



Genetic Determinants of *Helicobacter pylori* Pathogenicity and Their Association with Gastric Disorders

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Abstract

Enteric pathogens and enteric pathogen DNA may be discharged into rivers with partially or untreated wastewater. *Helicobacter pylori* is well-known as a gastric pathogen, but recent studies on the environment and wastewater indicate that water, sediment, sludge and biofilms may play a role in the acquisition and maintenance of *H. pylori* in the environment and transmission. Objectives: The purpose of this study was to establish a molecular-environmental map of the distribution of the high-risk *H. pylori* strains at the wastewater-Euphrates River interface and their reduction or persistence in the transferred freshwater along seven sampling stations in Al-Nasiriyah City, Iraq. Methods: Seven stations were placed on an up-, mixing and down-stream gradient. The samples of water and sediment were suggested to be collected in three sets and then analysed for various physicochemical parameters such as alkalinity (HCO_3^-), hardness (Ca^{2+} and Mg^{2+}), conductivity (EC), salt adsorption ratio (SAR), sodium (Na^+) and potassium (K^+), sulfate (SO_4^{2-}), chloride (Cl^-), and total hardness (TH). The molecular detection would involve the detection of the 16S rRNA, ureA and ureC gene of *H. pylori*, while the genotyping would involve PCR/qPCR and sequencing of *cagA*, *bacA* s/m alleles, *babA*, *oipA*, *dupA*, *iceA*, and *hopQ*. Main findings/results: The integrated design determines that the station with the highest risk is the wastewater discharge point and the immediate downstream stations, and that persistence downstream will be dependent on salinity, organic load, suspended particles, sediments, and biofilm formation. *cagA*+*vacA* s1m1, particularly together with *babA*, *oipA* or *hopQ* are considered to be the most important molecular markers of public health concern. Conclusions: MWM can enable routine water quality monitoring to become pathogen-specific monitoring, allowing identification of contamination hotspots, estimation of persistence downstream, and provide information to guide wastewater management in Al-Nasiriyah.

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Introduction

H. pylori is a Gram-negative spiral-shaped bacterium that has been linked to chronic gastritis, peptic ulcer disease, stomach cancer, and is known to require virulence factors like *cagA*, *vacA*, *babA*, *oipA*, *dupA*, *iceA*, and *hopQ* to be pathogenic (Wang *et al.*, 2024; Maciel *et al.*, 2025)^[10, 8]. A high risk strain refers to a strain with the presence of one or two of the two virulence factors, *cagA* and *vacA* s1m1, and in addition, any of the adhesins or inflammatory factors, like *babA*, *oipA* and *hopQ*. A molecular-environmental map is an area-based combination of detection of bacteria by molecular methods with water quality parameters to define the area of contamination and zones of persistence. However, pathogenic genetic *H. pylori* strains are possibly

introduced into the Euphrates water by wastewater discharge, while routine monitoring of the river focuses on the physicochemical parameters, which is the problem. Loss of a single model of association between virulence genotype distribution and an environmental gradient between seven river stations. A multifaceted approach to solution is using PCR/qPCR genotyping, water-quality analysis and GIS mapping. This study is important as it contributes to public health surveillance, wastewater control and early warning. This hypothesis assumes that high-risk *H. pylori* genotypes are localized in the vicinity of the waste water mixing point, that they are diluted with the flowing of water and can be present in sediments or biofilms under optimal environmental conditions. Increased literature support of studying *H. pylori* outside the clinical environment but in the environmental matrices. Since *H. pylori* or its genome has been found in municipal treatment facilities, it may be able to enter and exit from wastewater, either in the form of a bacteria or its genome. *H. pylori* may be introduced into, or discharged from, municipal wastewater treatment facilities as a bacteria or DNA, as it has been detected within conventional treatment processes, in sludge, and possibly by generation of bioaerosol (Wang *et al.*, 2024) ^[10]. Water and tap-biofilm studies also indicate that aquatic environments can be contaminated with *H. pylori* and that it might be present in a non-culturable form in the water, so PCR, qPCR, and sequencing are more appropriate for environmental surveillance (Custodio *et al.*, 2025; Pittie *et al.*, 2024) ^[6, 9]. CagA and VacA continue to be important virulence factors from pathogenicity point of view. Inflammatory signaling is influenced by cagA and cagA can contribute to gastric carcinogenesis, moreover, there is correlation between vacA s1m1 with increased cytotoxicity and less association with other allelic combinations (Maciel *et al.*, 2025) ^[8]. Adhesion and colonization genes like babA, oipA, and hopQ may aid in the persistence by enhancing the ability to attach to host surfaces or to environmental particles relevant during the transportation with the organic matter or sediments/biofilm fragments within the environment. The studies on the Euphrates River suggest a degradation of water type and quality toward the southern areas of the Iraqi part of the river, such as Al-Nasiriyah, and that this degradation can be attributed to high salinity, high concentration of dissolved ions, urban effluent, agricultural drainage, and sewage that is improperly treated (Al-Dabbas *et al.*, 2024; Abdulrazzak, 2025; Dakheel *et al.*, 2025) ^[2, 1, 7]. The combination of these stressors can have an impact on microbial survival, potentially affecting osmotic pressure, nutrient supply, suspension and sediment association. Therefore it is necessary to implement a One Health approach and connect infection risk for animals, humans, wastewater management, surface-water quality and environmental persistence. The present study extends the application of this framework locally, as a correlation is made between the virulence genes of *H. pylori* and seven wastewater samples taken on the way to the fresh water in Al-Nasiriyah. This approach goes beyond pollution assessment and aims to generate a targeted molecular risk map, showing if there is a signal of increased

