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Assessment of Physicochemical, Bacteriological Quality and Antibiotic-Resistant Bacteria in Groundwater Wells at Zawiyat Al-Mahjoub, Misrata

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تقييم الجودة الفيزيائية والكيميائية والبكتريولوجية والبكتيريا المقاومة للمضادات الحيوية في آبار المياه الجوفية في زاوية المحجوب، مصراتة

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Abstract:
Groundwater is the primary water source in Libya, yet its quality has deteriorated due to excessive extraction, lack of sewage infrastructure, and agricultural activities. In Zawiyat Al-Mahjoub, rising salinity, microbial contamination, and the emergence of antibiotic-resistant bacteria pose increasing public health risks. This study assessed the physicochemical properties, bacteriological quality, and antibiotic resistance profiles of groundwater wells in Zawiyat Al-Mahjoub, Misrata. Twenty wells from three geographical zones were sampled. Physicochemical parameters were analyzed following ASTM and WHO methods. Bacteria were isolated using selective differential media, and antibiotic susceptibility testing was performed using the Kirby–Bauer disk diffusion method according to CLSI guidelines. Electrical conductivity, total dissolved solids, hardness, sodium, and chloride exceeded WHO limits in most samples. Bacteriological contamination was detected in 70% of wells, yielding 25 bacterial isolates, including only 2 isolates of fecal E. coli. Several isolates showed high resistance to commonly used antibiotics, particularly Trimethoprim–Sulfamethoxazole (100%) and Ampicillin (88%). Groundwater in Zawiyat Al-Mahjoub is chemically degraded and microbiologically unsafe, with the presence of antibiotic-resistant bacteria representing an additional health concern. These findings highlight the urgent need for improved well protection and wastewater management strategies.

Keywords: Groundwater quality, Physicochemical parameters, Bacteriological contamination, Antibiotic resistance, E. coli, Salinity, Libya.

الملخص:
تُعدّ المياه الجوفية المصدر الرئيسي للمياه في ليبيا، إلا أن جودتها تدهورت بسبب الإفراط في استخراجها، ونقص البنية التحتية للصرف الصحي، والأنشطة الزراعية. في زاوية المحجوب، يُشكّل ارتفاع الملوحة، والتلوث الميكروبي، وظهور البكتيريا المقاومة للمضادات الحيوية مخاطر متزايدة على الصحة العامة. قيّمت هذه الدراسة الخصائص الفيزيائية والكيميائية، والجودة البكتيرية، وأنماط مقاومة المضادات الحيوية لآبار المياه الجوفية في زاوية المحجوب، مصراتة. أُخذت عينات من عشرين بئرًا من ثلاث مناطق جغرافية. حُللت المعايير الفيزيائية والكيميائية وفقًا لطرق ASTM وWHO. عُزلت البكتيريا باستخدام أوساط تفاضلية انتقائية، وأُجري اختبار حساسية المضادات الحيوية باستخدام طريقة انتشار قرص كيربي-باور وفقًا لإرشادات CLSI. تجاوزت الموصلية الكهربائية، والمواد الصلبة الذائبة الكلية، والصلابة، ونسبة الصوديوم، والكلوريد حدود منظمة الصحة العالمية في معظم العينات. تم الكشف عن تلوث بكتيري في 70% من الآبار، حيث تم العثور على 25 عزلة بكتيرية، منها عزلتان فقط من الإشريكية القولونية البرازية. أظهرت العديد من العزلات مقاومة عالية للمضادات الحيوية الشائعة الاستخدام، وخاصةً تريميثوبريم-سلفاميثوكسازول (100%) والأمبيسيلين (88%). المياه الجوفية في زاوية المحجوب متحللة كيميائيًا وغير آمنة ميكروبيولوجياً، ويمثل وجود البكتيريا المقاومة للمضادات الحيوية مصدر قلق صحي إضافي. تُبرز هذه النتائج الحاجة الملحة لتحسين حماية الآبار واستراتيجيات إدارة مياه الصرف الصحي.

الكلمات المفتاحية: جودة المياه الجوفية، المعايير الفيزيائية والكيميائية، التلوث البكتيري، مقاومة المضادات الحيوية، الإشريكية القولونية، الملوحة، ليبيا.

