

## Molecular identification of biological contaminants in different drinking water resources of the Jazan region, Saudi Arabia

Emad Abada, Zarrag Al-Fifi, Abdul Jabbar Al-Rajab, Mosbah Mahdhi and Mukul Sharma

### ABSTRACT

Drinking water quality plays a remarkable role in human infections and diseases. This study used polymerase chain reaction (PCR) techniques to detect bacterial pathogens. In addition, a physicochemical analysis was performed on drinking water samples from several sources. A total of 123 drinking water samples were collected from different areas in the Jazan region in Saudi Arabia: ground water (40 samples), bottled water (15 samples), tap water (52 samples), and water purification shops (16 samples). To isolate the bacterial pathogens, the water samples were spread on Nutrient and MacConkey agar media, and the grown pathogens were then identified by the 16S ribosomal RNA technique. In 87 (70.7%) of the 123 drinking water samples, there was no pathogen growth on the two-culture medium. However, 36 (29.3%) of the samples were found to be contaminated with bacteria. The physicochemical analysis indicated that the water samples were within the Saudi drinking water standards. The bacteria were resistant to Cefotaxime, Cefotaxime/Clavulanic acid, Erythromycin, Penicillin G, Rifampin and Sulfamethoxazole–Trimethoprim, respectively. The findings suggest that in Jazan, bottled water is a safer source of potable water than tap water. The contamination in the water may be occurring at the reservoirs rather than the water sources.

**Key words** | bacteria, drinking water, PCR, water quality

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### INTRODUCTION

Access to safe drinking water can improve health, productivity, and the ability to earn a living. Sustainable development and poverty reduction can be achieved by the availability and accessibility of clean, fresh water (Al-Bratty *et al.* 2017). The most suitable water for human use originates from rivers, ground water, aquifers, lakes, and waterways. Because water is in short supply in Saudi Arabia, groundwater is the main source of the water that is suitable for human ingestion (Alhababy & Al-Rajab 2015).

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Jazan is located in the southwestern part of Saudi Arabia (16°53'21"N 42°33'40"E). It is approximately 13,457 km<sup>2</sup>, and according to the 2010 census, the population is 1,365,110. It is considered to be the most densely populated area in Saudi Arabia (Al-Hatim *et al.* 2015). In Jazan, the potable options are mainly bottled water or water available in plastic containers after being processed at private water treatment stations (hereafter referred to as water purification shops). In addition, the local government in Jazan supplies treated desalinated sea water mixed with rain water to homes through pipes. Several studies have reported on the physical and chemical contamination of groundwater by wastewater, pesticides, fertilizers, and industrial waste

(Ashbolt 2004). The results of that study showed that the water quality of the samples was within the World Health Organization's (WHO's) guidelines.

Human enteric pathogens are known to cause waterborne diseases. Enteric pathogens fall into three major groups: viruses, bacteria, and protozoa (Desouky et al. 2003). Their fates can take many potential routes in the water environment. Bacteria are considered to be among the major microorganisms responsible for waterborne disease outbreaks. In many habitats, they are the most successful forms of life. A wide variety of bacterial pathogens have been detected in water sources, and many are enteric in origin. They include *Campylobacter jejuni*, enterotoxigenic *Escherichia coli*, *Shigella* spp., *Vibrio cholerae* O1, *Salmonella typhi*, enteropathogenic *E. coli*, *Aeromonas* spp., *V. cholerae* O139, and enterotoxigenic *Bacteroides fragilis* (Fricker 2003).

The common assessment of the microbial quality of drinking water has been based exclusively on culture techniques (Gantzer et al. 1998). Because these methods do not allow for the detection of specific water pathogens, 'indicator' bacteria exhibiting the possible presence of pathogens are monitored. Through the indicator method, which usually requires cultivation on Nutrient media, a reliable result can be obtained in less than 1 day (usually 3–7 days are required). However, by the time the results are available, the pathogens might have spread throughout the water distribution system. Therefore, the development of methods for the efficient monitoring of water supplies in order to detect the presence of microbial pathogens is essential for protecting public health and maintaining consumer confidence (Strauba & Chandlerb 2003).

Because of its high sensitivity and specificity, the polymerase chain reaction (PCR) is the most commonly employed molecular tool. It was developed for the detection of strictly opportunistic pathogenic bacteria (*Salmonella*, enterohaemorrhagic *Escherichia coli*, and *Aeromonas hydrophilia*) in raw and treated water (Tekpor et al. 2017). Compared to the traditional culture techniques, the PCR method has enhanced specificity, sensitivity, simplicity, and speed. The results are available in 24–48 h (WHO 2006).

Through the use of PCR techniques, the study aimed to develop a rapid, sensitive, and specific method for detecting specific pathogenic bacteria in the drinking water from sources such as groundwater, commercially bottled water,

tap water, residential water tanks, and water purification shops in Jazan. A review of the literature suggests that the present study is the first microbiological investigation of multiple drinking water sources in Jazan.

## MATERIALS AND METHODS

### Study area and water sample collection

Twelve locations in Jazan were selected for this study (Figure 1). One hundred and twenty-three samples were collected from several sources: ground water, water purification shops, commercially bottled water, and tap water from mosques, offices, and houses. All of the water samples were collected in sterile plastic containers as reported by the American Public Health Association (APHA) (1998) and transported in field coolers to the laboratory. The samples were immediately analysed and/or stored at 4 °C for up to 2 days until use.

### Physicochemical analysis of the water samples

The pH and water conductivity of the water samples were measured using a (Jenway, UK) electrode. Chloride and fluoride concentrations were estimated with a Sherwood chloride analyzer and EXTECH fluoride meter, respectively.

### Isolation and count of bacteria

A total of 100 µl of water samples was spread on Nutrient and MacConcky agar as described by Abada et al. (2018, 2019). The plates were incubated at 37 °C for 24 h; the grown bacterial colonies were counted as CFU/ml, and a single colony was streaked on Nutrient agar slants and kept at 4 °C.

