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Research Article

Detection of Some Antibiotic Resistance of beta lactam and flouroquinolines Gene Among Coliform Bacteria isolated from Al-Hussainiya river

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Abstract:

Background: Coliform bacteria are the most important bacterial contaminate the water bodies.

Objectives: the present study aimed to detect antibiotics resistance and biofilm formation activities among coliform bacteria isolated from different places on Al-Hussainiya river.

Method :- During a period of July 2023 to February 2024, 18 samples were collected from three different places (Baron, Al-Atishi area and the white arch) on Al-Hussainiya river in summer and winter (9 samples in summer and 9 samples in winter for isolation of coliform. Most probable number (MPN) methods were used for evaluated the numbers of coliform bacteria during two seasons. The isolated coliform were further identified using Biochemical tests and Vitek 2 technique. PCR method was used to detect some genes including SHV, TEM, CTX, *qur-A* and *Aac(6) Ib* in 15 isolates of *coliform* during summer and winter. The current data showed that the numbers of coliform were significantly higher in summer in the white arch and Baron hotel. In contrast coliform appeared to be significantly lower in winter compared to summer in Al-Atishi point. Two way anova showed significant differences between stations and seasons ($P \leq 0.05$).

The results :- of the antibiotics resistance test showed isolates of *E.coli* , *Klebsiella and Enterobacter spp* resistant to all tested types of antibiotic during summer, where's in winter season only *E. coli* were resistance to a few types of antibiotics.. The results of molecular identification showed that *SHV*, *TEM*, *CTX-M* *qur-A*, *Aac (6)Ib*, bla_{SHV}, bla_{TEM} *and* bla_{CTX-M} genes were detected in 3 isolates of *E. coli* during winter only. However, these genes were not identified *in Klebsiella* and *Enterobacter* spp. In contrast, no isolates were reported to have any genes during summer season. In addition, most isolates of coliform were found to be biofilm producer bacteria.

Conclusion: the concluded that the seasons and locations of samples collection determine of the coliform numbers. Additionally, seasons could be affected the ability of coliform to resistances for antibiotics . Finally, coliform bacteria were ability to biofilm formation and their resist or sensitive for antibiotics.

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Introduction

Water should be free of harmful microorganisms and indicator bacteria, which might be an indication of fecal contamination. [1]. Fecal contaminated drinking water is a major public health problem [2].Coliform bacteria are common pollutants found in a tap water. As a result, recognizing them as indications of human fecal contamination is critical for public health protection [3].Most coliforms are present in large numbers as the intestinal flora of humans and other warm blooded animals therefore found in fecal wastes [4].

Contamination of water in a developing countries is mostly due to the lack of awareness of the risks, and inadequate training of personnel and management working on drinking water systems. Such lack of awareness or training can result in avoidable waterborne disease outbreaks in various communities. Domestic garbage disposal in lakes is generating undesired changes in the physiochemical and biological features of these water bodies [5].

Microbes such as bacteria, viruses, and protozoa are the most common cause of disease. These pathogens enter water sources as a result of sewage and feces pollution. Bacteria and fungus predominate in aquatic systems, and microorganisms play highly particular functions in the recycling of materials and the purification of water in natural ecosystems. The principal human pathogens responsible for water contamination are Salmonella, *Acinetobacter, Chromobacterium, Alcaligens, Flavobacterium, Staphylococcus aureus, Pseudomonas aeruginosa, Clostridium botulinum, Vibrio cholerae,* and *Escherichia coli* [6].

The emergence of the carbapenemase producing enteric bacteria is a reflection of the heavy use of the third generation cephalosporins and the carbapenems as clinically relevant antibiotics that should only be limited to clinical environments. Hospital effluents have been known to be released into nearby aquatic environments that are further polluted with antibiotic resistant genes [7].

In order increase output and greatly meet the world's protein needs, aquaculture also uses antibiotics [8].This leads to the emergence of antibiotic resistant bacteria (ARB) carrying different antibiotic resistance genes (ARGs). The most common antibiotics used in aquaculture, the quinolones, tetracyclines, chloramphenicols, and sulfonamides [8,9].

