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Original Article

Microbial analysis and antimicrobial resistance screening of drinking water in the Qassim Region of Saudi Arabia via mass spectrometry technology

Ayman Elbehiry[1*], Musaad Aldubaib[2], Adil Abalkhail[1]

[1] Department of Public Health, College of Applied Medical Sciences, Qassim University, Buraydah, 51452, P.O. Box 6666, Saudi Arabia

[2] Department of Pathology and Laboratory Diagnosis, College of Veterinary Medicine, Qassim University, P.O. Box 6622, Buraydah, 51452, Saudi Arabia

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Abstract

Water intended for human consumption must be devoid of harmful bacteria that can lead to waterborne illnesses. Consequently, there is a pressing need for a rapid and precise method to identify bacterial contaminants in drinking water. The objective of this study was to investigate the protein profiles of various bacterial species present in water through the application of protein fingerprinting (PF) and real-time polymerase chain reaction (real-time PCR) techniques, as well as to evaluate their antimicrobial resistance. A total of two hundred water samples were collected from five distinct locations within the Qassim region of Saudi Arabia. The bacterial isolates were identified via the protein fingerprinting analytical technique (PFAT), which was subsequently confirmed via real-time PCR. The Kirby–Bauer method was employed to assess antibiotic resistance among the bacterial isolates. Among the 200 water samples analyzed, PFAT successfully identified 123 bacterial isolates, with the most frequently isolated species being 48 Pseudomonas aeruginosa (P. aeruginosa), 17 Staphylococcus aureus (S. aureus), and 16 Escherichia coli (E. coli). All the waterborne bacterial isolates were accurately identified 100% of the time, achieving a score of 2.00 or higher. The results from real-time PCR indicated that 87.5% of the P. aeruginosa isolates were positive for the oprI gene, all the S. aureus isolates were positive for the nuc gene, and 93.75% of the E. coli isolates were positive for the fliC gene. P. aeruginosa isolates presented a high level of resistance to aztreonam (64.6%), whereas S. aureus presented significant resistance to cefoxitin and cefepime (88.24%), followed by aztreonam (82.35%) and amoxicillin-clavulanate (70.6%). E. coli isolates were completely resistant to ampicillin (100%), with high resistance also observed against amoxicillin-clavulanate, cefoxitin (87.5%), and cefepime (81.25%). This study underscores the importance of utilizing PFATs for the microbiological identification of diverse water samples as a reliable and effective method. Furthermore, these findings emphasize the necessity for the regular surveillance and monitoring of antimicrobial-resistant bacteria in drinking water sources.

Keywords: Drinking water, Contaminated groundwater, Microbiological analysis, Water safety, Protein expression, Real-time polymerase chain reaction.

1. Introduction

Water constitutes a vital component of nearly all living organisms on Earth [1]. In arid regions such as Saudi Arabia, which are characterized by limited surface water, unpredictable precipitation patterns, and elevated evaporation rates, groundwater serves as the primary source of safe and reliable drinking water [2, 3]. Consequently, groundwater is integral to urban and suburban water supply systems and is regarded as the most economical source of water capable of sufficiently meeting the needs of households and agricultural operations in small towns and villages [4]. As of 2003, there were 106,370 privately owned multifunctional wells alongside 5,661 government-designated communal wells [5]. Tube wells have emerged as a significant source of water within the Kingdom of Saudi Arabia [3]. A decrease in the relative contribution of wells to fulfilling water demands in Saudi Arabia could facilitate enhanced water governance [6]. In Buraydah, which is located in the Qassim Province, the majority of the wells presented a high groundwater potential index, whereas the other wells were rated as having medium levels of contamination. Notably, the water quality of 50% of the groundwater wells was classified as inadequate or extremely low, necessitating substantial treatment interventions. According to the groundwater quality index [7], the overall water quality in the region is assessed as medium.

Contaminated water may contain harmful bacteria, viruses, and various pollutants that pose significant health risks, potentially leading to illnesses [8]. Furthermore, such contamination can result in the mortality of aquatic species, thereby adversely affecting the food chain and the overall health of the ecosystem [9]. The term "pathogenic

* Corresponding author.
E-mail address: ar.elbehiry@qu.edu.sa (A. Elbehiry).
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microorganisms" encompasses a diverse array of organisms, including bacteria, viruses, parasites, and protists. These microorganisms can induce a spectrum of diseases ranging from mild to severe and may also lead to infections that are challenging to treat, potentially resulting in long-term health complications. In most countries, water is sourced from natural water resources and subsequently transported to water treatment facilities. Following treatment, water is distributed through various systems to households, businesses, public buildings, and other entities [1]. The treated water is rigorously monitored to ensure compliance with established quality standards before its release for public consumption.

A multitude of factors can contribute to the contamination of water, encompassing various sources of pollutants and diverse strategies for their management [10]. Water may contain physical, chemical, and microbiological contaminants [1, 11]. The microbiological quality of water is essential for the prevention of waterborne diseases [12, 13]. The results of bacteriological assessments of total coliforms, fecal coliforms, and fecal streptococci serve as critical indicators of the presence of harmful enteric bacteria in water [14]. Since the early twentieth century, indicators of fecal contamination have been employed to evaluate the bacteriological properties of treated drinking water and to identify potential pathogenic microorganisms [13, 15]. Nevertheless, research has indicated that these indicators are detected in water before its consumption by the public [16]. Consequently, the World Health Organization has established guidelines for assessing the quality of treated drinking water [17].

Pathogenic bacteria, such as Vibrio cholerae, Escherichia coli (E. coli), Pseudomonas aeruginosa (P. aeruginosa), Salmonella typhi (S. typhi), and Staphylococcus aureus (S. aureus), can contaminate water, making it unsafe for drinking or other uses [13, 18]. In S. aureus, numerous virulence factors are expressed, including the nuc gene, which degrades the host's DNA and RNA, thereby increasing the survival of the bacterium. Moreover, this gene aids in the evasion of neutrophils, allowing bacteria to circumvent host immune defenses. In E. coli, the fliC gene encodes a type of flagellum that serves multiple functions in virulence, including the capacity to adhere to host cells and facilitate motility within the host environment [19]. Lipoprotein I (OprI) is a significant component of the outer membrane of P. aeruginosa and plays a critical role in maintaining the integrity of the microbial cell envelope, which is vital for its survival [20].

The virulence of certain bacteria can lead to severe illnesses, including cholera, dysentery, and typhoid fever, thereby presenting a significant risk to vulnerable populations, such as children, elderly individuals, and individuals with compromised immune systems [21, 22]. Notably, children under the age of five are particularly susceptible to waterborne infections resulting from contaminated water, especially in Asian and African countries [23]. This demographic is at increased risk for waterborne diseases because of factors such as a small body size, immature immune system, and limited access to adequate sanitation and hygiene practices [1]. Consequently, the early detection of pathogenic bacteria in water samples is critical for preventing the transmission of waterborne illnesses [24]. Furthermore, microorganisms can act as indicators of broader water quality issues, including the presence of heavy metals or other pathogens [25]. Thus, rapid identification of these microorganisms is essential for ensuring water safety. Traditional identification methods may require days or even weeks to yield results [24], whereas molecular techniques can deliver results in a significantly shorter timeframe. Molecular methods are also capable of detecting microorganisms in water samples that traditional techniques may overlook. For example, polymerase chain reaction (PCR) can identify a diverse array of species, including those that are nonculturable, and can detect pathogens that are not visible under microscopic examination. Additionally, PCR is relatively cost-effective and can be conducted within a brief period.

