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Antibiogram and Biofilm Production of *Escherichia coli* Isolated from Water Sewage Plants in Baghdad City

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Abstract:

The objective of this study is to investigate and determine the prevalence of antimicrobial-resistant *E. coli* in wastewater, before and after treatment, and the prevalence of *E. coli* strains capable of producing biofilms. Nineteen *E. coli* isolates were isolated from the inputs and outputs samples of AL-Rustamiya Sewage Treatment Plant on AL-Rusafa side (STP1) and the old AL-Karkh STP on AL-Karkh side (STP2). Antimicrobial susceptibility was assessed using VITEK-2, and isolates' ability to form biofilm was evaluated. According to the resistance level of STP1 and STP2, multiple drug resistance (MDR) was found 100% and 75% ,respectively, of output isolates. Extensive Drug Resistance (XDR) was found in STP1 (possible 2 isolates) more than in STP2 (one isolate). Biofilm formation analysis revealed two strong biofilm-producing isolates in the input samples of STP1, but the output samples showed its elimination and rising in the moderate type. The STP2 output samples revealed increasing in weak biofilm and decreasing in other types. Additionally, Shiga-like toxin–producing types of *E. coli* (*E.coli* O157:H7) were found frequently in STP2 than in STP1.

Key Words: *Escherichia coli,* Antibiogram, Biofilm, Sewage treatment Plants.

قياس حساسية المضادات الحيوية وانتاج الأغشية الحيوية لبكتريا *Escherichia coli* المعزولة من محطات مياه الصرف الصحي في مدينة بغداد

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الخلاصة:

هدف هذه الدراسة هو التحري وتحديد انتشار مقاومة مضادات الميكروبات للإشريكية القولونية في مياه الصرف الصحي قبل وبعد المعالجة وانتشار العزلات المنتجة للاغشية الحيوية لهذه البكتريا. تم عزل تسعة عشر عزلة من بكتيريا *E. coli* من عينات المدخلات والمخرجات لمحطة الرستمية من جهة الرصافة (STP1) ومحطة الكرخ القديمة من جهة الكرخ (STP 2). تم تقييم الحساسية للمضادات الميكروبية باستخدام VITEK-2، و تم تقييم قدرة العزلات على تكوين الأغشية الحيوية .وفقا لمستوى مقاومة STP1 وSTP2 ، تم العثور

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على مقاومة للأدوية المتعددة المتعددة (MDR) بنسبة 100% و 75٪ على التوالي من العزلات المخرجة. كما تم العثور على المقاومة الشاملة للأدوية (XDR) في STP1 (عزلتان محتملتان) أكثر من STP2 (عزلة واحدة). أظهر تحليل تكوين الأغشية الحيوية وجود عزلتين منتجتين للأغشية الحيوية من النوع القوي في العينات المدخلة لـSTP1 ، اما العينات المخرجة أظهرت القضاء عليهما وزيادة في النوع الحيوي المتوسط. كشفت عينات مخرجات STP2 عن زيادة في الأغشية الحيوية الضعيفة وانخفاض في الأنواع الأخرى. بالإضافة إلى ذلك، وجد نمط الإشريكية القولونية (*E.coli* O157:H7) المنتجة للسموم الشبيهة بالشيجا متكررة في STP2أكثر من STP1.

1. Introduction

As a vital component of life, water requires effective management to guarantee its optimal utilization, equitable distribution, and sustainable conservation, thereby protecting this precious resource for future generations [1]. In the aquatic environment, *E. coli* is considered a key indicator for studying pollution caused by antimicrobial-resistant bacteria derived from human and animal faeces. Bacterial communities, including *E. coli,* have been linked with antibiotic-resistance genes that can transfer to human and animal microbiota, significantly influencing the spread of resistance [2]. The spread of resistant bacteria in the community (even from healthy people) reflect the high risk of spreading infections [3]. A major finding from the Annual Report Antimicrobial Resistance Surveillance System of the Iraqi Ministry of Health, is the high prevalence of antibiotic resistance *E.coli* among other bacteria, showing significant resistance to important antibiotics. The report highlights that *E.coli* and other priority pathogens help focus attention and resources on addressing the most pressing AMR concerns in the country [4].

Escherichia coli in the environment, particularly those found in biofilms, can significantly contribute to the spread of antibiotic resistance. Biofilm is recognized as a hotspot for horizontal gene transfer (HGT) within and between bacterial species [5, 6]. Bacteria in biofilms have various advantages over planktonic bacteria, including higher protection against chemical, biological, and mechanical agents, allowing them to survive routine disinfection and cleaning treatments [7]. Sewage treatment plants are considered a hotspot for antibiotic-resistant bacteria, antibiotic resistance genes and mobile genetic elements [8, 9]. Unmetabolized and unused antibiotics are released into wastewater treatment facilities, creating selective pressure for the development and growth of Antibiotic-resistant bacteria. Additionally, the high microbial concentration, diverse microbial community and favorable growth conditions in wastewater treatment plants help in the vertical and horizontal transmission of resistance [10]. The objective of this study is to assess the antimicrobial resistance of the bacteria *E. coli* for selected antibiotics, and the ability of these isolates to form biofilms in two different treatment plants in Baghdad city, in order to evaluate the significance of the technologies used in these STP systems.

