Molecular Diagnosis and Health Risk Assessment (HRI) of Microbial Contaminants in Well Water in Rural Babil Governorate (2025–2026).

Abstract

The aim of this research is to study and understand the environmental and health status of groundwater in rural areas of Babylon Governorate in the period (2025-2026). The results indicated that water quality decreased and was unsuitable for direct human consumption because of the high level of microbial and parasitic contamination found in the field and laboratory. Bacteriological survey showed that the predominant bacteria isolated were enteric pathogens (Escherichia coli and Klebsiella pneumoniae) with high levels of multidrug resistance (MDR) and thus a huge challenge to the health care system. Aquatic ecosystem proved to be an important reservoir for the dissemination of resistance genetic factors between strains by molecular detection using polymerase chain reaction (PCR) showing its diagnostic accuracy to track and detect specific resistance genes (blaTEM, tetA, sul1). Microscopic examination also found the encysted stages of the parasites Giardia lamblia and Cryptosporidium parvum, which further enhances the risk of outbreaks of acute enteritis. The severity of the results is in the Health Risk Index (HRI) values which were above the international exposure limit in all of the study areas, and children had the highest exposure rates. There is a direct and continuous risk to the health and safety of the local population. The authors of this study suggest the need to implement periodic environmental monitoring of well water, molecular testing as an early warning system, the required use of home water filtration technologies, and the need to set up an engineered buffer zone that protects water sources from contamination by absorption pits and agricultural wastes for the protection of public health based on these data.

Keywords: Health risks, bacteria, well water, Babylon Governorate

1 Introduction

Well water is the key and immediate source for drinking, domestic and food production in many remote and rural regions, and groundwater is a fundamental element of water and health security in these regions. But, due to the growing environmental pressures and threats from rising human and farming activities, it is susceptible to contamination and reduction in its quality by microbes. The water, once a resource for life, can turn into a vector of many pathogens, endangering the physical lives and public health of inhabitants, especially the most vulnerable to health risks, as children and elderly.

The health challenge of parasitic contamination by bacteria and aquatic enteroprotozoa is all-encompassing as these organisms are known to survive and adapt in open aquatic environments and propagate themselves via the daily consumption chains. E. coli and K. pneumoniae are among the top enterobacterial pathogens, as well as the encysted stages of enteroparasites like G. lamblia and C. parvum. Moreover, the development and dissemination of antibiotic resistance in aquatic bacterial strains is one of the most intricate medical situations of the time. Resistance genes are spread through water resulting in hard to treat health infections of the intestinal tract, thereby raising the numbers of patients and burdening remote communities economically and health-wise. The traditional tests are no longer adequate in assessing the level of biological threat, and are needed to develop a precise and comprehensive understanding of the extent of this human risk. This is where the relevance of molecular detection methods using polymerase chain reaction (PCR) comes in. These methods are accurate and sensitive in diagnosis of pathogen's genotype and genotype of specific resistance genes like blaTEM, tetA, sul1. When these molecular methods are coupled with the Health Risk Index (HRI) model, [3] then the expected health effects and risk level of the human exposures to this water in the short and long term can be quantified. This field study in Babylon Governorate (2025-2026) is to show the environmental and health picture of well water, human health and living safety are put at the forefront to build a diagnostic and solid database that can be used by decision makers and health authorities to reduce pollution and provide environmentally and healthily safe water [4].

2 Literature Review

In many remote and rural communities, groundwater plays a key role in water and health security, with well water being the main and direct water source for drinking, domestic and agricultural uses. But this crucial resource is under threat from growing environmental pressures and risks as a result of the rise in human and agricultural activities and is susceptible to microbial contamination and degradation of quality. This water can become a vehicle for many pathogens and a threat to the physical wellbeing and public health of local people, especially those most susceptible to disease: children and elderly people.

This section introduces the scientific principles underlying groundwater contamination and the natural and human-induced degradation of groundwater quality in rural areas. It also addresses the molecular assessment, which illustrates the mechanisms of spread and transfer of antibiotic resistance genes among enteric pathogenic bacterial strains via the aquatic medium. In addition, it reviews scientific principles that have been established to measure the pathological and cumulative effects of exposure to microbial and parasitic contaminants, known as the Health Risk Assessment (HRA/HRI). This ensures an academic base in the integrated interpretation of the field results, in the objective and accurate analysis of the level of direct health risk at different social groups [5].

2.1 The Importance of Well Water as a Source of Water in Rural Areas

Water is regarded as the foundation for environmental stability and the major factor for human and health development, because it is directly connected to the maintenance of vital and physiological functions of organisms. Groundwater becomes a strategic resource and the only source on which many rural communities, without centralized pipe-borne water supply systems, fully rely to address the increasing demand for water for their daily activities. Well water is an essential lifeline vital to the life and livelihood of local populations as it is used for drinking, food preparation, household and livestock watering [6].

Therefore, the quality of this water is the most important factor determining the health and well-being of human beings; communities with pure water are protected from the outbreak of epidemic and communicable diseases. On the other hand, compromised biological or microbial water characteristics create a direct danger for physical safety because the individual immunity is compromised and pathological reactions are stronger particularly among the most vulnerable groups of people. The importance of this resource and its role to the environment and health is reflected in the following:

1. Water is an integral part of vital processes and continuation of human life and is one of the major pre-requisites for environmental and health stability for communities [7].

2. Well water is the sole source of water to cater all the rural household needs, drinking, agricultural and livestock purposes.

3. Contamination of clean water leads to serious health and epidemiological risks, while clean water helps to protect human health against communicable diseases [8].

2.2 Well Water Contamination and Its Impact on Public Health

Considering the lack of continuous disinfection treatment in rural areas, well water is a sensitive environmental reservoir, which can be easily impacted by the external pollutants. Reasons for its deterioration include the drainage of sewage from cesspools, the runoff of farm waste containing fertilizers and pesticides, and inadequate well design and inadequate well maintenance. Such contamination turns the water into a vehicle for resistant bacteria and intestinal parasites and so promotes the outbreak of epidemic diseases, the spread of intestinal and cutaneous infections and thus the direct and profound danger to public health and physical safety [9]. The following points can be used to illustrate this:

2.2.1 Well Water Contamination and Its Impact on Public Health (Continued)

1. Pathogenic bacteria and parasitic pollutants are transferred to the water from the leakage of sewage from pit latrines (cesspools) located near wells.