The protein fingerprinting analytical technique (PFAT) represents a highly innovative approach that has significantly transformed the processes of microbe identification and diagnostics [26]. This method has considerable potential within the drinking water sector, as it facilitates the identification of contaminants present in water. Consequently, health officials [27] are empowered to make timely decisions aimed at mitigating potential health risks associated with contaminated water. PFAT employs a combination of mass spectrometry and matrix-assisted laser desorption ionization (MALDI) to detect and identify microorganisms. This technique can identify microorganisms with both speed and accuracy, rendering it a valuable asset for microbiologists. Furthermore, PFATs can swiftly and accurately detect contaminants in drinking water and are relatively cost-effective compared with alternative methods [28]. Its user-friendly nature and minimal training requirements for operators further increase its practicality. A significant advantage of this method lies in its rapidity.

The extensive utilization of antibiotics in both veterinary and human medicine has resulted in the emergence of antibiotic-resistant bacterial strains. These organisms are introduced into the environment, particularly drinking water sources, through the discharge of wastewater [29]. The presence of residual antimicrobial substances in aquatic systems, along with other chemical pollutants, can further exacerbate the development of antimicrobial resistance [30]. The proliferation of antibiotic-resistant organisms in contaminated water can facilitate the horizontal transfer of resistance genes from environmental bacteria to pathogenic bacteria in humans, leading to infections that are challenging to treat [31]. Consequently, water acts as a significant conduit for the transmission of antibiotic-resistant organisms among individuals [30]. The implications of antimicrobial resistance for public health are particularly severe, especially in low- and middle-income countries [32]. International organizations, such as the World Health Organization (WHO), are actively engaged in addressing this issue, with one of their objectives being to monitor antimicrobial resistance [33]. Nevertheless, there is a notable deficiency in the attention given to tracking antimicrobial resistance in water, particularly in Middle Eastern countries, where research on this subject remains limited.

Globally, there has been a notable increase in multidrug resistance, which poses a significant threat to public health. Recent studies have documented the rise of multidrug-resistant bacterial infections originating from various sources, thereby underscoring the critical importance of appropriate antibiotic usage. In addition to screening for new multidrug-resistant strains, antimicrobial susceptibility testing is routinely employed to identify the most ef-

fective antibiotics. In Saudi Arabia, research on the monitoring of antimicrobial resistance has focused primarily on human and animal populations, while environmental factors have received inadequate attention. This oversight is particularly alarming given the growing concerns about the presence of antimicrobial-resistant microorganisms in drinking water sources. Addressing this knowledge gap is imperative; therefore, the objective of our study was to investigate microbial contamination in various drinking water sources within the Al-Qassim region of Saudi Arabia. This investigation employs protein fingerprinting technology, which is corroborated by real-time polymerase chain reaction (PCR) techniques. Furthermore, we evaluated antibiotic resistance among frequently identified bacterial species, including P. aeruginosa, S. aureus, and E. coli.

2. Materials and methods

2.1. Collection of water samples

Five locations within the Qassim region were selected for this study: Buraydah, Unayzah, Ar-Ras, Al-Bukayriyah, and Uyun Al-Jawa (Fig. 1). Water samples were collected from the specified localities, taking into account particular criteria such as the water source, treatment facility, storage systems, distribution network, and points of use. The sites were assessed for vulnerabilities, with a particular focus on contamination risks associated with unprotected sources, distribution loops, roof tanks, and filtered water storage. A total of 200 water samples were collected from various sources, including 50 samples from wells, 50 from tap water, 40 from commercially packaged water, 30 from filtered water stores, and 30 from rooftop tanks. The primary focus in collecting drinking water samples was to prevent contamination and ensure proper technician training. Therefore, the sample bottle lid was removed just before sampling, and the bottle was immediately sealed. To avoid cross-contamination, the covers were not placed on the surfaces. Before sample collection, each bottle was thoroughly cleaned with detergent, followed by the addition of concentrated hydrogen chloride (10.2 M). The final rinse was conducted with deionized water.

To counteract the bactericidal effects of chlorine during transportation, 208 µL of 3% sodium thiosulfate was added to each 250 mL sterile water sample bottle. The bottles were then sterilized in an autoclave at 121°C for 15 minutes, following the guidelines established by the American Public Health Association (APHA) [34]. Each bottle was labeled with time and location before being placed in a cool box for transport to the laboratory. After collection, the samples were stored in ice boxes and transported to the microbiology laboratory at Qassim University for bacteriological, mass spectral, and molecular analyses. In the laboratory, samples were logged into the Laboratory Information Management System and assigned a unique sample number. The samples were either analyzed immediately or refrigerated at 4°C for a maximum of two days before testing. They were examined for the presence of contaminants, including bacteria, to evaluate the prevalence of these contaminants within the region.

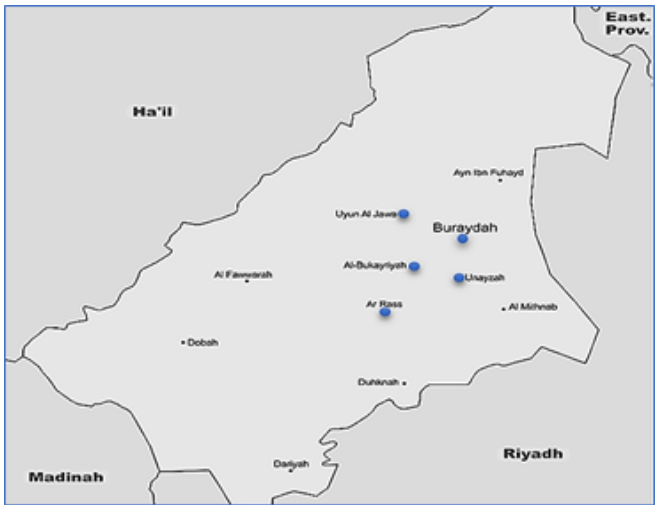

Fig. 1. Various locations designated for the collection of water samples in the Qassim region.

2.2. Contamination detection

To ascertain the presence of bacteria in the water samples, 100 µL of each sample was inoculated onto blood agar plates (Hardy Diagnostics, Santa Maria, United States) and incubated at 37°C for 24--48 hours. Following the incubation period, any colonies that developed on the plates were identified and enumerated. These colonies were subsequently compared to established bacterial species to determine the types of bacteria present in the water samples. A negative control was implemented via the addition of freshly autoclaved water to separate plates.

