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Prevalence and Antimicrobial Susceptibility of *Escherichia coli* and *Klebsiella pneumoniae* Isolated from the Tigris River, Baghdad, Iraq

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Abstract

The presence of antimicrobials and antimicrobial-resistant bacteria (ARB), in the aquatic environment is becoming a cause of great concern as the possibility of the development of antibiotic-resistant pathogens, even superbugs, is increasingly posing problems to the environment and human health. The main objectives of this study were to analyse the antibiogram profile, calculate the multiple antibiotic resistance indices, and assess the co-occurrence of resistance to several antibiotics in the isolates. Samples were collected from four sites: S1 Al-Muthana Bridge, S2 Al-Sarrafia Bridge, S3 Al-Senak Bridge, and S4 Al-Jadriyah Bridge from November 2023 until July 2024. This investigation included 32 *Escherichia coli* and 18 *Klebsiella pneumoniae* bacterial isolates, confirmed both phenotypically and genotypically. The isolation of *E. coli* and *K. pneumoniae* is determined by membrane filtration techniques and biochemical analysis followed by the VITEK2. Polymerase chain reaction (PCR) was used to establish the identity of the propagated isolates by identifying the β-galactosidase gene with *E. coli*-specific *lacZ3* primers, and the malate dehydrogenase *mdh* housekeeping gene. An antimicrobial susceptibility test was performed according to the Kirby-Bauer disk diffusion method against 15 selective antibiotics. The results showed *E. coli* exhibited complete susceptibility to Nitrofurantoin, Ampicillin, and Cefuroxime with average susceptibility (20%). While the susceptibility of *K. pneumoniae* was higher for: Nitrofurantoin, Ampicillin, Cefuroxime, Cefoxitin, Ceftazidime, Gentamycin, Amikacin and Imipenem with average susceptibility (53.3%) out of 15 antibiotics. In contrast, the results of resistance showed that most of the *E. coli* and *K. pneumoniae* isolates had a higher percentage of the resistance. Multiple antibiotic resistance (MAR) index values ranged from 33% to 93%, which indicates a high dose of the antibiotic used.

Keywords: Antibiotic resistance, Aquatic, *E. coli, K. Pneumoniae*, MAR, PCR

انتشار وحساسية مضادات الميكروبات لبكتريا الأشريكية القولونية والكليبسيلا الرئوية المعزولة من نهر دجلة، بغداد، العراق

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الخلاصة

إن وجود مضادات الميكروبات والبكتيريا المقاومة لمضادات الميكروبات (ARB) في البيئة المائية أصبح سبباً للقلق الشديد لأن إمكانية تطوير مسببات الأمراض المقاومة للمضادات الحيوية، وحتى الجراثيم المقاومة للمضادات الحيوية، تشكل بشكل متزايد مشاكل للبيئة والصحة البشرية. ان الأهداف الرئيسية لهذه الدراسة هي تحليل ملف المضادات الحيوية وحساب مؤشرات مقاومة المضادات الحيوية المتعددة وتقييم حدوث مقاومة العديد من المضادات الحيوية في العزلات. تم جمع العينات من أربعة مواقع: S1 جسر المثنى، S2 جسر الصرافية، S3 جسر السنك وجسر الجادرية S4 من شهر تشرين الثاني 2023 حتى شهر تموز 2024. شملت الدراسة 32 عزلة بكتيرية من الإشريكية القولونية و 18 عزلة من الكليبسيلا الرئوية، وتم تأكيدها ظاهريًا وجينيًا. تم تحديد العزلات البكتيرية للأشريكية القولونية والكليبسيلا الرئوية من خلال تقنيات الترشيح الغشائي والتحليل الكيميائي الحيوي متبوعًا بـ VITEK2. تم استخدام تقنية تفاعل البلمرة المتسلسل لتحديد هوية العزلات المنتشرة من خلال الكشف عن جين بيتا غالاكتوزيداز بأستخدام بادئات *lacZ3* الخاصة الخاصة بالأشريكية القولونية،وبادىء *mdh* الخاص ببكتريا الكليبسيلا الرئوية . تم إجراء اختبار حساسية المضادات الحيوية للميكروبات وفقًا لطريقة انتشار القرص Kirby–Bauer ضد 15 مضادًا حيويًا انتقائيًا. بينت النتائج أن الأشريكية القولونية أظهرت حساسية كاملة للنيتروفورانتوين والأمبيسلين والسيفوروكسيم بحساسية متوسطة (20٪). بينما كانت حساسية الكليبسيلا الرئوية أعلى في: نيتروفورانتوين، أمبيسلين، سيفوروكسيم، سيفوكسيتيم، سيفتازيدين، جنتاميسين، أميكاسين وإيميبينيم بمتوسط حساسية (53.3٪) من أصل 15 مضاداً حيوياً. في المقابل، أظهرت نتائج المقاومة أن معظم عزلات الأشريكية القولونية والكليبسيلا الرئوية كان لديها نسبة اعلى من المقاومة. أظهرت قيم مؤشر MAR أنها سجلت قيمًا عالية في المواقع الأربعة لنهر دجلة، تتراوح من 33٪ إلى 93٪، مما يدل على وجود جرعة عالية من المضاد الحيوي المستخدم.

