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Curing of *Citrobacter spp*. Strains Antibiotic Resistance Harboring Plasmids Using Novel Physical, Chemical and Natural Substances

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THE CURRENT study was conducted on four isolates of Citrobacter freundii identified previously molecularly and registered in the Global GenBank under the names ALGH1(accession No. OQ703592), ALGH2(accession No. OQ703593, ALGH3 (accession No. OQ703594), and ALGH4(accession No. OQ703595), Antibiotics profile analysis was achieved against 12 antibiotics, all isolates showed 100% resistance to (tetracycline, Doxycycline and oxytetracycline), whereas the isolates were sensitive to Amikacin, Trimethoprim, Nalidixic acid Norfloxaine, Levofloxacine and Ciprofloxacin. Curing experiments of these plasmids were achieved by physical(high temperature), chemical (urea) and natural substances by using both onion and garlic powder in three concentration (1%,5%,10%), urea was effective curing plasmids at concentrations of 200 and 400 µg/ml. 45°C successed in curing resistance plasmids. To our knowledge this study is considered the first one which recorded the using of onion and garlic powder in plasmid curing. As a result, the garlic in both the 5% and 10% concentrations was successful in curing the genes of both doxycycline and Tetracycline, whereas the onion failed in all concentrations. For all ALGH1,ALGH2&ALGH4 strains of the *Citrobacter* , while sensitivity appeared all bacterial isolate spresented against (Oxytetracycline) while the garlic as a curing agent at a concentration of (10%) resulted in the curing of antibiotic resistance genes (Tetracycline, Oxytetracycline, Doxycycline). This study aimed to cure plasmids from *Citrobacter* strains by using natural substances of onion and garlic at varying concentrations.

Keywords: Citrobacter spp, Antimicrobial susceptibility test, Curing plasmid.

Introduction:

Members of the genus *Citrobacter* belong to the family Enterobacteriaceae, which are gram-negative bacilli, with length ranges between (2-4µm) micrometers and width from (0.4-0.6 µm) micrometers [1].

Citrobacter bacteria are human opportunistic pathogens that can cause pathological infections such as urinary tract, respiratory tract, central nervous system, skin, and soft tissue infections [2]. The bacteria also can cause osteomyelitis, pyogenic arthritis, bacteremia, endocarditis, and intra-abdominal infections, especially in neonates and immunocompromised individuals [3].The ability of intestinal bacteria to spread from their normal habitat in the digestive tract to other parts of the body, such as the bloodstream, wounds, or urinary tract, determines their pathogenicity to humans and other living organisms [4].

The discovery of antibiotics was one of the most important medical interventions in the history of global health, and they were used to reduce morbidity and mortality caused by bacterial

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infections [5]. Antibiotics have been used for decades not only for medical purposes, but also as a preventive measure in a variety of fields, including animal husbandry and agriculture [6]. Antibiotics have prevented millions of deaths. However, a serious health and environmental problem, in particular the emergence of multiple antimicrobial resistance, has resulted from inappropriate use of antibiotics. More than 750,000 deaths annually are thought to be caused as a result to antimicrobial resistance [7].

A plasmid is a genetic component that has two strands and is found in most cases in a circular form separate from the chromosome. Plasmids can self-replicate without relying on the host chromosome because they contain the origin of replication called a replicon. The plasmid is characterized by being genetically stable and contains genes ranging from (1-300) [8].

Plasmids play an important role in the process of bacterial evolution, as they contribute to enhancing their resistance to antimicrobials and thus pathogenicity by carrying antimicrobial resistance genes. Therefore, their spread among bacterial communities constitutes a serious threat to public health globally [9]. Physical &chemical agents to cure plasmids, furthermore using natural materials such as onion &garlic which considered as supplementary or favor of diet as at temperature to cure plasmids by them.