2. Materials and Methods:

2.1. Sample collection and description of sewage treatment plants

A cross-sectional design was employed, with twenty samples collected in March 2022 from the input and the output of two sewage treatment plants (STPs) in Baghdad; AL-Rustamiya sewage treatment plant on AL-Rusafa side (STP 1) and the old AL-Karkh plant on AL-Karkh side (STP 2). Five samples were collected from input and five from output for each sewage treatment plant. The samples were collected at least two weeks after rainfall to minimize any potential errors in this study, because there is a connection between the rainwater drainage systems and the sewage collection systems.

Sewage Treatment Plant 1 and STP2 have a capacity of 300,000 and 205,000 m^3 per day, respectively. They receive a mixture of effluent from domestic wastewater and hospitals. The process involves using an upflow anaerobic sludge blanket reactor (UASBR), activated sludge biological treatment, and chlorine disinfection as a final step [11]. Water samples were collected from the selected STPs at a specific time of the day (10 a.m.).

2.2. Isolation of bacteria

Sterilized cups were used for sample collection, which were then transported to the laboratory of the College of Health and Medical Techniques / Baghdad. For culturing and analysis, MacConkey agar (HiMedia) and BHI agar (HiMedia) were used. To further diagnosis, a selective medium CHROM agar (HiMedia) was used to diagnose *E.coli* serotype O157:H7, followed by confirmation the diagnosis, VITEK-2 compact system (Biomérieux, France).

2.3. Antimicrobial Susceptibility Test

The Gram negative (GN) card was used in VITEK2- Compact system for the identification of *E. coli* and for conducting antibiotic sensitivity testing (AST-GN76 and GN222). The tested 37 antibiotics were related to 11 classes. Multidrug resistant (MDR) isolates were defined as those that resisted at least three drug classes, eXtensive Drug Resistance (XDR) is defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e., bacterial isolates remain susceptible to only one or two categories) [4, 12].

2.4. Biofilm formation assay (Microtiter plate method)

The biofilm assay was performed according to the protocol described by Risal *et al*. (2018) with some modifications. Each overnight culture in Tryptone Soy Broth (TSB) was diluted and adjusted by McFarland tube 0.5, and three 180µL aliquots pipetted into wells of a 96-well microplate, and incubated at 37ºC for 24 h. *E. coli* ATCC 25922 was used as positive control, and a medium devoid of bacteria that was incubated under the same conditions was used as the negative control. After discarding the used culture media from each well, the wells were carefully washed twice using phosphate buffered saline (PBS) and left in an incubator set to 37°C for at least 30 minutes to dry without a lid. Each well was treated with 200µl of 0.3% Crystal violet solution, followed by a 5-minute incubation period in a well-ventilated area. Distilled water was used to cleanse the wells twice, and any extra water was blotted out. To remove the biofilm stain, 180µL of a 33% glacial ethanoic acid solution was added and shaken at 100 rpm for 30 seconds [13]. In a microplate, absorbance was measured at OD630.

The isolates were classified as non-biofilm producer (OD≤ODc); weak biofilm producers (ODc<OD≤2xODc); moderate biofilm producers (2xODc<OD≤4xODc) and strong biofilm producers (OD>4xODc) [14].

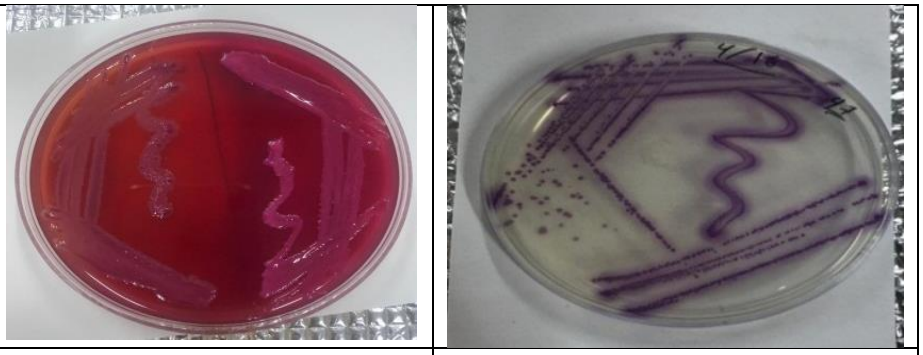
2.5. Statistical Analysis

The data generated were organized in Microsoft Excel 2021. Descriptive statistics including, significance (p- value) was conducted using SPSS- Fisher-Freeman-Halton Exact Test (because there was a count less than 5 for some samples). Categorical variables were expressed as count and percentages. The results of the analysis were presented using tables and graphs.