Examples of genes that have been found in aquaculture include *qnrA* and *qnrS* that confer resistance against fluoroquinolones [10].Broad-spectrum β-lactamase resistance genes, including *blaTEM-52* and *blaSHV-* [11].

The main mechanism of aminoglycoside resistance in staphylococci is attributed to drug inactivation caused by aminoglycoside-modifying enzymes (AMEs) encoded within mobile genetic elements. The most frequent AMEs are the bifunctional enzyme AAC (6')/APH (2') encoded by the gene aac (6')-Ie-aph (2')-Ia, APH (3')-III enzyme encoded by aph (3')-IIIa gene and ANT (4')-I enzyme encoded by ant (4')-Ia gene [12].

Genes encoding Aac (60)-Ib3 or Aac (60)-33 aminoglycoside acetyl transferases, Ant (20)-Ia aminoglycoside nucleotidyltransferase or the Aac (60)-Ib-cr enzyme, which acetylates fluoroquinolones as well as tobramycin and amikacin, were commonly adjacent to β-lactamase-encoding genes , The finding that aac (60) and ant(2")-Ia genes are adjacent to β-lactamase-encoding genes is consistent with earlier studies of *E. coli* [13].

Resistance to fluoroquinolones is also highly dependent on the ability of bacteria to decrease drug accumulation by efflux, though different efflux systems vary in their contribution to resistance for different fluroquinolones. For instance, AcrB expression is significantly higher in ciprofloxacin-resistant *E. coli*, 26% higher than in susceptible isolates [14]. Although it is unlikely to be the predominant mode of fluoroquinolone resistance. Specific quinolone efflux pump genes *qepA* and *oqxAB* as well as plasmid-mediated quinolone resistance (PMQR) genes, which

include *qnrA, qnrB, qnrC, qnrS, qnrD, qnrE,* and *qnrVC* are plasmid-encoded and can provide resistance in *E. coli* even in the absence of chromosomal mutations [14].

A study which looked at the distribution of *qnr* genes among ESBL-producing *E. coli* found that *qnrB* was present in 47.74%, *qnrS* was found in 47.10%, and *qnrA* was found in 2.58% of strains. These genes were highly associated with the ESBL genes bla_{CTX-M}, bla_{TEM}, bla_{SHV} [15].
Another study found *qnrS* in 21 of 43 isolates, *qnrB* in 6 of 45 isolates and *qnrA* in 2 of 43 isolates [16].They are highly transmissible and are transferred in tandem with additional β-lactams, gentamicin, and tetracycline resistance genes [17].

The most commonly reported PMQR mechanism is the AAC(6′)-Ib-cr enzyme which dually inactivates ciprofloxacin while additionally providing aminoglycoside resistance. The presence of it has been discovered in >70% of strains from the subgroup sequence type ST-131, an antibiotic resistant clone that has been reported to be rapidly spreading and responsible for high rates of UTI and bloodstream infections [18].

While the enzyme alone imparts a low-level MIC increase for ciprofloxacin in *E. coli*, it enhances resistance when combined with resistance-associated chromosomal mutations [19].

Therefore, the aim of the present study was to detect of antibiotics resistance and biofilm formation activities among coliform bacteria isolated from different places on Al-Hussainiya river.

Materials and methods

During the period of July 2023 to February 2024, 18 samples were collected from three places along *Al-Hussainiya river* distributed as (9 samples in the summer and 9 samples in the winter) for the detection of the antibiotic resistances genes and biofilm formation *among Enterobacter spp., Klebsiella spp., E. coli*,

1-Isolation and Identification of coliform bacteria:

1-1 Most probable number method .

MPN is MPN method was used to identify the coliform bacteria and it is contained contained from three stages as Presumptive test, Confirmed test and Completed test.
Presumptive test involves the primary presumption for the presence of Gram negative coliform bacteria in the samples demonstrated by the appearance of gas in the lactose fermentation broth. For the presumptive test procedure 15 sets of test tubes containing lactose fermentation broth Were prepared for each sample under analysis. Each test tube contained 10 ml of fermentation broth and inoculated with the water sample in a sequential order of 10 ml in five of each 2X lactose fermentation broth, 1ml in five of each 1x lactose fermentation broth and lastly 0.1 ml in five of each 10 ml 1X lactose fermentation broth. All the test tubes were incorporated with Derhum tubes for detection of gas formation by Gram negative coliform bacteria. Test tubes were incubated with half circled screw caps at 37 oC for 48 hours. This procedure was followed for all of the 75 samples individually [20]. The numbers of positive tubes in each set were compared with standard tables and the results were expressed as MPN index or presumptive coliform count per 100 ml of water..