### Molecular identification of bacterial isolates

#### Isolation of chromosomal DNA

One bacterial colony was suspended in 1 ml of lysis buffer and 15 µl of proteinase K (200 µg/ml), then the mixture was vortexed. The mixture was incubated at 56 °C for 30 min, followed by 95 °C for 10 min. An equal volume of

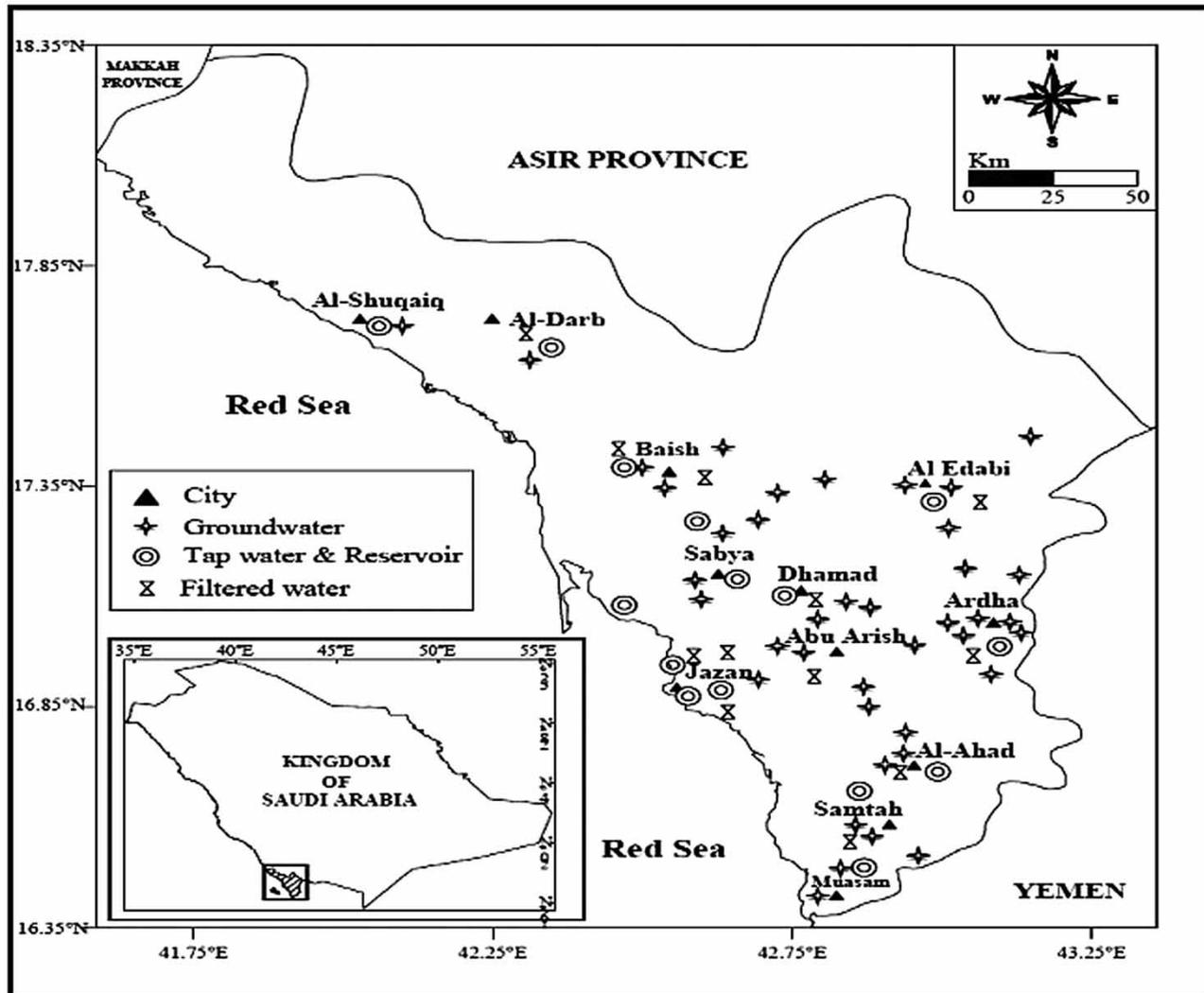


Figure 1 | Different locations for water sample collection of the Jazan region.

ice-cold isopropanol was used to precipitate the DNA. Then, the DNA pellet was washed twice with 70% ethanol, dried, and resuspended in 50  $\mu$ l of TE buffer (Abada et al. 2018, 2019).

### 16S rRNA identification of bacterial pathogens

The 16S rRNA universal primers named 27F 5'(AGAGTTT-GATCCTGGCTCAG)3' and 1492R 5'(TACGGTACCTG TTACGACTT)3' were used to amplify the genomic DNA of the bacterial isolates. The PCR was performed according to the instruction manual of Qiagen (PCR Kit). The PCR programme was 1 min of denaturation at 94  $^{\circ}$ C, followed by 25

cycles of 96  $^{\circ}$ C for 1 min, 55  $^{\circ}$ C for 1 min, and 72  $^{\circ}$ C for 1 min, with a final expansion under 72  $^{\circ}$ C for 10 min. The PCR product was analysed on 1% (w/v) agarose gels. A 100 kb marker was used as a DNA marker using TBE as a buffer. Finally, the PCR product was purified using a QIA-quick PCR purification kit (Qiagen) and eluted in 50  $\mu$ l Tris-HCl before the sequence (Abada et al. 2018, 2019).