2.3. Coliform identification and total bacterial count

Coliform bacteria were detected by incubating 100 µL of a positive water sample in 5 mL of Luria–Bertani (LB) medium (Evviva Sciences, Fremont, Canada) at 37°C for 24 hours. Following the decantation of the LB medium, 1 mL of the bacterial culture was collected and subsequently stored at -20°C for future analysis. Eosin Methylene Blue (EMB) medium (Fisher Scientific, Carolina, USA) was prepared and preserved to evaluate the growth of lactose fermenters on EMB plates and to identify the characteristic green sheen associated with Escherichia coli. The EMB plates were incubated at 37°C for 24 hours, and colonies exhibiting a green color were classified as E. coli. Additionally, the EMB plates were subjected to further analysis to quantify the number of lactose fermenters present. The total bacterial count was determined via pour plate and serial dilution techniques. After performing serial dilution with sterile normal saline solution, one milliliter (mL) of the sample was transferred to an empty, sterile petri dish. The sample was then evenly spread onto a Petri dish containing nutrient agar that had been melted and thoroughly mixed. The plate was incubated at 37°C for 48 hours until the entire mixture solidified. The number of colonies that developed was quantified as colony-forming units (CFUs)/mL.

2.4. Total coliform, fecal coliform, and fecal Streptococci counts

The total coliform count, fecal coliform count, and fecal streptococci count were determined via the most likelymost probable number (MPN) method, as previously described [34]. To evaluate the presence of coliform bacteria in the samples, lactose broth tubes (Neogen® Culture Media, Lansing, USA) were incubated at 35°C for several days. The fecal samples were subsequently subcultured with 2% bright green bile broth (Liofilchem, Roseto degli Abruzzi TE, Italy) and incubated at 37°C for one to two

days to identify fecal coliforms. Positive samples were then inoculated into E. coli broth supplied by Sigma–Aldrich, USA, and cultured at 44°C for 24--48 hours.

The presence of E. coli was confirmed through routine biochemical testing. Azide dextrose broth (HiMedia Laboratories Pvt. Ltd., Mumbai, India) was used to culture the fecal streptococci following the introduction of the water samples, which were subsequently incubated at 37°C for 24--48 hours. Cetrimide agar base and Slanetz and Bartley agar, obtained from HiMedia in Mumbai, India, were utilized for the detection of P. aeruginosa. Mannitol salt agar, sourced from Oxoid Ltd. in Cheshire, UK, was used to identify S. aureus. Cultures of the isolated bacteria were preserved via nutrient agar slants and nutrient broth, which were also procured from Oxoid Ltd. in Cheshire, UK. To maintain the purity of the bacterial cultures, nutrient agar slants stored at 4°C were replaced every 28 days.

2.5. Identification of bacterial isolates by PFAT on the basis of the Bruker library

The bacteria were subcultured to isolate them from potential contaminants that may have been introduced during the growth of the original culture. For the preparation of samples for PFAT, the recommended formic acid extended direct transfer procedure as outlined by Bruker Daltonics was employed [35]. PFAT identifies bacterial species by analyzing unique protein profiles. The method ionizes samples with a laser, converting proteins into charged ions that are separated by mass–charge ratios, creating a distinctive spectral fingerprint. This fingerprint is compared to a reference database of known microorganisms, facilitating the identification of unknown samples. In brief, a single colony from a fresh agar culture was directly spread onto a designated spot on a MALDI target plate, followed by the application of 1 µL of 70% formic acid and 1 µL of a matrix mixture, which consisted of a-synapto-4-hydroxycinnamic acid in a saturated solution within a standard solvent (Sigma–Aldrich). The matrix solution serves to stabilize the sample during laser application and to concentrate the laser energy on the sample, thereby increasing the detection efficacy. The Bruker Bacterial Test Standard was utilized on each plate (Bruker Daltonics, Germany) to calibrate the apparatus and validate the test results. E. coli DH5α was selected as the bacterial test standard because of its widespread application in research and its ease of handling.

In this study, the Microflex LT instrument and software version 3.1 (Bruker Daltonics, Germany), in conjunction with FlexControl software (Bruker Daltonics, Germany), were employed to automatically generate mass spectra for each isolate within the mass range of 2000--20000 Da. The software subsequently analyzed the spectra and compared them to reference spectra contained in an internal database, facilitating the identification of bacterial species present in the samples. The outcomes of this analysis were utilized to ascertain the bacterial composition of the samples. The identification results provided by Bruker were assigned a score reflecting the degree of correspondence between the mass spectra of the samples and those in the database. Following Bruker's recommendations, the identification categories were delineated as follows: unreliable identification is indicated in red (score < 1.700); probable genus identification is indicated in yellow (score 1.700--1.999); secure genus and probable species identification are indicated in green (score 2.000--2.299); and highly probable species identification is also indicated in green (score 2.300--3.000). Additionally, Bruker supplied guidelines for assessing the confidence level of each identification, which were based on factors such as result accuracy and the number of matching hits. A higher score correlates with increased confidence in the identification.

2.6. Validation of PFAT results via real-time PCR

In the present study, we selected the OprI, nuc, and fliC genes for the molecular identification of P. aeruginosa, S. aureus, and E. coli isolates, respectively. The OprI gene was selected based on Mokhtari and Amini's [36] study, which identified it as a biomarker for detecting P. aeruginosa. The nuc gene was chosen as a specific target for detecting S. aureus via PCR on the basis of its widespread use [37]. The fliC gene was also selected for detecting E. coli based on results from Machado et al. [38], who reported that the fliC gene was amplified in all tested E. coli strains.

2.6.1. Nucleic acid extraction

DNA extraction was performed via DNeasy kits following the manufacturer's instructions (Qiagen, Hilden, Germany). DNase treatment (Qiagen) was conducted at room temperature for 30 minutes to eliminate DNA molecules. The enzyme was subsequently inactivated by the addition of an equal volume of Stop Solution. Following centrifugation, the supernatant was collected, thereby preparing the sample for subsequent procedures. DNase treatment functions by hydrolyzing double-stranded DNA molecules into single strands, thereby disrupting the helical structure of the DNA. This step is essential for the further processing and application of DNA in molecular biology experiments.

2.6.2. Preparation of primers and standards for real-time PCR

An Applied Biosystems 7500 Fast Real-Time PCR System (USA) was used to validate the results of the PFAT analysis. Primers were selected based on the isolates that are commonly identified (Table 1). A total of 15 µL

Table 1. A set of primers for the identification of common bacterial species recovered from a variety of water samples.

Bacterial species	Primer name	Oligonucleotide sequence (5′ → 3′)	References
P. aeruginosa	*oprI*-F	ATGAACAACGTTCTGAAATTCTCTGCT	[39]
	oprI-R	CTTGCGGCTGGCTTTTTCCAG	
S. aureus	*nuc*-F	GCG ATT GAT GGT GAT ACGGTT	[40]
	nuc-R	AGC CAA GCC TTG ACG AAC TAA AGC	
E. coli	*fliC*-F	ATAATCTACGCCGCCAACT	[41]
	fliC-R	GACTCCATCCAGGACGAAA	

of OasigTM or PrecisionPLUSTM 2 qPCR Master Mix was utilized in this procedure, in conjunction with 1 µL of a primer and probe combination and 4 µL of RNase/DNase-free water to achieve the desired concentration of the reaction mixture. Each well contained a final volume of 20 µL, which was achieved by adding 15 µL of the solution mixture and 5 µL of the DNA template. For the negative control wells, 5 µL of RNase/DNase-free water was added. Five tubes were labeled, and each received 90 µL of template preparation buffer to establish a standard curve dilution series.