Introduction:

Groundwater represents the cornerstone of Libya’s water supply, accounting for nearly 95% of total consumption (Alfarrah & Walraevens, 2018). In the absence of permanent rivers, limited rainfall, and scarce surface reservoirs, groundwater extraction through wells remains the primary source for domestic, agricultural, and industrial needs. However, uncontrolled abstraction, inadequate sanitation infrastructure, and agricultural runoff have contributed to a progressive decline in groundwater quality. This deterioration is manifested not only in pollution but also in elevated concentrations of dominant salts such as sodium, magnesium, and calcium, often present as carbonates or sulfates, alongside increased levels of dissolved solids exacerbated by climate change (Abd El-Aziz, 2017).

Across North Africa and the Middle East, studies have consistently reported rising salinity, high total dissolved solids, and the presence of nitrates and heavy metals (Al-Barakah et al., 2017; Mallick et al., 2021). In Libya, most research has focused on the physico-chemical aspects of groundwater (Khairi & Abdulaziz, 2018; Amshaher & Ataf, 2021), while bacteriological safety and antibiotic resistance have received limited attention despite their direct implications for public health. Contaminated groundwater can transmit a wide range of diseases, including enteritis, gastritis, schistosomiasis, diarrhea, and urinary tract infections (Bharti et al., 2003). Fecal contamination, often originating from wild and domestic animals, septic systems, or inadequate treatment, is a major contributor to microbial pollution (Mzungu & Ebunam, 2022).

Globally, groundwater contamination by pathogenic and antibiotic-resistant microorganisms has emerged as a pressing concern (Wu et al., 2025). Human activities such as sewage leakage, disposal of untreated wastewater, and excessive antibiotic use in agriculture accelerate the spread of resistant bacteria into aquifers (Adhimi et al., 2022; Wang et al., 2025). These microorganisms can persist underground for extended periods, posing significant risks to populations dependent on untreated groundwater. Despite multiple studies on groundwater chemistry in Libya, integrated assessments that simultaneously address physico-chemical quality, microbial contamination, and antibiotic resistance remain scarce. This gap limits effective resource management and public health protection. Therefore, the present study aimed to evaluate the chemical, physical, and bacteriological properties of groundwater wells in Zawiya al-Mahjoub, Misrata region, and to assess the antibiotic susceptibility of the isolated bacteria.

Methodology:

Study Area:

The study was conducted in Zawiya al-Mahjoub, a rural community near Misurata, Libya (32.385° N, 14.972° E), with approximately 13,400 inhabitants relying almost exclusively on groundwater due to the arid climate and absence of surface water resources.

Sampling Strategy:

A stratified random sampling approach was adopted to select 20 wells distributed across northern, central, and southern zones, reflecting geographical and geological diversity. Wells were categorized by depth as shallow (<30 m), medium (30–60 m), and deep (>60 m). Well information was obtained from the Ministry of Water and Environment (2024).

Sample Collection:

Sampling was conducted between September 1st and 15th, 2025. From each well, 0.5 L of water was collected in sterile glass bottles, sealed, labeled, and stored at 4 °C until laboratory analysis. Sampling sites were georeferenced using GPS, and Figure (1) illustrates the spatial distribution of wells.

Figure (1): The geographical distribution of the sampled wells across northern, central, and southern zones.

Physico-chemical Analysis:

Parameters were determined according to international standards and WHO guidelines. Methods included:

- pH: ASTM D1293
- Electrical Conductivity: ASTM D1125
- Total Dissolved Solids: EPA Method 160.1
- Hardness, Ca, Mg: ASTM D2791, ASTM D511
- Major ions (Na^+, Cl^-, NO_3^-): ASTM D512, D1067, D3867

Analyses were performed at Al-Asas Al-Thameen Company Laboratory with quality control measures including duplicates, blanks, and calibration against certified standards.

Bacteriological Analysis:

Isolation and identification of bacteria were conducted at Alpha Medical Analysis Laboratory using selective differential media: Blood Agar (BA), Xylose Lysine Deoxycholate (XLD), Baird Parker Agar (BPA), Violet Red Bile Glucose (VRBG), and Tryptone Bile Glucuronic (TBX). Samples were incubated at 37 °C for 24–48 hours. Isolates were identified morphologically and biochemically, including lactose fermentation and hemolysis.