2. Runoff from agri-cultural activities and chemical pesticides from irrigated water degrade the quality and increase the toxicity of groundwater [10].

3. Local people suffer from acute intestinal diseases and frequent bacterial and parasitic infections due to drinking contaminated water.

4. Contaminated water with antibiotic resistant bacteria can be a very serious health problem and can jeopardize the medical system of the community [11].

3 Pathogenic Bacteria in Water Sources and Antibiotic Resistance

The groundwater is a dynamic system in which many pathogenic bacteria species can enter and grow, which are transported into groundwater through various environmental sources. The academic and health severity is doubly when these bacteria are possessing advanced resistance mechanisms against therapeutically used antibiotics [12]. The main dilemma lies in the ability of aquatic isolates to horizontally exchange and transfer Antibiotic Resistance Genes (ARGs) among microorganisms within the aquatic ecosystem. This dangerous phenomenon turns water into an active biological reservoir and a major source of propagation of recalcitrant strains which makes the therapeutic alternatives of the clinicians more difficult, and constitutes a great direct threat to the health and physical well-being of the rural population. The following aspects are summarized as the risks and drivers of this phenomenon [13]:

1. Constant microbial contamination and human activities contaminate the groundwater source and spread pathogenic bacteria.

2. Antibiotic-resistant bacteria are a major health risk that make drugs less effective.

3. The resistance genes are horizontally passed on between bacterial strains in the aquatic environment, so increasing the hazard.

4. The contaminated water becomes the main active source of the outbreak of antibiotic resistance genes in the population [14].

5. Infections in the gut and systemic health infections are caused by ingestion of bacterially contaminated water, and are hard to treat and control.

6. Resistance can be spread through water, resulting in higher therapeutic and economic costs in the health care system [15].

4 Parasitic Pollutants in Well Water

Parasitic pollutants in well water pose a significant health risk to rural people, as parasite cysts and eggs can survive for long periods of time in water. Untreated use of this water results in chronic intestinal infections and acute digestive disorders which have negative effects on human health and living well-being [16].

Well water is contaminated by mixing with human and animal wastes, which carry parasitic cysts and eggs.

2. Waterborne parasites (Giardia and Cryptosporidium) lead to acute intestinal inflammation and chronic diarrhea, putting at risk health vulnerable population groups.

3. Parasitic pollutants also have an impact on health including malnutrition and impaired growth in children through loss of fluid and nutrients [17].

5. Molecular Detection of Pathogens in Water:

The new paradigm is the molecular techniques including the Polymerase Chain Reaction (PCR) which has revolutionized microbiological water testing and is well recognized as a much more qualitative and decisive approach than the traditional culture-based techniques. The conventional assays are based on the cultivation of microorganisms, which can take several days and sometimes give a false negative result because of the bacteria in a VBNC state. Molecular diagnostics, on the other hand, are able to detect and describe the nucleic acids (DNA/RNA) of bacterial and parasitic pathogens with extremely high precision and sensitivity in record time [18].

These modern techniques are not only valuable for microbe detection, but will also be useful if used for the diagnosis of virulence genes, and genes for antibiotic resistance (ARGs), directly in water samples. The use of molecular detection gives a scientifically sound and diagnostic accurate database, which can help researchers and health authorities to predict early epidemiology risk and trace the contamination origin with a high accuracy to protect public health [19].

Health Risk Index (HRI) Assessment Associated with Water Contamination

One of the internationally accredited quantitative academic models is the Health Risk Index (HRI) model, which is used to convert the environmental and microbiological data to a number to represent the level of actual risk to human health [20]. This is a model to estimate exposure to bacterial and parasitic water pollutants on a daily basis with respect to consumption rate and age group. The use of the HRI helps to predict potential adverse health impacts over both the short and long term, moving beyond mere description of manifestations of environmental pollution towards a human centred, applied health assessment that can be used to inform decisions to prevent adverse health effects in the population [12]. Worthwhile points about the significance of adopting this index are:

The Health Risk Index is a system that converts the levels of contamination of the microorganisms to exact figures, which represent the percentage of the risk of contamination to the human being.

The HRI model takes into account the level of exposure and age exposed in every day in order to calculate the risk associated with drinking.

3. The index calculation can aid in the prediction of future epidemiological risks, and can give strong evidence for decision-makers to safeguard community health [21].

Justifications and Rationale for Conducting the Study:

The necessity of assessing the reality of well water in the rural areas of Babylon Governorate, where local people depend on it directly in the absence of clean water and the absence of filtration network in the area, has necessitated this study. Scientific significance of the study is due to the lack of specialized studies combining the molecular analysis of the presence of antibiotic-resistant bacterial pollutants and the quantitative calculation of the Health Risk Index (HRI) in these areas. The research is designed to fill this knowledge gap by delivering accurate molecular and environmental information which can be used to assess the safety of water for consumption and the magnitude of direct health risks and provide a sound scientific basis for both health and environmental institutions to take immediate preventive actions [22]:

5 The aim of this study

The main goal of this research is to evaluate by molecular techniques bacterial pollution and parasites resistant to antibiotics in well water, throughout the rural region of Babylon, and to estimate the health risks. This is followed by the following specific objectives:

• Molecular diagnosis of antibiotic-resistant bacteria and parasitic contaminants.

• Health Risk Index (HRI) for human consumption.