### Sequencing of the PCR product and gene homology

The purified PCR products were sequenced using a PRISM BigDye Terminator v3.1 Cycle Sequencing Kit. The sequencing primers were 785F 5'(GGATTAGATACCCTGGTA)3'

and 907R 5'(CCGTCAATTCTTTAAGTTT)3'. DNA samples containing the extension products were added to Hi-Di formamide. The mixture was incubated at 95 °C for 5 min, followed by 5 min on ice and then analysed by the ABI Prism 3730XL DNA Analyzer (Applied Biosystems). The gene homology and related sequences were carried out by public databases BLAST at the NCBI server (<http://www.ncbi.nlm.nih.gov/blast/>) (Abada *et al.* 2018, 2019).

### Antibiotic sensitivity test

The antibacterial sensitivity test was performed by the disc diffusion method on Nutrient agar plates, as described by Begum *et al.* (2017). A bacterial isolate was overlaid on a Nutrient agar plate and left to dry for 15 min. The antibiotic discs were placed on the surface of the Nutrient agar plate, and the plates were then incubated at 37 °C for 24 h. The following eight commercially available antibacterial discs (HiMedia Laboratories, India) were used: Cefotaxime (CTX, 30 µg), Cefotaxime/Clavulanic acid (CEC, 10 µg), Erythromycin (E, 15 µg), Gatifloxacin (GAT, 10 µg), Penicillin G (P, 10 µg), Rifampin (R, 25 µg) and Streptomycin (S, 10 µg), and Sulfamethoxazole–Trimethoprim (SXT, 30 µg). The sensitivity or resistance to the antibiotics was evaluated in accordance with the instructions in the HiMedia instruction manual.

## RESULTS

A safe water supply is essential. It is one of the eight principal components in primary health care. However, the inaccessibility and insufficient supply of this precious resource are still a significant issue in several communities (WHO 2004). The goal of this study was the evaluation of the quality of the drinking water in Jazan. PCR techniques were used for the detection of specific pathogenic bacteria in the potable water from sources such as groundwater, commercially bottled water, tap water, residential water tanks, water purification shops, and tap water in houses. Such information could facilitate the determination of the presence of contamination and the possible influence of drinking water on infection and disease in the community.

### Physicochemical analysis

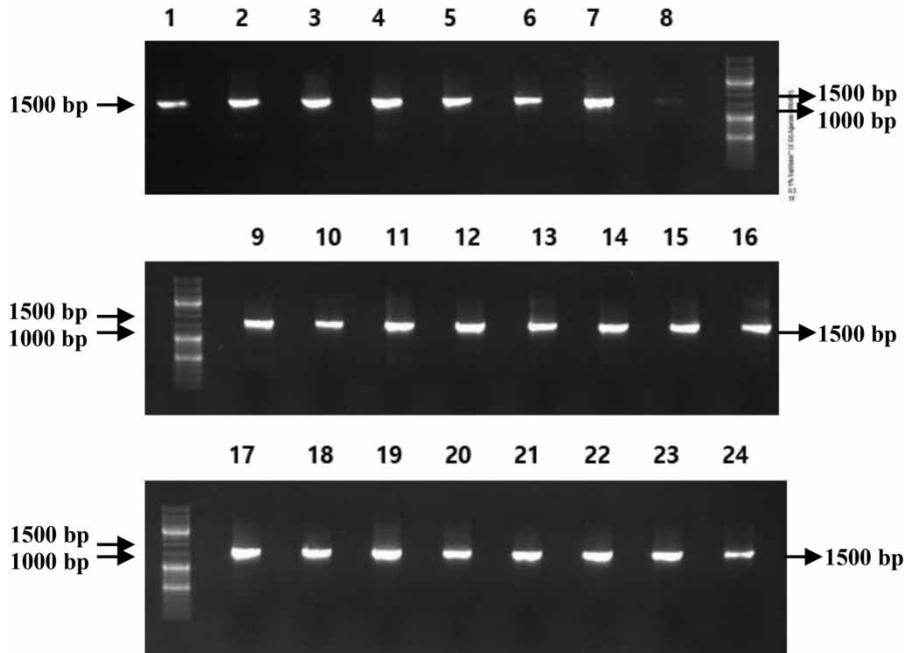
All of the water samples were investigated with regard to the physicochemical parameters, such as pH, water electrical conductivity, and chloride and fluoride concentrations. The pH values were 6.91–8.33, and the electrical conductivity was 169–1,809 µS/cm. While the chloride content was as high as 25 mg/l, the fluoride content was in a range of 0.1–0.5 mg/l. The parameters measured in this study were within the guidelines of the WHO (2003) and the Gulf Cooperation Council Standardization Organization (2008).

### Plate count of bacterial isolate

One hundred and twenty-three water samples were collected from the following sources in the Jazan region: water purification shops (16 samples, 13%), tap water from mosques (17 samples, 14%), tap water from offices (20 samples, 16%), tap water from houses (15 samples, 12%), commercially bottled water (15 samples, 12%), and underground water (40 samples, 33%). The results revealed that 81% of the total water purification shop samples were contaminated by bacteria. This constituted 10.5% of all samples. Bacterial contamination was found in 17.6% of the tap water sampled from the mosques. This represented 2.4% of all samples. For the tap water in offices, the bacterial contamination was 35%, i.e., 5.6% of all samples. The bacterial contamination in the tap water in houses was 20%, i.e., 2.4% of all samples. The tests of the underground water showed contamination at 25%, i.e., 8.1% of all samples. The commercially bottled water was free of bacteria.