Antibiotic Resistance Testing:

Antibiotic susceptibility was assessed using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar. Tested antibiotics included Cefoxitin (Fox, 30 µg), Amoxicillin–Clavulanic acid (Amc, 30 µg), Trimethoprim–Sulfamethoxazole (Sxt, 25 µg), Doxycycline (Do, 30 µg), Cefuroxime (Cxm, 30 µg), Ciprofloxacin (Cip, 5 µg), and Imipenem (Imp, 10 µg). Results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines (Barry, 2007).

Statistical Analysis:

Descriptive statistics were used to summarize physico-chemical and bacteriological parameters. Pearson's correlation coefficient was applied to evaluate relationships between variables, with statistical significance set at $p < 0.05$. Analyses were performed using SPSS version 25, and Microsoft Excel 16 was used to generate tables and figures.

Result:

The results of this study provide a comprehensive overview of the structural, physicochemical, and bacteriological characteristics of groundwater wells in Zawiyat Al-Mahjoub, Misrata. Data were collected from 20 wells distributed across northern, central, and southern zones, reflecting geographical diversity. The findings reveal that most wells are shallow and located in close proximity to septic tanks, which increases their susceptibility to contamination. Physicochemical analysis demonstrated that several parameters, including electrical conductivity, total dissolved solids, hardness, sodium, and chloride, exceeded both WHO and Libyan standards, indicating significant salinity and mineral enrichment. Bacteriological examination confirmed contamination in 70% of wells, with isolates including fecal indicators such as *E. coli* and *Enterobacter spp.*, as well as opportunistic pathogens like *Klebsiella spp.* and *Streptococcus spp.*. Antibiotic susceptibility testing further revealed alarming resistance patterns, with complete resistance to Trimethoprim–Sulfamethoxazole and high resistance to Ampicillin, alongside emerging resistance to Imipenem. Together, these results highlight the combined chemical and microbiological risks associated with groundwater use in the study area and underscore the urgent need for improved well protection and wastewater management.

Data obtained from groundwater wells:

Table 1 summarizes the structural characteristics of the sampled wells, including depth, age, and distance from septic tanks. The predominance of shallow domestic-use wells and their close proximity to septic tanks highlight their vulnerability to contamination.

Table (1): Data obtained from groundwater wells

Sample Number	Purpose of Use	Well age	Well Depth (meters)	The distance between the well and the septic tank	Locations
1	Daily Use	8 years	7	8 meters	14.992341E 32.387571N
2	Daily Use	3 years	10	20 meters	14.990388E 32.385484N
3	Daily Use	2 years	15	20 meters	14.983308E 32.393264N
4	Daily Use	18 years	13	25 meters	14.981062E 32.395596N
5	Daily Use	2 years	15	50 meters	14.971436E 32.394053N
6	Daily Use	5 years	12	55 meters	14.971662E 32.381627N
7	Daily Use	10 years	13	50 meters	14.991593E 32.383289N
8	Daily Use	1 year	14	30 meters	14.986723E 32.38118N
9	Daily Use	16 years	35	25 meters	15.002689E 32.378746N
10	Daily Use	1 year	22	20 meters	14.995897E 32.373853N
11	Daily Use	9 years	13	38 meters	14.989004E 32.389446N
12	Daily Use	8 years	10	20 meters	14.982574E 32.385059N
13	Daily Use	13 years	12	20 meters	14.975706E 32.387240N
14	Daily Use	1 year	12	10 meters	14.973703E 32.382950N
15	Daily Use	9 years	9	30 meters	14.985017E 32.386783N
16	Daily Use	1 year	15	10 meters	14.989309E 32.378698N
17	Daily Use	9 years	17	25 meters	14.964179E 32.386161N
18	Daily Use	13 years	9	30 meters	14.967126E 32.384619N
19	Daily Use	17 years	6	30 meters	14.980306E 32.399340N
20	Daily Use	10 years	14	20 meters	14.973254E 32.392222N

Table 2 presents the general characteristics of the wells. Depth ranged from 6 to 35 m (mean 17.4 ± 8.3 m), with 85% of wells less than 10 years old. The distance from septic tanks ranged between 8 and 55 m (mean 25.3 ± 10.7 m), and 60% of wells were located within 25 m. All wells were used for domestic purposes.