Confirmed test.

From each positive tubes a loopful of suspension was inoculated into lipid-free biomass(LFB) 1X media containing Durham tube to re-confirm the positive lactose fermentation. with the presence of inverted Durham tube. Tubes were then incubated for 24 h at 44.5 °C and examined for gas production. Tubes were be negative for gas production after 24 h were further incubated and examined again at 48 h [21].The LFB broth tubes which are show positive gas production were further used for complete test for *E. coli*.

Completed test.

Positive samples with the production of gas in the lactose fermentation broth were selected for the confirmed test procedures to detect the indicator bacteria origin

Escherichia coli. EMB media was used to differentiate other Gram negative coliform bacteria from the *Escherichia coli* by the production of green metallic sheen in the media. The presence of green metallic sheen in EMB confirms the presence the indicator bacteria *E. coli*. One loopful sample from the positive test tubes was inoculated on EMB by streaking and incubated at 37 oC for 24 hours and then observed for the production of green metallic sheen [21].This was the final stage of the MPN method where in the decision of water quality as potable or non-potable, could be made after confirmation and completion of the study. All bacterial isolates were further identified by Vtek 2 systemThe identifications were confirmed by the.

1- Antibiotic sensitivity test

Follow its the manufacturer's instructions Vitek 2 System was used to evaluate the resistance activity of the bacterial coliform for wide range of antibiotics.

Table (1) types of antibiotics used in this research

Antibiotic	Abbreviation	Concentration
Ampicillin	AMP	<=2
piperacillin/tazo	TZP	<=4
cefotaxime	FEP	<=0.25
cefteazidime	CAZ	<=0.12
ceftazidim/Avibactam	CAZ/AVI	<=0.12
ceftolozane/Tazobactam	C/T	<=0.4
Cefopime	PEP	<=0.12
Carbapenem	C	-
Meropenem	Mpm	<=0.25
Amikacin	AMK	2
Gentamicin	GEN	<=1
Ciprofloxacin	CIP	<=0.06
Tigecycycline	TET	<=0.5
Colistin	CA	-
Trimethoprim/Sulfamethoxazole	TMP-SMX	<=20
Amoxicillin acid	AMC	<=2
Ertapenem	ETP	<=0.12
Cefazoline	CFZ	<=4
Cefuroxime	CXM	2
Cefuroxime Axetil	CRO	2
Ceftriaxone	CTX	<=0.25
Fosfomycin	FO	<=16
Nitrofurantion	NIT.	32

2- Detection of antibiotics gene resistance

A:-Preparation of bacterial DNA:

The DNA to be amplified was extracted from pure colonies by boiling method. The bacteria were harvested from 1.5 ml of an overnight Luria-Bertani broth culture, suspended in sterile distilled water, and incubated at 95 C◦ for 10 min. Following centrifugation of the lysate, the supernatant was stored at -20 C◦ as a template DNA stock [22].

B:- Amplification of genes encoding for antibiotics resistance

Aac(6) Ib, blaTEM, blaSHV, bla CTX-M, and *qnr-A* internal regions were amplified using primer sets for these genes as shown in Table 2. They were purchased from Alpha DNA Company (USA).