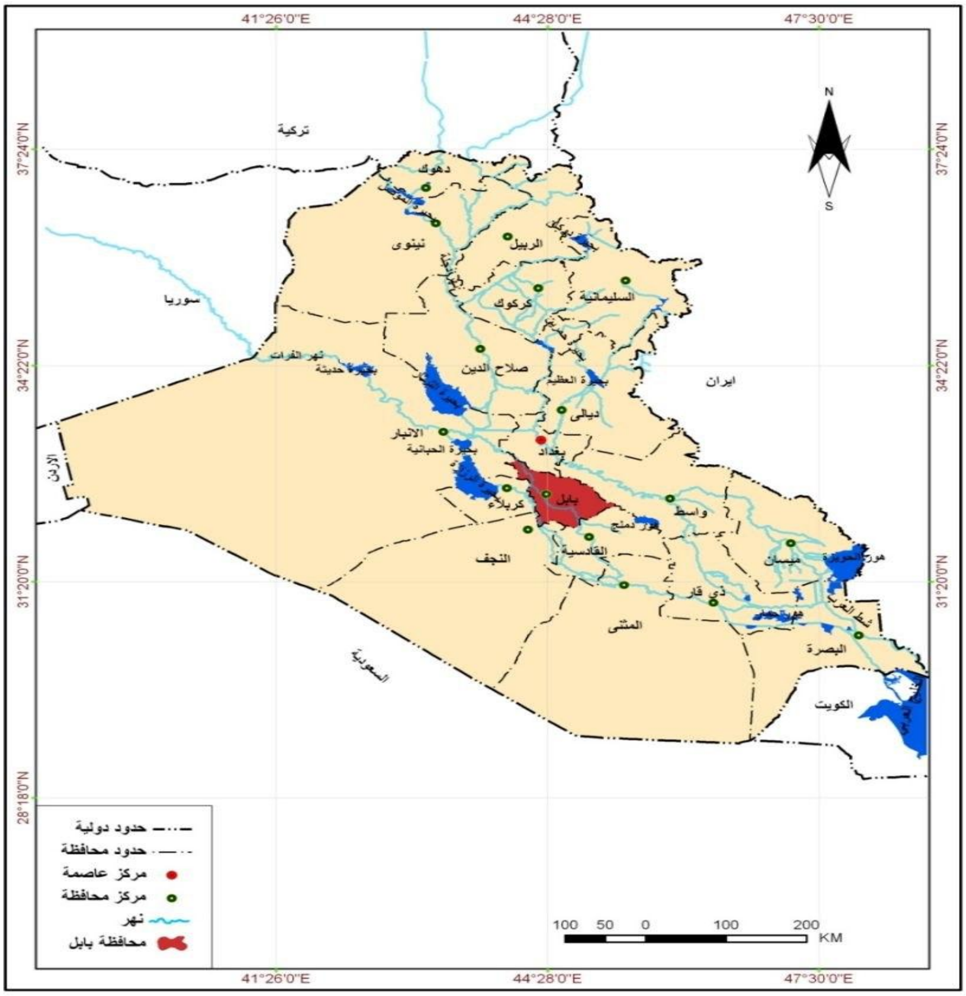
Well water quality assessment for seasonal changes during the period (2025-2026).

Materials and Methods

1. Study Area

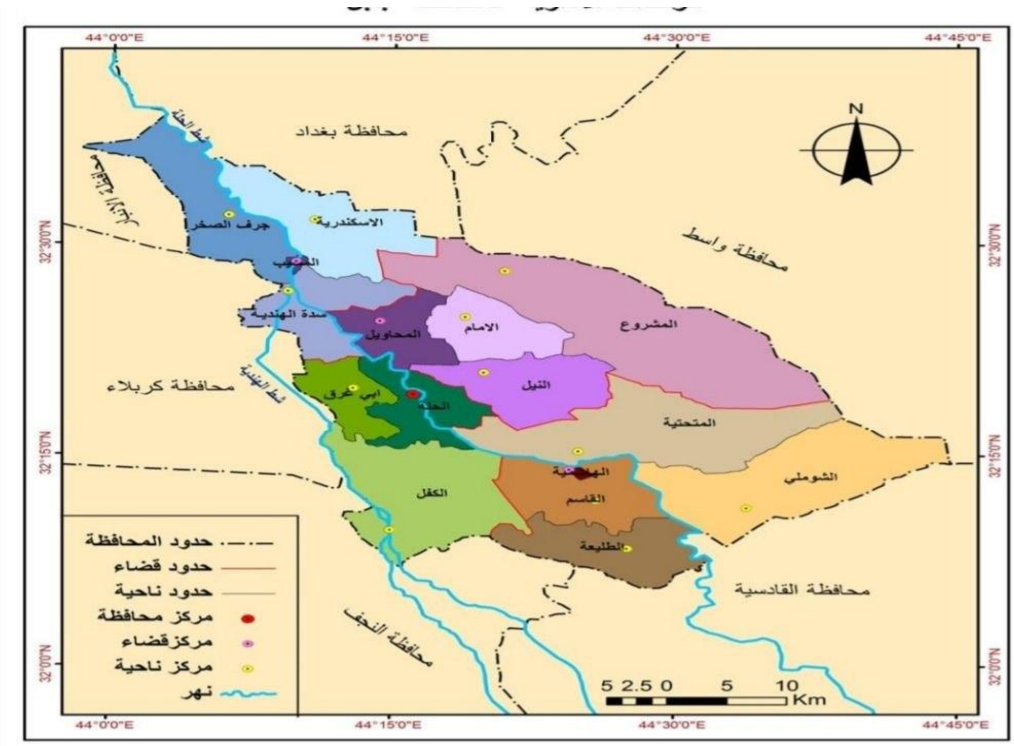
The study area included four major rural zones (Al-Midhadiya, Al-Kifl, Al-Saddah and Jubbala) from Babylon Governorate which were chosen for their geographic variation and for the fact that their inhabitants are using bank filtration and shallow well water directly for drinking and domestic needs. These areas are of great environmental and humanitarian importance because they are remote areas where no advanced centralized water purification networks exist, which makes the inhabitants particularly vulnerable to salinity and ever-increasing water contamination [23]. The governorate is located in the Mesopotamian alluvial plain with a semi-arid climate, in which geomorphological features and aquifer properties are playing a vital role in controlling the groundwater quality and its suitability for human and agricultural purposes. Map (1) presents the geographic and administrative position of Babylon Governorate in regard to the Republic of Iraq and Map (2) presents the full details of adopted administrative and the boundaries between districts and sub-districts in the Babylon Governorate.

Map (1): Location of Babylon Governorate in Iraq



Source: Republic of Iraq, Ministry of Water Resources, General Directorate of Survey, Map Production Department, Administrative Map of Iraq.