1. Introduction

Antimicrobial resistance (AMR) is a serious and increasing public health concern [1]. It represents a grand societal challenge with important dimensions in the water environment that contribute to its evolution and spread [2]. The vast amounts of trash that surface water receives from both human and animal sources create the perfect environment for the growth, development, and spread of bacteria resistant to antibiotics [1]. Antibiotics are ecological agents that propel microbial evolution by altering the composition of bacterial communities, preventing or enhancing their ecological roles, and enhancing and preserving drug resistance. The exposure of aquatic ecosystems to antibiotic contamination is a significant consequence of the rising use of antibiotics [3]. As a result, appropriate usage of existing antibiotics is required. Furthermore, educational activities and annual testing of antimicrobial sensitivity are vital for minimizing antibiotic resistance rates [4]. Gram-negative bacteria (GNB) pose the greatest threat to (AMR). GNB are particularly significant in hospitals because they put patients at risk and generate a high morbidity and death rate. This implies that antibiotic resistance intensifies treatment problems, demanding actions [5,6]. There are few local and regional reports of antibiotic-resistant microorganisms and antibiotic-resistant genes, notably in metropolitan receiving and wastewater systems [7]. Despite being a serious worldwide health problem, antibiotic resistance is least addressed in developing countries [8].

Coliform bacteria are bacteria present in the environment in the faeces of warm-blooded animals, including humans [9]. They include three different groups of bacteria (total coliform, fecal coliform, and *Escherichia coli*), and they function as risk-level-based indicators of the quality of drinking and recreational water. People's intestines contain huge levels of *Escherichia coli*, a subgroup of fecal coliforms that are mostly innocuous [10]. And it can

enter the environment through fecal material excretion. Thanks to the availability of quick, simple, sensitive, and targeted analysis techniques, it is simple to detect in water [11]. *E. coli* isolated from water sources were discovered to be pathogenic and antibiotic-resistant [12]. This is linked to various reasons, such as mutations, improper use or overuse of antibiotics, and *E. coli* dynamic capacity to exchange genetically resistant genes with one another [13,14]. Regarding of *Klebsiella pneumoniae* is a Gram-negative bacterium capable of colonizing, invading, and causing infections in different anatomical sites of the human body [15]. *K. pneumoniae* is a member of Enterobacteriaceae, which causes many infectious diseases, including blood, lung, wound, burn, and urinary tract infections. It is found in sewage and river water as a coliform bacterium [16]. The rise of antibiotic-resistant *K. pneumoniae*, primarily due to the creation of antibiotic-inactivating enzymes, is currently responsible for most treatment failures, jeopardising the efficiency of antibiotic classes that have been used for decades [17].

To fill this research gap, the primary goals of this study were to look at the prevalence of antibiotic resistance in isolates of *E. coli* and *K. pneumoniae* from diverse Tigris River sites. Specifically, by measuring the multiple antibiotic resistance (MAR) indices of the isolates, identify the co-occurrence of resistance to several antibiotics, and determine the isolates' antibiogram profiles. This information can help in understanding the occurrence of antibiotic resistance in aquatic environments. It can also be used to draft for the improvement of the water environment.

2. Materials and Methods

2.1. Study area

The study area included four sites on the Tigris River within Baghdad city chosen to apply the current study approach **:** The first site was Al-Muthana Bridge at the coordinate (33.429255°N 44.346125°E), the second site, Al-Sarrafia Bridge at the coordinate (33.353972° N,44 .372625°E), the third site, Al-Senak Bridge at the coordinate (33.328607° N, 44.399351°E), and the fourth site, Al-Jadriyah Bridge at the coordinate (33.282625° N, 44.375470°E), as shown in (Figure 1) and locations coordinate points have determined.

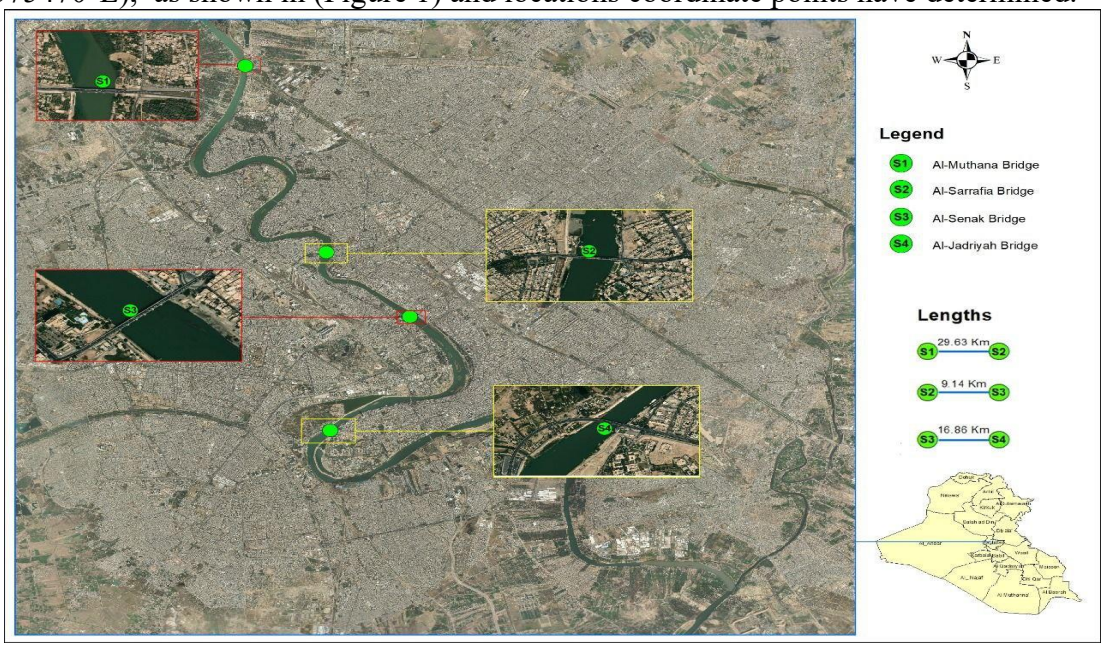

Figure 1: Satellite image of selected sampling sites along the Tigris River, Baghdad (S1-S4) representing the study sites codes.