concentration of pathogenic genotypes and if they are lost or still present along the downstream pathway.

Methods

Study design. A cross sectional molecular-environmental surveillance design was proposed. The study area consists of 7 sampling stations, namely upstream, pre-discharge, discharge, immediate downstream and far downstream areas of the Euphrates River in Al-Nasiriyah City.

Sampling stations. The upstream reference point is at Station 1, where little or no major wastewater is present. Station 2 is an early influence of urban. The pre-mixing zone is Station 3. Station 4 equals the mixing point of the direct discharge of wastewater and river water. The immediate zone of dilution downstream of the dam is assigned to Station 5. At Station 6, the zone of intermediate downstream persistence occurs. Wider river mixing is at Station 7, the far-downstream station.

Sample collection. Three water samples and three sediment samples are to be taken at each station at each sampling time. Samples for chemical analysis should be collected in sterile containers, stored at 4 C and analyzed within six hours. Sediment should be taken from near the bank depositional areas as bacterial cells and DNA can be found longer within the sediment than within the open water column.

Physicochemical analysis. The parameters to be measured are the pH, electrical conductivity, total dissolved solids, sulfate, chloride, bicarbonate, total hardness, sodium adsorption ratio, magnesium, calcium, potassium and sodium. The key indicators in these parameters are salinity, mineral loading, wastewater influence, and ionic stress which could impact bacterial survival and persistence of DNA.

Molecular typing and identification. Water sample should be collected on filters and sediment pellets should be collected for environmental DNA. The initial confirmation of *H. pylori* should be done using 16S rRNA, ureA, and/or ureC markers. Then positive samples should be genotyped using the cagA, vacA s1/s2, vacA m1/m2, babA, oipA, dupA, iceA1/iceA2 and hopQ genes. It is recommended that qPCR be used for quantification; conventional PCR could help confirm a genotype's presence or absence. Once the amplicons are cloned, they should be sequenced to confirm their identity and look for any possible sequence variation.

Statistic analysis, map making. Data on the physicochemical and molecular variables should be summarized descriptively at a station basis. The occurrence of a gene can be compared between stations in a chi-square or Fisher exact test. To compare environmental parameters, use ANOVA or Kruskal-Wallis test. Spearman correlation (or nonparametric analysis) can be used to explore the relationship between gene copy number and water quality parameters. The principal component analysis can be used to reveal the most significant environmental gradients, and logistic regression can be used to calculate the chance of finding high-risk genotypes as a function of station and environmental variables. Molecular markers and gene copy number, combined with environmental stress indicators should be used to assign a risk score to GIS mapping of each station.