Table (2) primers sequences used for amplification of amplification of genes encoding for antibiotics resistance

Gene		Primers sequence	bp	References
blaTEM	F	5-TTGGGTGCACGAGTGGGTTA-3	467	Gangoue-pieboji *et al*.,2005
	R	5- TAATTGTTGCCGGGAAGCTA-3		
blaSHV	F	5-AGGATTGACTGCCTTTTTG-3	392	Colom *et al*.,2003
	R	5-ATTTGCTGATTTCGCTCG-3		
CTX-M	F	5- ACCGCCGATAATTCGCAGAT-3	588	Kaftandzieva *et al*.,2011
	R	5-GATATCGTTGGTGGTGCCATAA-3		
qnr-A	F	5-ATTTCTCACGCCAGGATTTG-3	516	Robicsek *et al*.,2006
	R	5-GATCGGCAAAGGTTAGGTCA-3		
Aac(6) Ib	F	5-TTGCGATGCTCTATGAGTGGCTA-3	482	Robicsek *et al*.,2006
	R	5-CTCGAATGCCTGGCGTGTTT-3		

Each PCR tube included the following reagents: 1 µL of primer F and 1 µL of primer R (10 pmol), 3 µL of DNA template, 12.5 µL of ReadyMix Taq PCR Reaction, and deionized water were used to produce the final volume of 25 µL. A thermal cycler (Labnet Edison, NJ, USA) was used for the amplification process and PCR cycling was performed under conditions the reactions mixtures included an initial denaturation at 95°C for 5 min consisted of 35 cycles of 95 ºC for 30 seconds , specific annealing temperature 55-60 C◦ for 35 seconds , extension 73 ºC for 1 min, and a final extension at 73ºC for 5 min for all genes. The detection of PCR products was carried out on 0.8 to 1% agarose gels by electrophoresis alongside with (100-l500) bp ladder (Promega) and visualized under UV light.

2- Biofilm formation

To investigate the ability of coliform bacteria for biofilm forming, isolates were tested according to the method described by [23]. With some modification, isolates were grown in 10 ml of nutrient broth medium by inoculating the medium with a full loop of each isolate individually, and tubes were incubated at a temperature of 37°C for 24 hours. The cells were collected from the test tubes by centrifuge was then poured, and the tubes were washed with normal saline solution. Thereafter, tubes were left to dry then they were stained with 0.1% of Crystal Violet dye and left for 5-10 min. Then tubes were turned over to dry until a layer appeared. Visible staining on the walls and base of the tube indicates a positive result.

Statistical analysis

All results were presented as (mean± SD). Two-way analysis of variance (ANOVA) followed by Tukey's HSD multiple range post hoc testing were used for detection any significant differences between coliform numbers between stations and seasons. The level for accepted statistical significance was

$P < 0.05$. Statistical analysis were performed using MiniTab statistical software version 17, IBM (Pennsylvania, USA).

Result and Discussion

Most probable number

In summer season, the results of MPN were 500, 500 and 170 MPN per 100ml in the white arch, Al-Atishi point and Al-Baron hotel, respectively as presented in Figure (1). These results showed that the coliform bacteria were significantly higher in the white arch in comparison to other two places, where's their intensity was singfintly lower in the Al-Baron hotel ($P \leq 0.05$). This study was agree with study [24]. In other place in Ganga Rivers showed that the results obtained from the microbial analysis of the Ganga River water signified the occurrences of the bacterial pathogens at the sites indicated fecal contamination in the holy river. The high load of coliform bacteria in the river sites is possible, as the human population in northern region has grown considerably due to presence of Ganga River, became polluted. Bacteriophages present in River Ganga kill or infect bacteria which gave Ganga River its bactericidal affect

In contrast, the data obtained from MPN during winter showed that the coliform number in the white arch significantly higher 900 MPN per 100 ml compare to other stations, while the lower number of coliform was shown in Al-Atishi point at ($P \leq 0.05$).This study was agreed with study [25]. showed that higher organisms in winter months than in summer but disagree with study [26], showed that during summer months, the maximum density of coliform bacteria reached values of more than 10^4 bacteria per 100 ml in these oligotrophic environments, not only in the surface but also in raw water (used for drinking water production). This represents an increase of 4 orders of magnitude compared to the winter season.