### Molecular characterization of bacterial isolates

The 16S rRNA universal primers, 27F 5'(AGAGTTT-GATCCTGGCTCAG)3' and 1492R 5'(TACGGYTACCTGTTACGACTT)3', were used to amplify the genomic DNA of the bacterial isolates. The PCR products were electrophorized by 1% agarose gel, stained by ethidium bromide, and visualized by a UV transilluminator. According to the results, PCR products of approximately 1,500 bp were visualized (Figure 2). The PCR products were purified with a PCR purification kit (Qiagen). They were then sequenced with an



**Figure 2** | PCR product of the amplified 16S rRNA region of the bacterial isolates.

Applied Biosystems Prism 3730xl DNA analyzer (Applied Biosystems). The sequence results of the PCR product, on the basis of the BLAST public database of homologous genes on the National Center for Biotechnology Information (NCBI) server (<http://www.ncbi.nlm.nih.gov/blast/>), showed that there were 16 bacterial isolates belonging to 14 genera (Figure 3). The isolates included *Acidovorax*, *Acinetobacter*, *Aeromonas*, *Arthrobacter*, *Bacillus*, *Chryseobacterium*, *Cronobacter*, *Exiguobacterium*, *Klebsiella*, *Paenibacillus*, *Pseudomonas*, *Shigella*, *Sphingomonas*, and *Rheinheimera*. All of the sequences have been deposited in GenBank (<http://www.ncbi.nlm.nih.gov/>), with the corresponding accession numbers and the human-associated diseases (Table 1).

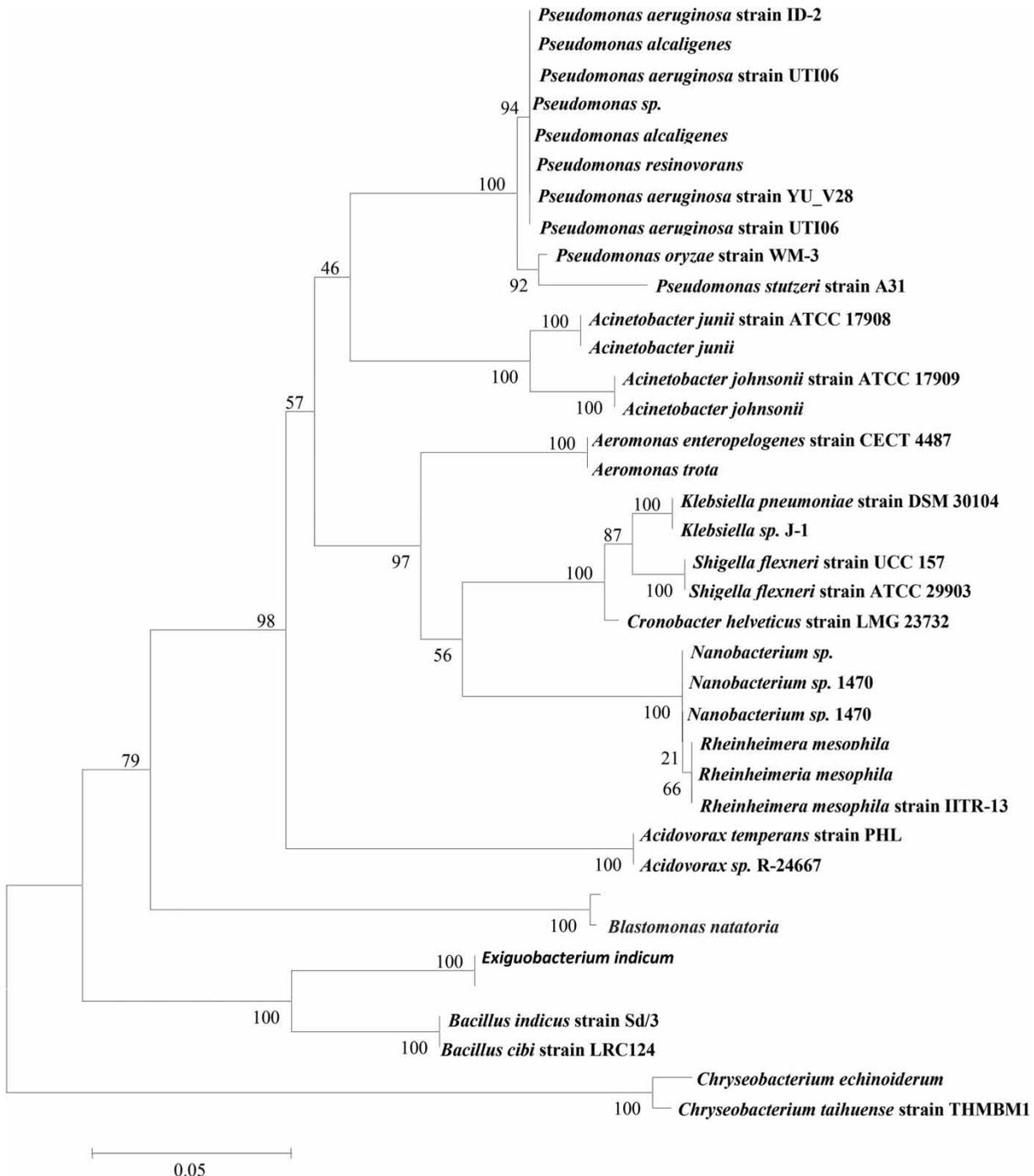
### Antibiotic sensitivity test

The antibiotic susceptibility tests found various levels of antibiotic resistance in the bacterial isolates in the water samples. All of the bacterial isolates showed 100% resistance to CEC and *P. Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Shigella resinivorans* exhibited 75% resistance. *Aeromonas enteropelogenes*, *Chryseobacterium echinoideorum*, *Shigella flexneri* and *Sphingomonas*

*ursincola* showed 62.5% resistance. *Acidovorax temperans*, *Acinetobacter junii*, *Acinetobacter* sp., *Pseudomonas alcaligenes*, and *Pseudomonas oryzae* showed 50% resistance. *Cronobacter helveticus* and *Rheinheimera mesophila* exhibited 37.5% resistance. Finally, the lowest rate of resistance, 12.5%, was observed for *Bacillus indicus* (Table 2).

### DISCUSSION

The conventional methods for identifying bacteria in clinical and environmental samples rely on morphological, biochemical, and serological tests. The genetic information (genes) stored in the rRNA were found in conservative quantities in all of the bacteria. In addition, the mutation rate was slow (Woese 1987). The most famous gene used in phylogenetic studies of the genera of archaea and bacteria is the 16S rRNA. Recently, universal primers have been used to amplify the conserved region of 16S rRNA through the PCR technique (Leight et al. 2018). The most common health hazards associated with drinking water result from contamination by pathogenic microorganisms, such as viruses, bacteria, and protozoa.