The positive control template from tube 1 was thoroughly mixed, and 10 µL was subsequently transferred to tube 2. This process was continued, with 10 µL transferred from tube 2 to tube 3, and so forth until all the tubes were utilized. Each well received 5 µL of the standard template, resulting in a final volume of 20 µL. In the subsequent step, amplification was performed via the 7500 Fast Real-Time PCR system. To mitigate the risk of PCR contamination, AmpErase® Uracil-N-glycosylase (UNG) was incubated for 15 minutes at 37°C, followed by a 2-minute incubation at 95°C. This treatment allowed UNG to degrade any uracil residues present in the DNA, thereby preventing sample contamination and eliminating contaminating primers from the reaction. Additionally, it removes carryover from previous reactions. A total of 40 amplification cycles were conducted, with each cycle comprising 10 seconds of denaturation, followed by annealing and extension processes at 60°C for 60 seconds. The resulting data were analyzed via Sequence Detection System software, and the amplification results were interpreted by plotting the delta Rn (ΔRn) against the cycle number.

2.7. Antibiotic susceptibility testing

Following the guidelines established by the Clinical Laboratory Standards Institute (CLSI), pure isolates of P. aeruginosa, S. aureus, and E. coli were evaluated for antibiotic resistance via the Kirby–Bauer disc diffusion method on Mueller–Hinton agar (Sigma–Aldrich, Darmstadt, Germany). This assessment aimed to ascertain the resistance profiles of the isolates to various antibiotics. The diameters of the inhibition zones were measured and recorded in millimeters to determine the susceptibility or resistance of each isolate. A range of antibiotics at varying concentrations was employed for the bacteria under investigation, as detailed in Table 2. All antibiotic discs utilized in this study were procured from Oxoid, Hampshire, UK.

2.8. Statistical analysis

All calculations derived from the data collected in our study were conducted via the Statistical Package for the Social Sciences (SPSS) version 20.0.

3. Results

A total of 200 distinct water samples were collected from five locations in the Qassim region of Saudi Arabia, including Buraydah, Unayzah, Ar-Ras, Al-Bukayriyah, and Uyun Al-Jawa, from December 2023 to April 2024. From these samples, 123 bacterial isolates were preliminarily identified via the culture method, which was subsequently confirmed through PFAT in collaboration with real-time PCR. Furthermore, the susceptibility and resistance of commonly isolated species, including P. aeruginosa, S. aureus, and E. coli, to various antibiotics were evaluated via the Kirby–Bauer disc diffusion method on Mueller–Hinton agar.

3.1. Phenotypic identification via the culture method

Using conventional culture techniques (as shown in Table 3), a total of 9 out of 50 samples (18%) were collected from wells, 11 out of 50 samples (22%) from tap water, 4 out of 40 samples (10%) from packaged water, 5 out of 30 samples (16.67%) from filtered water stores, and 7 out of 30 samples (23.33%) from roof tank water were positive for total coliforms. Escherichia coli was identified in four samples (8%) from tap water and twelve samples (40%) from roof tanks. Furthermore, only one sample (3.33%) from filtered water stores and four samples (13.33%) from roof tanks tested positive for S. faecalis. The aforementioned results indicate that a total of 36 samples (18%) tested positive for total coliforms, 16 samples (8%) tested positive for E. coli, and 5 samples (2.5%) tested positive for S. faecalis.

3.2. Analysis of the protein profile in a positive bacterial culture

Table 4 presents the microorganisms identified in various water samples through protein analysis via PFAT. A

Table 2. References for zone diameters as established by the National Committee for Clinical Laboratory Standards for various antimicrobials employed against isolates of *P. aeruginosa*, *S. aureus*, and *E. coli* obtained from a range of water sources.

Antibiotic disc	Conc. (µg)	*P. aeruginosa* Inhibition Zone (mm)			*S. aureus* Inhibition Zone (mm)			*E. coli* Inhibition Zone (mm)		
		S	I	R	S	I	R	S	I	R
Ampicillin	10	≥27	20-26	≥19	≥36	27–35	≤26	≥15	16–22	≤15
Amoxicillin–clavulanate	20/10	-	-	-	≥37	28–36	≤27	≥25	18–24	≤17
Gentamicin	10	15	13-14	≤12	≥28	19–27	≤17	≥27	19–26	≤18
Aztreonam	30	≥ 50	17-49	≤16	-	-	-	≥37	28-36	≤27
Chloramphenicol	30	-	-	-	≥18	13–17	≤12	≥18	13–17	≤12
Trimethoprim–sulfamethoxazole	1.25/23.75	-	-	-	≥19	16–18	≤15	≥16	11–15	≤10
Amikacin	30	≥22	16-21	≤15	≥27	20–26	≤19	≥27	19–26	≤18
Cefepime	30	≥ 18	15-17	≤14	≥30	23–29	≤22	≥38	31–37	≤30
Cefoxitin	30	-	-	-	≥30	23–29	≤22	≥30	23–29	≤22
Piperacillin–tazobactam	100/10	≥ 128/4	32/4	≤16/4	≥31	24–30	≤23	≥31	24–30	≤23

S = susceptible, I = intermediate, and R = resistant

Table 3. Analysis of various potable water sources collected in the Al–Qassim area of Saudi Arabia via microbiological analysis.

Source of water samples	No. of samples	Total coliforms		*E. coli*		*Streptococcus faecalis*	
		No. of Positive culture	% of Positive culture	No. of Positive culture	% of Positive culture	No. of Positive culture	% of Positive culture
Wells	50	9	18%	0	0%	0	0%
Tap water	50	11	22%	4	8%	0	0%
Packaged water	40	4	10%	0	0%	0	0%
Filtered water stores	30	5	16.67%	0	0%	1	3.33%
Roof tank water	30	7	23.33%	12	40%	4	13.33%
Total	**200**	**36**	**18%**	**16**	**8%**	**5**	**2.5%**

Table 4. A list of different microorganisms isolated from various water sources via PFATs directly from bacterial cultures.

Bacterial species	Examined water source					Log score values				Total
	Wells (N=50)	Tap water (N=50)	Packaged water (N=40)	Filtered water stores (N=30)	Roof tank water (N=30)	0.00-1.69	1.70-1.99	2.00-2.29	2.30-3.00	
	No. of +ve isolates	No. of +ve isolates	No. of +ve isolates	No. of +ve isolates	No. of +ve isolates					
Pseudomonas aeruginosa	10/50	12/50	0/40	0/30	26/30	0	0	42	6	48 (39.02%)
Staphylococcus aureus	6/50	2/50	0/40	2/30	7/30	0	0	12	5	17 (13.82%)
Escherichia coli	2/50	4/50	0/40	2/30	8/30	0	0	14	2	16 (13%)
Klebsiella oxytoca	1/50	0/50	1/40	1/30	2/30	0	1	5	0	6 (4.88%)
Enterobacter cloacae	2/50	1/50	1/40	1/30	1/30	0	0	4	2	6 (4.88%)
Raoultella orthinolytica	1/50	0/50	0/40	1/30	3/30	0	0	4	1	5 (4.1%)
Delftia acidovorans	0/50	2/50	1/40	0/30	2/30	0	0	5	0	5 (4.1%)
Streptococcus faecalis	0/50	0/50	0/40	1/30	4/30	0	0	4	1	5 (4.1%)
Acinetobacter johnsonii	0/50	0/50	0/40	1/30	2/30	0	0	3	0	3 (2.44%)
Salmonella enterica	1/50	0/50	0/40	0/30	2/30	0	0	2	1	3 (2.44%)
Serratia rubidaea	0/50	0/50	0/40	1/30	2/30	0	0	3	0	3 (2.44%)
Leliottia amnigena	0/50	0/50	0/40	1/30	1/30	0	0	2	0	2 (1.63%)
Serratia marescens	0/50	0/50	0/40	0/30	2/30	0	0	2	0	2 (1.63%)
Acinetobacter hemolyticus	0/50	0/50	0/40	1/30	1/30	0	0	2	0	2 (1.63%)