Table (2): Characteristics of Groundwater Wells.

Parameter	Range	Mean ± SD	Observation / Note
Well Depth (m)	6 – 35	17.4 ± 8.3	Majority shallow (<20 m)
Well Age (years)	1 – 18	8.5 ± 4.2	85% of wells <10 years old
Distance from Septic Tank (m)	8 – 55	25.3 ± 10.7	60% ≤ 25 m
Purpose of Use	—	—	All for domestic use
Location Zones	North, Central, South	—	Stratified sampling across zones

Physico-chemical Characteristics:

Table 3 shows the physicochemical parameters compared with WHO and Libyan standards. The mean pH (7.25 ± 0.42) was within acceptable ranges, but electrical conductivity (5189.6 ± 2301.4 µS/cm), total dissolved solids (3711.4 ± 1395.1 mg/L), hardness (1204.1 ± 401.3 mg/L), sodium (825.0 ± 191.8 mg/L), and chloride (1578.0 ± 752.4 mg/L) exceeded permissible limits. Nitrate levels (48.8 ± 0.7 mg/L) were near the WHO limit.

Table (3): Physico-chemical parameters compared with WHO and Libyan standards.

Parameter	Mean ± SD	WHO Limit	Libya Limit	Status
pH	7.25 ± 0.42	6.5–8.5	6.5–8.5	Within range
Electrical Conductivity	5189.6 ± 2301.4	≤1500	500–1500	Exceeds limit
Total Hardness	1204.1 ± 401.3	≤500	500	Exceeds limit
Sodium	825.0 ± 191.8	≤200	30–150	Exceeds limit
Chloride	1578.0 ± 752.4	≤250	200–250	Exceeds limit
Nitrate	48.8 ± 0.7	≤50	10–45	Near limit
TDS	3711.4 ± 1395.1	≤1000	500–1000	Exceeds limit

Correlation analysis confirmed strong positive relationships between EC and mineral salts, particularly magnesium ($r = 0.944$, $p < 0.001$), supporting the interpretation that salinity is closely linked to mineral enrichment.

Bacteriological Contamination:

Bacteriological analysis showed contamination in 70% of wells, with 25 isolates identified (Figure 2, Figure 3). The most frequent organisms were *Bacillus* spp. (7), Klebsiella spp. (5), Streptococcus spp. (4), Enterobacter spp. (3), and E. coli (2).

Bacillus spp. are typically environmental organisms, indicating soil or agricultural infiltration. In contrast, *E. coli* and Enterobacter spp. are fecal indicators, confirming wastewater contamination. Klebsiella spp. and Streptococcus spp. are opportunistic pathogens capable of causing respiratory and urinary infections, making their presence in domestic-use wells a notable health concern.

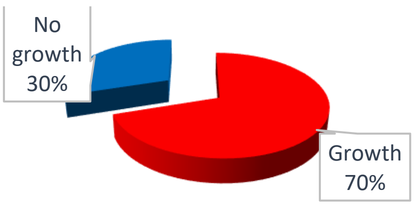

Figure (2): shows that 70% of wells were contaminated, while 30% remained free of detectable bacterial pollution.

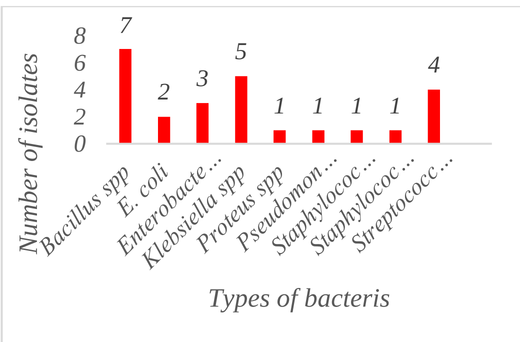

Figure (3): The distribution of the 25 bacterial isolates, with *Bacillus spp.* being the most frequent (7 isolates), followed by *Klebsiella spp.* (5), *Streptococcus spp.* (4), *Enterobacter spp.* (3), and *E. coli* (2).