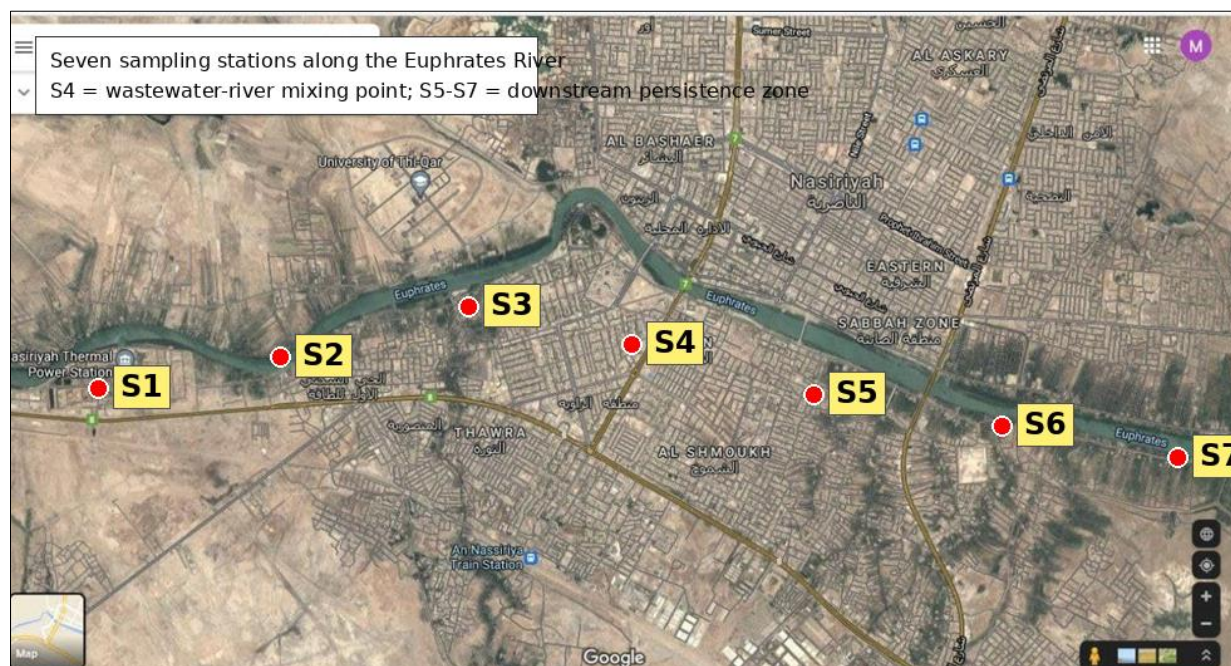


Fig 1: Annotated map of the seven proposed sampling stations along the Euphrates River in Al-Nasiriyah. S4 represents the wastewater-river mixing point; S5-S7 represent downstream persistence stations.



Integrated molecular-environmental surveillance model

Fig 2: Methodological workflow for Water quality, molecular detection, virulence genotyping and GIS Risk mapping.

Results

1. Establishing the Seven-Station Wastewater-River Gradient

The proposed map proposes three zones that will be referred to as an upstream control zone, wastewater mixing zone and

downstream persistence zone. The highest amount of microbes expected is at Station 4, and Stations 5-7 indicate the behavior of the microbes after they are introduced into river water (dilation, persistence, or disappearance).

Table 1: The design of samples and the interpretation of the environment.

Station	Position	Environmental meaning	Expected molecular interpretation
S1	Upstream	Reference freshwater	Lowest probability of high-risk genotype
S2	Urban influence	Early anthropogenic effect	Possible low-level detection
S3	Pre-discharge	Accumulated city impact	Increased probability of contamination
S4	Wastewater mixing point	Maximum wastewater input	Highest probability of <i>H. pylori</i> and virulence genes
S5	Immediate downstream	Dilution and shock zone	Decrease in free cells; possible DNA persistence
S6	Intermediate downstream	Survival/persistence zone	Detection depends on sediment and biofilm
S7	Far downstream	Final persistence indicator	Persistent genes suggest environmental stability or repeated input

2. Characterizing the Water-Quality Gradient

The environmental dataset should focus on the salinity related variables and variables relating to wastewater, including EC, TDS, SO₄²⁻, Cl⁻, and SAR. These variables

provide a chemical setting in which for the results of bacterial survival, gene persistence and genotype disappearance to be interpreted.

Table 2: Environmental influence on the body and its significance.