total of 123 bacterial isolates were obtained, with P. aeruginosa being the most frequently isolated species, accounting for 39.02% (48 isolates). This was followed by S. aureus at 13.82% (17 isolates) and E. coli at 13% (16 isolates). Additionally, Klebsiella oxytoca and Enterobacter cloacae each represented 4.88% (6 isolates), whereas Raoultella ornithinolytica, Delftia acidovorans, and S. faecalis each accounted for 4.1% (5 isolates). Acinetobacter johnsonii constituted 2.44% (3 isolates), and both Salmonella enterica and Serratia rubidaea represented 2.44% (3 isolates). Furthermore, Lelliottia amnigena, Serratia marcescens, and Acinetobacter hemolyticus each accounted for 1.63% (2 isolates). The findings indicate that all 123 isolates of waterborne bacteria were accurately identified with a 100% success rate, with a minimum score of 2.00. All the spectra were analyzed via the Compass software of the Microflex LT system. The line spectra exhibited several prominent ion peaks within the mass range of 2000–14000 Da. According to the Bruker taxonomy, multiple reference bacterial strains (see Figs. 2 and 3) presented intensity peaks that were closer to 4000 Da than to 13000 Da. During this investigation, 16 bacterial species were accurately identified with a 100% success rate, yielding scores ranging from 2.00--3.00. The identification of the bacteria was accomplished by comparing the protein fingerprints of various bacterial types with those cataloged in the MALDI Biotyper database, which encompasses over 5989 strains maintained in the National Type Culture Collection.

3.3. Molecular analysis of common proteomic-identified bacteria

The PFAT results were verified via the SYBR Green real-time PCR method to ensure the accuracy of the data.

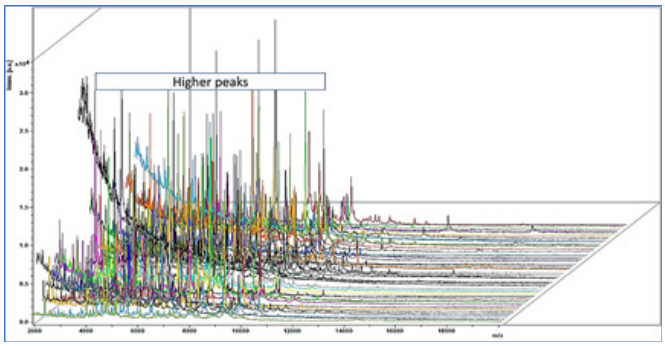

Fig. 2. The spectral protein profiles of 123 bacterial isolates were characterized from diverse water samples. A significant number of prominent-ion peaks were detected in the initial bands of the spectrum, with sizes ranging from 4000 to 13000 Daltons.

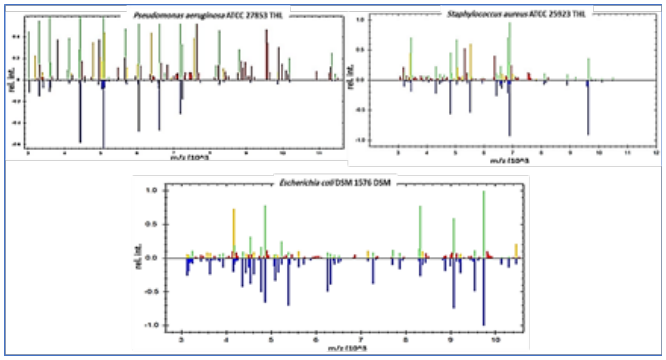

Fig. 3. The mass spectral protein profiles of bacteria present in drinking water, including S. aureus, E. coli, and P. aeruginosa, were analyzed and compared with reference strains cataloged in the IVD Compass program of the MALDI Biotyper. In the resulting spectra, blue lines represent the archived spectral proteins, green lines denote matching peaks, red lines indicate mismatched peaks and yellow lines signify intermediate peaks.

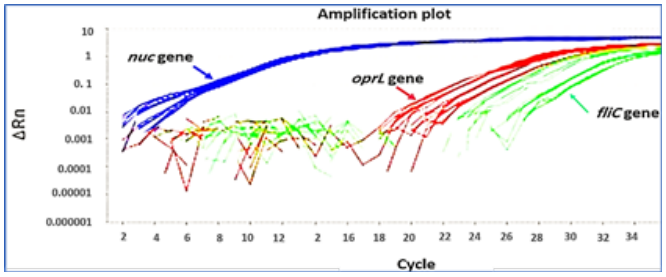

Fig. 4. Using a real-time PCR amplification plot, the nuc gene of S. aureus (blue lines), the oprL gene of P. aeruginosa (red lines), and the fliC gene of E. coli isolates (green lines) were detected.

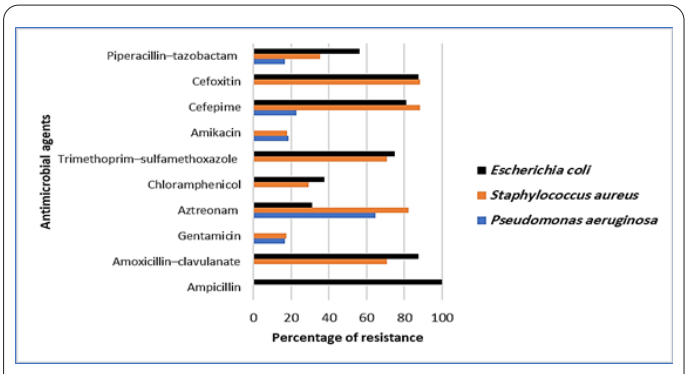

Fig. 5. Resistance percentages of P. aeruginosa, S. aureus, and E. coli to different antimicrobial agents.

A range of commonly isolated bacteria, including P. aeruginosa, S. aureus, and E. coli, were identified via specific gene primers: oprI, nuc, and fliC, respectively. As shown in Fig. 4, the findings of this study revealed that 42 out of 48 P. aeruginosa isolates tested positive for the oprI gene (87.5%), all S. aureus isolates tested positive for the nuc gene, and 15 out of 16 E. coli isolates (93.75%) tested positive for the fliC gene. A comparison of the results obtained from SYBR Green real-time PCR and PFAT indicated a high level of agreement among the bacterial isolates; thus, the real-time PCR results effectively corroborated the protein analysis results.