2.2 Water Samples Collection

Water samples were collected seasonally from November 2023 until July 2024 from all sampling sites on the same day, and the sample time on average was between 07: 00 AM and 12:00 PM [18]. Each water sample was taken from 20-30 cm below the water surface in three replications using sterile and well-labelled polyethylene bottles (1 litters). The volume of each water sample was 500 ml. Sampling in each site was carried out from different points at the river and then mixed to get the best representation of the site's environmental reality. The samples were preserved in an icebox for transportation. They were promptly conveyed to the microbiological laboratory. Preliminary microbiological testing was conducted early to prevent unforeseen fluctuations (ideally within 1-2 hours of arrival).

2.3 Detection and isolation of E. coli and K. Pneumoniae by membrane filtration techniques

The membrane filtering approach and the numerous tube fermentation method are two distinct techniques [19], the first approach was applied in this work. To filter all materials, two volumes of approximately 100 mL were passed through a 0.45 µm pore-sized nylon membrane filter (Sarrtorius, Gottingen, Germany), using a sterile filtration unit (Sartorius Filtration Products, Gottingen, Germany) and vacuum pump (Sartorius). Using sterile forceps, the membranes were aseptically extracted, and transferred onto HiCrome^{TM} *E. coli* agar and HiCrom^{TM} *Klebsiella* selective agar base for *E. coli* and *Klebsiella* (Hi-media, India). These media are highly selective for recovery of *Klebsiella sp.* and *E. coli,* but not inhibitory for other genera of Enterobacteriaceae. The plates were then incubated at 37 °C for 24 h. The presumptive colonies of *Klebsiella sp.,* with violet colour morphology, and *E. coli* is distinguished by green colour on HiCrome agar.

2.4 Identification of E. coli and K. pneumoniae

Presumptive bacterial colonies were selected and sub-cultured to facilitate additional bacterial identification. *E. coli* and *K. pneumoniae* were identified by colony morphology and Gram staining. Under illumination microscopy, *K. pneumoniae* and *E. coli* micelles are frequently Gram negative and have a short, rod-shaped morphology. Traditional biochemical assays, include (methyl red, indole, Vogas-Proskauer, triple sugar iron, Simon Citrate Agar, motility, oxidase, urea hydrolysis, catalase, and sulfur production) [20,21]. The identities of the isolates were further confirmed using the VITEK 2 compact system (BioMérieux, Marcy, l'E'toile, France), a standardized colorimetric identification system is a method that combines conventional tests with carbohydrate utilisation assays [22,23].

2.5 Confirmation of E. coli and K. pneumoniae by Polymerase chain reaction

To validate the identification of the propagated isolates, PCR was conducted for the detection of the β-galactosidase gene using *lacZ3* primers specific to *E. coli* [24,25], and the malate dehydrogenase (mdh) housekeeping gene to *K. pneumoniae* [26]. The template DNA for PCR was prepared using FavoPrep™ genomic DNA extraction Mini kit, according to the manufacturer's instruction (FAVOR GEN biotech corp, Taiwan). PCR amplification conditions and reaction components of the target genes shown in (Table 1) were performed using Mj Mini Gradient Thermal Cycler (Bio-Rad, USA). The amplified PCR products were analysed utilizing electrophoresis in a 1.5% agarose gel for 30 minutes at 100V. (Cleaver Scientific, UK), and finally visualized with Gel documentation (Bio-Rad, USA) [27].

Table 1: Targeted genes, Primers, PCR conditions, Compositions, and product size utilized in this study.

Primer's name	Sequence 5'-3'	PCR conditions	Composition of PCR mixtures (25 µl)	Amplicon size
LacZ3	**F:** 5' TTGAAAATGGTCTGCTGCTG 3' **R:** 5' TATTGGCTTCATCCACCACA 3'	3 minutes at 95°C, 35 cycles of 95 °C for 30s, 58 °C for 30s and 72°C for 1minute.	12.5 µl master mix, 2 µl of DNA sample, 1 µl forward ,1µ reverse primer, 8.5 µl water	243 bp
Mdh	**F:** 5'GCGTGGCGGTAGATCTAAGTCATA 3' **R:**5' TTCAGCTCCGCCACAAAGGTA 3'	5 minutes at 95°C, 30 cycles of 95 °C for 60s, 53 °C for 60s. and 72 ° C for 5minute.	12.5 µl master mix, 2 µl of DNA sample, 1 µl forward ,1µ reverse primer, 8.5 µl water	364 bp