3. Results:

From 20 sewage samples collected, 19 *E.coli* isolates were identified using differential and selective media (one sample related to output STP2 was missed during isolation steps) and

confirmed by identification using VITEK2- Compact system. Figure 1 shows the detection of *E.coli* isolates on culture media.



A: *E.coli* on MacConkey agar incubated at 37C for 24h.

B: *E.coli* O157:H7 on CHROM agar incubated at 37C for 24h

Figure 1: Detection of *E.coli* isolates on culture media

3.1. Types of isolates:

The results indicated a variation in numbers and percentages of Shiga-like toxin–producing types of *E. coli* (*E.coli* O157:H7) and *E.coli* (non *O157:H7)* in the both sewage treatment plants (STP1 and STP2). Eight *E. coli* isolates in STP2 were *E.coli O157:H7* (88.8%), while STP1 had only two isolates of the *E.coli O157:H7,* representing 20%, as shown in table 1.

Table 1: The distribution of bacteria in two sewage treatment plants (STP1and STP 2)

Sewage plant	Samples	*E.coli O157:H7* No. (%)	*E. coli* (non *O157:H7)* No. (%)
STP1	Input (5)	2 (40%)	3 (60%)
	Output (5)	0 (0%)	5 (100%)
STP2	Input (5)	4 (80%)	1 (20%)
	Output (4)	4 (100%)	0 (0%)

3.2. Antimicrobial susceptibility

Cephalosporin class recorded the highest resistance level (the highest level for Cefsulodin) in the nineteenth isolates (89.47%) followed by Penicillins (63.15%).

Escherichia coli collected from AL-Rustamiya sewage treatment plant (STP1) exhibited a high resistance to the tested antibiotics. These isolates were found to be more resistant in output samples compared to input samples (table 2). Notably, all isolates, regardless of whether they were input or output isolates, exhibited 100% sensitivity to a range of antibiotics, including Doripenem (a carbapenem), amikacin and tobramycin (aminoglycosides), minocycline (a tetracycline), and Ciprofloxacin (a fluoroquinolone). Regarding the input isolates, most of them were highly susceptible to most antibiotics.

Table 2: Antimicrobial resistance against 37 antimicrobials for *E. coli* isolates in AL-Rustamiya sewage treatment plant on AL-Rusafa side.

	Sample no.	Resisted antimicrobials	No. (%) of resisted antimicrobials
Input	1	Ce (Cefsulodin)	1 (2.70%)
	2	Ce (Cefsulodin, Cefadroxil, Cefazolin, Cefradine,Cefotaxime) M (Aztreonam) Car (Loracarbef) P (Amoxicillin, Ampicillin, Carbenicillin, Pipracillin) Cin (Cinoxacin) F (Levofloxacin) Ph (Chloramphenicol)	14 (37.84%)
	3	β (Cefotaxime, Ceftazidine/Avibactam, Meropenem/Vabobactam) T (Doxycycline)	4 (10.81%)
	4	Ce (Cefsulodin)	1 (2.70%)
	5	T (Doxycycline, Tetracycline) Ce (Cefsulodin) Cin (Cinoxacin)	4 (10.81%)
Output	1	Ce (Cefsulodin, Cefadroxil, Cefazolin, Cefradine, Cefditoren, Cefixime, Cefotaxime, Cefepime) Cem (Cefotetan, Cefoxitin) P (Amoxicillin, Ampicillin, Carbenicillin, Pipracillin) β (Amoxicillin/ Clavulanic Acid, Ampicillin/ Sulbactam, Piperacillin/Sulbactam, Piperacillin/Tazobactam, Piperacillin/Sulbactam M (Aztreonam) Car (Loracarbef, Imipenem) T (Doxycycline, Tetracycline)	25 (67.57%)
	2	P (Amoxicillin, Ampicillin, Carbenicillin, Pipracillin) Ce (Cefsulodin, Cefadroxil, Cefazolin, Cefradine, Cefotaxime) Car (Loracarbef) M (Aztreonam) Cin (Cinoxacin) F (Levofloxacin) Ph (Chloramphenicol)	14 (37.84%)
	3	β (Amoxicillin/ Clavulanic Acid, Ampicillin/ Sulbactam, Piperacillin/Sulbactam, Piperacillin/Tazobactam,Ceftolozane/Tazobactam, Meropenem/Vabobactam) Ce (Cefsulodin, Cefadroxil, Cefazolin, Cefradine, Cefxime, Cefotaxime) Cem (Cefoxitin) M (Aztreonam) Car (Loracarbef, Imipenem) P (Amoxicillin, Ampicillin, Carbenicillin, Pipracillin) Cin (Cinoxacin)	21 (56.76%)
	4	P (Amoxicillin, Ampicillin, Carbenicillin, Pipracillin) β (Temocillin, Amoxicillin/ Clavulanic Acid, Ampicillin/ Sulbactam, Piperacillin/Sulbactam, Piperacillin/Tazobactam, Meropenem/Vabobactam) Ce (Cefsulodin, Cefadroxil, Cefazolin, Cefradine, Cefditoren, Cefxime, Cefotaxime, Cefepime) Car (Loracarbef, Imipenem) T (Doxycycline, Tetracycline) Cem (Cefotetan, Cefoxitin)	24 (64.86%)
	5	P (Amoxicillin, Ampicillin, Carbenicillin, Pipracillin) β (Temocillin, Amoxicillin/ Clavulanic Acid, Ampicillin/ Sulbactam, Piperacillin/Sulbactam,Piperacillin/Tazobactam, Meropenem/Vabobactam) Ce (Cefsulodin, Cefadroxil, Cefazolin, Cefradine, Cefditoren, Cefxime, Cefotaxime, Cefepime) M (Aztreonam) Car (Loracarbef, Imipenem) T (Doxycycline, Tetracycline) Cem (Cefotetan, Cefoxitin)	25 (67.57%)