3.4. Antimicrobial susceptibility and resistance profiles of commonly isolated bacterial species

Following the Kirby–Bauer method, ten antimicrobial agents were evaluated against 48 strains of P. aeruginosa, 17 strains of S. aureus, and 16 strains of E. coli, which are commonly isolated from various water sources. The results indicated that 64.6%, 22.92%, and 18.75% of the P. aeruginosa isolates were resistant to aztreonam, cefepime, and amikacin, respectively, as presented in Table 5. Furthermore, 16.67% of the P. aeruginosa strains exhibited resistance to gentamicin and piperacillin-tazobactam. In terms of antimicrobial resistance, S. aureus was found to be highly resistant to cefoxitin (88.24%) and cefepime (88.24%), followed by aztreonam (82.35%), amoxicillin-clavulanate (70.6%), trimethoprim-sulfamethoxazole (70.6%), piperacillin-tazobactam (35.29%), chloramphenicol (29.41%), amikacin (17.65%), and gentamicin (17.65%). In contrast, ampicillin was classified as having intermediate resistance (94.12%). E. coli isolates presented a high degree of resistance to ampicillin (100%), followed by amoxicillin-clavulanate (87.5%), cefoxitin (87.5%), trimethoprim-sulfamethoxazole (75%), cefepime (81.25%), piperacillin-tazobactam (56.25%), chloramphenicol (37.5%), and aztreonam (31.25%). As illustrated in Figure 5, certain isolates of P. aeruginosa, S. aureus, and E. coli were resistant to aztreonam, cefepime, and piperacillin-tazobactam.

4. Discussion

The identification of bacterial species in potable water indicates the presence of pathogenic microorganisms that can lead to waterborne diseases [42, 43]. The results of the total coliform count from the study revealed that 18% of the well water samples exceeded the drinking water standards established by both national standards and international standards [44]. A prior comprehensive investigation into the quality of water samples from over 1,000 wells across the seven provinces of the Kingdom of Saudi Arabia revealed that fecal streptococci were present in 8% of the samples [45]. Additionally, a study conducted in the Khamis Mushait region of Southwest Saudi Arabia reported that 57.6% of 33 well water samples were contaminated with fecal streptococci, whereas 87.9% exhibited coliform contamination, as determined by the analyzed bacterial properties [46]. Given that the wells were not treated with chlorine before use, these findings were anticipated. The evidence suggests that the presence of coliform bacteria may result from contamination originating from residential areas, particularly among farmers and animal han-

Table 5. The effectiveness of 10 antimicrobial agents against 48 *P. aeruginosa*, 17 *S. aureus*, and 16 *E. coli* strains recovered from different water samples was evaluated.

Antimicrobial agents	*P. aeruginosa* (N=48)						*S. aureus* (N=17)						*E. coli* (N=16)					
	S		I		R		S		I		R		S		I		R	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Ampicillin	46	95.8	2	4.2	0	0.00	1	5.88	16	94.1	0	0.00	0	0.00	0	0.00	16	100
Amoxicillin–clavulanate	-	-	-	-	-	-	5	29.4	0	0.00	12	70.6	2	12.5	0	0.00	14	87.5
Gentamicin	39	81.3	1	2.1	8	16.67	2	11.76	12	70.6	3	17.6	0	0.00	16	100	0	0.00
Aztreonam	17	35.4	0	0.00	31	64.6	3	17.65	0	0.00	14	82.35	11	68.75	0	0.00	5	31.25
Chloramphenicol	-	-	-	-	-	-	12	70.59	0	0.00	5	29.41	9	56.25	1	6.25	6	37.5
Trimethoprim–sulfamethoxazole	-	-	-	-	-	-	5	29.4	0	0.00	12	70.6	4	25	0	0.00	12	75
Amikacin	37	77.1	2	4.17	9	18.75	3	17.65	11	64.7	3	17.65	1	6.25	15	93.75	0	0.00
Cefepime	38	79.17	0	0.00	11	22.92	2	11.76	0	0.00	15	88.24	3	18.75	0	0.00	14	81.25
Cefoxitin	-	-	-	-	-	-	2	11.76	0	0.00	15	88.23	2	12.5	0	0.00	14	87.5
Piperacillin–tazobactam	40	83.33	0	0.00	8	16.67	11	64.71	0	0.00	6	35.29	7	43.75	0	0.00	9	56.25

dlers who are frequently exposed to such contaminants. Furthermore, delivery nozzles from these systems are vulnerable to airborne particles that may be dispersed during storm events [47]. Several previous studies have indicated that elevated levels of bacteria are often found in proximity to exterior wells, which is attributed to atmospheric dust storms and the movement of animals [48, 49]. These findings are particularly concerning, as certain regions of Qassim continue to depend on well water as their primary source of water.

According to both national and international standards regarding total coliform levels, 23.33% of the water samples collected from tankers in this investigation presented elevated concentrations of total coliform bacteria. This finding aligns with the research conducted by Alqahtani et al. [50], who reported that 33% of water samples from tankers were contaminated with coliform bacteria. In contrast, a study conducted in the Shebaa region of southwestern Saudi Arabia reported that only 2.6% of 39 water samples collected from tankers tested positive for total coliform bacteria [51]. The researchers in that study concluded that the contamination of desalinated water in their area was not predominantly due to water tankers but rather resulted from a combination of factors. Conversely, another investigation revealed that 60–68.8% and 31.2–37.5% of bacterial samples obtained from a tanker in Makkah tested positive for fecal coliforms and total coliforms, respectively [52]. Throughout the shipping process, biofilms are present in the water, and tankers may contain contaminated pouring equipment, as well as dust [53]. Furthermore, our study demonstrated that inadequate or nonexistent water treatment facilities are likely contributing factors to the poor microbiological quality of the community tanker water examined in our research [54]. Concerns regarding the sanitation of water tankers have also been raised [55]. Accessing communal tankers can be challenging, resulting in infrequent cleaning and maintenance of their interiors. Additionally, prolonged storage of water within tankers promotes the growth of biofilms on the water surface.

During the microbiological examination of water samples collected from roof tanks for this study, a substantial number of microbial indicators were present in the majority of samples, surpassing the limits established by national and international guidelines. Abu-Zeid and colleagues reported that contamination was detected in 26.4% of the 200 samples taken from household tanks [51]. Researchers have hypothesized that the elevated incidence of diarrhea among residents in the Shebaa area may be associated with water pollution resulting from the storage of water in domestic reservoirs. Our research suggests that water quality deteriorates more rapidly at the point of use than at the source, potentially because of the presence of biofilms in domestic tanks. The relationship between diarrhea and the maintenance of water tanks has been extensively investigated in prior studies [56]. This study revealed alarming findings regarding wastewater disposal during both the summer and the winter months. Wastewater seepage may occur as a result of deficiencies in home drainage systems, leading to bacterial contamination of water in household containers and wells [57, 58]. Therefore, the implementation of effective educational and promotional campaigns is essential to ensure the provision of high-quality drinking water in roof tanks sourced from the supply.