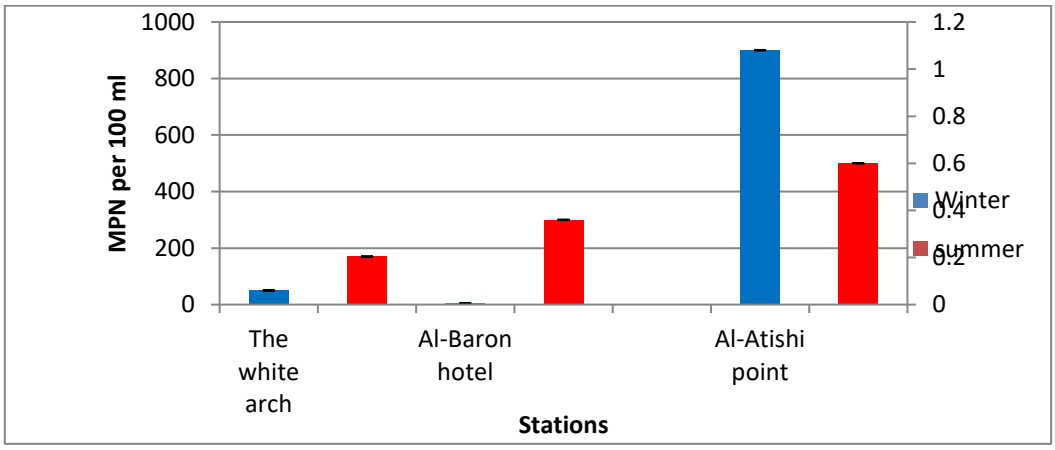

Figure (1): Coliform bacteria MPN per 100ml isolated from tested stations during summer and winter are significantly different ($P \leq 0.05$).

Figure showed that there were significant differences between stations and seasons about the numbers of isolated coliform (P=0.000).

Isolation of coliform group

The results of the present study showed that all samples of water were positive for *Escherichia coli* showing metallic green growth in EMB media . Fifteen isolates of coliform were identified and enumerate for evaluate of antibiotics resistance in each season. These isolates were distributed as 5 isolates of each genera of *E. coli*, *K. pneumonia* and *Enterobacter* spp. However, the identification isolates were isolated from all places during summer and winter seasons and these data are 5 (33%) for *Klebsiella* and 5(33%) for *Enterobacter* isolated in winter

and summer from water in agreement with study of [27], and also differ finding was reported by [28].

Antibiotic sensitivity

The present data showed that all isolates of *Klebsiella and Enterobacter* spp. in the winter season were sensitive to all used antibiotic Table (3) . The present results are disagreement with study of [29], who found that 54 % of the coliform isolates were resistant to at least one type of antibiotic, and 46 % of the isolates were sensitive to the tested antibiotics. Some bacteria were multi-drug resistant against two, three or four antibiotics (4 %, 5 %, and 1 %). It is noticeable that all bacterial isolates that were resistant against the combination of trimethoprim and sulfamethoxazole were at least resistant to one more antimicrobial substance. Also eight of the 11 tetracycline resistant coliform bacteria were multi drug resistant. The result of this study showed that 3 isolated of *E.coli* were resistances to other antibiotic cefazoline , cefuroxime , cefuroxime Axetil and ceftriaxone and sensitive to other antibiotic .

In table (3) show that the *E. coli* , *Klebsilla* and *Enterobacter* isolated were sensitive to all antibiotic except resistance to Amoxicillin acid , Ertapenem, Cefuroxime , Cefuroxime Axetil, Fosfomycin and Nitrofurantion.

Table (3) : Number of coliform bacteria isolated from river water during winter showed antibiotic resistance activity against range of antibiotics.

Antibiotic	*E.coli* res in wate	*Klebsilla* res in wate	*enterobacter* res in water
Ampicillin	0	0	0
piperacillin/tazo	1	0	0
cefotaxime	0	0	0
cefteazidime	2	0	0
ceftazidim/Avibactam	0	0	0
ceftolozane/Tazobactam	0	0	0
Cefopime	2	0	0
imipenem	0	0	0
Meropenem	0	0	0
Amikacin	0	0	0
Gentamicin	1	0	0
Ciprofloxacin	1	0	0
Tigecycycline	0	0	0
Colistin	0	0	0
Trimethoprim/Sulfamethoxazole	2	0	0
Amoxicillin acid	0	0	0
Ertapenem	0	0	0
Cefazoline	3	0	0
Cefuroxime	3	0	0
Cefuroxime Axetil	3	0	0
Ceftriaxone	3	0	0
Fosfomycin	0	0	0
Nitrofurantion	0	0	0

Table (4) Number of coliform bacteria isolated from river water during summer showed antibiotic resistance activity against range of antibiotics.