A: Aminoglycosides, β: - β lactam, Car: carbapenems, Ce: Cephalosporins, Cem: Cephamycin, Cin: Cinnoline, F: Fluoroquinolones, M: Monobactam, P: Penicillins, Ph: Phenicols, T: Tetracyclines

The antimicrobial susceptibility results for *E. coli* isolates from the old AL-Karkh sewage treatment plant on AL- Karkh side, showed less resistance to antibiotics compared to STP1 (Table 3). However, these isolates demonstrated the highest susceptibility (100% sensitivity) to Doripenem (Carbapenem), amikacin (aminoglycosides) and Ciprofloxacin (Fluroquinolones).

Table 3: Antimicrobial resistance against 37 antimicrobials for *E. coli* isolates in the old AL-Karkh sewage treatment plant on AL- Karkh side.

	Sample no.	resisted antimicrobials	No. (%) of resisted antimicrobials
Input	1	P (Amoxicillin, Ampicillin, Pipracillin) Ce (Cefazolin, Cefotaxime) A (Tobramycin) T (Doxycycline, Minocycline, Tetracycline)	9 (24.32%)
	2	P (Amoxicillin, Pipracillin) Ce (Cefazolin) Cem (Cefoxitin) T (Doxycycline, Tetracycline)	6 (16.22%)
	3	Ce (Cefsulodin)	1 (2.70%)
	4	P (Ampicillin)	1 (2.70%)
	5	Ce (Cefotaxime) β (Ceftazidime/Avibactam, Ceftolozane/Tazobactam,Meropenem/Vabobactam) M (Aztreonam)	5 (13.51%)
Output	1	β (Temocillin, Ampicillin/Sulbactam, Piperacillin/Sulbactam) P (Carbenicillin, Pipracillin) Ce (Cefazolin, Cefotaxime) Cem (Cefoxitin) F (Levofloxacin) T (Doxycycline, Minocycline, Tetracycline, Tigecycline)	13 (35.14%)
	2	P (Amoxicillin, Ampicillin, Pipracillin) Cem (Cefoxitin) Ce (Cefotaxime) F (Levofloxacin) T (Doxycycline, Minocycline, Tetracycline, Tigecycline) Ph (Chloramphenicol)	11 (29.73%)
	3	β (Temocillin, Amoxicillin/ Clavulanic Acid, Ampicillin/ Sulbactam, Piperacillin/Sulbactam, Piperacillin/Tazobactam, Ceftazidime/Avibactam, Meropenem/Vabobactam) P (Amoxicillin, Ampicillin, Carbenicillin, Pipracillin) Ce (Cefsulodin, Cefadroxil, Cefazolin, Cefradine, Cefditoren, Cefxime, Cefotaxime, Cefepime) Car (Loracarbef, Imipenem) M (Aztreonam) Ph (Chloramphenicol) Cin (Cinoxacin) Cem (Cefotetan, Cefoxitin) A (Tobramycin)	27 (72.97%)
	4	Ce (Cefsulodin)	1 (2.70%)

A: Aminoglycosides, β: - β lactam, Car: carbapenems, Ce: Cephalosporins, Cem: Cephamycin, Cin: Cinnoline, F: Fluoroquinolones, M: Monobactam, P: Penicillins, Ph: Phenicols, T: Tetracyclines

Three resistance patterns were determined for isolated *E. coli* in both STP1 and STP2 (Table 4).