In the present study, among the various bacterial species identified in water samples, P. aeruginosa, S. aureus, and E. coli accounted for 81 out of 123 isolates, representing 65.85% of all detected bacteria. This identification was achieved through proteomic analysis of bacteria isolated from diverse water samples via PFAT. These bacterial species are recognized for their pathogenic characteristics and have the potential to contribute to waterborne diseases, underscoring the necessity of their identification to mitigate associated risks. Testing water samples for contaminants is imperative for public health and safety [59, 60]. PFAT is widely acknowledged as an effective technique for the accurate identification of bacteria in water samples [61]. Its rapidity and precision facilitate prompt and comprehensive detection and identification of bacteria, thereby providing critical structural information. This technology enables the determination of appropriate treatment strategies for water samples to ensure optimal safety. Sala-Comorera et al. reported that protein fingerprinting represents a promising technology for the identification of bacteria in drinking water, contingent upon its proper development [35]. Owing to its robustness, speed, and user-friendliness, the PFAT is deemed suitable for both occasional and routine applications. However, researchers have highlighted a significant challenge in the development of mass spectral databases, as most existing databases concentrate on a limited number of clinically relevant species, prompting the creation of numerous new databases. To increase the accuracy of PFAT for environmental applications in the future, expanding the database to achieve more precise results is essential.

PFAT has recently been employed to identify bacterial populations in tap water and mineral water, which represent the two most prevalent sources of drinking water globally [62]. In a study conducted by Sala-Comorera et al. [63], eleven samples of tap water were collected from various locations, whereas ten samples of mineral water were sourced from different brands. The findings revealed a significant disparity in bacterial diversity between the two types of drinking water. Specifically, bacteria associated with Alphaproteobacteria were more prevalent in tap water, whereas Gammaproteobacteria were more frequently isolated from mineral water. In the context of groundwater utilized for drinking purposes, PFAT is also employed in the United States for monitoring [64]. Groundwater can harbor a variety of opportunistic pathogens, including Aeromonas hydrophila, Bacillus cereus, P. aeruginosa, and Stenotrophomonas maltophilia, which can be identified through high-throughput methodologies. The identification of these pathogens underscores the potential health risks associated with the consumption of contaminated groundwater. A similar investigation was conducted by Suzuki et al. [65], who utilized PFAT to identify various microorganisms and assess their responses to human activities during the study. Additionally, Kacaniova et al. [66] performed a series of experiments on water samples to develop a reliable and accurate method for distinguishing Pseudomonas species via matrix-assisted laser desorption/ionization time–of–flight mass spectrometry (MALDI–TOF MS). They successfully isolated and identified 161 strains of Pseudomonas species, including P. extremorientalis, P. fluorescens, P. fragi, P. proteolytica, and P. veronii. The results indicated that MALDI-TOF MS is a highly sensitive technology for detecting Pseudomonas in environmental samples owing to its superior discrimina-

tory capabilities.

Differentiating bacteria with similar traits with traditional methods is difficult, often leading to misidentification. PFAT, which analyzes highly conserved microbial proteins, improves accuracy in species differentiation. The main difference between traditional methods and PFATs lies in the time and financial resources needed for sample identification. PFATs for bacterial identification cost approximately $1.43 per sample, whereas conventional methods cost $4.60 to $8.23. Cherkaoui et al. [67] reported that reagents for phenotypic identification with automated tools cost approximately $10 per isolate, whereas PFATs cost less than $0.50 per sample. However, the costs and maintenance of PFAT may limit its use. The proteomic identification process was efficient, taking approximately 30 minutes per isolate and nearly 2 hours for a complete 96-spot target plate. Compared with traditional approaches, the PFAT method is more reliable for the routine identification of various bacterial types because of its straightforward procedures, large sample capacity, high accuracy, enhanced sensitivity, and reproducibility. PFAT is accurate in identifying closely related species in clinical laboratories, but it has limitations. The similarity among microorganisms can hinder differentiation, and a limited database may result in misidentification, leading to incorrect detections or failures in identification.

The SYBR Green real-time PCR method developed in this study has demonstrated efficacy in validating the presence of bacteria commonly found in various water samples from diverse sources. The results obtained from PFAT and real-time PCR indicate a robust correlation between the detection of the operI, nuc, and fliC genes, irrespective of the origin of the isolates. The integration of these methodologies facilitates the rapid and precise identification and characterization of different bacterial strains. PFAT provides expedited protein profiling, whereas real-time PCR enables the identification of specific genes, thereby significantly enhancing diagnostic accuracy [61, 65]. By combining these two approaches, microbiological analyses can be conducted with greater thoroughness and confidence [68]. In our investigation, we identified various bacterial species in drinking water samples. The identification of these bacteria through proteomic and molecular techniques is crucial, as they may be associated with waterborne diseases, which pose a significant threat to public health. Determining the types of bacteria present in drinking water is vital for safeguarding public health and safety. The early detection of harmful microorganisms that can lead to waterborne illnesses allows timely intervention and management of potential threats. The application of advanced techniques such as real-time PCR and protein fingerprinting, in conjunction with established methods, enhances the efficiency of water quality monitoring. This proactive approach aids in preventing the spread of potential epidemics and ensures the overall quality of water resources.

Throughout the world, there is extensive documentation demonstrating the presence of antibiotic-resistant microorganisms in drinking water [69]. A significant portion of vulnerable bacteria are eliminated when selection pressures, such as the use of antibiotics at specific concentrations, are introduced [70]. However, some bacteria have the ability to survive under these conditions by developing resistance mechanisms that are encoded through genetic processes. These mechanisms are usually found on chromosomes, plasmids, and other mobile genetic elements [71]. Despite this, there is no system in place to monitor the levels of antibiotic-resistant bacteria in drinking water, leading to a lack of guidelines on safe consumption levels. This study investigated the development of antibiotic resistance among isolates of S. aureus, E. coli, and P. aeruginosa obtained from various sources of drinking water in the Al-Qassim region of Saudi Arabia. The results indicated that the E. coli isolates exhibited significant resistance to ampicillin (100%), amoxicillin-clavulanate (87.5%), cefoxitin (87.5%), and trimethoprim-sulfamethoxazole (75%), among other tested antibiotics. Additionally, P. aeruginosa isolates presented notable resistance to aztreonam (64.6%), whereas S. aureus isolates presented considerable resistance to cefoxitin (82.33%), cefepime (82.33%), aztreonam (82.35%), trimethoprim-sulfamethoxazole (70.6%), and amoxicillin-clavulanate (70.6%).

Previous studies have indicated that 86.5% of E. coli isolates from water sources in northern Ghana were sensitive to ciprofloxacin, whereas 96.2% were resistant to cefuroxime [72]. In the study conducted by Larson et al. [73], the antibiotic resistance patterns of E. coli isolates obtained from drinking water in rural Andean households in Cajamarca, Peru, were evaluated. The results revealed that the E. coli isolates demonstrated a certain degree of resistance to tetracycline (37.6%), ampicillin (34.2%), sulfamethoxazole-trimethoprim (21.4%), and nalidixic acid (13%). Daly et al. [74] identified 53 E. coli isolates, 37 Serratia isolates, and 32 Enterobacter isolates from public and drinking water sources in the Republic of Ireland. Antimicrobial susceptibility testing revealed that 55% of all the isolates were resistant to amoxicillin and 22% were resistant to amoxicillin-clavulanic acid, whereas resistance to the other tested antibiotics remained below 10%. Ferro et al. [75] identified seven strains of E. coli from water sources designated for human consumption in the Bagua region of Amazonas. These findings revealed that all identified E. coli strains were resistant to nalidixic acid, gentamicin, chloramphenicol, amoxicillin combined with clavulanic acid, and ciprofloxacin.

