-kind antibiotics from which their antibiotic resistance profiles and multiple antibiotic resistance phenotypes were recorded. The obtained consequences are represented in **Table 5**. The outcomes discovered that each isolate was proof against nine to 12 antibiotics from 15 tested extraordinary antibiotics as proven in desk one and five. The bottom antibiotic resistance of all examined isolates seemed with Gentamycin (6/14) accompanied through kanamycin (7/14), Amoxyclav (eight/14), and Chloramphenicol (8/14), respectively. The best antibiotic resistance of all isolates appeared with Erythromycin (eleven/14), Ciprofloxacin (eleven/14) and Levofloxacin (eleven/14) accompanied using Tetracycline (10/14), Cefixime (10/14), Penicillin (10/14), Tobramycin (10/14), and Doripenem (10/14), respectively as represented in desk five. Those findings, of our understanding, are first recoded for these examined antibiotics.

Table 5. Multiple drug resistant of bacterial isolates against different antibiotics

	Ampicillin	**Chloramphenicol**	**Erythromycin**	**Tetracycline**	**Cefixime**	**Nalidixic acid**	**Gentamicin**	**Amoxyclav**	**Penicillin**	**Ciprofloxacin**	**Kanamycin**	**Tobramycin**	**Levofloxacin**	**Doripenem**	**Amikacin**	**Multiple drug resistance (MDR)**
ISO 19	R	I	R	R	S	R	S	S	R	R	S	R	S	R	R	9/15
ISO 23	S	R	R	R	S	S	S	R	S	R	R	I	R	R	R	9/15
ISO 35	S	R	S	R	R	R	S	R	R	S	R	R	R	I	R	10/15
ISO 39	S	S	R	S	R	R	R	S	R	R	S	R	R	R	R	10/15
ISO 42	R	S	R	S	R	R	R	I	R	R	S	R	R	R	R	11/15
ISO 10	R	R	R	R	R	R	S	R	S	S	R	S	S	S	R	9/10
ISO 15	R	I	R	R	I	R	R	S	R	R	R	R	I	R	S	10/15

ISO 7	R	R	S	R	R	R	S	R	R	R	I	R	R	R	S	11/15
ISO 12	I	R	R	R	R	I	S	R	R	S	R	R	R	R	S	10/15
ISO 8	R	R	R	S	R	R	S	I	R	R	R	S	R	R	S	10/15
ISO 11	R	S	R	R	R	S	R	R	I	R	I	R	R	I	R	11/15
ISO 16	I	R	I	R	I	R	R	S	R	R	R	S	R	R	R	10/15
ISO 14	R	I	R	R	R	R	S	R	I	R	I	R	R	R	S	10/15
ISO 17	R	R	R	S	R	R	R	R	R	R	S	R	R	I	R	12/15
No. R/S	9/14	8/14	11/14	10/14	10/14	11/14	6/14	8/14	10/14	11/14	7/14	10/14	11/14	10/14	9/14	

ISO: Isolate; R: resistant; S: susceptible; I: intermediate

Using ribosomal RNA sequence and SEM analysis to explore microbial variety inside and between groups, and to find new strains, is each an implicit and a one-of-a-kind tactic (Magray, Kumar, Rawat, & Srivastava, 2011). The *16S rRNA* gene, which has been notably conserved and hypervariable regions used for strain identification, is observed as a minimum one replica in all bacterial species. Assessment of the bacteria *16S rRNA* gene sequence with a recognized bacterial collection within the database is a permitted genetic approach. Furthermore, the *16S rRNA* gene collection has been widely used to deduce the phylogenetic relationships among bacteria (Sujatha, Kumar, & Kalarani, 2012). The isolates were recognized exactly and definitively, in step with the results of this investigation. This confirmed that every one of the bacterial isolates belonged to extraordinary genera (Dai *et al*., 2020). Whilst in this look at five of the fourteen bacterial isolates were *Escherichia coli*, seven were *Bacillus species*, two were *Klebsiella variicola* and *Pseudomonas fragi*, respectively. A few former researchers have determined a flaw in traditional/nontraditional identification based on phenotypic trends (Brumfield *et al*., 2020; Shahraki, Chaganti, & Heath, 2021; Wragg *et al*., 2009).

The amplicon of *16S rDNA* of all fourteen bacterial isolates changed into positioned at the equal role on stained agarose gel recorded almost 1450 bp compared with 1-kbp ruler for instance as proven in Fig. 2. The assembled bacterial isolate sequences had been deposited within the NCBI GenBank database beneath the subsequent accession numbers: KY296349.1, KY296350.1, KY296352.1, KY296351.1, KY296355.1, KY296353.1, KY296354.1, KY296356.1, KY296357. 1, and OL589572.1-OL589576.1 as displayed in Fig. 3 and 4 as well as Table 6. The alignment DNA sequence results discovered that the modern bacterial isolates taxonomy belonged to firmicutes and proteobacteria phyla. Firmicutes, bacterial isolates, had been *Bacillus subtilis*, *Bacillus paralicheniformis*, *Bacillus licheniformis*, *Bacillus tequilensis*, *Bacillus velezensis*, and *Bacillus jeotgali* as represented in Table 4 and 6 as well as Fig. 3. While proteobacteria bacterial isolates were *Escherichia coli*, *Pseudomonas fragi*, and *Klebsiella variicola* as represented in tables three and six and Fig. 4. Phylogenetic trees confirmed that every isolate was placed in a distinctive clade in keeping with the genetic distance as shown in Fig. 3 and 4. The acquired bacterial isolates expressed identity for the closest traces deposited within the GenBank database that ranged from 98.73% to 99.90% as represented **in Table 6.**

Table 6. the similarity ratios among the current bacterial isolates and the nearest deposited ones in NCBI database.