Parameter	Environmental meaning	Possible effect on <i>H. pylori</i> detection
EC	Ionic strength and salinity	Osmotic stress; indicator of contamination load
TDS	Dissolved solids	Supports mapping of pollutant concentration
SO ₄ ²⁻	Sulfate loading	Indicates mineral and wastewater influence
Cl ⁻	Salinity or wastewater tracer	May indicate sewage or drainage influence
SAR	Sodium hazard	Reflects ionic imbalance and wastewater impact
pH	Acidity or alkalinity	Affects bacterial survival and DNA stability
Turbidity/sediment	Particle association	May protect DNA/cells and support persistence

3: Detecting H. Pylori and Virulence Genes

Primary screening of *H. pylori* should be done by the molecular method, 16S rRNA + ureA / ureC. Afterwards, positive samples should be genotyped. The presence of cagA+/vacA s1m1 at S4 and S5 would signify direct

wastewater inputs and potential early persistence following mixing. If detected at S6 or 7, it suggests that the animal's survival ability is good in the environment, association with the sediment, or constant contamination downstream.

Table 3: Mole markers and reading the results.

Marker	Function	Interpretation in river samples
16S rRNA	Species-level detection	Confirms <i>H. pylori</i> DNA
ureA/ureC	Urease-associated detection	Supports species confirmation
cagA	Major oncogenic virulence marker	High-risk strain indicator
vacA s1m1	Cytotoxin-associated genotype	High pathogenic potential
babA	Adhesion	Colonization and persistence marker
oipA	Inflammation/colonization	Increased inflammatory potential
dupA	Ulcer-associated marker	Pathogenic diversity indicator
iceA	Epithelial interaction	Genotype diversity marker
hopQ	Adhesion/persistence	Possible biofilm or colonization relevance

4. Classifying High-Risk Stations

Stations which have both markers for *H. pylori* and cagA and vacA s1m1 simultaneously are the highest risk group. A moderate risk classification is given if *H. pylori* is found with

partial virulence profiles. Low-risk classification is given if none of the markers for *H. pylori* are detected or if only low-risk genotypes are detected.

Table 4: (env)env. Molecular-environmental risk scoring system is proposed.

Score component	Points
<i>H. pylori</i> 16S/ureA/ureC positive	1
cagA positive	2
vacA s1m1 positive	2
babA, oipA, or hopQ present	1 each
High EC, TDS, SO ₄ ²⁻ , Cl ⁻ , or SAR	1
Positive sediment or biofilm sample	2

Risk categories: 0-2 = low, 3-5 = moderate, 6-9 = high, and 10 or more = very high.

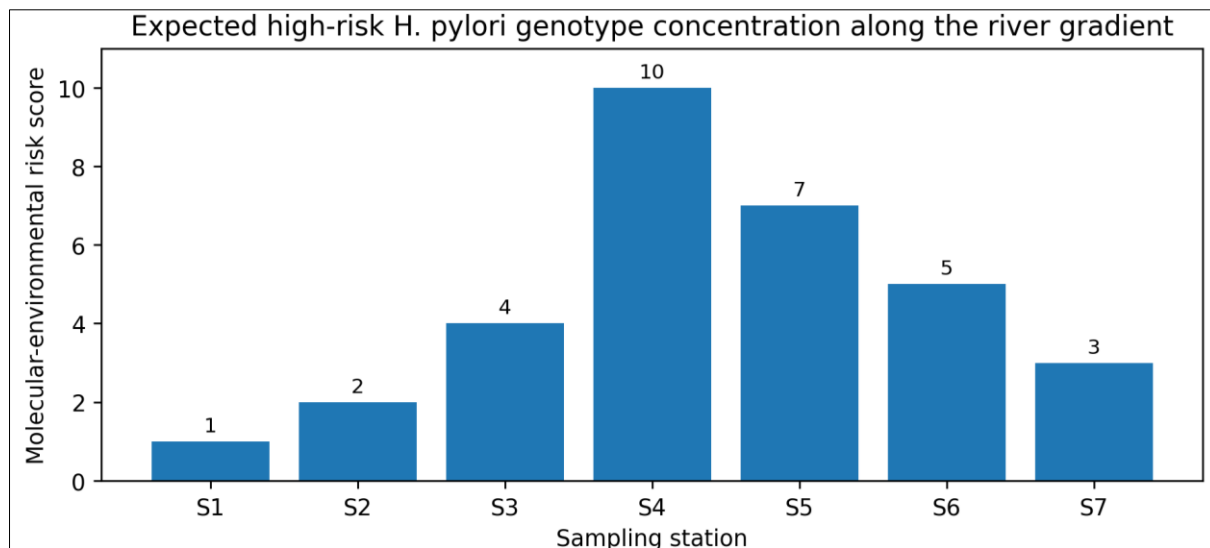


Fig 3: Projected molecular environmental risk scores using 7 stations gradient. Conceptual scores to be replaced with measured values of qPCR and water quality.