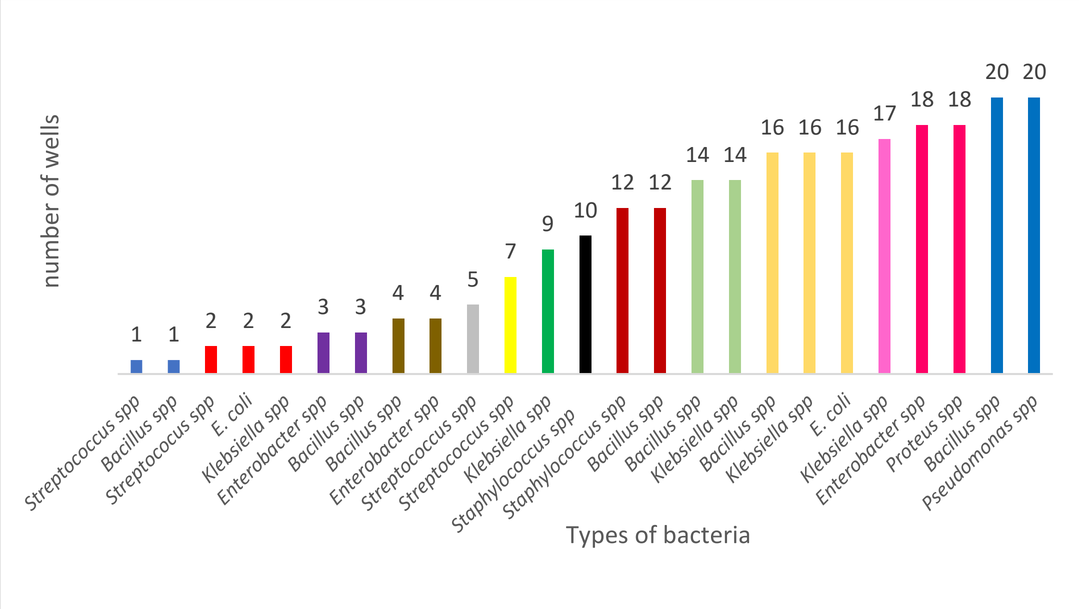

Figure (4): Types of bacteria isolated from each well, highlighting the presence of fecal indicators in shallow wells.

Table 6 presents total coliform and *E. coli* counts. *E. coli* was detected in wells W2 and W16, confirming fecal contamination.

Table (6): Total coliform and *E. coli* counts.

Wells	W1	W2	W3	W4	W9	W16	W17	W18	W20
Total coliform	700	2500	300	2300	2500	10000	300	800	3100
E. coli	-	2	-	-	-	3	-	-	-

Well Depth and Contamination:

Table 7 demonstrates the relationship between well depth and contamination. Shallow wells (<30 m) showed the highest contamination (89%), medium-depth wells (30–60 m) had moderate contamination (57%), while deep wells (>60 m) had the lowest contamination (25%).

Table (7): Well depth and contamination status.

Category	Wells	Avg Depth (m)	Mean Distance from Septic (m)	Contamination (%)
Shallow (<30 m)	9	17.3	18.6	89
Medium (30–60 m)	7	42.5	31.4	57
Deep (>60 m)	4	68.2	49.0	25

Antibiotic Resistance Profiles:

Table 8 summarizes antibiotic resistance patterns. All isolates were resistant to Trimethoprim–Sulfamethoxazole (100%), while 88% were resistant to Ampicillin. Moderate resistance was observed to Amoxicillin–Clavulanic acid (72%), Cefuroxime (60%), and Ciprofloxacin (52%). Emerging resistance to Imipenem (48%) is particularly concerning.

The high resistance to Trimethoprim–Sulfamethoxazole and Ampicillin reflects their widespread and often uncontrolled use in human and veterinary medicine in Libya.

Table (8): Antibiotic resistance patterns.