Map 2: Administrative Units of Babylon Governorate



The map of Babylon Governorate for the year 2024 was used as a source for this work.

Summarized Water Sample Collection 24 water samples were taken periodically from 8 designated well in each rural area (two well per rural area) in the period of December 2025 to February 2026. Once the pumps were allowed to run for a few minutes, sample was poured into 1,000mL sterile glass bottles and transported in cooler boxes at 4°C for laboratory processing as shown in the table below (1).

Table 1. The results of the samples collected from the tested wells throughout the study period (2025-2026).

Month / Time Span	Field Period	Field Campaign	Number of Wells	Number of Samples	Targeted Rural Sectors
December	December 1–15	First	8	8	Al-Midhadiya, Al-Kifl, Jubbala, Al-Saddah
January	January 1–15	Second	8	8	Al-Midhadiya, Al-Kifl, Jubbala, Al-Saddah
February	February 1–15	Third	8	8	Al-Midhadiya, Al-Kifl, Jubbala, Al-Saddah

3. Physicochemical Analysis Field parameters were measured such as pH, temperature and electrical conductivity (EC) using multi-

parameter field meter and turbidity and total dissolved solids (TDS) were measured spectrophotometrically in laboratory.

4. Microbiological Detection of Bacteria; Bacterial strains were isolated and identified using the membrane filtration method by culturing on differential and selective media such as MacConkey Agar, Nutrient Agar and EMB Agar and biochemical identity was confirmed by using the VITEK-2 system.

5. Molecular and Parasitic Detection The DNA extraction of the bacteria was performed using commercial DNA Extraction Kit and conventional Polymerase Chain Reaction (Conventional PCR) was used for the detection of antibiotic resistance genes: blaTEM, tetA and sul1. The amplified gene products were separated and resolved in 1.5% Agarose Gel Electrophoresis, they were visualized and documented under UV Transilluminator. The remaining water samples were concentrated using filtration-sedimentation techniques; and then purified using the Zinc Sulfate Flotation technique to improve the recovery efficiency for parasitic examination. The sediment obtained was subjected to fine microscopic analysis to look for the presence of Giardia lamblia cysts and Cryptosporidium parvum oocysts to provide a complete and accurate microbiological evaluation of the water safety.

6. Antibiotic Susceptibility and Resistance Pattern Mueller Hinton Agar was used to determine the susceptibility and resistance pattern of the bacteria to 8 most commonly used clinical antibiotics by Kirby-Bauer Disc Diffusion method. Microbial isolates which were obtained from water samples were cultured under standard conditions and the diameters of the inhibition zones were measured with a high degree of accuracy to determine levels of resistance. Laboratory scientific results were interpreted based on internationally accredited standards from the Clinical and Laboratory Standards Institute (CLSI). The goal of this test is to find out if there are any environmental hazards because antibiotic-resistant bacteria are spreading in water sources, and to accurately determine what health risks these bacteria pose to the population.

Health Risk Index (HRI) Calculation: The Health Risk Index (HRI) was calculated to assess the risk of human exposure to microbial pollutants every day, based on the formula adopted by the United States Environmental Protection Agency (US-EPA):

$$HRI = \frac{CDI}{RfD}$$

CDI is the Chronic Daily Intake rate of pollutants calculated using an average daily drinking water consumption rate (2 L/day for adults and 1 L/day for children) and average body weight (70 kg for adults and 15 kg for children). RfD is the expected (reference) dose allowable. The HRI values of >1 (HRI > 1) show a direct health risk to consumers.

9. Statistical Analysis SPSS software was used for statistical analysis of data. One-way ANOVA and paired T-tests were used to determine significant differences at a

A P value of < 0.05 was considered statistically significant.

6 Results

2. General well water contamination Results from the bacteriological tests of 24 water samples obtained from 8 wells in four rural areas of Babylon Governorate showed that bacteriological contamination rate was 75.00% (18 samples) and the parasitological samples contamination rate was 75.00% (18 samples).

Table (2).The geographical distribution and contamination rate in the study areas.

Rural Region	Number of Wells	Number of Samples	Contaminated Samples	Contamination Rate(%)	Mean pH	Mean NTU
Al-Midhadiya	2	6	5	83.33%	7.8	6.4
Al-Kifl	2	6	5	83.33%	7.6	5.8
Jubbala	2	6	4	66.67%	7.9	6.1
Al-Saddah	2	6	4	66.67%	7.5	5.2
total	8	24	18	75.00%	7.7	5.87