2.6 Phenotypic antibiotic susceptibility testing

Antibiotic susceptibility testing (AST) was performed by the disc diffusion method on Muller Hinton (MH) agar using the Kirby Bauer disc diffusion method and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guideline of 2023 [28,29]. Bacterial suspensions were prepared and adjusted to an opacity corresponding to 0.5 red using a McFarland standard densitometer, and the inoculum was placed onto well-dried Mueller Hinton agar plates [8]. Sterile nontoxic cotton swabs dipped into the standardized inoculum were used to streak the entire surface of Mueller–Hinton agar plates. Gram-negative bacteria were tested against 15 antibiotics as follows: Ampicillin(10µg),Tetracycline(30 µg), Cefuroxime (30 µg), Chloramphenicol (30 µg), Ceftriaxone (25µg), Azithromycin (15 µg) , Ciprofloxacin (5 µg),Amikacin(30µg),Ceftazidime(30µg),Cefoxitin(30µg),Gentamicin(10µg),Imipenem(10µg) , Nitrofurantoin(100 µg) , Ofloxacin (5 µg), Trimethoprim/Sulfamethoxazole (25 µg) [30,31] , and these antibiotic concentrations selected based on the Clinical & Laboratory Standards Institute (CLSI) guidelines 2023. After that, the plates were incubated for 24 hours (overnight) at 37 °C. Following the inhibition zone diameter was measured using a ruler, and the results were categorised as sensitive, intermediate sensitive, or resistant based on the 2023 CLSI guidelines [28,29].

2.7 Multiple Antibiotic Resistant Index Value (MAR)

MAR index for each bacterial isolate against the antibiotics tested was computed using the formula (It was mathematically expressed) illustrated below. The multiple antibiotic resistance index is computed by dividing the number of resistant antibiotics to which an isolate is resistant by the total number of antibiotics tested against the organism. The MAR index is a technique for analysing health risks; values equal to or less than 0.20 indicate a low dose of antibiotics used, while values more than 0.20 indicate a high dose of antibiotics used [32,33].

Equation (1) illustrates how the Multiple Antibiotic Resistant (MAR) index of bacteria is calculated. Where (a) is the number of antibiotics that the isolate is resistant to, and (b) is the total number of antibiotics tested against the isolate to derive the value of the MAR index [32,33].

$$\text{MAR Index value} = a/bX100 \quad (1)$$

2.8 Statistical Analysis

The data were statistically analysed using the Joint Applications Statistical Package (JASP) and R statistical programming. One-way analysis of variance (ANOVA) was used to determine statistically significant differences at the significance level ($p < 0.05$) for the sensitivity of the studied bacteria in the sampling sites used in the study. Cluster analysis was used to analyse the sensitivity of bacteria in the study sites as well as to antibiotics by identifying groups close in their effect on bacteria, represented by dendrogram figures [34].

3. Results

3.1 Detection and isolation of E.coli and K.pneumoniae from a River

A total of 50 bacterial isolates were identified throughout the research period. The most commonly occurring bacteria isolated from the water samples was *E. coli* 32/50 (64%), followed by *K. pneuomoniae*, which is the second most commonly observed bacteria 18/50 (36%). Biochemical tests showed identical positive and negative results for *E.coli* and *K.pneumniae* as described by Brenner and Farmer ; Cappuccino and Welsh [20,21], as shown in figure 2. The Vitek 2 compact system outcomes appeared with a similartiy of 95% for *E.coli*.

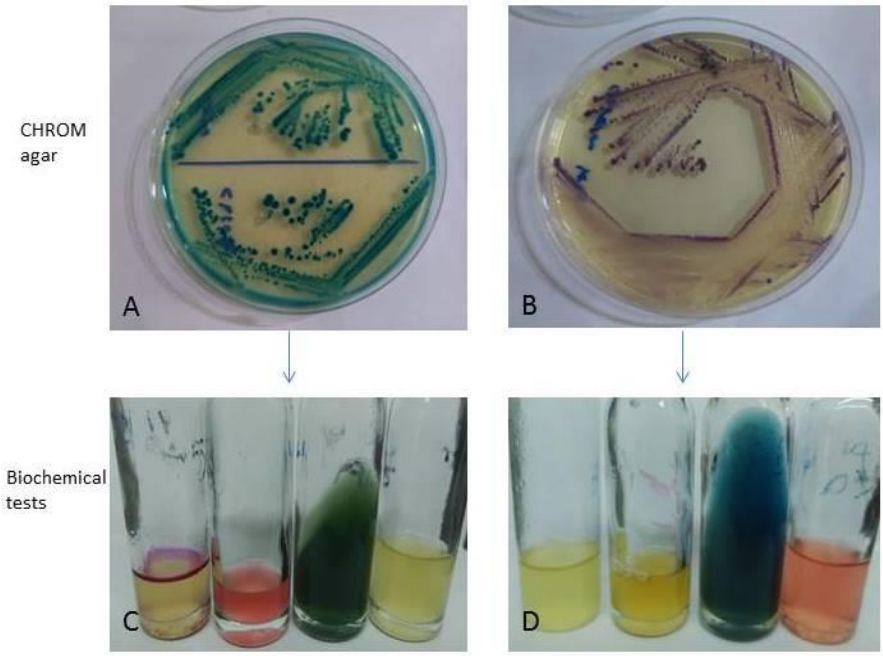

Figure 2: (**A**) Green colonies of *Escherichia coli* on HiCrom *E.coli* agar. (**B**) Violet colonies of *Klebsiella pneumoniae* on HiCrom *Klebsiella* selective agar. (**C**) *E.coli* biochemical tests: Indol (+), Methyl red (+),Citrate (-) , Urea (-). (**D**) *K. pneumoniae* biochemical tests: Indol (-), Methyl red (-),Citrate (+) , Urea (+).