Material and Methods

Bacterial Isolates:

Four clinical isolates of *Citrobacter spp.* were obtained from the Department of Biology/ College of Sciences/University of Mosul All of them were molecularly diagnosed and registered in the National Center for Biotechnology Information(NCBI) under accession numbers

C . freundii strain ALGH1 under accession number OQ703592

Citrobacter sp strain ALGH2 under accession number OQ703593

C. freundii strain ALGH3 under accession number OQ703594

C. freundii strain ALGH4 under accession number OQ703595

Antibiotic Susceptibility Test

A sensitivity test was conducted for the isolates of *Citrobacter spp*. using the Kirby-Bauer method, where 12 antibiotics were used, prepared by the Turkish company Bioanalyse, as shown in Table (1). Several pure bacterial colonies were transferred to a test tube containing a saline solution to obtain the bacterial suspension at a concentration of 1.5 x 10^8 colony-forming unit/ ml, compared to a McFarland tube No. 0.5, 100 microliters of the suspension were spread on solid Mueller-Hinton agar medium. The discs were placed using sterile forceps on the medium (6 discs in each Dish), and the dishes were incubated at 37°C for 24 hours. After completing the incubation period, the zone of sensitivity was read by Ruller and compared with the standard diameter of inhibition zones for each antibiotic as shown in Table (1).according to [10]. (The sensitivity or resistance of this bacteria to the antibiotics was determined.

Detection of the presence of plasmids:

Extraction and purification of plasmid DNA:

The plasmid DNA of the four bacterial isolates of *Citrobacter spp*. were extracted using a plasmid extraction kit according to (Promega, USA)

Curing Plasmid experments:

Curing using high temperature curing using:

The procedures followed by [11] have been achieved following**:**

1. Bacterial colonies were grown in 5 ml of Brain heart infusion broth (BHI) and incubated at 37°C for 24 hours.
2. 100 µl of the bacterial suspension were placed in 5 ml of BHI broth and incubated at 45 °C for 24 hours.
3. 100 µl of the six dilutions from previous suspension was spread on Mueller-Hinton plates to be used as master plates, which incubated at 37 °C for 24 hours, then all 50 colonies from these plates were transferred to another plates containing the antibiotics in the medium: Tetracycline 10µg, Oxytetracycline 30µg, and Doxycycline 10µg. The plates were incubated at 37 °C for 24 hours, then the only number of grown colonies were counted.

Curing plasmids using urea:

The method of [12] was followed different concentrations of urea (100, 200, and 400 µmol) were used.

5ml of the BHI broth was inoculated with a *C. freundii* bacterial colony and incubated at 37 ºC for 24 hours. Then 0.1 ml of the bacterial suspension was added to 5 ml of the BHI broth containing urea with the three concentrations separately. The medium was incubated at 37°C for 24 hours. Then the bacterial cultures were diluted to the sixth dilution using physiological saline solution, and 0.1ml of the last dilution was spread on the the Mueller-Hinton agar to be used as master plates and incubated at 37 °C for 24 hours. Transferring 50 colonies onto Mueller-Hinton agar and considering it the master plate, then were subcultured it again on the medium containing the antibiotics Tetracycline 10µg, Oxytetracycline30µg, and Doxycycline 10µg, and the number of not inhibited colonies by the antibiotics were counted.

Preparation of onion and garlic alcoholic extracts

Both dry onion and garlic powders in concentrations 1%, 5% and 10% of each were soaking in absolute ethanol for 24 hours. Then was extracted using a vacuum pump. The extracts were filtered using a Whatman NO.1 filter paper and the extracts were concentrated using a device Rotary evaporator at 50°C under vacuum pressure. The color turned yellow, then it was placed in an electric oven at a temperature of 40°C until it dried and the alcohol volatilized, then they were stored in glass bottles until use. The extracts were dissolved with DMSO to obtain liquid solutions [13]**.**