Antibiotic	Resistance (%)	Sensitivity (%)
Trimethoprim–Sulfamethoxazole	100	0
Ampicillin	88	12
Cefoxitin	1	99
Amoxicillin–Clavulanic acid	72	28
Cefuroxime	60	40
Ciprofloxacin	52	48
Doxycycline	16	84
Imipenem	48	52

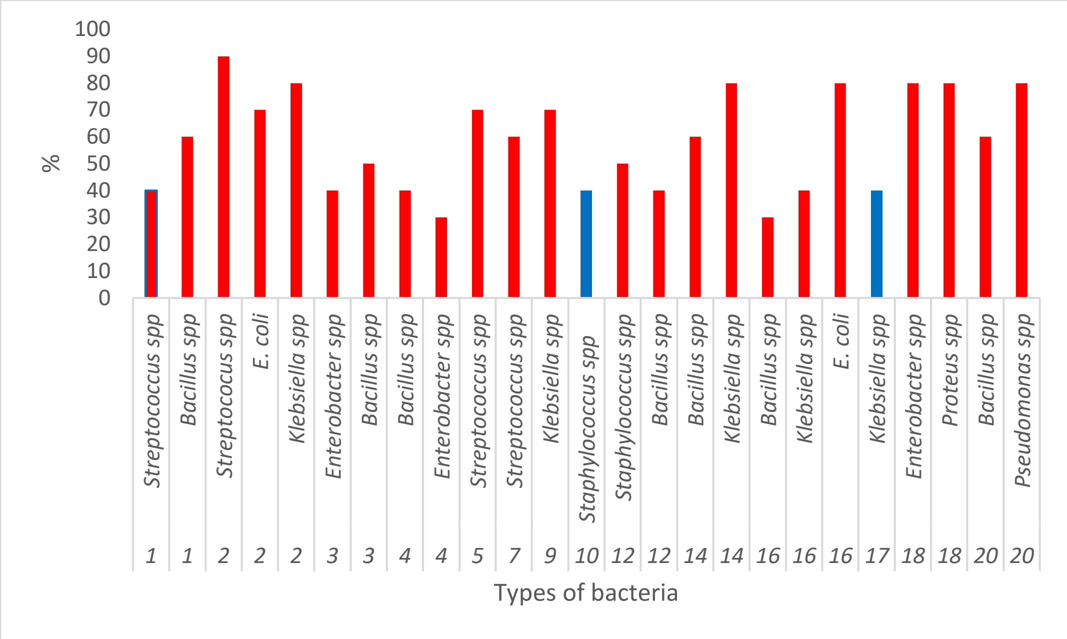

Figure (5): shows that 12 wells contained antibiotic-resistant bacteria, while 8 wells did not.

Discussion:

The present study demonstrates that groundwater in Zawiyat Al-Mahjoub is increasingly vulnerable to combined chemical deterioration, microbial contamination, and the presence of antibiotic-resistant bacteria. This multifactorial degradation reflects the interplay of hydrogeological processes, anthropogenic activities, and inadequate wastewater management. Elevated concentrations of sodium, chloride, and total dissolved solids observed in the samples are consistent with saline intrusion and mineral leaching. Similar findings have been reported in Tunisian coastal aquifers, where seawater intrusion has been linked to excessive pumping and reduced hydraulic gradients (Ayadi et al., 2018), and in Egypt's Nile Delta, where comparable increases in salinity have been documented in shallow wells subjected to over-extraction (El-Sayed, 2019). The coexistence of high hardness and bicarbonate levels in our samples suggests carbonate dissolution, a process typical of carbonate-rich formations in western Libya (Mubarak & Sherif, 2015). These geochemical signatures highlight the dual influence of natural processes and human-driven abstraction on groundwater quality, consistent with deterioration patterns previously reported in arid North African aquifers (Ben Salem et al., 2014; Gad, 2019).