Antibiotic	*E.coli* sen in wate	*Klebsilla* sen in wate	*enterobacter* sen in w ter
Ampicillin	5	5	0
piperacillin/tazo	5	5	5
cefotaxime	5	5	5
cefteazidime	5	5	5
ceftazidim/Avibactam	5	5	5
ceftolozane/Tazobactam	5	5	5
Cefopime	5	5	5
imipenem	5	5	5
Meropenem	5	5	5
Amikacin	5	5	5
Gentamicin	5	5	5
Ciprofloxacin	5	5	5
Tigecycycline	5	5	5
Colistin	0	0	0
Trimethoprim/Sulfamethoxazole	5	5	5
Amoxicillin acid	0	0	0
Ertapenem	0	0	0
Cefazoline	5	5	5
Cefuroxime	0	0	0
Cefuroxime Axetil	0	0	0
Ceftriaxone	5	5	5
Fosfomycin	0	0	0
Nitrofurantion	0	0	0

sen=sensitive

Amplification of genes encoding for antibiotics resistance

In the present study PCR product of *aac(6')-Ib-cr* and *qnr-A* genes were amplified by using specific primers. The PCR product (band) of *aac(6')-Ib-cr* and *qnr-A* genes were 482 bp and 516 bp, respectively Figure (2).

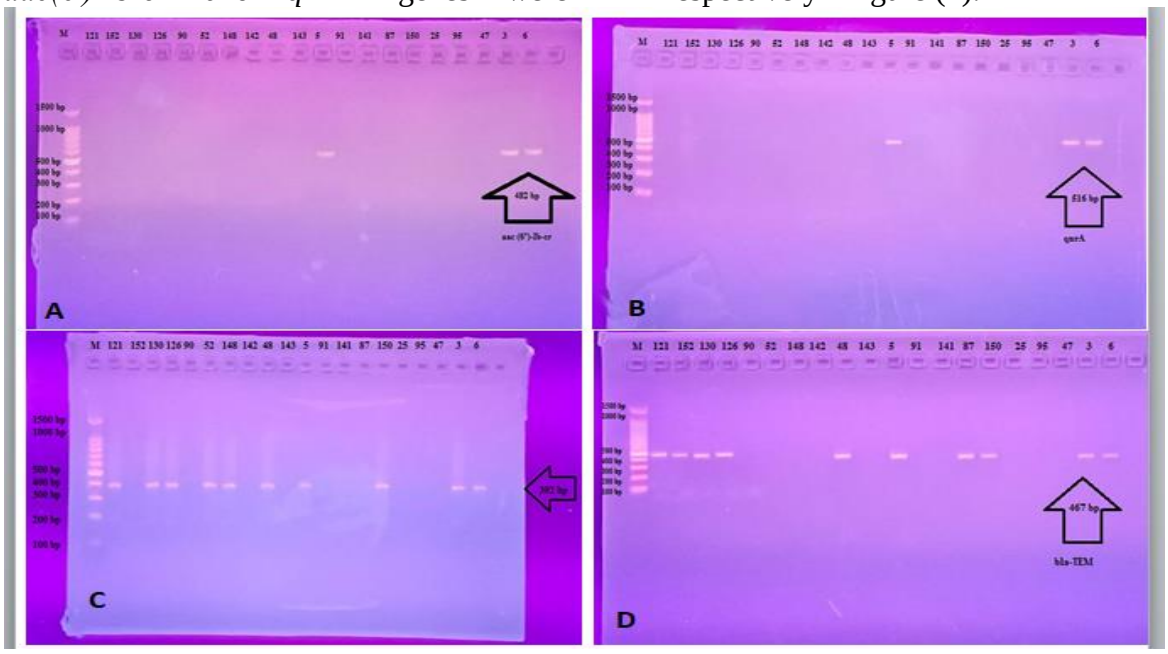

Figure (2): Electrophoresis of the PCR product of the antibiorics encoding genes of coliform isolates in 1% agarose and 100 V for 30 min. A- aac(6′)-Ib-cr gene (482 bp), B- a qnr-A gene 516(bp), C- bla_{SHV} gene (392bp) and D- bla_{TEM} gene (467 bp).