The current bacterial isolate	Accession No.	The nearest one (GenBank)	Accession No.	Identity ratio
***Bacillus subtilis,* HMOS7, isolate 7**	**KY296349.1**	***Bacillus subtilis* strain F1F4**	**MK208590.1**	**98.73%**
***Bacillus paralicheniformis,* HMOS12, isolate 12**	**KY296350.1**	***Bacillus paralicheniformis* strain SA25**	**MT525268.1**	**99.52%**
***Bacillus licheniformis,* HMOS8, isolate 8**	**KY296352.1**	***Bacillus licheniformis* strain IND706**	**MT642946.1**	**99.52%**
***Bacillus tequilensis,* HMOS11, isolate 11**	**KY296351.1**	***Bacillus tequilensis* strain DHL27**	**MN833520.1**	**99.52%**
***Bacillus velezensis,* HMOS16, isolate 16**	**KY296355.1**	***Bacillus velezensis* strain XC1**	**MT649755.1**	**99.61%**
***Bacillus subtilis,* HMOS14, isolate 14**	**KY296353.1**	***Bacillus subtilis* strain BECMYCR5**	**MK286957.1**	**99.90%**
***Pseudomonas fragi,* HMOS10, isolate 10**	**KY296354.1**	***Pseudomonas fragi* strain A2H8**	**KY940315.1**	**99.43%**
***Bacillus jeotgali,* HMOS17, isolate 17**	**KY296356.1**	***Bacillus jeotgali* strain CQB-24**	**KR347228.1**	**99.32%**
***Klebsiella variicola,* HMOS15, isolate 15**	**KY296357.1**	***Klebsiella variicola* strain SA006**	**MN725749.1**	**99.13%**
***Escherichia coli,* HMOS19, Isolate 19**	**OL589572.1**	***Escherichia coli strain UFV 59***	**MN557358.1**	**96.57%**
***Escherichia coli,* HMOS23, Isolate 23**	**OL589573.1**	***Escherichia coli strain EHEC***	**MF919606.1**	**99.66%**
***Escherichia coli,* HMOS35, Isolate 35**	**OL589574.1**	***Escherichia coli* strain IRQBAS14**	**LN867523.1**	**98.16%**
Escherichia coli, HMOS39, Isolate 39	**OL589575.1**	***Escherichia coli* isolate L1/II-A-2**	**LT548254.1**	**97.12%**
***Escherichia coli,* HMOS42, Isolate 42**	**OL589576.1**	***Escherichia coli***	**Z83204.1**	**99.66%**