Table 4: Antibiotic susceptibility patterns of *E. coli* isolates in STP1 and STP2

Pattern of resistance	STP1 No. (%) of isolates		STP2 No. (%) of isolates	
	Input	Output	Input	Output
Resistant to one antibiotic class (non-MDR)	2 (40%)	0 (0%)	2 (40%)	1 (25%)
Resistant to two antibiotics classes	1 (20%)	0 (0%)	0 (0%)	0 (0%)
Resistant to ≥3 antibiotics classes (MDR)	2 (40%)	5 (100%)	3 (60%)	3 (75%)
Total	5	5	5	4

MDR: Multiple Drug Resistance.

The third pattern revealed a concerning trend, where 68.4% (13/19) of the isolates exhibited MDR, defined as resistance to three or more classes of antibiotics, indicating a high-risk status. All output isolates of STP1 were MDR, one of them (figure 2) is coming close to being XDR, because it was susceptible to only 3 classes of antibiotics. However, there was a same possibility (XDR) to one of the input isolates. No isolate was susceptible to all antibiotics.

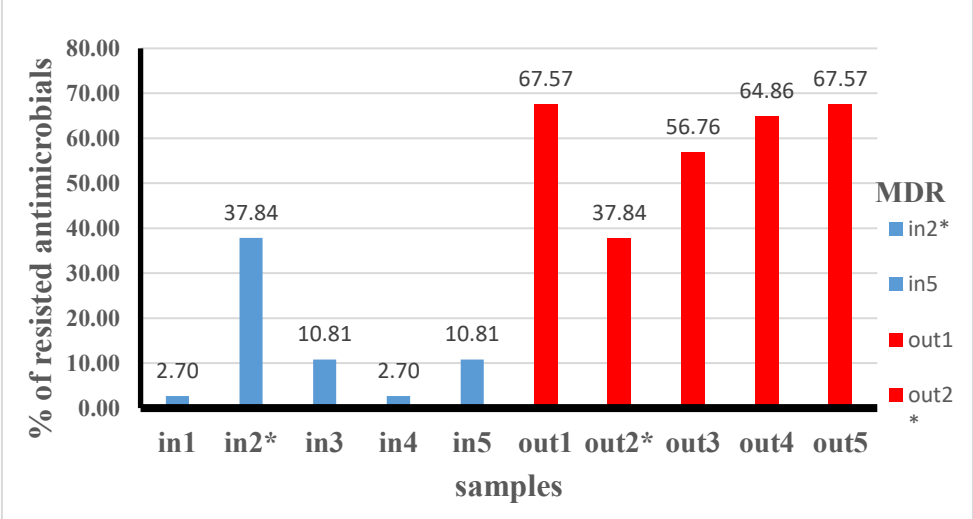


Figure 2: Antimicrobial resistance against 37 antimicrobials for *E. coli* isolates in AL-Rustamiya sewage treatment plant (STP1) on AL-Rusafa side. MDR: Multiple Drug Resistance, in: input, out: output, *: possible Extensive Drug Resistant (XDR).

The old AL-Karkh sewage treatment plant (STP2) has the same number of MDR isolates in input and output samples (Table 4). One of the outputs MDR isolates was XDR (Figure 3), it was susceptible to only two classes of antibiotics.