For further identification, the molecular confirmation of the *lacZ3* gene and *mdh* in the identified *E.coli* and *K.pneumoniae* isolates respectively, has been detected by PCR. The *lacZ3* gene present in *E.coli* that encodes the beta-galactosidase protein responsible for the breakdown of lactose [24,25],-and targeting of the *mdh* gene[26] for *K.pneumoniae* (Figure 3).

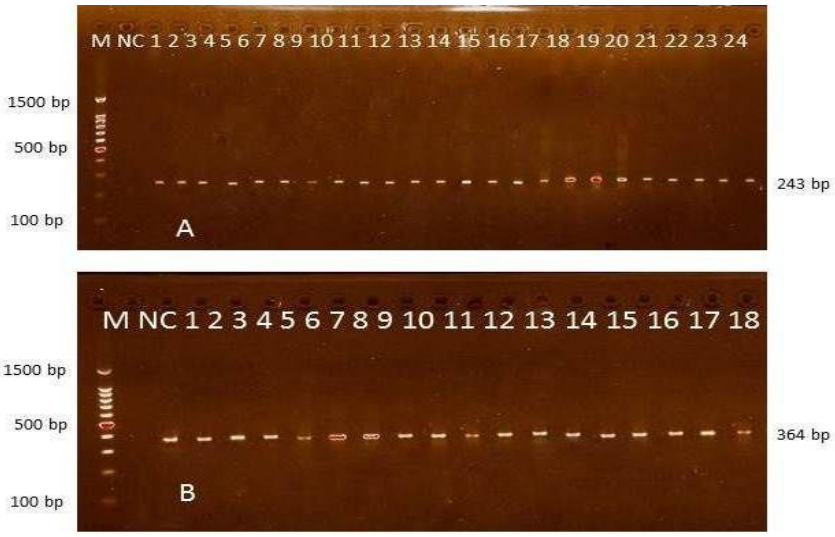

Figure 3: Products of PCR. (A) Lane M; DNA Ladder, Lane NC; Negative control, Lanes 1-24; positive *E.coli*. (B) Lane M; DNA Ladder, Lane NC; Negative control, Lanes 1-18; positive *K.pneumonae*.

3.2 Profiles antibiogram

The antibiogram profiles (resistant, intermediate, and susceptible) of the 50 bacterial isolates (32 *E.coli* and 18 *K.pneumoniae*) against 15 antibiotics are presented in (Table2). The susceptibility test was interpreted according to the criteria of the CLSI. Antibiotics resistance was observed in both *E.coli* and *K.pneumoniae*. *E.coli* exhibited complete susceptibility to Nitrofurantoin, Ampicillin, and Cefuroxime, with an average susceptibility of 20% . The susceptibility of *K.pneumoniae* was higher as fellow: Nitrofurantoin, Ampicillin, Cefuroxime, Cefoxitin, Ceftazidime, gentamycin, Amikacin, and Imipenem with an average susceptibility of 53.3% (Table 2), as noted that these antibiotics mentioned above are dividing between Beta-lactam and aminoglycosides groups.

Table 2: Antibiotic resistance pattern of *E. coli* (n=32) *and K. pneumoniae* (n = 18) isolates displaying phenotype profile (resistant, intermediate, and susceptible) against (15) types of antibiotics

Name of antibiotics	***E.coli***			***K.pneumoniae***		
	Risistant n (%)	**Intermediate n (%)**	**Susceptible n (%)**	**Risistant n (%)**	**Intermediate n (%)**	**Susceptible n (%)**
Nitrofurantoin (NIT)	32 (100)	0(00)	0(00)	18 (100)	0	0
Ampicillin (AMP)	32 (100)	0(00)	0(00)	18 (100)	0	0
Cefuroxime (CXM)	30 (93.8)	2 (6.3)	0(00)	18 (100)	0	0
Cefoxitin (CX)	30 (93.8)	1 (3.12)	1 (3.12)	18 (100)	0	0
Ceftazidime (CAZ)	30 (93.8)	1 (3.12)	1 (3.12)	18 (100)	0	0
Gentamycin (GEN)	26 (81.3)	4 (12.5)	2 (6.3)	14 (77.8)	4 (22.2)	0
Amikacin (AK)	27(84.4)	3 (9.4)	2 (6.3)	15(83.3)	3 (16.7)	0
Imipenem (IPM)	23 (71.9)	6(18.8)	3 (9.4)	12 (66.7)	6 (33.3)	0
Ceftriaxone (CTR)	19 (59.4)	11 (34.4)	2 (6.3)	11(61.11)	5 (27.8)	2 (11.11)
Ciprofloxacin (CIP)	14 (43.8)	**12 (**37.5)	6 (18.8)	13 (72.2)	3(16.7)	2 (11.11)
Azithromycin (AZM)	10 (31.3)	0(00)	22 (68.8)	9 (50)	0	9 (50)
Tetracycline (TE)	10 (31.3)	9 (28.1)	13 (40.6)	5 (27.8)	9 (50)	4 (22.2)
Ofloxacin (OF)	7 (21.9)	5(15.6)	20 (62.5)	7 (38.9)	5 (27.8)	6 (33.3)
Trimethoprim/Sulfamethoxazole (COT)	5 (15.6)	5 (15.6)	22 (68.8)	2 (11.11)	5 (27.8)	11(61.11)
Chloramphenicol (C)	0	11 (34.4)	21 (65.6)	0	11(61.11	7 (38.9)

In contrast, the results of resistance revealed that most of the *E.coli* and *K.pneumoniae* isolates were resistant; the examined isolates of *E.coli* (32) and *K.pneumoniae* (18) showed resistance to one or more antimicrobial agents. Although, the percentage of resistant isolates was higher, more than 50% of them (61.487%) and (65.928%) *E.coli* and *K.pneumoniae* , respectively as shown in Figure 4.