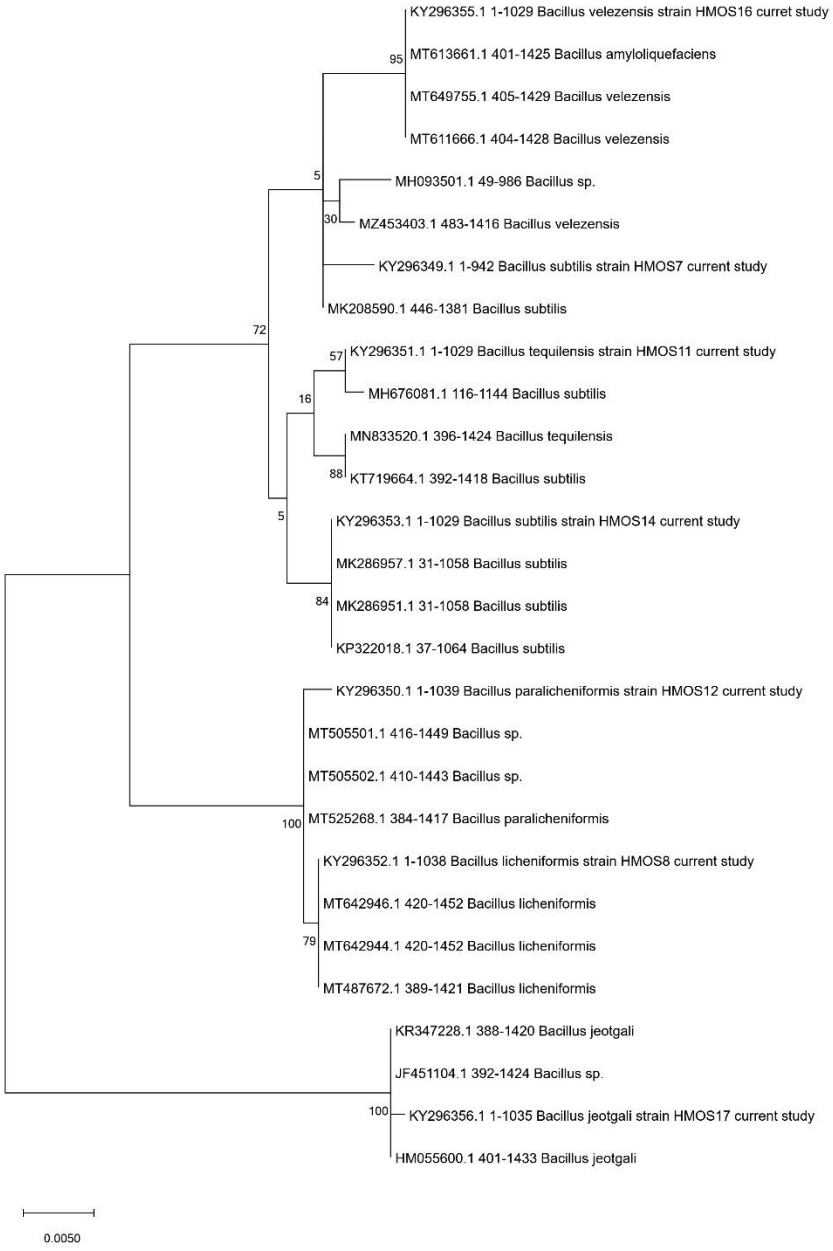

Fig.3. the created phylogenetic tree of the gram-positive bacterial isolates using MEGA X software and Maximum likelihood method. The phylogenetic tree displays *Bacillus subtilis*, HMOS7, isolate 7 *Bacillus paralicheniformis*, HMOS12, isolate 12; *Bacillus licheniformis*, HMOS8, isolate 8; *Bacillus tequilensis*, HMOS11, isolate 11; *Bacillus velezensis*, HMOS16, isolate 16; *Bacillus subtilis*, HMOS14, isolate 14 and *Bacillus jeotgali*, HMOS17, isolate 17 in different clades.