**Figure 3** | Phylogenetic tree analysis of the 16S rRNA gene sequence of the bacterial strain.

Maintaining the quality of water sources and protecting them from contamination pose serious challenges (Aksever et al. 2015). In the present study, the 16S rRNA analysis of the commercially bottled water showed it to be free of bacteria.

The highest bacterial contamination, 11% of all samples, was found in the water purification shop samples. Five bacterial isolates were found to belong to the genera *Acidovorax*, *Rheinheimera*, *Acinetobacter*, and *Pseudomonas*. A study by

**Table 1** | GenBank accession number of the bacterial strains isolated from the drinking water samples collected from different locations of the Jazan region and their human-associated diseases

	Total water samples	No. of samples contaminated with bacteria	No. of samples not contaminated	% of contamination with bacteria	16S rRNA strain homology	GenBank accession number	Human-associated diseases
Water shops	16	13	3	10.5	<i>Acidovorax temperans</i>	MK184288	Wound infection
					<i>Rheinheimera mesophila</i>	MK184289	Meningitis, UTI
					<i>Acinetobacter junii</i>	MK184296	Wound infection, Abscesses
					<i>Paenibacillus</i> sp.	Submitted	Pneumonia, Septicaemia
					<i>Acinetobacter</i> sp.	Submitted	Wound infection, Abscesses
					<i>Pseudomonas aeruginosa</i>	MK184298	Wound infection, Abscesses
					<i>Pseudomonas alcaligenes</i>	MK184299	UTI, Conjunctivitis
Tap water of mosques	17	3	14	2.4	<i>Rheinheimera mesophila</i>	MK184295	Urinary tract infection
Tap water of government offices	20	7	13	5.6	<i>Sphingomonas ursincola</i>	MK203061	Skin, Gastrointestinal and urinary tract
					<i>Rheinheimera mesophila</i>	MK184295	Bacteriemia, Abdominal sepsis
					<i>Chryseobacterium echinoideorum</i>	MK184292	Skin redness, Abscess
					<i>Arthrobacter sanguinis</i>	MK203062	Corneal infection
					<i>Bacillus indicus</i>	MK184294	Skin infection
					<i>Pseudomonas alcaligenes</i>	MK184299	
Tap water of houses	15	3	12	2.4	<i>Shigella flexneri</i>	MK184303	Wound and enteric infection
Bottled water	15	–	15	–			
Underground water	40	10	30	8.1	<i>Pseudomonas resinovorans</i>	MK184290	Wound infection, Redness
					<i>Exiguobacterium indicum</i>	MK184291	Wound infection
					<i>Pseudomonas oryzae</i>	MK184293	Eye infection
					<i>Acinetobacter johnsonii</i>	MK184297	Wound infection, Abscesses
					<i>Klebsiella pneumonia</i>	MK184300	
					<i>Cronobacter helveticus</i>	MK184301	Necrotizing enterocolitis
					<i>Aeromonas enteropelogenes</i>	MK184302	Wound infection
	123	36	87	29%			

Pant et al. (2016) found *Pseudomonas* spp. and *Acinetobacter* spp. to be the most prevalent bacteria in tap water samples. Jazan residents use the water from water purification shops for cooking, washing, and drinking. Usually, these private shops sterilize underground water after several physical treatments with ozone. Plastic bottles are filled in the open air and not under sterile conditions. In addition, the bottles are not sterilized; they are merely washed with soap. Consequently, there is a high possibility of recontamination after ozone sterilization.

The published data have identified the bacterial genera that are associated with human diseases, such as wound

infections, abscesses, septicaemia, and pneumonia. Of the total underground water samples, 8.1% were contaminated with the genera *Acinetobacter*, *Aeromonas*, *Cronobacter*, *Exiguobacter*, *Klebsiella*, and *Pseudomonas*. The bacterial contamination of the groundwater collected from different areas in Jazan could be attributed to poorly constructed wells, septic tanks, and uncased wells. Small communities that use sewage tanks are particularly vulnerable to bacterially contaminated drinking water. The groundwater supply of these communities is routinely monitored for compliance with the drinking water standards, and it is generally

**Table 2** | Sensitivity test of the bacterial isolates against different antibiotics

Isolate name	CEC	CTX	E	GAT	P	RIF	S	SXT	% of R
<i>Acidovorax temperans</i>	R	R	S	S	R	R	S	S	50%
<i>Acinetobacter junii</i>	R	R	I	S	R	R	S	I	50%
<i>Acinetobacter</i> sp.	R	R	I	S	R	R	S	I	50%
<i>Aeromonas enteropelogenes</i>	R	R	R	S	R	R	S	I	62.5%
<i>Bacillus indicus</i>	I	I	I	S	R	S	S	S	12.5%
<i>Chryseobacterium echinoideorum</i>	R	R	R	S	R	S	S	R	62.5%
<i>Cronobacter helveticus</i>	R	I	I	S	R	R	S	I	37.5%
<i>Klebsiella pneumoniae</i>	R	R	R	S	R	R	S	R	75%
<i>Pseudomonas alcaligenes</i>	R	I	R	S	R	R	S	I	50%
<i>Pseudomonas aeruginosa</i>	R	R	R	S	R	R	S	R	75%
<i>Pseudomonas oryzae</i>	R	I	I	S	R	R	S	R	50%
<i>Pseudomonas resinovorans</i>	R	R	R	S	R	R	S	R	75%
<i>Rheinheimera mesophila</i>	R	S	S	S	R	S	S	R	37.5%
<i>Shigella flexneri</i>	R	R	R	S	R	R	S	S	62.5%
<i>Sphingomonas ursincola</i>	R	R	R	S	R	I	S	R	62.5%

R, resistant; S, sensitive; I, intermediate.

free of pollution (Ministry of Health 2000). To overcome the problems of contaminated underground water, leaks in pipes and tanks must be repaired. In addition, the tops of wells must be sealed, and animals must be kept away. Well holes should be no less than 1 m above the water table.