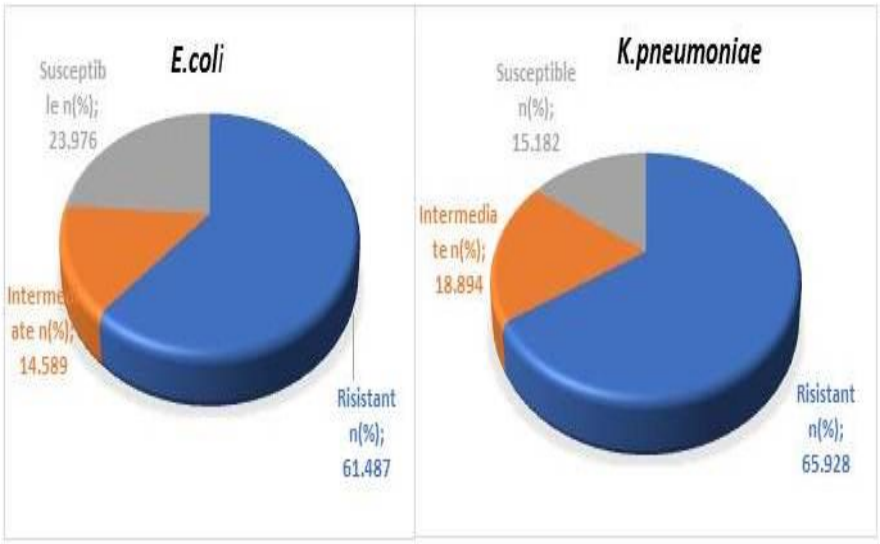

Figure 4: Frequency of resstance of *E.coli* and *K.pneumoniae* isolated from the Tigris River to 15 antibiotics.

3.3 MAR index value

The value of the multiple antibiotic resistance index to *E.coli* and *K.pneumoniae* for all sites in the current study recorded a high dose of the antibiotic used, which is illustrated in Figure 5.

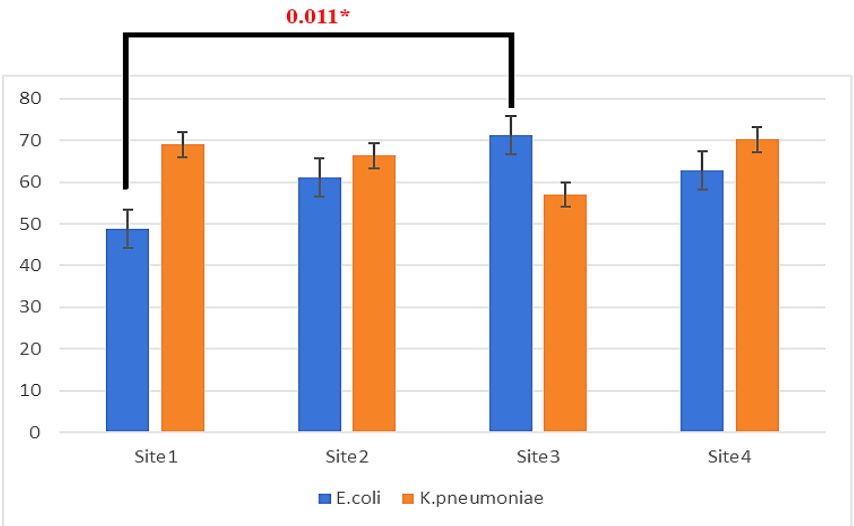

Figure 5: MAR Index for *E. coli* and *K. pneumoniae* isolated from four sites along the Tigris River.

3.4 Various resistance patterns were detected for E.coli and K.pneumoniae as the following :
3.4.1 Escherichia coli

Various resistance patterns were detected for *E.coli* (32 isolates), and the results showed 14 resistance patterns for 15 antibiotics (Figure 5). The results showed that the highest resistance pattern detected of all isolates (32) was (100%) for the antibiotics Nitrofurantoin (NIT) and Ampicillin (AMP). As well as, it was noted that isolates number (E14IS) and (E29IS) were similar in their resistance pattern to each of the following antibiotics: CXM, AK, GEN, CX, CAZ. In addition, the two isolates (E18IS) and (E44IS) were identical in their resistance pattern to the same antibiotics above within cluster A . While the isolates (E30IS) and (E40IS) in cluster B had the same resistance pattern to the CXM, AK, GEN, CX, CAZ , as shown in Figure 6.