The prevalence of *qnr and aac(6')-Ib-cr* genes appears to vary considerably in different studies depending on the criteria used to select the bacterial strains. The overall prevalence of *qnr* in Enterobacteriaceae has been reported to range from 0.2% to 50%, and *aac(6')-Ib-cr* may be more prevalent than *qnr* [30].The distribution of qnr genes in Enterobacter isolates has been investigated in the UK and Spain [31,32]. The prevalence of *qnr* genes, especially *qnrB*, has also been reported for clinically isolates of *K. pneumonia* and other Enterobacteriaceae genera in Asian countries [33,34].

In China, *qnr* and *aac(6')-Ib-cr* genes were detected in 8.0% and 9.9% of extended-spectrum β-lactamase (ESBL)-producing *E. coli* and *K. pneumonia* isolates, respectively, that were collected from six provinces or districts [35].

The results obtained from the current study showed that the *qnr-A* and *aac(6')-Ib-cr* genes were detected in 3 isolates of *E. coli* in Table (5) and this study was no similar with study of [36]. in Japan who found that twenty-four *E. coli* isolates obtained from surface water and wastewater were detected to carry aac(6′)-Ib-cr gene.

Table (5) The distribution of antibiotics resistance genes among the isolates of coliform bacteria from the Al-Hussainiya river during winter season

Gens	*E. Coli*	*Klebsiella*	*Enterobacter*
qnr-A	3	_	_
Aac(6) Ib	3	_	_
bla shv	3	-	-
bla TEM	3	-	
bla CTX-M	3	-	-

The PCR products of *bla shv* , *bla TEM* and *bla CTX-* were 392 bp, 476bp and 200 bp, respectively Figure

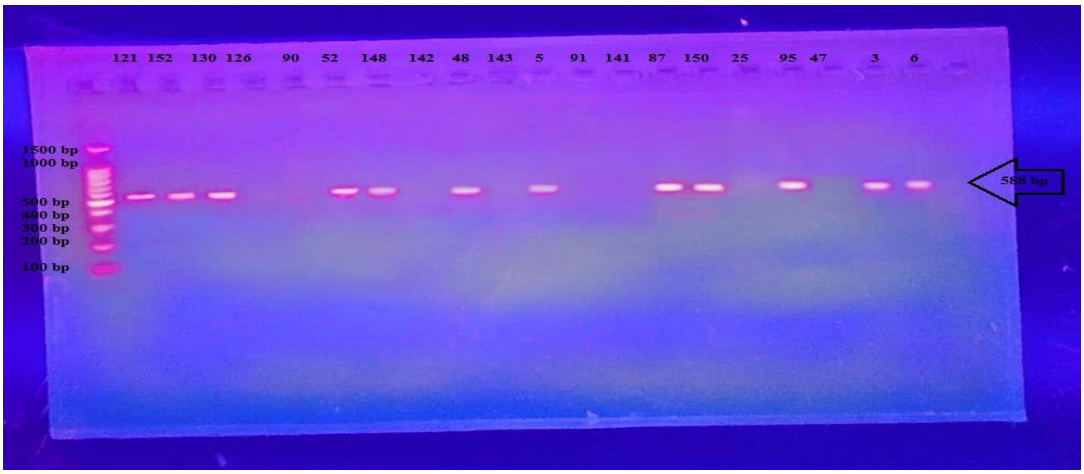

Figure 3: Electrophoresis of the PCR product for the bla CTX gene size product (band 200bp) of coliform isolates in 1% agarose and 100 V for 30 min.