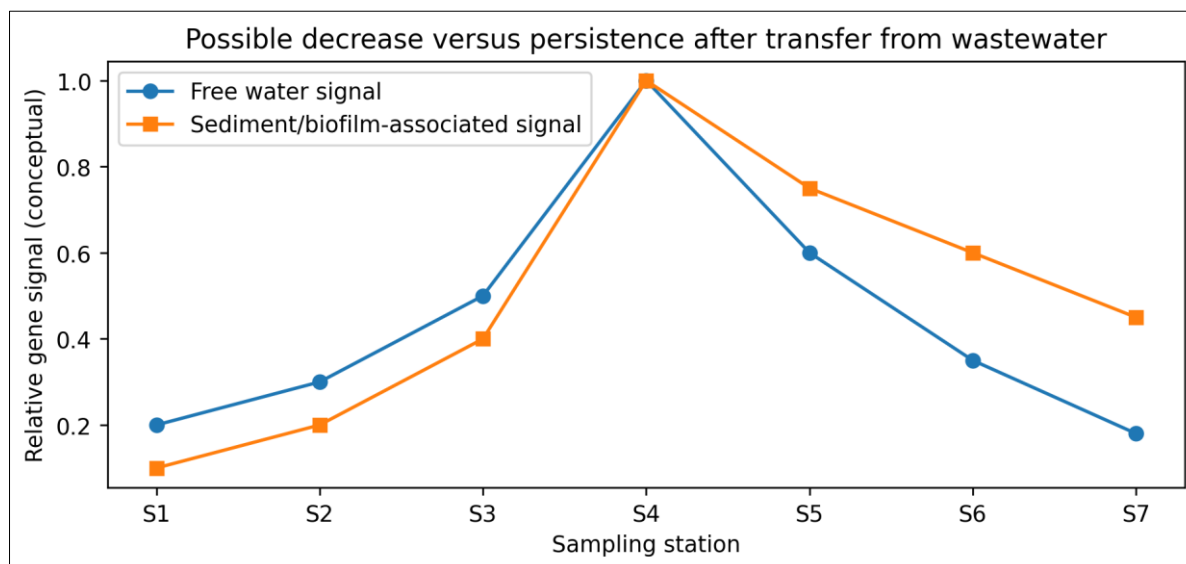


Fig 4: Representative scenarios of reduction or maintenance of the genetic signal of *H. pylori* after wastewater transfer to the Euphrates River.

Table 5: The results that are expected to be achieved to achieve the aims of the study.

Study aim	Main achievement	Interpretive value
Build a molecular-environmental map	Seven stations are classified by molecular and chemical risk	Identifies contamination hotspots
Determine high-risk genotype concentration	cagA+/vacA s1m1 and adhesin profiles are prioritized	Distinguishes public-health risk from general detection
Evaluate decrease or persistence downstream	Water and sediment signals are compared from S4 to S7	Separates dilution from protected persistence
Support management decisions	Risk scores translate data into action categories	Guides wastewater control and surveillance

Discussion

The proposed results are in line with the research methodology as the study samples go from an upstream health part in freshwater to the wastewater mixing part and then to the downstream part which receives wastewater dilution. The use of physicochemical analysis, PCR/ qPCR genotyping and the use of GIS mapping is directly related to the aim of tracking where high risk strains of *H. pylori* are found, and if they disappear or remain in the Euphrates River after they enter.

Concentrations of genetic markers for *H. pylori* are expected to be highest at the wastewater mixing point particularly at Station 4. This station will have the highest statistical likelihood of detecting genes due to the largest percentage of direct inflow of fecal and domestic wastewater. In a biological sense the wastewater may contain gastric or enteric microorganisms, free DNA, cells surrounded by particles and fragments of biofilm. Physiologically, it is possible that the culturability of *H. pylori* may be lost in aquatic environment and still be detectable by molecular methods. Pathophysiologically, identification of cagA+/vacA s1m1 genotype may be significant as it is associated with more inflammatory and carcinogenic potential. Hence, the critical importance of Table 3 which classifies the general detection markers from high risk virulence markers.