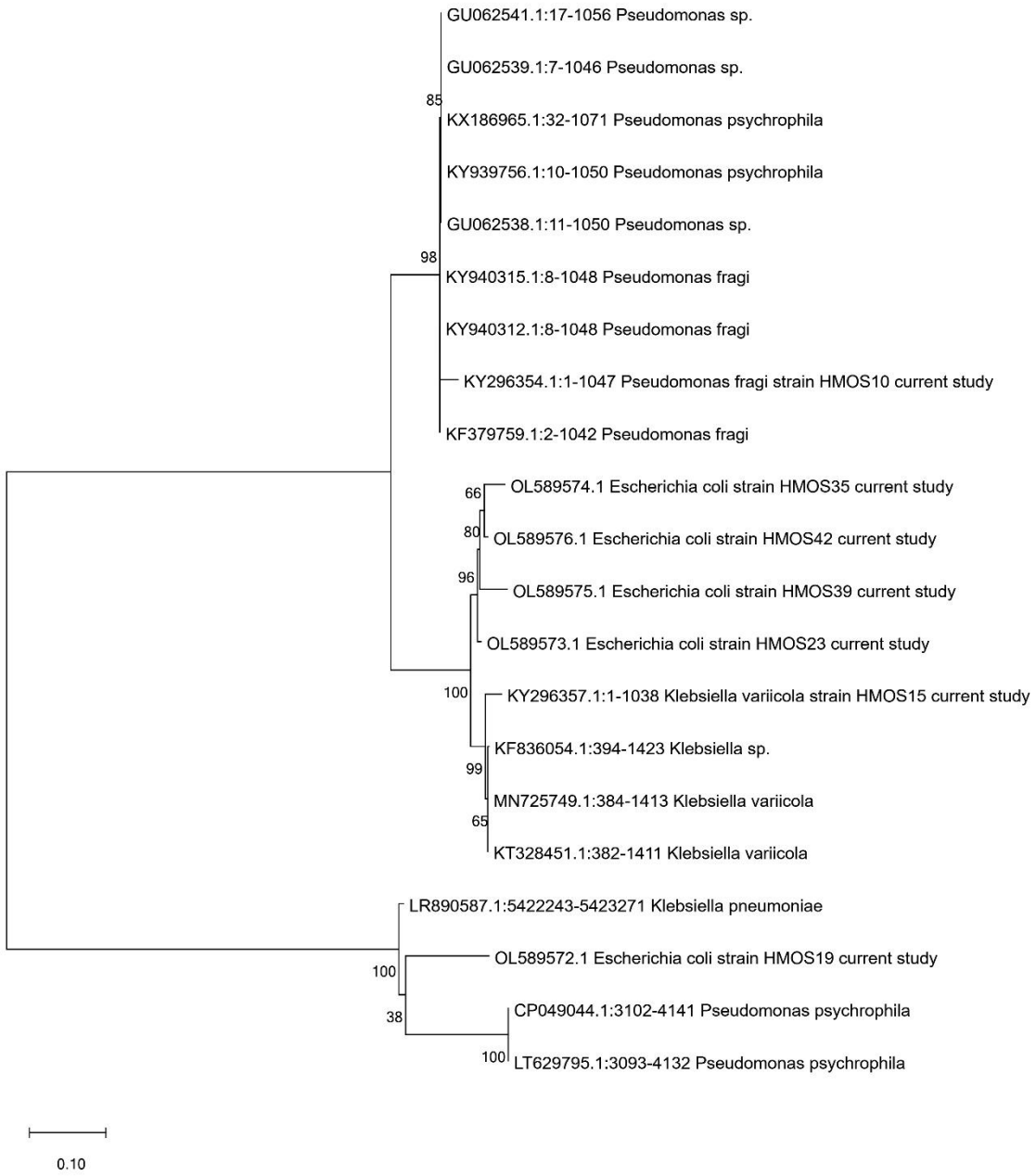

Fig.4. the created phylogenetic tree of the gram-negative bacterial isolates was made by using MEGA X software and Maximum likelihood method. The phylogenetic tree displays *Escherichia coli*, HMOS19, isolate 19, *Escherichia coli*, HMOS23, isolate 23, *Escherichia coli*, HMOS35, isolate 35, *Escherichia coli*, HMOS39, isolate 39, *Escherichia coli*, HMOS42, isolate 42, *Pseudomonas fragi*, HMOS10, isolate 10 and *Klebsiella variicola* HMOS15, isolate 15 in a separate clade.

The SEM assessment demonstrated the deep structure of the bacterial cells (Mei *et al*., 2020) of all nine present bacterial isolates and distinguished amongst themselves based on cell shape and length at ultrahigh intensification. The prevailing SEM results confirmed the similarity of the modern-day nine bacterial isolates with *Bacillus subtilis*, *Bacillus paralicheniformis*, *Bacillus licheniformis*, *Bacillus tequilensis*, *Bacillus velezensis*, *Bacillus jeotgali*, *Pseudomonas fragi*, and *Klebsiella variicola* as proven in Fig. 5.

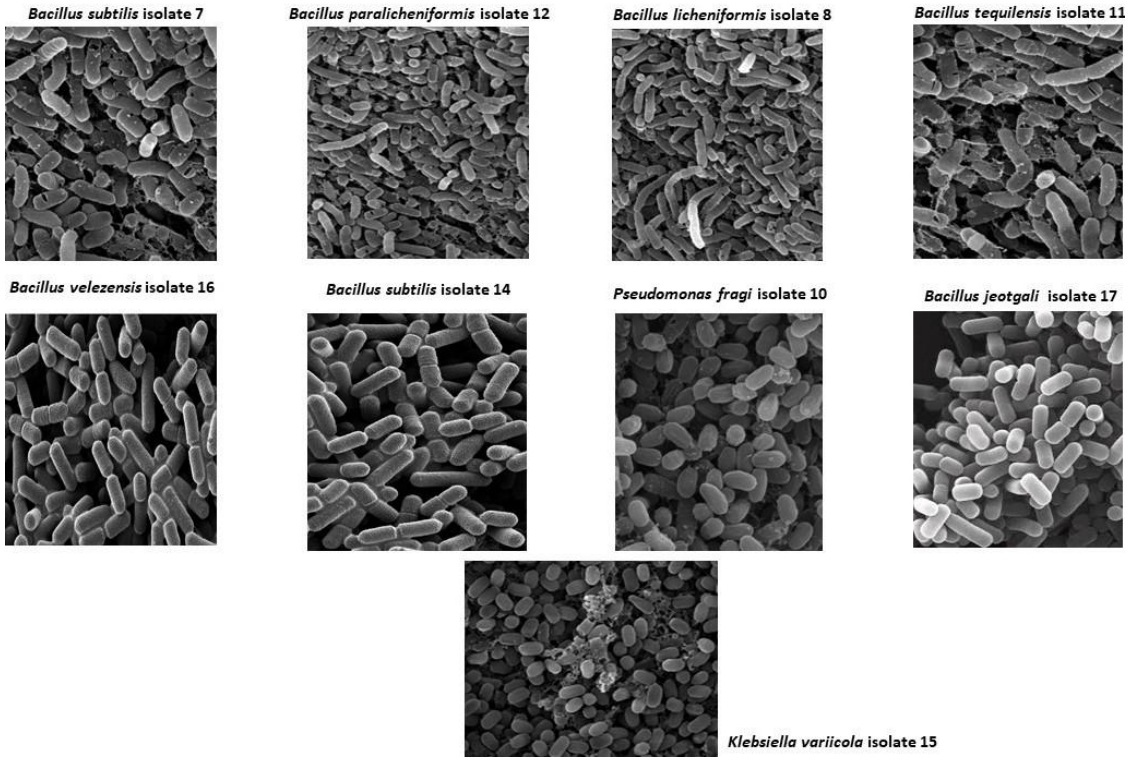

Fig.5 the SEM i
of the obtained n
bacterial
isolates fr
left-right as follows: *Bacillus subtilis*, HMOS7, isolate 7; *Bacillus paralicheniformis*, HMOS12, isolate 12; *Bacillus licheniformis*, HMOS8, isolate 8; *Bacillus tequilensis*, HMOS11, isolate 11; *Bacillus velezensis*, HMOS16, isolate 16; *Bacillus subtilis*, HMOS14, isolate 14; *Pseudomonas fragi*, HMOS10, isolate 10; *Bacillus jeotgali*, HMOS17, isolate 17 and *Klebsiella variicola* HMOS15, isolate 15**.**