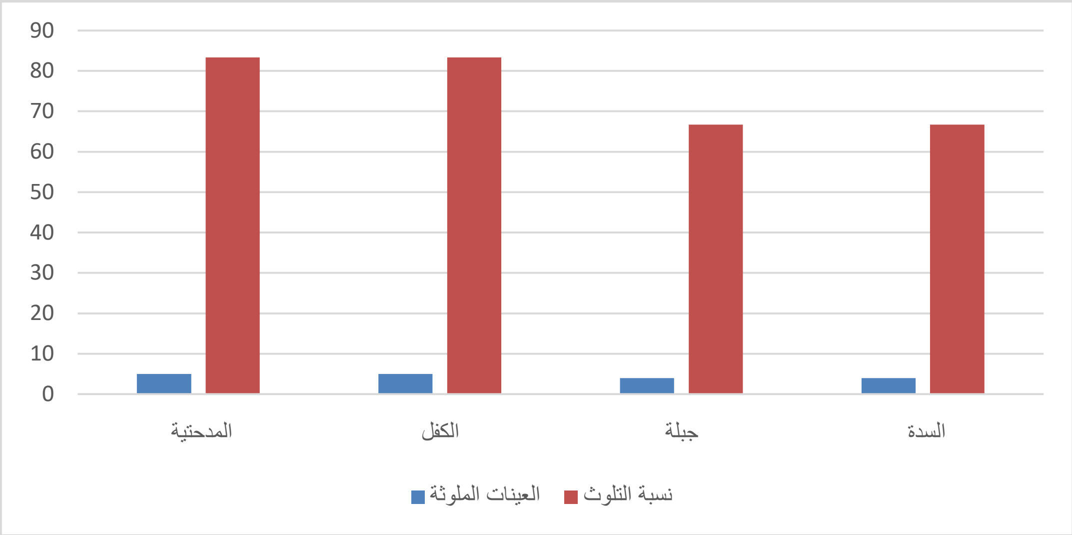


Table (2): Geographical distribution and contamination rate in the study areas

Geographical Distribution Results

The geographical distribution of results in the above table and graph shows that there is a distinct difference between the level of bacterial contamination of the four study areas. Al-Midhadiya and Al-Kifl showed the highest infection rate with a contamination rate of 83.33% (5 contaminated samples out of 6 tested samples for each region), while Jubbala and Al-Saddah had the same contamination rates (4 contaminated samples out of 6 tested samples for each region). This overall high contamination rate is caused by the close proximity of majority of the targeted wells to environmental pollution sources, agricultural activities and domestic sewage, and also by the presence of favourable environment for the persistence and proliferation of the bacterial pollutants due to variations in the physicochemical characteristics of wells including pH and turbidity (NTU) [25]. High water turbidity (NTU) provides a chemical and physical barrier to the stress factors in the environment and improves the ability of microorganisms to survive in aquatic media.

2. Microbiological Isolation and Identification Results for Bacteria

Results from microbiological examination of the well water samples indicated that there were differences in the number of different bacterial species that contaminated the water samples. A summary of the counts and prevalence rates of the isolated bacterial strains and the level of statistical significance between the strains are given in the following table:

Table 3: Distribution of counts and percentages of bacterial strains isolated from well water

Isolated Bacterial Species	Number of Isolates	Percentage(%)	Significant Differences	Prevalence Rate
Escherichia coli	11	39.29%	a	high
Klebsiella pneumoniae	8	28.57%	b	moderate
Pseudomonas aeruginosa	5	17.86%	bc	low
Enterococcus faecalis	4	14.28%	c	Very low
Total Number of Isolates	28	100%	-	-

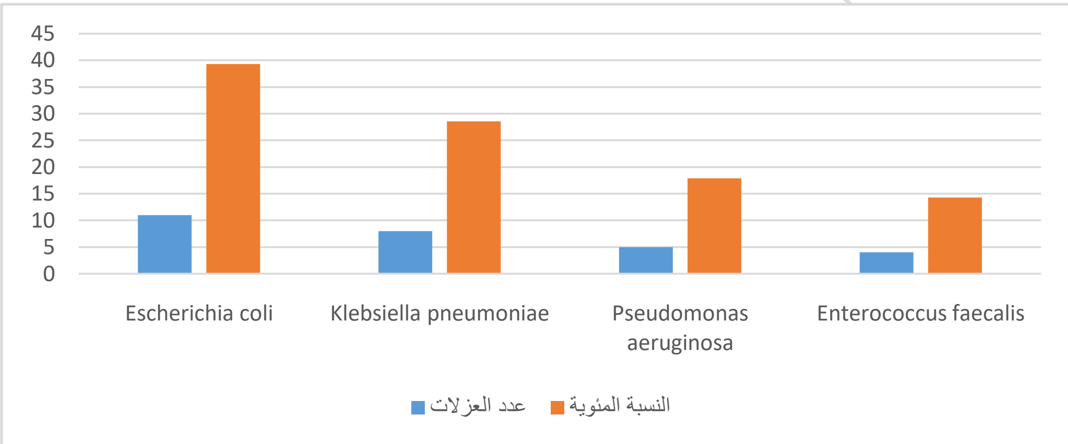


Figure 3: Distribution of counts and percentages of isolated bacterial strains

The above table and graph illustrate the microbiological isolation and identification results showing that 28 bacterial isolates were recovered from the targeted well water samples and that there was a significant statistical difference in the prevalence of each of the isolates species. E. coli was the most common species with 11 isolates (39.29%), followed by Klebsiella pneumoniae with 8 (28.57%) and Pseudomonas aeroguinosa with 5 (17.86%) and the lowest prevalence rate was obtained for Enterococcus faecalis with 4 (14.28%). This distribution reflects well water contamination by sewage and fecal contamination from seepage of waste water and agricultural activities around the wells in the area as well as the high ability of Gram-negative enteric bacteria to colonize aquatic environments and form biofilms.

3. Molecular Detection (PCR) Results for Resistance Genes The results of the molecular detection by using the Polymerase Chain Reaction (PCR) technique showed differences in the prevalence of antibiotic resistance genes. The number of bacteria isolated with each target gene and the percentage of isolates positive for each target gene are shown in the table below:

Table 4: Positive bacterial isolates and resistance genes numbered and percentage.

Resistance Gene	Resistance Type	Numberof Positive	Isolates Percentage(%)
bla_{TEM}	Penicillin Resistance	9	81.82%
tetA	Tetracycline	7	63.64%
Sul1	Sulfonamide	5	45.45%

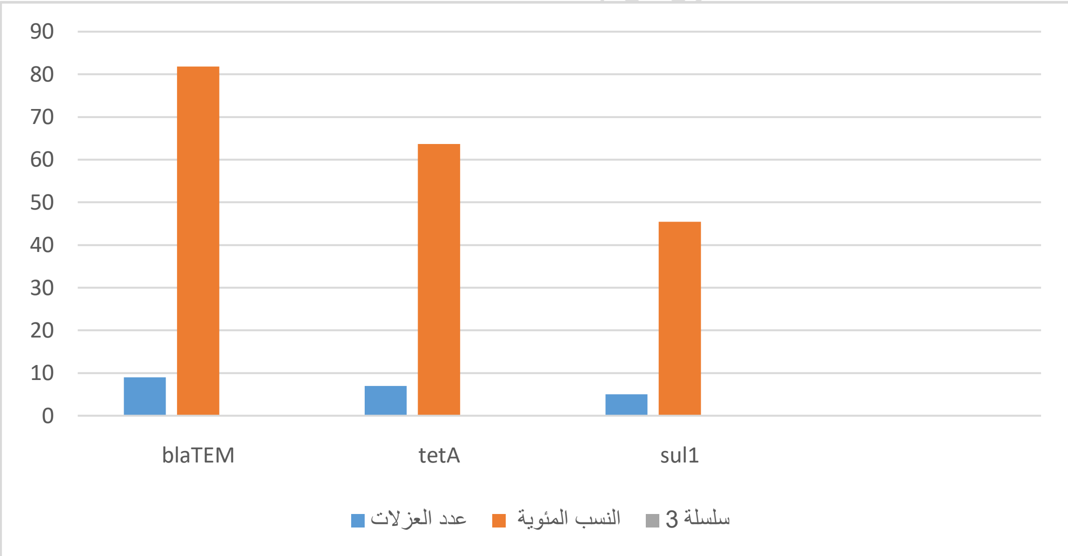


Table 4: Counts and percentages of positive bacterial isolates and resistance genes

Molecular Analysis (PCR) Results: The results are sent home in a letter.

