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Marine Science Centre, University of Basrah, Iraq

New registration of three bacterial strains isolated from water samples in Al-Qurna/Basrah Governorate

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Abstract

The purpose of the current work was the isolation and identification of bacterial strains that may be associated with disease and pollution of water. Three sites around Al-Qurna Hospital were selected to collect water samples monthly from March 2026 to May 2026. Samples were frequently taken between 9 a.m. and 12 p.m. Water samples were collected from the surface at a depth of 30 to 40 cm in autoclaved amber bottles. The bottles were pre-washed twice with water samples before being filled. The findings suggested the isolation of three types of isolates namely Enterobacter bugandensis, Enterobacter asburiae and Enterobacter kobei with the percentages 38.2%, 31.7% and 30.1% respectively. In the genetic analysis, the 16S rRNA gene was investigated using PCR approach. A single band with a molecular weight of 1500 bp was used to determine the presence of a gene.

Keywords: New registration, Three isolates, 16s rRNA, Bacteria, Qurna provenance.

Introduction

Water is essential for maintaining an adequate food supply and a productive environment for the human population, animals, plants and microbes worldwide (Gavrilescu, 2021). Increased water usage coupled with growing populations and economies would affect biodiversity throughout the global ecosystem and drastically reduce per-person access to (Shukla *et al.*, 2021). It is estimated that 20% of the world population does not have access to clean drinking water and about 50% lack appropriate sanitation. This is a major concern since in many developing nations more than 95% of untreated urban sewage is dumped into surface waterways. Iraq is one of nine Middle

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Eastern nations facing a lack of drinkable water (Todd, 2024). Runoff, irrigation, and non-point source waste water contribute to surface water body problems. Impacts depend on soil contact, water body type, planned use, hydraulic retention duration, and waste water's function in ecosystems (Hashem and Qi, 2021). Hospital wastewaters are extremely complex effluents that potentially contain multidrug-resistant (MDR) bacterial isolates (Kelly *et al.*, 2023). As well as bacteria, excrement from patients, disinfectants, and metabolized pharmaceuticals (Queen *et al.*, 2022). Therefore, the exchange of antibiotic-resistant genes is likely to occur in hospital wastewaters, hotspots for antibiotic resistance (More and more bla genes like blaNDM, blaKPC, blaCTX-M, and blaSHV are seen in hospital-associated gram-negative bacteria (Kandemir *et al.*,2026; López-Hernández *et al.*, 2026). Thus, the aim of the present investigation was to register the novel bacterial strains that were isolated from hospital wastewater at NCBI.

Materials and Methods

Samples collection

Water samples were collected for three months from March 2026 to May 2026 from hospital effluents at Al-Qurna city hospital for Pediatrics and Maternity in three sites. Collecting samples began at 9 a.m. and ended at 12 p.m. For surface water (30–40 cm depth), autoclaved amber bottles were pre-washed twice with water samples before filling.

Bacterial Identification

The conclusion it was due to bacteria was based on the following: Morphological diagnosis and media characteristics: Colonies grown on blood agar, EMB and MacConkey agar were identified after incubation for 24 hours at 37°C according to the growth of the bacteria. Next, we examined the isolates under the microscope and employed the Gram stain to identify the bacterial cells' morphological characteristics, especially their response to the stain, which indicates the form and organization of the bacterial cells colonies. Biochemical reactions and motility tests including methyl red, citrate, urease, Voges-Proskauer, catalase, oxidase, and indole were used to identify bacteria. The isolates were identified further by VItEK2 as bacteria. The gold standard in microbiological detection, calorimetric technique, has been improved to VITEK 2. The instructions of Biomerieux were adhered to.

DNA Extraction

Chromosomal DNA was extracted from 23 S. aureus isolates with the greatest antibiotic resistance and hemolysin production. Equipment manufacturer guidelines were followed for extraction. As a standard, agarose gel electrophoresis separated DNA fragments. Gel concentrations ranged from 0.8% for extracted DNA to 1.2–2% for visual confirmation of PCR results. An agarose gel was prepared by combining agarose granules with 1X TBE buffer and heated in a microwave oven until completely melted. DNA samples were mixed with 1/10 loading buffer and loaded into the sample wells. Connect the lid and some power cables. Apply a current to the device. For 1.5 hours for the PCR product and 2 hours for

the plasmid DNA, the gel is typically run at 50 volts. For documentation, digital cameras are used to take pictures of the DNA bands after they have been visualized with a 365 nm UV trans illuminator.