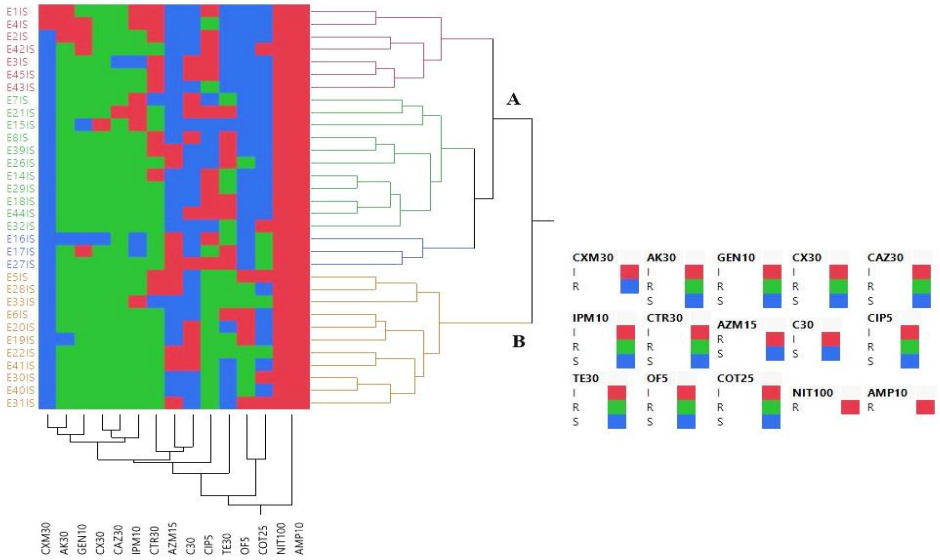

Figure. 6: Dendrogram two-way of *E.coli* isolated and 15 antibiotics is shown by a Heat map: CXM Cefuroxime**,** NIT Nitrofurantoin**,** AMP Ampicillin**,** CX Cefoxitin**,** CAZ Ceftazidime, GEN gentamycin , AK Amikacin**,** IPM Imipenem**,** CTR Ceftriaxone**,** CIP Ciprofloxacin**,** AZM Azithromycin**,** TE Tetracycline**,** OF Ofloxacin**,** , C Chloramphenicol , COT Trimethoprim/Sulfamethoxazole

Regarding *K.pneumoniae*, the results revealed various resistance patterns among the 18 isolates .They showed 14 resistance patterns against 15 antibiotics (Figure 7). The results illustrated that the highest resistance pattern detected among all isolates (18) was 100% for the antibiotics NIT, AMP, CXM, CX, and CAZ. Additionally, It was noted that isolates (K9IS) , (K12IS), and (K35IS) in cluster (A) exhibited similar resistance patterns to most of the antibiotics utilized in this study . It was noted that the isolates (K34IS) and (K36IS) in cluster (A) were identical in their resistance pattern to most antibiotics above within cluster A . While the isolates (K23IS) , (K47IS), and (K50IS) in cluster B had the same resistance pattern to the most antibiotics, as shown in Figure 7.

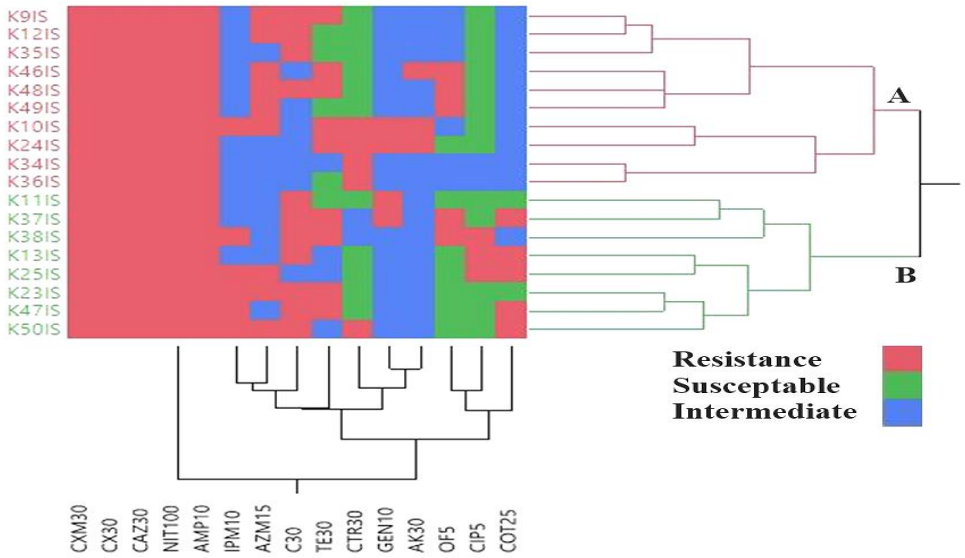

Figure 7: Dendrogram two way of *K.pneumoniae* isolated and 15 antibiotics is shown by a Heat map: CXM Cefuroxime**,** NIT Nitrofurantoin**,** AMP Ampicillin**,** CX Cefoxitin**,** CAZ Ceftazidime, GEN gentamycin , AK Amikacin**,** IPM Imipenem**,** CTR Ceftriaxone**,** CIP Ciprofloxacin**,** AZM Azithromycin**,** TE Tetracycline**,** OF Ofloxacin**, ,** C Chloramphenicol , COT Trimethoprim/Sulfamethoxazole

4. Discussion

The majority of the *E.coli* and *K.pneumoniae* isolates investigated in this study had and MDR resistance pattern to the antibiotics used. The fact that each study evaluates a distinct set of antibiotics. It is not possible to compare the resistance patterns of waterborne *E.coli* and *K. pneumoniae*.

K.pneumoniae ssp., *pneumoniae* being the most predominant species in the water sample for *Klebsiella* species (Fig.2). It is unclear negative detection of other *Klebsiella* species in this work is attributed to the low density of the other *Klebsiella* species or the inability of HiCrom selective agar to isolate other species, However, Merlino and co-workers [35] , indicated the possibility of isolating other *Klebsiella* species using CHROM agar. The prevalence of Gram-negative bacteria in the aquatic environment has been already reported, especially in the coilform group [36]. Environmental contamination with antibiotic-resistant bacteria is directly linked to population growth, anthropogenic impacts on rivers, primarily due to sewage and chemical compound discharge, as well as antibiotics and hormones, which are widely used in human and veterinary clinical practice and have since become common in Iraq. Increased levels of antibiotic resistance in microorganisms isolated from aquatic habitats, indicate abuse or misuse of antimicrobial treatments [37,38].