In a related investigation, isolates of P. aeruginosa and E. coli obtained from a receiving stream and a wastewater treatment facility in Accra presented increased resistance to amoxicillin-clavulanate, cefuroxime, and aztreonam [76]. Previous studies have indicated that clinical strains of P. aeruginosa typically exhibit drug resistance. Silva et al. [77] isolated 30 P. aeruginosa strains from drinking water, all of which demonstrated resistance to one or more antimicrobial agents. The highest resistance levels among P. aeruginosa isolates were found for chloramphenicol, gentamicin, and trimethoprim-sulfamethoxazole, while only a few environmental isolates were resistant to cefotaxime. This bacterium exhibits intrinsic resistance to many antimicrobial agents due to the interaction of multidrug efflux systems or type 1 AmpC β-lactamase with its outer membrane's reduced permeability [78]. In contrast, Wei et al. [79] assessed the resistance of 77 P. aeruginosa isolates obtained from mineral and spring water in China to 14 different antimicrobial agents and reported that none of the isolates were resistant to the antibiotics tested. The discrepancies in these findings may be attributed to the different sources of the samples analyzed.

Adesoji et al. [80] identified 45 strains of S. aureus

from 150 water samples collected from various sources in Dutsin-Ma, Katsina State, Nigeria. This study assessed antimicrobial resistance and revealed 100.0% resistance to cloxacillin and 48.0% resistance to gentamicin. All the isolated strains presented multidrug resistance with various antibiotic resistance patterns. While S. aureus is not typically used as an indicator of fecal contamination, its presence in drinking water can pose serious public health risks. High levels of this bacterium, especially strains with antibiotic-resistance genes [81], have the potential to be transferred to the intestinal microbiota through mobile genetic elements. In South African water sources, S. aureus is known to be highly virulent and resistant to multiple drugs [81], and it can form biofilms within distribution systems, creating reservoirs for pathogens and resistance genes [82]. The detection of resistance to at least one antibiotic in more than fifty percent of bacterial isolates from drinking water suggests that drinking water may play a significant role in the transmission of antibiotic-resistant bacteria between urban and rural populations in the Qassim region.

The contamination of drinking water sources is likely attributable to the discharge of untreated human and animal waste into the environment. Traditional water treatment methods have been reported to be inadequate for effectively eliminating antimicrobial-resistant bacteria [31]. In Saudi Arabia, chlorine is predominantly employed for tap water treatment; however, studies have indicated that certain bacteria exhibiting antibiotic resistance may demonstrate tolerance to chlorine, which could facilitate the emergence of antibiotic-resistance genes [83]. Research has shown that the combination of ultraviolet (UV) light and chlorine is more effective in eliminating antibiotic-resistant bacteria than the use of either method in isolation [31, 84]. Access to clean drinking water is essential for safeguarding human and animal health, as well as for mitigating the prevalence of antibiotic resistance and waterborne diseases.

Water quality is a significant public health concern, especially in areas where microbial contamination is prevalent. Regular monitoring of water quality is essential for identifying pathogens such as E. coli and S. aureus, which can harbor antibiotic-resistant strains. Advanced technologies, such as mass spectrometry technology, allow for rapid identification of bacteria and resistance profiles, providing crucial public health information [85, 86]. Exposure to contaminated water during childhood and old age can lead to increased morbidity and mortality rates. Therefore, this study underscores the importance of local health authorities implementing strict water quality monitoring and management protocols.

Effective public health interventions require a deep understanding of antibiotic resistance, as the overuse of antibiotics in human and veterinary medicine can worsen infectious disease outbreaks. Research has emphasized the importance of integrated surveillance systems for the early detection of microbial contamination. It is crucial to prioritize the coordination of water management, healthcare, and agriculture in public health policies to improve community health outcomes in the Qassim Region [11, 30]. To address microbiological contamination and antibiotic resistance, targeted public health initiatives are essential. The development of good water hygiene practices necessitates community participation and education. Investments in infrastructure, such as water filtration systems and enhanced wastewater management, can significantly enhance water quality. Local health authorities must implement evidence-based policies informed by microbiological analysis and antibiotic resistance screening to guide local public health strategies and contribute to broader efforts for sustainable water management and improved public health.

Future studies on microbial identification and antibiotic resistance will greatly benefit from the incorporation of mass spectrometry methods. These methods not only allow for the isolation of bacterial populations but also provide a deeper understanding of microbial interactions and resistance mechanisms. Another important area for future research is the investigation of the environmental factors that contribute to microbial resistance. By analyzing the physicochemical parameters of drinking water, such as pH, turbidity, and nutrient levels, valuable insights can be gained into the relationships between these factors and the prevalence of antibiotic-resistant bacteria. The use of geographic information systems (GISs) can enhance the spatial analysis of resistance patterns, enabling targeted public health interventions to improve water quality in specific regions. Research should also focus on exploring how socioeconomic factors influence water quality and microbial resistance in the future. Understanding how community behaviors, such as antibiotic use in agriculture and medicine, contribute to environmental antibiotic pressure is crucial. Furthermore, raising public awareness and educating individuals about appropriate antibiotic use can help engage them in the mitigation process. By actively involving the community in efforts to combat antibiotic resistance, we can work toward a healthier and more sustainable future.

5. Conclusions

This study posits that certain pathogenic bacteria may be disseminated through drinking water sources, potentially resulting in severe diseases associated with antibiotic-resistant bacteria. The detection of microbial pathogens in water samples collected from various locations represents a significant environmental concern with considerable health implications. Among the bacteria frequently identified were P. aeruginosa, S. aureus, and E. coli, each exhibiting varying degrees of resistance to different antibiotics. The application of the protein fingerprinting analytical technique (PFAT) in conjunction with real-time PCR enables the accurate and rapid identification of diverse bacterial species present in a variety of drinking water sources. This study underscores the necessity for regular surveillance and monitoring of water sources in the Al-Qassim region to evaluate the presence of bacteria. To ensure consistent water quality, health officials must implement a strategy for periodic microbial monitoring to identify effective methods and measures for preventing the deterioration of water quality.

Limitations of the study

This study was conducted only during the dry season, limiting its ability to capture the full range of water quality variations throughout the year. The wet season can introduce diverse pollution levels and microbial profiles that were not assessed. Additionally, the findings are not generalizable, as the study focused on specific drinking water sources in the Qassim region of Saudi Arabia, which may

not reflect overall water quality conditions in the country or globally. The study also excluded harmful microorganisms such as Vibrio cholerae, viruses, fungi, and parasites, including protozoa and helminths.

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Authors' contributions
AE, MA, and AA developed the project idea, carried out all the experiments, analyzed the results, and wrote the manuscript. The work has been read and approved by all the authors.

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Conflicts of interest
The authors declare no conflicts of interest regarding the publication of this manuscript.

Availability of data and materials
The datasets used and analyzed during the current study are available from the corresponding author upon request.

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