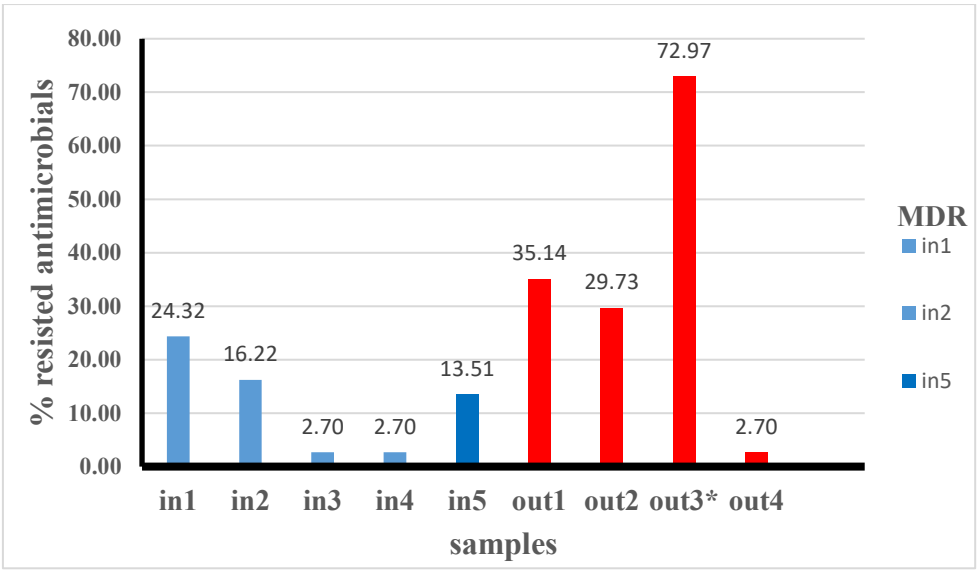
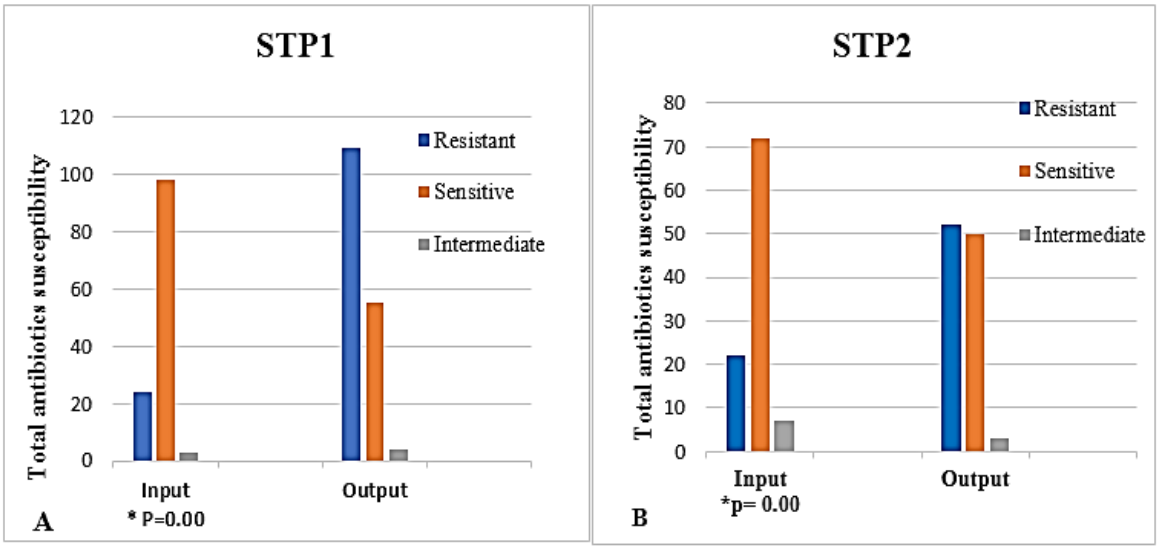


Figure 3 : Antimicrobial resistance against 37 antimicrobials for *E. coli* isolates in the old AL-Karkh sewage treatment plant (STP2) on AL- Karkh side. MDR: Multiple Drug Resistance, in: input, out: output, * Extensive Drug Resistant (XDR).

Statistically, there was a significant difference (p =0.00) between the resistance pattern of the input and output samples of tested antibiotics for both STP1 and STP2, but no significant association (P> 0.05) between the two sewage plants (STP1 and STP2) regarding the resistance (figure 4).



* P≤ 0.05: significant association.

Figure 4: Antimicrobial resistance associations for *E. coli* isolates between input and output samples for A: STP1 on Al-Rusafa side and B: STP2 on AL- Karkh side

3.3. Biofilm Analysis

Seventeen isolates (89.47%) showed biofilm formation in a microtiter plate method across both sewage treatment plants (STP1and STP 2) and revealed different formations in *E.coli* biofilm types including strong, moderate, weak, and no-biofilm respectively as shown in table 5. Strong biofilm was not found in STP2.

Table 5: Biofilm formation analysis for *E. coli* isolates in STP1 samples and in STP2 samples.

	NO.	Isolates	Biofilm OD630	interpretation
Control			0.068	negative
AL-Rustamiya sewage treatment plant on AL-Rusafa side STP1	1	Input *E.coli*	0.145	weak
	2	Input *E.coli*	0.288	moderate
	3	Input *E.coli*	0.509	strong
	4	Input *E.coli* O157:H7	0.392	strong
	5	Input *E.coli* O157:H7	0.324	moderate
	1	Output *E.coli*	0.222	moderate
	2	Output *E.coli*	-	No-biofilm
	3	Output *E.coli*	0.226	moderate
	4	Output *E.coli*	0.290	moderate
	5	Output *E.coli*	0.172	weak
the old AL Karkh plant on AL Karkh side STP2	1	Input *E.coli* O157:H7	0.088	No-biofilm
	2	Input *E.coli*	0.163	weak
	3	Input *E.coli* O157:H7	0.259	moderate
	4	Input *E.coli* O157:H7	0.109	weak
	5	Input *E.coli* O157:H7	0.238	moderate
	1	Output *E.coli* O157:H7	0.198	weak
	2	Output *E.coli* O157:H7	0.104	weak
	3	Output *E.coli* O157:H7	0.155	weak
	4	Output *E.coli* O157:H7	0.107	weak

When comparing the inputs and outputs of both STP 1 and STP2 (Figures 5,6), the results of STP1 indicated a decrease in strong biofilm but an increase in moderate biofilm. In contrast, the STP2 revealed increasing in weak biofilm and decreasing in other types. This suggests that the treatments at plants were useful in decreasing the biofilm types especially strong biofilm, and that will eliminate the danger of *E.coli* from treated plants.