The results of the current study showed the high resistance antibiotic of *E.coli* against Ceftazidime (93.8%), Amikacin (84.4%) and Gentamycin (81.3%), and low resistance against Trimethoprime (15.6%) and Ciprofloxacin (43.8%), and these results agree with the findings reported by Mahdi *et al*., [39] , which recorded high resistance for Ceftazidime 70% and low resistance for Trimethoprime (27.5%) and Ciprofloxacin (17.5%) while recorded low resistance against aminoglycosides as Amikacin and Gentamycin (5%) and (7.5%), respectively. Also, our results agreed with Younus [40] in Gentamycin only and disagreed with other antibiotics.

The rates of Trimethoprime- Sulfamethoxazole and Ciprofloxacin resistance for *E.coli* were (15.6%) and (43.8%), respectively; these results are in line with other findings reported by [41,42]. The high rate of Gentamycin resistant *E.coli* and *K.pneumoniae,* as shown in (Table 2) was in disaccordance with previous research [41] . It was reported that (16%) of fecal coliform isolated from river water was multiresistant. In a study published by Mckeon *et al*., 87% of coliform bacteria isolated from ground water in the USA were multiresistant; of these (14%) were *E.coli* [43]. In Egypt 100% of *E.coli* isolated from water exhibited multiple resistance [44] . In another study conducted in Iraq, *E.coli* was shown to be the mjority MDR at (78.5%) [45].

The current results revealed the values of the MAR index, which showed that they recorded high values in the four sites of the Tigris River, ranging from 33% to 93%, which indicates a high dose of the antibiotic used. No MAR value was recorded in the current study equal to or less than 20%. The statistical description of the MAR rate for the two types of bacteria within the sites showed significant differences between *E. coli* bacteria in the first site (Al-Muthana Bridge) and the third site (Al-Senak Bridge) , where the probability value was($P= 0.011$). As for the rest of the sites, there were no significant differences between them for the bacteria under study. Al Muthana Bridge, is situated near the first point of the Tigris River entering Baghdad city. This location characterises the northern section of the Tigris River, a natural region shaped mainly by fishing, agriculture, and poultry, with minimal industrial presence (Figure 4).

A high-risk source of antibiotic contamination is indicated by a MAR index value greater than 0.2 [46]. The first site is situated near the start of the Tigris River entrance to Baghdad city, which explains the notable variations in *E. coli* bacteria between the first and third sites. This location symbolises the northern portion of the Tigris River, which is a natural region devoid of industrial activity and primarily impacted by farming, fishing, and chicken operations. Regarding the third location, is situated in the heart of Baghdad city and is roughly 9.14 kilometres away from the second site, which is full of human activities such as restaurants, fish traps, residential buildings, sewage, etc., in addition to the presence of industrial activities. It is located a short distance from the Medical City Hospital in Baghdad, where many medical pollutants, sewage pollutants and medical waste are released into the river. Also the differences in water flow, temperature, and sedimentation between the two sites can influence *E. coli* populations. Faster water flow can dilute bacterial concentrations, while stagnant or slow-moving water can lead to higher concentrations. As previously indicated, cross-resistance and cross-sensitivity in *E.coli* from environmental isolates originate from concurrent exposure to various medicines, resulting in coselection[47] . Compared to other methods of bacteria source tracking , the MAR indexing method is less cost-effective, rapid and easy to apply, and it does not require specialized training[48] . These results are consistent with Salikan *et al.,* [32], who showed that the multiple antibiotic resistant (MAR) index of all of the isolates from Ibai River and Terengganu River was more than 20%, and agreed with Odonkor *et al*.,[30], who recorded high MAR index values, which suggesting that there may be a risk connected to the water sources that were sampled, especially the surface waters under investigation. This risk could arise from the surface waters being contaminated by unsanitary conditions.

5. Conclusion

According to the findings, most of the isolates are resistant to antibiotics widely used in medical practice, such as Ampicillin and Cefuroxime. And provide an overview of the prevalence and abundance of multidrug- resistant bacteria in many sites in the Tigris River. The results presented in this paper provide evidence that river water may be significant

crucially in the spread of antibiotic resistance in *K. pneumonia* and *E. coli* as fecal coliform bacteria. Once introduced to natural waterways, these bacteria may possess a distinct advantage over antibiotic-sensitive coliform bacteria. They can be accumulated by filter-feeding animals and freshwater fish, therefore heightening the potential risk to public health. Prioritizing the consistent use of spatial data on the development of antibiotic resistance is crucial for antimicrobial surveillance and establishing a practical framework for monitoring the transmission of antimicrobial resistance. In conclusion, based on these findings, the majority of the isolates exhibited resistance to widely used antibiotics, including Ampicillin and Cefuroxime. It gives a general overview of the frequency and quantity of bacteria resistant to many drugs in various Tigris River locations.

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7. Competing interest

The authors declare that they have no conflicts of interest

Ethics approval and consent to participate

Authors attest to the approval of ethical considerations. The study received ethical clearance from the local ethical council at the University of Baghdad, and the study protocol was approved by the Scientific Research Commission, Research & Technology Centre of Environment, Water & Renewable Energy / Ministry of Higher Education & Scientific Research Disclosure and conflict of interest.

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