The study found that 5.6%, 2.4%, and 2.4% of the total tap water samples collected from governmental buildings, mosques, and houses, respectively, in Jazan were contaminated with the genera *Arthrobacter*, *Bacillus*, *Chryseobacterium*, *Pseudomonas*, *Rheinheimera*, *Shigella*, and *Sphingomonas*. Species of *Acinetobacter*, *Aeromonas*, *Alcaligenes*, *Chryseobacterium*, *Comamonas*, *Elizabethkingia*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Microvirgula*, *Providencia*, *Pseudomonas*, *Serratia*, and *Yokenella* have been isolated from freshwater environments in Korea (Kim et al. 2015). These genera cause bacteraemia. They also cause corneal, skin, gastrointestinal, enteric, and urinary tract infections in humans. Haseena et al. (2017) concluded that bacterial diseases, such as cholera, typhoid, gastrointestinal, and skin infections, are spread by polluted water. However, the Jazan tap water, which comes from the government distribution system, is potable. It is in accordance with the WHO guidelines (unpublished report).

Currently, many government buildings, mosques, and houses have water tanks (reservoirs) for storage and as a solution to the problem of low water pressure. The water supplied through the government distribution pipes is not used directly. It is stored in tanks prior to use. Although these storage tanks have many benefits, they can become contaminated by bacteria if they are not cleaned or maintained appropriately. For example, the sediment that collects at the bottom of unmaintained tanks has been found to be biologically contaminated (Khan et al. 2015). The build-up of biofilms and the sediment over a year could affect water palatability (Stewart et al. 2016). In addition, the storage of water in cisterns results in a decrease in the disinfectant concentration. The extended time for which the water is held facilitates bacterial growth. Therefore, the regular care of tanks and related equipment can have a positive effect on water quality.

The contamination of drinking water with the bacterial pathogens that infect immunocompromised individuals, children under 5 years old and pregnant women cannot be ignored (Chandra et al. 2016). In this study, the presence of *Klebsiella* sp. and *Pseudomonas* sp. in drinking water sources was concerning because these microorganisms comprise a majority of the coliform microorganisms. Another

microorganism of public health interest is the *Aeromonas* sp., which is known to be the cause of gastroenteritis and septicemia in the immunocompromised and the elderly (Mina *et al.* 2018).

In the present study, the bacteriological quality of bottled water was found to be better than that of tap water. This confirmed the findings of Islam *et al.* (2010) in Dhaka, Kassenga (2007) in Tanzania, and Yasin *et al.* (2012) in Rawalpindi and Islamabad, Pakistan. Studies by Mythri *et al.* (2010) in Karnataka, India, and Ahmad & Bajahlan (2009) in Yanbu, Saudi Arabia, found no significant difference in the bacteriological quality of tap and bottled water. These findings were not confirmed in the present study. In contrast, tap water was found to be superior by Zamberlan da Silva *et al.* (2008) in Brazil and Abed & Alwakeel (2007) in Riyadh, Saudi Arabia. A review of the literature indicates that the bacteriological contamination of drinking water is a significant problem not only in Jazan but also in South Asia and other parts of the world, such as Sudan (Rai *et al.* 2009), Makkah al-Mokaarama (Abdelrahman & Eltahir 2011), Egypt (Mihdhdhir 2009), and Canada (Ennayat *et al.* 1988).

In water quality testing, pH is a critical parameter. It facilitates the interpretation of water chemical data. The pH values of the water samples from the study area ranged from neutral to alkaline (pH 6.91–8.33); however, they were within the WHO recommended values and lower than those reported by Halim *et al.* (2009) and Muhammad *et al.* (2010). Electrical conductivity (EC) was measured for all of the samples. International standards for EC have not been provided by the WHO guidelines, the National Standards for Drinking Water Quality (NSDWQ), or the United States Environmental Protection Agency. The EC values for the water samples in the current study were lower and also higher than those reported by Baig *et al.* (2009).

The concentrations of chloride in the current study were higher than those reported by Farooqi *et al.* (2007) in the Lahore and Kasur regions (chloride: 20.4–299 mg/l, fluoride: 2.47–21.1 mg/l); however, the fluoride concentrations were lower. The isolation and identification of bacteria at the point of use indicate that the water supply network systems may play a role in their distribution. The system environment can also be a contributing factor (Hong *et al.* 2010).

A well-known contributing factor to the presence of antibiotic-resistant bacteria on the consumer end is likely the presence of biofilms in the plumbing and distribution systems of the buildings from which the water samples were taken. The present study examined the antibiotic resistance of the bacteria isolated from multiple water sources. The presence of antibiotic-resistant bacteria in the water supply systems, despite disinfection, could be the result of ageing infrastructure and, thus, the formation of biofilms on the water surfaces (Abe *et al.* 2012). According to Shi *et al.* (2013), the disinfection process itself could lead to the increased concentration of antibiotic-resistant bacteria in drinking water, as was found in the present work. The presence of antibiotic-resistant bacteria in the Jazan water sources has public health significance. The promotion of antibiotic-resistant organisms in humans through the possible colonization of the gastrointestinal tract and the conjugal transfer of antibiotic resistance to the normal flora could lead to increases in the number of multi-antibiotic resistance organisms (McKeon *et al.* 1955). The prevalence of drug-resistant organisms poses a major challenge for clinicians. The consumption of water containing these antibiotic-resistant organisms could prolong the treatment time for waterborne diseases. Thus, the treatment of waterborne diseases with these antibiotics may be inappropriate and new, expensive antibiotics will need to be developed.