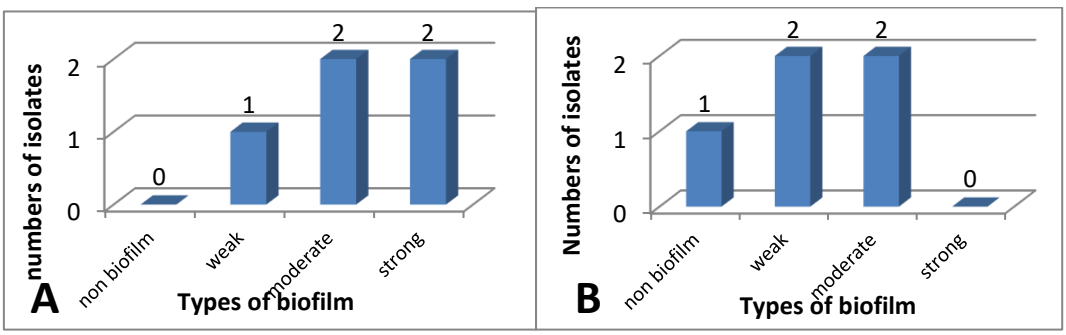


Figure 5: Numbers and types of biofilm forming isolates in (A) input STP1 and (B) input STP2 samples

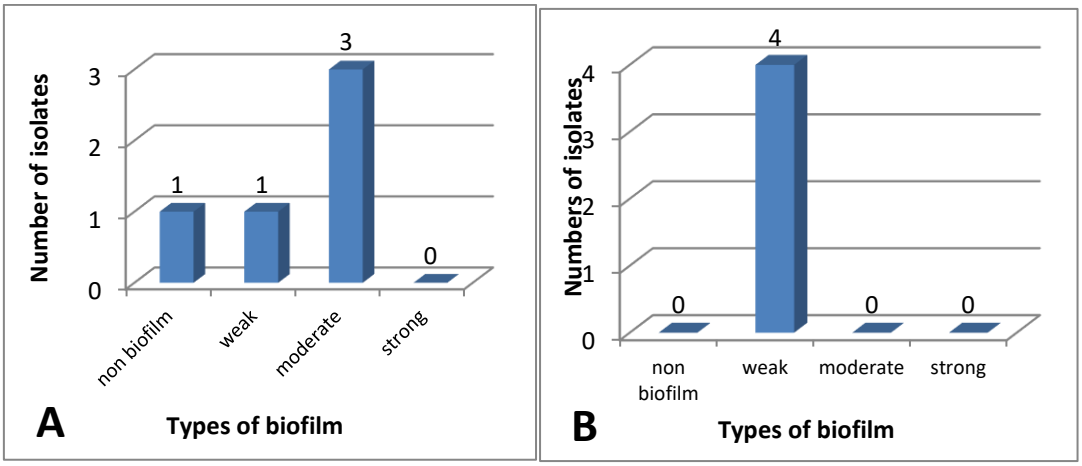


Figure 6: Numbers and types of biofilm forming isolates in (A) output STP1 and (B) output STP2 samples**.**

4. Discussion

Sewage treatment plants were established as central units to reduce the pollutant loads to acceptable limits prior to the release of the resulting effluent into receiving water bodies. Consequently, the presence of harmful bacteria in STP effluents is concerning, particularly for individuals working in public health and water administration [15].

In the present study, *E. coli* O157:H7 was diagnosed when examined by Chromagar. STP2 had 8 (88.8%) isolates of this type, while STP1 had only two (20%). It is a Shiga-like toxin–producing type of *E. coli*, causing a disease, typically foodborne illness, it must integrate environmental cues to travel the GIT and fine-tune virulence determinants [16,17] Infection with this type of pathogenic bacteria may lead to hemorrhagic diarrhoea, and kidney failure; these have been reported to cause the deaths of children younger than 5 years of age, of elderly patients, and of immunocompromised patients [18]. The findings of a study conducted in Al-Najaf 2022, revealed that there was a significant prevalence in children with diarrhea, which could be the case caused by a variety of factors. Contaminated food and water have been identified as major contributors to significant outbreaks of *E. coli* O157:H7, serving as a primary vehicle for the transmission of this pathogen, and are considered among the leading causes of its spread [19].

Presence and dissemination of Antimicrobial Resistance (AMR) bacteria and their determinant genes in environmental compartments, such as lakes, rivers and sediments associated with wastewater discharge [20, 21]. The fact that isolates of *E. coli* O157:H7 are also MDR, as seen in almost all Al-Karkh samples, adds to its hazard.