PCR Technique and Detection of Bacteria

According to Table (1) and the PCR reaction continents below, universal 16S rRNA primers were used to target the gene sections that are unique to each bacterial species and conserved across strains.

Table (1): Universal 16 S rRNA gene primers Sequence of primers.

Name	Nucleotides sequence	bp Number
27 F	5-AGAGTTTGATCCTGGCTCAG-3	20 bp
1492 R	5-GGTTACCTTGTTACGACTT-3	19 bp

Reagents used to amplify the 16 S rRNA gene were measured using the final volume of 50 µl in the PCR (Promega master mix). Reagent Go taq green master mix volume (10 µl) DNA template (5 µl), primer forward (2 µl), primer reverse (2 µl), and nuclease-free water (31 µl) total volume of 50. One cycle of initial denaturation at 95°C is used to amplify the 16 S rRNA gene. This is followed by a cycle of all PCR steps, including product denaturation, for five minutes at 95°C for thirty seconds. Annealing for 30 seconds at 55°C. 35 Extension for one minute at 72°C and 1500 bp. Last extension for five minutes at 72°C and cooling to 4°C.

Results and Discussion

The 16S rRNA gene was verified in this investigation using standard PCR. Figure (1) shows a single band at 1500 bp for the 16S rRNA marker, indicating the gene's presence. The current results revealed that all isolates which subjected to 16S rRNA were *Enterobacter bugandensis , Enterobacter asburiae and Enterobacter kobei.* (Sahoo *et al*., 2025). The difficulties in bacterial taxonomy are demonstrative and common to other bacterial genera specially in *Enterobacter spp*. (Raeisi *et al*., 2026). Genome alterations and behavioral changes were seen when a ground-based strain of *Enterobacter bugandensis , Enterobacter asburiae and Enterobacter kobei* were compared to a control strain following spaceflight, providing insight into the genetic basis of drug resistance (Cirkovic and Brkic, 2025). Amplification of the 16S rRNA gene is a highly accurate and versatile way to identify bacteria for species identification, even when the species is notoriously difficult to identify using biochemical methods (Silva *et al*., 2026). It is evident that the specificity and accuracy of identification are enhanced by the circumvention of biochemical tests and the reduction of time via 16S rRNA sequence-based identification. The origin of microorganisms may be ascertained, and clinical as well as environmental specimens can be distinguished and analyzed.