Bacillus subtilis raises in slight temperatures. Starvation and pressure are common in this environment; therefore, *Bacillus subtilis* has several advanced strategies that allow survival under harsh conditions, such as the formation of strain-resistant endospores. Every other method is the absorption of extracellular DNA, to adapt the microorganism by recombination. However, these techniques take a long time. *Bacillus subtilis* can also shield itself faster against many disturbing conditions together with acidic, alkaline, osmotic or oxidative situations, and warmth (Zaghloul, Abdelaziz, & Mostafa, 1994). *B. subtilis* has no recognized pathogenic interactions with humans or animals; in contrast to the benefits of Gram-negative bacteria, the recombinant products produced by the cells of *B. subtilis* might not be contaminated with

lipopolysaccharide endotoxins (Zaghloul *et al*., 1994). Additionally, *Bacillus velezensis* changed into first located by Spanish researchers in Magaraga, southern Spain, recognized by constructing a phylogenetic tree for *16S rDNA* gene collection evaluation (Meng & Hao, 2017). Some scientific researchers have shown that *Bacillus velezensis* can synthesize various digestive enzymes together with amylase, protease, gelatinase, glucanase, and cellulase as well as degrade the robust decomposition of the natural count (Irorita Fugaban, Vazquez Bucheli, Holzapfel, & Todorov, 2021). Furthermore, *Bacillus velezensis* can inhibit the boom and replica of dangerous microorganisms and is capable of running down organics, organic sulphides, and natural nitrogen (Saravanabhavan, Govindasamy, Natesan, & Gopal, 2020).

Then again, *Pseudomonas fragi* is a psychrotrophic, Gram-negative bacterium that can increase at temperatures between 2°C and 35°C (Ercolini *et al*., 2010). Similarly, it may produce several styles of enzymes, including lipases and proteases (Wang, Wang, *et al*., 2017). Those enzymes are accountable for the deterioration of meat, fish, veggies, and dairy products. Over the past decades, many food spoilage problems have been associated with *P. fragi* around the world. Similarly, *Pseudomonas* species have been categorised as strictly cardio, *Pseudomonas* also can live to tell the tale in microaerophilic and anaerobic environments (Ercolini, Russo, Torrieri, Masi, & Villani, 2006; Filiatrault, Picardo, Ngai, Passador, & Iglewski, 2006).

Tap water is a large reservoir of waterborne pathogens (Bridge *et al*., 2010). Low water satisfaction, sanitation, and hygiene are accountable for 1.7 million deaths in line with 12 months worldwide, particularly, owing to infectious diarrhea (Baker *et al*., 2016). The five main environmental causes of mortality in children beneath five years, 361,000 youngsters beneath five years die of diarrhea, owing to confined get right of entry to safe ingesting water, sanitation, and hygiene (Hutton & Varughese, 2016). It is expected that waterborne ailments motive extra than million deaths and four billion cases of diarrhea per year. Infectious diarrhea is answerable for the best burden of this morbidity and mortality, and kids under five years are the most severely affected populations. Pathogens are made of bacteria, protozoa, and viruses (Girones *et al*., 2010). The maximum common waterborne bacterial illnesses are typhoid fever, bacillary dysentery, salmonellosis, *E. coli* contamination, campylobacteriosis, botulism, cholera, legionellosis, leptospirosis, and others (El-Kowrany *et al*., 2016).

Bacillus jeotgali is a facultative, Gram variable, and motile anaerobic rod. Its colonies are cream-orange, easy, abnormal, and flat. It grows in the presence of 13% NaCl. The boom does not arise inside the presence of more than 14% sodium chloride (Green-Ruiz, Rodriguez-Tirado, & Gomez-Gil, 2008). The growth temperature is 30°C–35°C. The choicest pH for the boom is seven to 8 ± 0. The boom is inhibited below pH 5 ± 0 (Yoon *et al*., 2001). The preceding findings agree with the outcomes of the present examination. Additionally, some publications have mentioned that *Bacillus tequilensis* may to produce biosurfactants (Nayarisseri *et al*., 2019).

Further, *Bacillus velezensis* may be used to do away with chemical oxygen demand based on simultaneous adsorption and biodegradation for wastewater treatment in further studies, improvement and use of genetic resources of *Bacillus velezensis* should be investigated further (Li *et al*., 2018). In assessment, *Klebsiella variicola*, a denitrifying bacterium, has been isolated from the scientific surroundings. The isolation of *K. variicola* from the natural surroundings is rarely suggested, and the maximum of these isolates originate from the endophytic environment (Feng *et al*., 2018).