The results of susceptibility to the antibiotic classes in the current study were similar to the results of Sulaymaniyah (Iraq) [22] and Nepal [23] on *E. coli* isolates isolated from patients with urinary tract infections. However, wastewater surveillance has shown the geographical variabilities of antibiotic resistance in different countries [24]. In western Kenya, Ampicillin (Penicillins) found the highest levels of resistance 85.3% in sewage plants whereas amikacin and gentamycin (aminoglycosides) showed 100% sensitivity [25]. It was diagnosed that 40 out of 67 isolates of *E. coli* from 3 sites in Tigris River (Iraq), were resistant to β-lactam group of antibiotics [26].

None of the *E. coli* isolates were susceptible to all tested antibiotics, indicating that each isolate were resistant to at least one antibiotic; Therefore, the sensitivity rate for all antibiotics was zero. A high number of resistant isolates of *E.coli* was found in the effluent (output) samples compared to input isolates. It has been suggested that wastewater can even increase the antibiotic resistance in the effluent [27]. Therefore, studying the impact of the treatment process on antibiotic resistance is important [20]. Excessive antibiotic use of broad-spectrum antimicrobials in treating human and animal infections has been linked to the spread of MDR microorganisms [28]. In a study on patients with Cystitis in Hilla city (Iraq), 62% (31/50) of *E.coli* isolates were MDR [29].

Additionally, it has been demonstrated that *E. coli* is a substantial source of genes responsible for antimicrobial drug resistance, making it a valuable marker of resistance in bacterial communities [30, 31].

A high percentage (68.4%) of MDR was found in studied samples, and an XDR was also found represented in one sample in Al-Karkh, in addition to two samples in Al-Rusafa that were possible to be XDR. It has not been possible to apply definitions for MDR, XDR, and PDR (Pandrug Resistant) globally, due not only to the various definitions used but also to differences in the antimicrobial agents used for routine antimicrobial susceptibility testing in microbiology laboratories [32].
Exposure to chlorine disinfection in STPs can enhance the resistance of *E. coli* to antibiotics [33]. In addition to causing DNA damage, chlorine can also trigger the expression of several genes linked to antibiotic resistance. Furthermore, by eliminating susceptible bacteria and leaving behind resistant ones, chlorine disinfection can favor microorganisms with higher levels of antibiotic resistance [34]. Moreover, the interaction between natural organic compounds in water and disinfectants like chlorine produces disinfection byproducts (DBPs), which may have further consequences on antibiotic resistance [35]. However, the precise mechanisms by which chlorine contributes to *E. coli*'s resistance to antibiotics remain unclear [36].

There was a significant association between the resistance pattern of the input and output samples of tested antibiotics for both STP1 and STP2, but no significant association between the two sewage plants (STP1 and STP2) regarding the resistance. Large amounts of waste are sent to sewage treatment plants from a variety of sources. This can result in the effluent containing high concentrations of antibiotics and bacteria resistant to antibiotics [31]. This can then get into the environment and possibly cause other bacteria to develop an antibiotic resistance. Moreover, plasmid exchange and other horizontal gene transfer pathways can permit the spread of antibiotic resistance genes among bacteria in high population densities [37].
Biofilms form as a defense mechanism, making the microbial community stronger and more difficult to eliminate. Bacterial species, including *E. coli,* can develop biofilms as early as two hours and survive for up to 10 years in food sectors, even with routine cleaning and sanitation [38].

The present study found two isolates with strong biofilm in STP1 inputs only, but none of these isolates in both STP1 and STP2 outputs. Shear forces produced by the water flow in STPs are one reason why the biofilm output is lower than the input [39]. In addition, the biofilm may be broken up and eliminated from the system by the turbulent flow that occurs during the treatment procedure [40]. Another factor that can contribute to the reduction in biofilm output is the use of disinfectants within the treatment process [41]. In addition to specific strain traits

and their sources, the physicochemical conditions that exist in each STP may have a significant impact on biofilm development, which is a stress-dependent response [42].

Conclusions

The presence of MDR bacteria, biofilm and Shiga- like toxin producing *E. coli* in wastewater treatment plants, particularly in the effluent, poses risks to humans, animals, and the environment. To mitigate the dangers associated with pathogen maintenance and transmission as well as the spread of MDR characteristics, responsible government institutions must assess the significance of the technologies used in STP systems.

Although this focused on a small sample size, this study revealed results were very similar to previous studies with a larger number of samples. The results can offer practical and actionable guidance for the wastewater treatment industry on the role of the treatment process. There are numerous opportunities for extension of this work. First and foremost, along with current work, a large scale molecular -based study, and microbial based study on a Gram-positive bacteria indicator such as Enterococci can give a better insight on the role of wastewater treatment plants on the fate of antibiotic resistance and the dissemination of these elements to the environment.

6. Conflict of Interest: The authors declare that they have no conflicts of interest.

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