## CONCLUSION

Water is a precious commodity, and the supply of potable water is fundamental to the socioeconomic development of communities. The detection of bacterial pathogens in the water samples collected from different areas in Jazan is an important environmental issue because of the public health consequences. Using the PCR method, this study facilitated the detection and identification of bacteria in a shorter time than that achieved with conventional methods. The results indicate that the drinking water quality from the investigated sources was within the WHO recommended limits. The evaluation of total water quality will require additional investigations to determine the presence of other kinds of pollutants, such as pesticides, polycyclic

aromatic hydrocarbons, pharmaceuticals, and personal care products. This study revealed the presence of antibiotic-resistant bacteria in the Jazan water sources. Because of the public health implications, stringent measures must be taken to prevent the outbreak of disease. Finally, the findings suggest that bottled drinking water is the safest source of drinking water in Jazan; therefore, regular monitoring studies are highly recommended.

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## REFERENCES

- Abada, E., Al-Faifi, Z. & Osman, M. 2019 Bioethanol production with carboxymethylcellulase of *Pseudomonas poae* using castor bean (*Ricinus communis* L.) cake. *Saudi J. Biol. Sci.* **26** (4), 866–871.
- Abada, E., Masrahi, Y. S., Al-Abboud, M., Alnashiri, H. M. & El-Gayar, K. E. 2018 Bioethanol production with cellulose enzyme from *Bacillus cereus* isolated from sesame seed residue from the Jazan region. *BioResources* **13** (2), 38832–38845.
- Abdelrahman, A. A. & Eltahir, Y. M. 2011 Bacteriological quality of drinking water in Nyala, South Darfur, Sudan. *Environ. Monit. Assess.* **175** (1–4), 37–43.
- Abe, Y., Skali-Lami, S., Block, J. C. & Francius, G. 2012 Cohesiveness and hydrodynamic properties of young drinking water biofilms. *Water Res.* **46** (4), 1155–1166.
- Abed, K. F. & Alwakeel, S. S. 2007 Mineral and microbial contents of bottled and tap water in Riyadh, Saudi Arabia. *Middle-East J. Sci. Res.* **2** (3–4), 151–156.
- Ahmad, M. & Bajahlan, A. S. 2009 Quality comparison of tap water vs bottled water in the industrial city of Yanbu, Saudi Arabia. *Environ. Monit. Assess.* **159** (1–4), 1–14.
- Aksever, F., Karaguzel, R. & Mutluturk, M. 2015 Evaluation of groundwater quality and contamination in drinking water basins: a case study of the Senirkent-Uluborlu basin (Isparta-Turkey). *Environ. Earth Sci.* **73**, 1281–1293.
- Al-Bratty, M., Arbab, I. A., Alhazmi, H. A., Attafi, I. M., Javed, S. A. & Al-Rajab, A. J. 2017 ICP-MS determination of trace metals in drinking water sources in Jazan area. *Saudi Arabia Curr. World Environ.* **12** (1), 6–16.
- Alhababy, A. M. & Al-Rajab, A. J. 2015 Groundwater quality assessment in Jazan Region, Saudi Arabia. *Curr. World Environ.* **10** (1), 22–28.
- Al-Hatim, H. Y., Alrajhi, D. & Al-Rajab, A. J. 2015 Detection of pesticide residue in dams and well water in Jazan Area, Saudi Arabia. *Am. J. Environ. Sci.* **11** (5), 358–365.
- APHA 1998 *Standards Methods for the Examination of Water and Wastewater*. American Public Health Association, New York.
- Ashbolt, N. J. 2004 Microbial contamination of drinking water and disease outcomes in developing regions. *Toxicology* **198**, 229–238.
- Baig, J. A., Kazi, T. G., Arain, M. B., Afridi, H. I., Kandhro, G. A., Sarfraz, R. A., Jamal, M. K. & Shah, A. Q. 2009 Evaluation of arsenic and other physico-chemical parameters of surface and groundwater of Jamshoro. *Pakistan J. Hazard. Mater.* **166**, 662–669.
- Begum, K., Mannan, J. S., Rahman, M. M., Mitchel-Antoine, A. & Opoku, R. 2017 Identification of antibiotic producing bacteria from soil samples of Dhaka, Bangladesh. *J. Microbiol. Exp.* **4**, 00134–00139.
- Chandra, S., Saxena, T., Nehra, S. & Mohan, K. M. 2016 Quality assessment of supplied drinking water in Jaipur city, India, using PCR-based approach. *Environ. Earth Sci.* **75**, 153–167.
- Desouky, A. H., Kheiralla, Z. H., Zaki, S., Rushdy, A. A. & Abd-El-Raheim, W. 2003 Multiplex-PCR and PCR-RFLP assays to monitor water quality against pathogenic bacteria. *J. Environ. Monit.* **5**, 865–870.
- Ennayat, M. D., Mekhael, K. G., El-Hossany, M. M., Abd-El, K. & Arafa, R. 1988 Coliform organisms in drinking water in Kalama village. *Bull Nutr. Inst. Arab Republic of Egypt* **8**, 66–81.
- Farooqi, A., Masuda, H. & Firdous, N. 2007 Toxic flouride and arsenic contaminated ground water in the Lahore and Kasur districts Punjab, Pakistan, and possible contaminant sources. *Environ. Pollut.* **145** (3), 839–849.
- Fricke, C. R. 2003 The presence of bacteria in water after regrowth. *Appl. Environ. Microbiol.* **43**, 49–60.
- Gantzer, C., Maul, A., Audic, J. M. & Chwartzbrod, L. 1998 Detection of infectious enteroviruses, enterovirus genomes, somatic coliphages, and *Bacteroides fragilis* phages in treated wastewater. *Appl. Environ. Microbiol.* **64** (11), 4307–4312.
- Gulf Cooperation Council Standardization Organization (GSO) 2008 GSO5/FDS/1025, 22 pages.
- Halim, M. A., Majunder, R. K., Nessa, S. A., Oda, K., Hiroshiro, K., Saha, B. B., Hussain, S. M., Latif, S. A., Islam, M. A. & Jinno, K. 2009 Groundwater contamination with arsenic in Sherajdikhan, Bangladesh: geochemical and hydrological implications. *Environ. Geol.* **58**, 73–84.
- Haseena, M., Mlaik, M. F., Javed, A., Arshad, S., Asif, N., Zulfiqar, S. & Hanif, J. 2017 Water pollution and human health. *Environ. Risk Assess. Remediat.* **1** (3), 16–19.
- Hong, P. Y., Hwang, C., Ling, F., Andersen, G. L., LeChevallier, M. W. & Liu, W. T. 2010 Pyrosequencing analysis of bacterial