The molecular analysis (PCR) result presented in the table and Figure 4 show a widespread dissemination of the multidrug resistance (MDR) genes among the bacteria isolated from targeted water samples. The gene blaTEM for penicillin resistance was the most prevalent one, 81.82% (9 out of 11 isolates), and this may be due to the constant selective pressure due to the overuse and misuses of beta-lactam antibiotics. The tetracyclines resistance gene, tetA, was the second most common gene (63.64%, 7 isolates), followed by the sulfonamide's resistance gene sul1 (45.45%, 5 isolates). The results have important environmental and public health implications because the majority of these genes are commonly found on mobile genetic elements like plasmids, suggesting that the aquatic environment is contaminated with therapeutic and pharmaceutical residues. As a result, the impaired water bodies become environmental reservoirs and also potential reservoirs of resistant bacteria, reducing the efficacy of available therapeutic options and the need for strict policies to be applied to water resource management and public health.

4. Antibiotic Susceptibility Assessment Results The Kirby-Bauer disc diffusion test demonstrated advanced levels of multidrug resistance (MDR).

Table (5): Antibiotic resistance rates of *E. coli* and *K. pneumoniae* isolates

المضاد الحيوي	E. coli(%)	K. pneumoniae(%)	Overall percentage	Assessment
Ampicillin	81.82%	87.50%	84.21%	high
Tetracycline	63.64%	62.50%	63.16%	moderate
Sulfamethoxazole	45.45%	50.00%	47.37%	moderate
Ciprofloxacin	36.36%	37.50%	36.84%	low

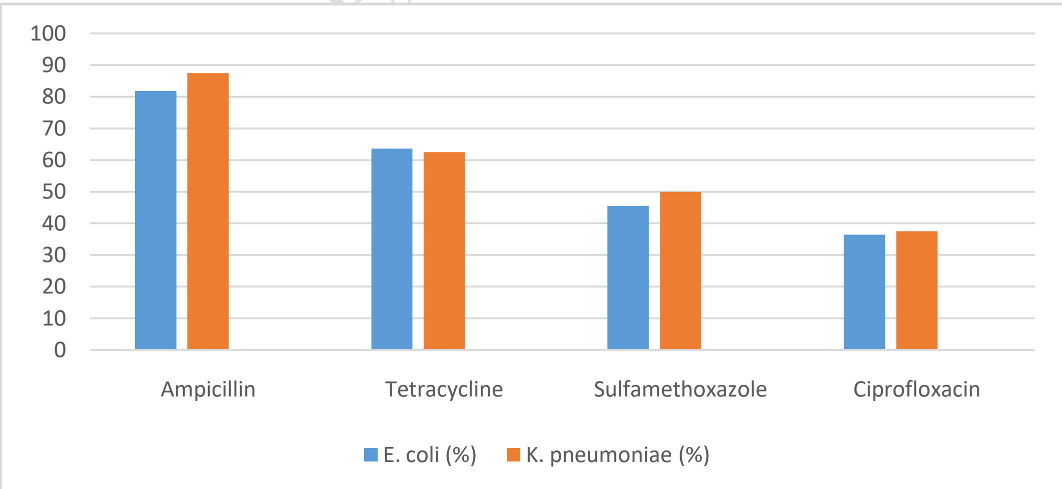


Figure (5): Comparison of antibiotic resistance rates between *E. coli* and *K. pneumoniae* isolates

Antibiotic Susceptibility Assessment Results

Table and Figure 5 shows the evaluation results on antibiotic susceptibility patterns (Kirby-Bauer) which showed the critical multidrug resistance (MDR) pattern of E. coli and K. pneumoniae isolates. The percentage of resistance (84.21 % of E. coli and 87.50 % of K. pneumoniae) of the bacteria to ampicillin was found to be very high since the genes that mediate beta-lactamase enzymes are widely distributed on plasmids. This is followed by significant resistance to tetracycline (63.16%) and sulfamethoxazole (47.37%) while the least resistance is recorded for ciprofloxacin (36.84%). These field data validate the magnitude of the selective pressure exerted by indiscriminate, overuse of antibiotics and validate high-risk isolates contamination of aquatic environment, which is a real therapeutic challenge and requires strict control of medical treatment governance.

5. Microscopic Detection Results for Parasitic Contaminants

Microscopic examination of the sediment revealed clear parasitic contamination in the tested well water samples. Table (5) illustrates the counts and prevalence rates of the detected parasitic species:

Table 6: Prevalence rates of parasitic contaminants detected in well water samples (N=24)

Detected parasite	Number of positive sample	Prevalence Rate
Giardia parasite cysts	6	25.00%
Cryptosporidium	3	12.50%
Total Number of samples	9	37.50%