Microbiological analysis further revealed contamination pathways that are closely tied to human activity. The detection of *E. coli* and *Enterobacter spp.* provides direct evidence of fecal contamination, most likely originating from septic tanks located in close proximity to wells. Opportunistic pathogens such as *Klebsiella spp.* and *Streptococcus spp.* raise additional concerns, as they are capable of causing respiratory and urinary tract infections. Comparable contamination patterns have been reported in Tunisia, where *E. coli* and *Klebsiella* were associated with wastewater infiltration into shallow aquifers (Mahjoubi et al., 2016), and in Egypt, where uncontrolled septic systems contributed to similar microbial pollution (El-Shafai et al., 2008). The predominance of *Bacillus spp.* in our samples indicates infiltration from agricultural soils, underscoring the combined impact of domestic and agricultural activities on groundwater microbiology.

Resistance profiles revealed in this study are alarming. Complete resistance to Trimethoprim–Sulfamethoxazole and high resistance to Ampicillin reflect the widespread and often uncontrolled use of these antibiotics in both human and veterinary medicine in Libya (Adhimi et al., 2022). Moderate resistance to Amoxicillin–Clavulanic acid, Cefuroxime, and Ciprofloxacin suggests emerging challenges in clinical treatment. Particularly concerning is the detection of resistance to Imipenem, a last-resort antibiotic, which indicates the potential spread of multidrug-resistant organisms into community water supplies. These findings align with regional evidence from Tunisia and Egypt, where resistant bacteria have been isolated from groundwater and wastewater sources (Mahjoubi et al., 2016; El-Shafai et al., 2008). From a public health perspective, the presence of resistant pathogens in domestic-use wells represents a tangible risk of waterborne outbreaks and hard-to-treat infections. Communities relying on untreated groundwater may face increased incidence of gastrointestinal diseases, urinary tract infections, and respiratory illnesses, compounded by limited treatment options due to antibiotic resistance (Wu et al., 2025).

Although this study was cross-sectional, seasonal variability is likely to influence groundwater quality. Previous research in arid regions has shown that microbial loads typically increase during warmer months due to enhanced microbial activity, while chemical concentrations fluctuate with recharge and evaporation dynamics (Gad, 2019; Ayadi et al., 2018). Incorporating seasonal sampling in future studies would provide a more comprehensive understanding of temporal changes in both chemical and bacteriological parameters.

By combining physicochemical analysis, bacteriological assessment, and antibiotic-resistance profiling, this study advances prior research in Libya and neighboring countries. Most earlier studies focused solely on chemical parameters (Khairi & Abdulaziz, 2018; Amshaher & Ataf, 2021), overlooking microbial safety and resistance. The integrated approach adopted here provides a more robust evidence base for policy development, resource management, and public health protection.

Conclusion:

This study demonstrates that groundwater in Zawiyat Al-Mahjoub is increasingly at risk due to combined chemical deterioration, microbial contamination, and the presence of antibiotic-resistant bacteria. These findings pose significant public-health risks, particularly the potential for waterborne diseases, infections caused by multi-drug-resistant pathogens, and long-term exposure to elevated salinity and chemical constituents. The detection of E. coli and resistant Klebsiella strains in drinking-water sources underscores the urgency of improving sanitation systems and strengthening groundwater-protection measures.

By integrating physicochemical analysis, bacteriological assessment, and antibiotic-resistance profiling, this study advances prior research in Libya and neighboring countries. Such a comprehensive approach provides a deeper understanding of the interconnected factors driving aquifer degradation and offers a more reliable evidence base for policy development and water-resource management. Continued monitoring, combined with improved wastewater handling and controlled groundwater abstraction, is essential for safeguarding public health and ensuring sustainable access to safe drinking water in the region.

Recommendations:

- Septic tank design and construction: Septic tanks should be fully sealed to prevent leakage and groundwater contamination.
- Buffer zones: Maintain a minimum distance of ≥30 m between wells and septic tanks, in line with international guidelines.
- Well construction: Encourage deeper well drilling and proper casing to reduce vulnerability to surface contamination.
- Water quality monitoring: Implement routine monitoring of salinity, mineral content, and microbial indicators to track aquifer health.
- Antibiotic resistance surveillance: Establish regular testing for antibiotic resistance, with particular attention to MRSA antibiotics, to mitigate public health risks.

Limitations:

This study was limited by the number of sampled wells, the absence of seasonal sampling, the restricted range of antibiotics tested, and reliance on culture-based methods, which may underestimate microbial diversity. Consequently, causal relationships between well location and contamination levels could not be fully established.

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