- biofilm communities in water meters of a drinking water distribution system. *Appl. Environ. Microbiol.* **76** (16), 5631–5635.
- Islam, S., Begum, H. A. & Nili, N. Y. 2010 Bacteriological safety assessment of municipal tap water and quality of bottle water in Dhaka city: health hazard analysis. *Bangladesh J. Med. Microbiol.* **4** (1), 9–13.
- Kassenga, G. R. 2007 The health-related microbiological quality of bottled drinking water sold in Dar es Salaam, Tanzania. *J. Water Health* **5** (1), 179–185.
- Khan, S., Knapp, C. W. & Beattie, T. K. 2015 Antibiotic resistant bacteria found in municipal drinking water. *Environ. Process.* **3**, 541–552.
- Kim, T. W., Joung, Y., Han, J.-H. & Jung, W. 2015 Antibiotic resistance among aquatic bacteria in natural freshwater environments of Korea. *J. Water Health* **13** (4), 1085–1097.
- Leight, A. K., Crump, B. C. & Hood, R. R. 2018 Assessment of fecal indicator bacteria and potential pathogen co-occurrence at a shellfish growing area. *Front. Microbiol.* **9**, 384–397.
- McKeon, D. M., Calabrese, J. P. & Bissonnette, G. T. 1955 Antibiotic resistant gram-negative bacteria in rural groundwater supplies. *Water Res.* **29**, 1902–1908.
- Mihdhir, A. A. 2009 Evaluation of bacteriological and sanitary quality of drinking water stations and water tankers in Makkah Al-Mokarama. *Pak. J. Biol. Sci.* **12** (4), 401–405.
- Mina, S. A., Marzan, L. W., Sultana, T. & Akter, Y. 2018 Quality assessment of commercially supplied drinking jar water in Chittagong City, Bangladesh. *Appl. Water Sci.* **8**, 24–32.
- Ministry of Health 2000 *Drinking Water Standards for New Zealand*. Ministry of Health, Wellington.
- Muhammad, S., Tahir, S. M. & Khan, S. 2010 Arsenic health risk assessment in drinking water and source apportionment using multivariate statistical techniques in Kohistan region, northern Pakistan. *Food Chem. Toxicol.* **48** (10), 2855–2864.
- Mythri, H., Chandu, G., Prashant, G. & Subba, R. W. 2010 Fluoride and bacterial content of bottled drinking water versus municipal tap water. *Indian J. Dent. Res.* **21** (4), 515–517.
- Pant, N. D., Poudyal, N. & Bhattacharya, S. K. 2016 Bacteriological quality of bottled drinking water versus municipal tap water in Dharan municipality, Nepal. *J. Health Popul. Nutr.* **35**, 17–23.
- Rai, S. K., Ono, K., Yanagida, J. I., Kurokawa, M. & Rai, C. K. 2009 Status of drinking water contamination in Mountain Region, Nepal. *Nepal Med. Coll. J.* **11** (4), 281.
- Shi, P., Jia, S., Zhang, X. X., Zhang, T., Cheng, S. & Li, A. 2013 Metagenomic insights into chlorination effects on microbial antibiotic resistance in drinking water. *Water Res.* **47** (1), 111–120.
- Stewart, C., Kim, N. D., Johnston, D. M. & Nayerloo, M. 2016 Health hazard associated with consumption of roof-collected rainwater in urban area in emergency situations. *Int. J. Environ. Res. Public Health* **13** (10), 1012–1039.
- Strauba, T. M. & Chandler, D. P. 2003 Towards a unified system for detecting waterborne pathogens. *J. Microbiol. Methods* **53**, 185–197.
- Tekpor, M., Akrong, M., Asmah, M. H., Banu, R. A. & Ansa, E. D. O. 2017 Bacteriological quality of drinking water in the Atebubu-Amantin district of the Brong-Ahafo region of Ghana. *Appl. Water Sci.* **7**, 2571–2576.
- WHO 2003 *Chloride in Drinking Water*. WHO/SDE/WSH/03.04/16.
- WHO 2004 *Guidelines for Drinking Water Quality*, Vol. 1, 3rd edn. World Health Organization, Geneva. Available from: [http://www.who.int/water\\_sanitation\\_health/dwq/gdwq3\\_contents.pdf](http://www.who.int/water_sanitation_health/dwq/gdwq3_contents.pdf).
- Woese, C. R. 1987 Bacterial evolution. *Microbiol. Rev.* **51** (2), 221–271.
- World Health Organization (WHO) 2006 *Guidelines for Drinking-Water Quality: First Addendum to Third Edition*, Vol. 1. World Health Organization, Geneva.
- Yasin, N., Shah, N., Khan, J., Saba, N. & Islam, Z. 2012 Bacteriological status of drinking water in the peri-urban areas of Rawalpindi and Islamabad-Pakistan. *Afr. J. Microbiol. Res.* **6** (1), 169–175.
- Zamberlan da Silva, M. E., Santana, R. G., Guilhermetti, M., Filho, I. C., Endo, E. H. & Ueda-Nakamura, T. 2008 Comparison of the bacteriological quality of tap water and bottled mineral water. *Int. J. Environ. Health* **211** (5–6), 504–509.

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