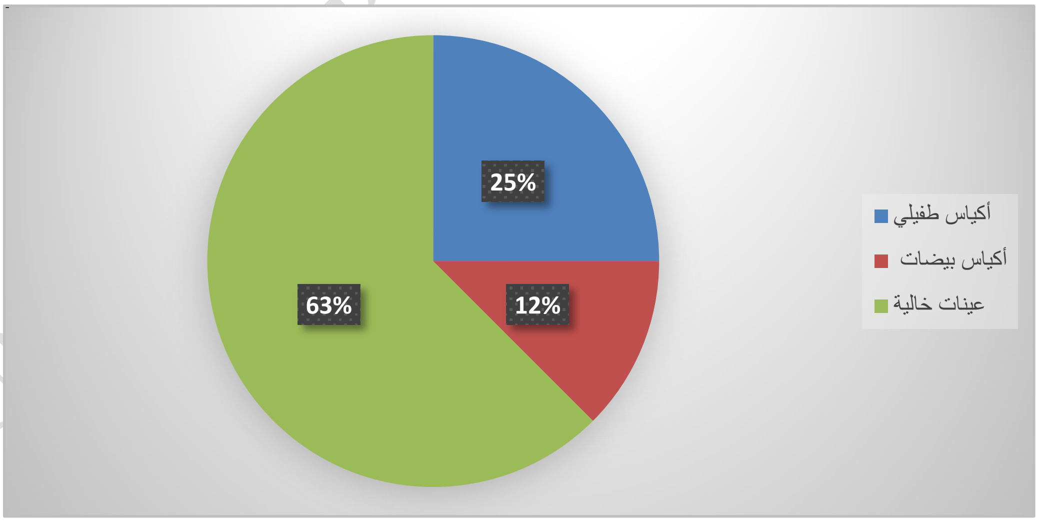


Figure 6: Relative distribution of prevalence rates of parasitic contaminants in well water samples

Microscopic Detection Results

The microscopic results obtained in the attached table and graph are an actual/realized parasitic contamination of the well water samples examined with

37.50% (9 positive samples out of 24 water samples) prevalence rate. Giardia lamblia cysts were most dominant (6 samples), Cryptosporidium parvum oocyst (3 samples) and the remaining 62.50% (15 samples) were parasite free. This variation is a direct result of environmental and health risk and the presence of these protozoan cysts suggests contamination from the mixing of groundwater with surface water or the presence of fecal contamination from agricultural and livestock operations. The implications of these findings are of major concern because these encysted stages are highly resistant to the effects of commonly used water disinfectants like chlorination and therefore, strict requirements for well water disinfection and filtration are necessary to prevent harm to the local communities who depend on these wells.

6. Health Risk Index (HRI) Results

Assessment of chronic daily exposure and evaluation of reference dose indicated that there may be health risks associated with the use of well water. Table 2 shows the Health Risk Index (HRI) values and their classifications in each of the study areas:

Table 7: HRI values and health risk level across study regions

Region	HRI values for children	HRI values for adults	Risk Level classification
Al-Midhadiya	2.45	1.35	High
Al-Kifl	2.10	1.18	High
Jubbala	1.85	0.98	High
Al-Saddah	1.62	0.87	High
Overall Average	2.01	1.10	Health Risks

Health Risk Assessment Results

Results of the health risk assessment as presented in Table 7 indicate the high and direct health risks of the local community members due to their use of these well waters as the Health Risk Index (HRI) values are greater than the environmentally and medically permissible safety values (HRI > 1) in most geographical sectors studied. Comparative analysis shows that there is a significant difference in sensitivity and age response, with the children category having higher risk levels than adults in all regions, and an overall average of 2.01 for children, and 1.10 for adults. The highest risk rates were found in Al-Midhatiya (2.45 for children and 1.35 for adults), Al-Kifl (2.10 for children and 1.18 for adults), Jebala (1.85 for children and 0.98 for adults) and the lowest in Al-Saddah (1.62 for children and 0.87 for adults). This increased sensitivity in children is due to their lower body weight and increased water consumption and metabolism per kilogram of body weight and, therefore, to a greater bioaccumulation in the environment and increased toxic effects, which requires stringent precautions in water treatment and risk assessment on health effects.

7 Statistical Analysis

One-Way Analysis of Variance (One-Way ANOVA) showed that there were statistically significant differences ($P < 0.05$) between the seasonal variations and the contamination rates of the bacteria with the highest contamination rate in summer than in winter. In addition, there were significant differences ($P = 0.012$) between the Health Risk Index (HRI) value of children and that of adults, showing the sensitivity and vulnerability of children to health risks.

8 Discussion

The results of the field work shows that the quality of the ground water in the rural areas of Babylon Governorate is decreasing. This overall bacterial contamination rate (75%) and predominance of Escherichia coli (39.29%) and Klebsiella pneumoniae (28.57%) are due to the direct proximity of shallow wells to the sewage channels and the cesspools, the surface run-off of animal wastes and the agricultural activities, in the absence of any environmental oversight and enclosing protection structures. These contaminants combine with high turbidity levels (NTU) to insulate bacteria from environmental stress factors in the water column and to help them survive.

In molecular detection, the presence of resistance gene blaTEM in most of the samples (81.82%) and resistance to ampicillin (84.21%) may be explained by the indiscriminate use of these antibiotics in human and veterinary medicine. These resistance genes spread horizontally between pathogenic strains through horizontal gene transfer with the help of mobile genetic elements, like plasmids, and by using groundwater as an environmental reservoir.

As for parasitic contamination (37.50%), Giardia lamblia cysts and Cryptosporidium parvum oocysts were detected, which can be attributed to their high resistance to harsh environmental conditions, conventional disinfectants, thus increasing their potential to pose public health threat when they reach daily consumption networks.

Health Risk Index (HRI) analysis revealed that the values were above the internationally permissible values (HRI > 1) in most of the study areas (2.01 for children and 1.10 for adults) which indicates the human and environmental significance of the study. This difference is because children have a lower body weight and daily water needs per kg of body weight, and thus are more susceptible to intestinal disease, growth problems, and effects of toxic substances over time. Such results highlight the important need in rural regions to have sustainable treatment solutions, domestic filtration technologies, and the early warning system for protection of community health infrastructure.

9 Conclusion

This study aims to conduct an integrated assessment of the environment and health status based on the groundwater state of wells in the countryside of Babylon

Governorate. The results show that the water sources are not suitable for human consumption without treatment because of high level of microbial and parasitic contamination. The analyses showed the occurrence of multidrug resistant (MDR) pathogenic enteric bacterial strains and the presence of the resistance genes (blaTEM, tetA and sul1) was confirmed by molecular detection (PCR), highlighting the aquatic environment as a source for transfer of these resistance genes. Microscopic examination also revealed the presence of encysted parasitic forms which are public health hazards (Giardia lamblia and Cryptosporidium parvum) with Health Risk Index (HRI) values above internationally safety thresholds (HRI > 1) especially among the vulnerable pediatric patients. Therefore, the use of untreated well water is an immediate threat to the health of the rural population and routine environmental monitoring with effective treatment and filtration solutions must be implemented to protect public health.