Conclusions

This observes to the first-class of our know-how, reports the superiority of microorganisms in faucet water sources. As a result, the usage of morphophysiological, multidrug resistance, *16S rRNA* gene sequencing, and SEM evaluation in these findings has substantially aided in distinguishing and figuring out similar bacterial isolates belonging to other genera. The alignment and phylogenetic analysis revealed that the acquired bacterial isolates belongs to the one-of-a-kind species as follows *Escherichia coli*, *Bacillus subtilis*, *Bacillus paralicheniformis*, *Bacillus licheniformis*, *Bacillus tequilensis*, *Bacillus velezensis*, *Pseudomonas fragi*, *Bacillus jeotgali*, and *Klebsiella variicola*. In this research, the acquired bacterial isolate *Klebsiella variicola* can use within the elimination of nitrogen from wastewater in future research.

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Ethics approval

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated during the current study are available from the corresponding author on reasonable request.

Code availability

Not applicable.

Conflict of interest

The authors declare no competing interests.

Author Contributions Statement AE, MM, HE and MB, conceived, designed, and directed the research work, provided an explanation of the results, and created the figures. AE, MM, HE and MB shared in improving the data and developed some techniques. FE, AO, and FN drafted the original manuscript. All researchers provided essential feedback, helped shape the manuscript, argued the DATA, and contributed to the final version of the article.

References

Abdel-Dayem, S. (2011). Water quality management in Egypt. *International Journal of Water Resources Development*, 27(1), 181–202. https://doi.org/10.1080/07900627.2010.531522.

Al Moosa, M. E. A., Khan, M. A., Alalami, U. & Hussain, A. (2015). Microbiological quality of drinking water from water dispenser machines. *International Journal of Environmental Science and Development*, 6(9), 710–713. https://doi.org/10.7763/IJESD.2015.V6.685.

Allam, M. N. & Allam, G. I. (2007). Water resources in Egypt: future challeges and opportunities. *Water International*, 32(2), 205–218. https://doi.org/10.1080/02508060708692201.

Amarasiri, M., Sano, D. & Suzuki, S. (2020). Understanding human health risks caused by antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARG) in water environments: current knowledge and questions to be answered. *Critical Reviews in Environmental Science and Technology*, 50(19), 2016–2059. https://doi.org/10.1080/10643389.2019.1692611.

Aram, S. A., Saalidong, B. M. & Osei Lartey, P. (2021). Comparative assessment of the relationship between coliform bacteria and water geochemistry in surface and ground water systems. *PLOS ONE*, 16(9), e0257715. https://doi.org/10.1371/journal.pone.0257715.

Ashour, M. A., El Attar, S. T., Rafaat, Y. M. & Mohamed, M. N. (2009). Water resources management in Egypt. *JES. Journal of Engineering Sciences*, 37(2), 269–279. Available from: http://www.aun.edu.eg/journal_files/20_J_5125.pdf . https://doi.org/10.21608/jesaun.2009.121215.

Ateba, C. N. & Bezuidenhout, C. C. (2008). Characterisation of Escherichia coli O157 strains from humans, cattle and pigs in the North-West Province, South Africa. *International Journal of Food Microbiology*, 128(2), 181–188. https://doi.org/10.1016/j.ijfoodmicro.2008.08.011.

Ayad, A. R., Khalifa, A., & ElFawy, M. (2021). An Assessment of the integrated water resources management in Egypt. *Journal of Environmental Treatment Techniques*, 9(4), 751–768. https://doi.org/10.1016/j.ijfoodmicro.2008.08.011Baker, K. K., O'Reilly, C. E., Levine, M. M., Kotloff, K. L., Nataro, J. P., Ayers, T. L., Farag, T. H., Nasrin, D., Blackwelder, W. C., Wu, Y., Alonso, P. L., Breiman, R. F., Omore, R., Faruque, A. S., Das, S. K., Ahmed, S., Saha, D., Sow, S. O., Sur, D., Zaidi, A. K., Quadri, F. & Mintz, E. D. (2016). Sanitation and hygiene-specific risk factors for moderate-to-severe diarrhea in young children in the global enteric multicenter study, 2007–2011: case-control study. *PLOS Medicine*, 13(5), e1002010. https://doi.org/10.1371/journal.pmed.1002010.

Bridge, J. W., Oliver, D. M., Chadwick, D., Godfray, H. C. J., Heathwaite, A. L., Kay, D., Maheswaran, R., McGonigle, D. F., Nichols, G., Pickup, R., Porter, J., Wastling, J. & Banwart, S. A. (2010). Engaging with the water sector for public health benefits: waterborne pathogens and diseases in developed countries. *Bulletin of the World Health Organization*, 88(11), 873–875. http://doi.org/10.2471/BLT.09.072512.

Brumfield, K. D., Hasan, N. A., Leddy, M. B., Cotruvo, J. A., Rashed, S. M., Colwell, R. R. & Huq, A. (2020). A comparative analysis of drinking water employing metagenomics. *PLOS ONE*, 15(4), e0231210. https://doi.org/10.1371/journal.pone.0231210.