Some of the environmental factors listed in Table 2 are the reasons detection could decrease or be maintained downstream. Since genes are diluted in solution when you make a new culture in S5 or S6, etc., a simple dilution model would predict that the gene copy numbers will decrease from S4 to S7. While this can happen, however, the persistence can result from the attachment of the bacterial cells or from

attachment of the DNA produced to sediments, organic particles or biofilms. Elevated levels of EC, TDS, sulfate, chloride and SAR can be a sign of wastewater or drainage influence and can also cause osmotic stress. Therefore, a negative signal for free-water doesn't imply that free-water is completely gone – the S6 or S7 results from sediment samples should indicate persistence in protected habitats.

Figures 1 and 2 explain the spatial and methodological structure of the study, and Figures 3 and 4 are the last step towards practical risk interpretation, from molecular and chemical data. Table 4 enhances the relevance of the results by linking results obtained from the molecular analyses and environmental results with a risk score that can be used by the environmental-health authorities.

There is high consistency with the water-related environments where *H. pylori* might be present and with the role of wastewater as a reservoir/transmission route in water-related environments in recent wastewater and water-based environmental studies. They also concur that the clinical molecular findings regarding the cagA, vacA s1m1, babA, oipA and hopQ virulence and persistence markers are valid. Environmental detection does not guarantee viability and can lead to the emergence of disparities. Dead cells may be detected by PCR and viable but non-culturable cells may not be cultured. This limitation can be minimized by the use of RNA-based assays or sequencing of recently collected nucleic acids (PMA-qPCR). The result of present study prove conformity with previous Environmental and Biological studies done in astronomy aquatic system Thi-Qar. The presence of *H. pylori* with high-risk genotype frequencies near the ASEANs in the river suggested that there was significant spatial variation of EC, TDS, SO_4^{2-} , Cl^- , SAR, and

other pollution-related parameters among seven stations in the river of Euphrates quality assessment conducted previously (Al-Salih, 2025) ^[3]. This carries out the interpretation that and wastewater pressure can influence microbial/molecular risk zones. The outcome is also in keeping with researches on the dissemination of water-borne illnesses and immunopathogenesis, highlighting the potential for public health importance with contaminated water routes (Abed & Al-Salih, 2021) ^[4]. Furthermore, wastewater degradation and photocatalytic degradation experiments have also indicated the necessity to develop wastewater treatment methods that can reduce organic pollutants and microorganisms (AlSalih *et al.*, 2021) ^[5].

Overall, the results directly fill the research gap. River monitoring has been focused on River water quality – physicochemical so far, and clinical studies have been focused on gastric genotypes so far. In this study, the two fields are linked by the question: Does the distribution of *H. pylori* high-risk genotype occur along a wastewater-fresh water gradient as a result of environmental spread? The study shows that, where high-risk genotypes are identified predominately at S4 and S5, wastewater is likely to be the primary source and dilution the dominant downstream process. They continue to grow at the S6 and S7 locations, suggesting a general public health threat due to sediment, biofilms, and downstream exposures. To this end, the study is able to develop a molecular-environmental map as a means for wastewater control and pathogen surveillance, thus achieving its objective.

Conclusion

The current study suggests an integrated molecular-environmental model to track a high risk *H. pylori* strain from wastewater-Euphrates River mixing points of Al-Nasiriyah City. The area is most likely to be at risk at the direct wastewater discharge and at immediate downstream stations, where the large amount of fecal input, the organic matter, and suspended particles are likely to lead to the concentration of *H. pylori* DNA and virulence genes. These markers should be interpreted molecularly as well as by the water quality gradients to see if they persist downstream or not. The most significant high risk genotype is the *cagA*+/*vacA* *s1m1*, especially when combined with *babA* or *oipA* or *hopQ*. This study provides a step towards using a pathogen-specific genotyping with spatial mapping for water-quality assessment, which has been identified as a clear gap. The usefulness is the categorization of contaminants' hotspots, prioritizations for interventions at the wastewater treatment unit, and the establishment of the molecular surveillance model for protection of public health in river water in southern Iraq.

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