The author would like to thank and appreciate all those who have helped support this research and the laboratory and molecular analyses.

Funding This study was conducted at the personal expense of the author and has not received any external funding.

Conflict of Interest: There are no conflict of interest declarations to make in this research.

References

[1] Al-Hasnawi, S. A. (2021). *Molecular identification of drug-resistant Enterobacteriaceae from rural environmental sources* [Master's thesis, University of Babylon]. Babylon Academic Repository.

[2] Al-Jiboori, H. A., & Al-Basrawi, N. H. (2015). Hydrogeological map of Iraq (2nd ed., Scale 1:1,000,000). *Journal of Geological Survey of Iraq*, 11(1), 23–45.

[3] Al-Khafaji, M. S. (2023). *Application of US-EPA health risk index model for microbial waterborne pollutants in Central Euphrates basin* [Doctoral dissertation, University of Baghdad]. Baghdad Academic Research Portal.

[4] Al-Mayah, S. H., & Al-Taee, A. M. (2020). Microbiological evaluation of well water quality in central Iraq regions. *Iraqi Journal of Science*, 61(4), 780–791.

[5] Al-Ouqaili, M. T., & Al-Alany, M. M. (2019). *Molecular characterization of extended-spectrum β-lactamases genes (blaTEM) isolated from water resources in Iraq* [Doctoral dissertation, University of Anbar]. Anbar University Repository.

[6] Al-Rubaie, H. M. (2018). *Prevalence and microscopic diagnosis of Giardia lamblia cysts in raw water in Babylon Governorate* [Master's thesis, Al-Furat Al-Awsat Technical University]. ATU Digital Library.

[7] APHA (American Public Health Association). (2017). *Standard methods for the examination of water and wastewater* (23rd ed., pp. 921–985). American Public Health Association.

[8] Baldursson, S., & Karanis, P. (2011). Waterborne transmission of protozoan parasites: Review of worldwide outbreaks – An update 2004–2010. *Water Research*, 45(20), 6603–6614.

[9] Clinical and Laboratory Standards Institute (CLSI). (2023). *Performance standards for antimicrobial susceptibility testing* (33rd ed., CLSI supplement M100, pp. 12–48). Clinical and Laboratory Standards Institute.

[10] Edberg, S. C., Rice, E. W., Karlin, R. J., & Allen, M. J. (2000). *Escherichia coli*: The best biological indicator of drinking water safety. *Journal of Applied Microbiology*, 88(S1), 106S–116S.

[11] Fayer, R., & Xiao, L. (2008). *Cryptosporidium and Cryptosporidiosis* (2nd ed., pp. 201–245). CRC Press.

[12] Green, M. R., & Sambrook, J. (2012). *Molecular cloning: A laboratory manual* (4th ed., Vol. 1, pp. 115–150). Cold Spring Harbor Laboratory Press.

[13] Hassan, R. M., & Al-Zubaidi, A. K. (2024). Assessment of microbial and parasite contamination in rural groundwater supplies: A case study in central Iraq. *Environmental Monitoring and Assessment*, 196(2), 145–158.

[14] Kavcar, P., Sofuoglu, A., &Sofuoglu, S. C. (2009). A health risk assessment for exposure to trace elements via drinking water consumption. *Ecotoxicology and Environmental Safety*, 72(5), 1518–1525.

[15] Khan, S., Cao, Q., Zheng, Y. M., Huang, Y. Z., & Zhu, Y. G. (2008). Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing, China. *Environmental Pollution*, 152(3), 686–692.

[16] Larsson, D. G., & Flach, C. F. (2022). Antibiotic resistance in the environment. *Nature Reviews Microbiology*, 20(5), 257–269.

[17] Leclerc, H., Mossel, D. A., Edberg, S. C., & Struijk, C. B. (2001). Indicators of fecal pollution in water: Dear demise of a traditional paradigm. *Critical Reviews in Microbiology*, 27(4), 320–340.

[18] Pitondo-Silva, A., & Stehling, E. G. (2016). Polymerase chain reaction (PCR) multiplex for detection of resistance genes in Gram-negative bacteria. *Journal of Microbiological Methods*, 128(2), 44–49.

[19] Ryan, U., Hijjawi, N., & Xiao, L. (2019). Food and waterborne *Cryptosporidium*, *Giardia* and *Cyclospora*. *Veterinary Parasitology*, 275(1), 35–42.

[20] Sultana, M. S., Rana, S., & Yamazaki, S. (2017). Assessment of health risk index (HRI) of microbial contamination in rural groundwater supplies. *Environmental Monitoring and Assessment*, 189(8), 412–425.

[21] Tacconelli, E., Carrara, E., Savoldi, A., Harbarth, S., Mendelson, M., & Monnet, D. L. (2018). Discovery, research, and development of new antibiotics: The WHO priority list of antibiotic-resistant bacteria. *The Lancet Infectious Diseases*, 18(3), 318–327.

[22] United States Environmental Protection Agency (US-EPA). (2011). *Exposure factors handbook 2011 edition* (Final Report, EPA/600/R-09/052F, pp. 3-1–3-35). US-EPA.

[23] World Health Organization (WHO). (2022). *Guidelines for drinking-water quality: Fourth edition incorporating the first and second addenda* (pp. 45–112). World Health Organization.

[24] Zhang, L., & Li, X. (2024). Molecular surveillance of antibiotic resistance genes (*blaTEM*, *tetA*) in groundwater ecosystems under high agricultural pressure. *Science of the Total Environment*, 912(2), 204–215.

[25] Zhao, Y., Wang, J., & Chen, H. (2023). Integrated health risk assessment (HRI) of microbial pathogen exposure from rural drinking water sources. *Journal of Environmental Management*, 345(1), 102–115.