The present study showed that *bla* $_{shv}$, *bla* $_{TEM}$ and *bla*$_{CTX-M}$ genes were detected in 3 isolates of *E. coli* identified from winter and the current data is in agreement with study [37], which showed that a higher prevalence of ESBLs encoding genes by bacterial isolates has been noticed, posing a threat to public health across the world. The dissemination of ESBLs encoding genes, *blaTEM, blaSHV, blaCTX-M* and *blaOXA-1* from the surface waters could be a crucial and potential health risk to communities. Therefore, regular monitoring and urgent water quality control measures and practices are necessary to avoid infections due to ESBLs producing organisms. Moreover, further studies including different surface waters across the highly industrial areas are necessary to scrutinize the emergence of ESBLs encoding genes resistance among bacterial species

But the study appeared these gene were no detected in Klebsiella and Enterobacter this disagreed with study (Khan *et al*.,2021)(37) showed that Klebsiella sp was found to possess TEM (28.6%, 2/7) and SHV (50%, 2/4) ESBL genes.

Biofilms formation

Figure (4) showed that during summer season (5(100%) of *E. coli* isolates were biofilm formation distributed into 2 (40%) were strong biofilm production and 3(60%) were moderated biofilm production . Previous study conducted at Baghdad city, illustrated that 5 out of the 10 *E. coli* isolates isolated from Tigris River revealed , biofilm formation [38]. The same authors found that , 3 (30%) of isolates of *Klebsiella* spp. were able to form biofilm 1 (33%) of them was strong biofilm and 2 (67%) were moderate biofilm formation , 2(20%) isolates of *Enterobacter* spp. were detected to produce biofilm 1 (50%) isolate was strong and the other one was moderated biofilm producer as this study is similar to the present study .

Biofilm producing ability of *K. pneumonia* was detected by agar method in the current study, 3(30%) isolates *K. pneumonia* were found as biofilm producers. in consistence with the present data, higher percent biofilm producing isolates (62.5%), were reported by [39]. (81.8%) , [40].and (67.3%) by [41]. In contrast, the present results are not in agreement with study lower adjust f biofilm producers 16.7%.which were reported by [42]. It is worth to mention that all biofilm formation isolates was sensitive to used antibiotics, antibiotics resistance bacteria were not detected to have ability for biofilm formation.

The relationship between antibiotic resistance and biofilm-production has attracted considerable interests from researchers. Previous studies have pointed out that biofilm formation can be induced by certain antibiotics under conditions of low doses, suggesting that regulation of biofilms may be involved in the global response to external stress, including antibiotics [43]. Nevertheless, previous studies regarding quantitative correlation between biofilm formation and antibiotic resistance have yielded different and inconsistent results between different bacterial species, leaving researchers further explore the association [43,44].

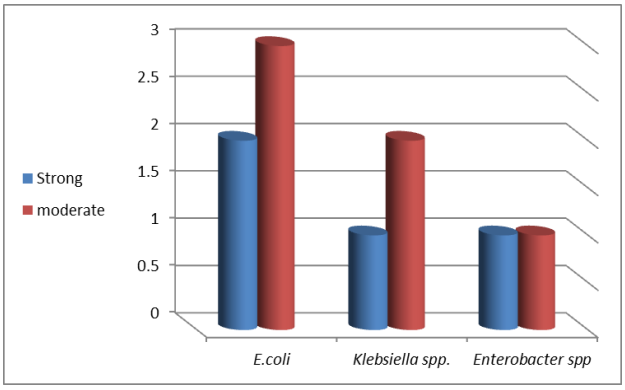

Figure (4) Coliform bacteria isolated from the *Al-Hussainiya river* in summer to form biofilm

The results of biofilm formation isolates isolated from *Al-Hussainiya river* during winter are all isolates of *E.coli* were strong biofilm formation 3 of them 60% were antibiotics resistance and the two were antibiotics sensitive . In addition 2 (100%) isolates of *klebsilla* were exhibited strong formation biofilm, 3 (100%) isolates *Enterobacter* were biofilm formation distributed as (2 (67%) were strong and 1(33%) was moderate biofilm formation.

Conclusion

It can be concluded that seasons and locations of samples collection influenced of the coliform numbers. Additionally, seasons could be affected the ability of coliform in antibiotics resistance. Finally, coliform bacteria were detected to be biofilm formation bacteria regardless of their ability to resist or sensitive for antibiotics .the *SHV, TEM, CTX-M qur-A, Aac (6)Ib, bla$_{SHV}$, bla$_{TEM}$ and bla CTX-M* genes were detected in 3 isolates of *E. coli* during winter only

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