Cissé, G. (2019). Food-borne and water-borne diseases under climate change in low-and middle-income countries: Further efforts needed for reducing environmental health exposure risks. *Acta tropica*, 194, 181–188. https://doi.org/10.1016/j.actatropica.2019.03.012.

Cotruvo, J. A. (2017). 2017 WHO guidelines for drinking water quality. 1st addendum to 4th ed.. *Journal-American Water Works Association*, 109(7), 44–51. Available from: https://www.who.int/publications-detail-redirect/9789241549950.

Dai, Z., Sevillano-Rivera, M. C., Calus, S. T., Bautista-de Los Santos, Q. M., Eren, A. M., Van Der Wielen, P. W. J. J., Ijaz, U. Z. & Pinto, A. J. (2020). Disinfection exhibits systematic impacts on the drinking water microbiome. *Microbiome*, 8(1), 42. https://doi.org/10.1186/s40168-020-00813-0.

El Bedawy, R. (2014). Water resources management: alarming crisis for Egypt. *Journal of Management*

and Sustainability, 4(3), 108. https://doi.org/10.5539/jms.v4n3p108.

Elarabawy, M., Tosswell, P. & Attia, B. (2000). Integrated water resources management for Egypt. *Journal of Water Supply: Research and Technology-Aqua*, 49(3), 111–125. https://doi.org/10.2166/aqua.2000.0010.

El-Gayar, K. E. (2017). Isolation, identification and characterization of Bacillus subtilis FROM tap water, 19(4), 817.

El-Kowrany, S. I., El- Zamarany, E. A., El-Nouby, K. A., El-Mehy, D. A., Abo Ali, E. A. A., Othman, A. A., Salah, W. & El-Ebiary, A. A. (2016). Water pollution in the Middle Nile Delta, Egypt: an environmental study. *Journal of Advanced Research*, 7(5), 781–794. https://doi.org/10.1016/j.jare.2015.11.005.

El-Rawy, M., Abdalla, F. & El Alfy, M. (2020). Water resources in Egypt. In:. *Regional Geology Reviews*. Springer. Available from: https://link.springer.com/chapter/10.1007/978-3-030-15265-9_18, (687–711). https://doi.org/10.1007/978-3-030-15265-9_18.

El-Sadek, A. (2010). Virtual water trade as a solution for water scarcity in Egypt. *Water Resources Management*, 24(11), 2437–2448. https://doi.org/10.1007/s11269-009-9560-9.

Ercolini, D., Casaburi, A., Nasi, A., Ferrocino, I., Di Monaco, R., Ferranti, P., Mauriello, G. & Villani, F. (2010). Different molecular types of Pseudomonas fragi have the same overall behaviour as meat spoilers. *International Journal of Food Microbiology*, 142(1–2), 120–131. https://doi.org/10.1016/j.ijfoodmicro.2010.06.012.

Ercolini, D., Russo, F., Torrieri, E., Masi, P. & Villani, F. (2006). Changes in the spoilage-related microbiota of beef during refrigerated storage under different packaging conditions. *Applied and Environmental Microbiology*, 72(7), 4663–4671. https://doi.org/10.1128/AEM.00468-06.

Ewida, A. Y., Hegab, A. F., Zaghloul, R. A., Salem, A. A. & Abdel-Rahman, H. M. (2021). Environmental impact assessment of different water resources in Egypt in comparison with antibiotic resistance activity of their bacterial communities. *Journal of American Science*, 17(4). https://doi.org/10.7537/marsjas170421.04.

Exum, N. G., Betanzo, E., Schwab, K. J., Chen, T. Y. J., Guikema, S. & Harvey, D. E. (2018). Extreme precipitation, public health emergencies, and safe drinking water in the USA. *Current Environmental Health Reports*, 5(2), 305–315. https://doi.org/10.1007/s40572-018-0200-5.

Farhat, N., Kim, L. H. & Vrouwenvelder, J. S. (2020). Online characterization of bacterial processes in drinking water systems. *npj Clean Water*, 3(1), 1–7. https://doi.org/10.1038/s41545-020-0065-7.

Feng, Y., Feng, J. & Shu, Q. L. (2018). Isolation and characterization of heterotrophic nitrifying and aerobic denitrifying Klebsiella pneumoniae and Klebsiella variicola strains from various environments. *Journal of Applied Microbiology*, 124(5), 1195–1211. https://doi.org/10.1111/jam.13703.

Filiatrault, M. J., Picardo, K. F., Ngai, H., Passador, L. & Iglewski, B. H. (2006). Identification of Pseudomonas aeruginosa genes involved in virulence and anaerobic growth. *Infection and Immunity*, 74(7), 4237–4245. https://doi.org/10.1128/IAI.02014-05.