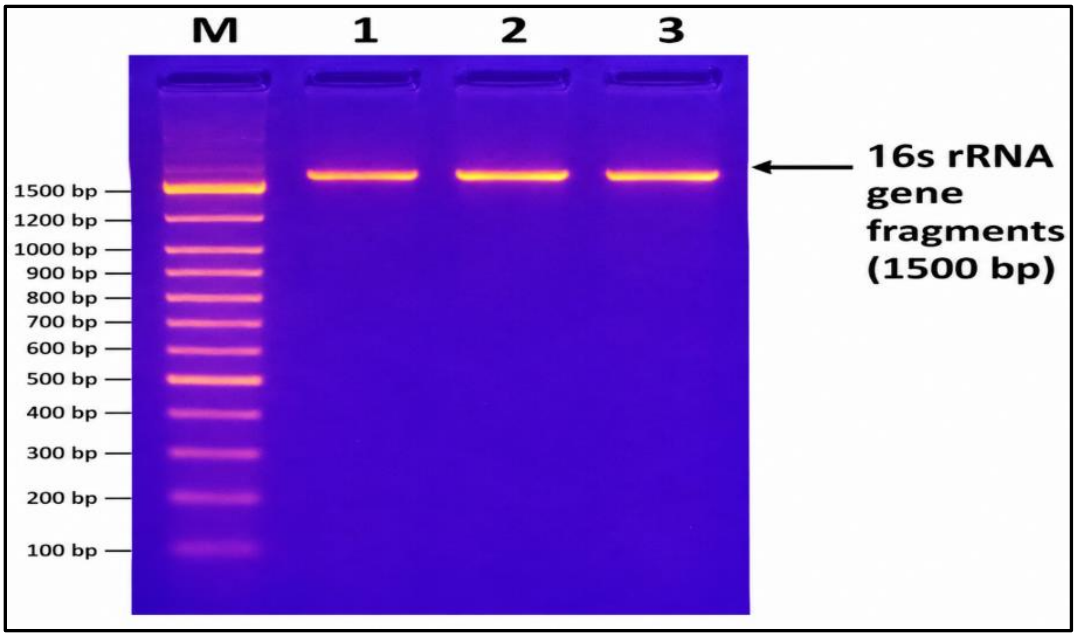


Figure 1. 16S rRNA gene. The existence of the gene was verified by looking for a single band with a certain molecular weight of 1500 bp in agarose gel electrophoresis with 1.5%.

Conclusions

This study sought to extract and identify disease-causing microorganisms that may contaminate water.The results of this study verified the existence of a pathway between the hospital effluents and the drain water. However, the relatedness of clinical and environmental strains is depend on the genetic similarity between strains from the both sources. Typically, samples were taken monthly from three locations, near to Al-Qurna ospital. The results demonstrated three isolates of new NCBI registered were *Enterobacter bugandensis*, *Enterobacter asburiae* and *Enterobacter kobei* with each accession No PZ882195.1 for HAB 51, PZ886181 for HAB52 and PZ886266.1 for HAB53.

Ethics approval and consent to participate

We have obtained approval to conduct our study from the ethics committee of University of Basrah, Marine Science Centre –Marine Biology Department, also College of Science– Biology Department which provided written informed consent.

Consent for publication

This manuscript does not contain any individual personal data, rendering it inapplicable.

Availability of data and materials

All data and materials are incorporated in this investigation. For additional information, please reach out to the author regarding data requests.

Competing interests

The authors declare no competing interest

Funding
The study is self-funded. Availability of data and materials In this study, all the data and materials are included. If more information is needed, please contact the author for data requests.

Authors' contributions
HF was involved in the study's design. AS and HF were responsible for the collection and interpretation of the samples. All individuals who participated in the data refining process were responsible for the manuscript preparation. The final manuscript was reviewed and approved by the authors.

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تسجيل جديد لثلاث سلالات بكتيرية معزولة من عينات مياه في قضاء القرنة/محافظة البصرة

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المستخلص

هدفت الدراسة الحالية إلى عزل سلالات بكتيرية وتشخيصها، والتي تكون مسببة للأمراض وتساهم في زيادة تلوث المياه. جُمعت العينات شهرياً في الفترة من مارس 2026 إلى مايو 2026 من ثلاثة مواقع قريبة من مستشفى قضاء القرنة. وقد بدأت عمليات أخذ العينات عادةً بين الساعة التاسعة صباحا والساعة الثانية عشرة ظهراً. أُخذت عينات المياه من الطبقة السطحية على عمق يتراوح بين 30 و40 سم باستخدام قناني زجاجية كهرمانية معقمة بالأوتوكليف، ومغسولة مسبقاً مرتين بعينة المياه نفسها قبل ملئها. أظهرت النتائج عزل ثلاثة انواع من العزلات البكتيرية، وهي: *Enterobacter bugandensis* و *Enterobacter asburiae* و*Enterobacter kobei*، بنسب مئوية بلغت 38.2% و31.7% و30.1% على التوالي. ولأغراض التشخيص الجزيئي، جرى تحليل جين 16S rRNA باستخدام تقنية تفاعل البلمرة المتسلسل (PCR)، حيث تم الكشف عن وجود الجين من خلال ظهور حزمة واحدة عند وزن جزيئي محدد قدره 1500 زوج قاعدي (bp).

الكلمات المفتاحية: تسجيل جديد، ثلاث عزلات، بكتيريا، 16S rRNA، قضاء القرنة.

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