Franco-Duarte, R., Černáková, L., Kadam, S., Kaushik, K. S., Salehi, B., Bevilacqua, A., Corbo, M. R., Antolak, H., Dybka-Stępień, K., Leszczewicz, M., Relison Tintino, S., Alexandrino de Souza, V. C., Sharifi-Rad, J., Coutinho, H. D. M., Martins, N. & Rodrigues, C. F. (2019). Advances in chemical and biological methods to identify microorganisms-from past to present. *Microorganisms*, 7(5), 130. https://doi.org/10.3390/microorganisms7050130.

Girones, R. Ferrus, M. A., Alonso, J. L. Rodriguez-Manzano, J., Calgua, B., de Abreu Correˆa, A., . . . Bofill-Mas, S. (2010). Molecular detection of pathogens in water–the pros and cons of molecular techniques. *Water Research, 44*(15), 4325-4339. https://doi.org/10.1016/j.watres.2010.06.030.

Gleick, P. H. & Palaniappan, M. (2010). Peak water limits to freshwater withdrawal and use. *Proceedings of the National Academy of Sciences of the United States of America*, 107(25), 11155–11162. https://doi.org/10.1073/pnas.1004812107.

Green-Ruiz, C., Rodriguez-Tirado, V. & Gomez-Gil, B. (2008). Cadmium and zinc removal from aqueous solutions by Bacillus jeotgali: pH, salinity and temperature effects. *Bioresource Technology*, 99(9), 3864–3870. https://doi.org/10.1016/j.biortech.2007.06.047.

Hanrahan, G., Gardolinski, P., Gledhill, M. & Worsfold, P. (2002). Environmental monitoring of nutrients. In: Burden FR, Mckelvie ID, Forstner U, Guenther A, Eds. Environmental monitoring handbook. New York: McGraw-Hill.

Havelaar, A. & Melse, J. (2003). Quantifying public health risk in the WHO Guidelines for drinking-water quality: A burden of disease approach.

Hutton, G. & Varughese, M. (2016). The costs of meeting the 2030 sustainable development goal targets on drinking water, sanitation, and hygiene.

Irorita Fugaban, J. I., Vazquez Bucheli, J. E., Holzapfel, W. H. & Todorov, S. D. (2021). Bacteriocinogenic Bacillus spp. Isolated from Korean fermented cabbage (kimchi)—beneficial or hazardous?

Fermentation, 7(2), 56. https://doi.org/10.3390/fermentation7020056.

Javid, P., Zadabbas Shahabadi, H. Z., Amirkhani, H., Amrollahi, N. & Ranjbar, M. S. (2020). Isolation and identification of halophilic and halotolerant bacteria from the sediments of the Qeshm Island mangrove forest. *Advances in Oceanography and Limnology*, 11(1). https://doi.org/10.4081/aiol.2020.8743.

Jeena, M. I., Deepa, P., Mujeeb Rahiman, K. M., Shanthi, R. T. & Hatha, A. A. (2006). Risk assessment of heterotrophic bacteria from bottled drinking water sold in Indian markets. *International Journal of Hygiene and Environmental Health*, 209(2), 191–196. https://doi.org/10.1016/j.ijheh.2005.11.003.

Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, 35(6), 1547–1549. https://doi.org/10.1093/molbev/msy096.

Lee, S. H., Yun, J. W., Lee, J. H., Jung, Y. H. & Lee, D. H. (2021). Trends in recent waterborne and foodborne disease outbreaks in South Korea, 2015–2019. *Osong Public Health and Research Perspectives*, 12(2), 73–79. https://doi.org/10.24171/j.phrp.2021.12.2.04.

León-Buitimea, A., Morones-Ramírez, J. R., Yang, J. H. & Peña-Miller, R. (2021). Editorial: Facing the upcoming of multidrug-resistant and extensively drug-resistant bacteria: novel antimicrobial therapies (NATs). *Frontiers in Bioengineering and Biotechnology*, 9, 636278. https://doi.org/10.3389/fbioe.2021.636278.

Li, W., Jia, M. X., Deng, J., Wang, J. H., Lin, Q. L., Liu, C., Wang, S. S., Tang, J. X., Zeng, X. X., Ma, L., Su, W., Liu, X. Y., Cai, F. & Zhou, L. Y. (2018). Isolation, genetic identification and degradation characteristics of COD-degrading bacterial strain in slaughter wastewater. *Saudi Journal of Biological Sciences*, 25(8), 1800–1805. https://doi.org/10.1016/j.sjbs.2018.08.022.

Magray, M. S. U. D., Kumar, A., Rawat, A. K. & Srivastava, S. (2011). Identification of Escherichia coli through analysis of *16S rRNA* and 16S-23S rRNA internal transcribed spacer region sequences. *Bioinformation*, 6(10), 370–371. https://doi.org/10.6026/97320630006370.

Mao, D. P., Zhou, Q., Chen, C. Y. & Quan, Z. X. (2012). Coverage evaluation of universal bacterial primers using the metagenomic datasets. *BMC Microbiology*, 12(1), 66. https://doi.org/10.1186/1471-2180-12-66.

Federation, W. E., & APH association. (2005). Standard methods for the examination of water and wastewater. Washington, DC: American Public Health Association (APHA).