The experiment

1. 5ml of BHI broth were inoculated by colony of *Citrobacter spp.* and incubated at 37 ºC for 24 hours, then fixing the cell concentration at 1.5 x 10^8 CFU/ml
2. (1 ml) from three concentrations of onion and garlic extract separately was diluted into 5 ml of the previously prepared standard bacterial suspension and incubated at 37°C for 24 hours.
3. Six dilutions of the bacterial culture were prepared using physiological saline solution, and 100 µl of the last dilution was we spread and culture on Mueller- Hinton to obtain single colonies, the plates were then incubated at 37°C for 24 hours
4. 50 colonies were transferred to plates on Mueller- Hinton agar considering it a master plate and then subcultures again on the medium containing the antibodies (Tetracycline 10µg) ,(Oxytetracycline30µg) and (Doxycycline10µg) and incubated for 24 hours at 37°C then the number of colonies were counted.

Results and Discussion

Susceptibility Of C. freundii strains to antibiotics:

The results of susceptibility test for antibiotics in Table (2) showed that ALGH1, ALGH2, ALGH3, ALGH4 of *Citrobacter* strains showed 100% resistance to antibiotics (Tetracycline, Doxycycline and Oxytetracycline), exhibited 100% sensitivity to (Ciprofloxacin, Levofloxacine, Norfloxaine, Trimethoprim-sulfamethoxazole, Amikacin, Gentamycin and Nalidixic acid). The strains also showed moderate sensitivity to antibiotics (Ceftazidime and Ceftriaxone). Our result of the resistant to Doxycyclin agree with [14] who said that one bacterial isolate of *Citrobacter spp.* was multidrug resistant (MDR) which resistance against Doxycyclin, Piperacillin and Cefotaxime, while nine strains were extensively drug resistance (XDR).

Tetracycline is a broad-spectrum antibiotic that inhibits bacterial protein synthesis. by inhibiting translation. It prevents aminoacyl-tRNA from interacting or associating with bacterial ribosomes. This antibiotic done this work by binding to the 16s unit of the 30s ribosomal unit and prevents the tRNA molecule charged from binding to an amino acid to the A site of the ribosome [15].

According to Roberts and Schwarz [16], there are three main ways in bacteria exhibit resistance to tetracycline: enzymatic inactivation of the compound, excretion of the compound outside the bacterial cell through efflux pumps, and genes present on chromosomes or plasmids that encode for the production of cytoplasmic proteins that protect ribosomes from tetracycline's action and provide ribosomal protection.

The plasmid DNA of the isolated *Citrobacter* species was investigated using a special screening kit through which the plasmid DNA was obtained Isolates ALGH1 and ALGH 2 each had two bands of plasmids in the first and second well, respectively. the third well, represented by isolate ALGH3, it contained three plasmid bands, lastly

the fourth well, represented by the ALGH4 had no band. The results of estimating the plasmid content of bacterial species belonging to the Enterobacter family from previous studies by [17] indicated that most of them contain large and small plasmid bands, as some genera possessed three plasmid bands of different sizes, while others possessed two or one bands. Many studies have been found regarding the prevalence and distribution Plasmids of different sizes among intestinal bacteria, another study conducted by [18] on the isolation and sequence analysis of the genus *Citrobacter spp* contains one small plasmid.

Curing the Plasmid:

The Plasmid Curing Using Heat:

Citrobacter strains grew at 37°C, while higher temperatures had a significant impact on the Plasmids carrying the genes responsible for antibiotic resistance, such that most bacterial isolates lost their resistance to antibiotics at a temperature of 45°C, as shown in Table (3).

It is noted from Table (3) that curing occurred in the plasmids of *Citrobacter* bacterial isolates for all antibiotics (Tetracycline, Oxytetracycline, Doxycycline) at a temperature of at 45°C, the sensitivity to the antibiotic (T 30µg) was moderate, as the number of growing colonies ranged between (23-25) colonies, while the sensitivity to antibiotics (DO10µg), (TE10µg) were high, and the number of growing colonies ranged between 8-20 colonies .This result is consistent with the result of the researcher [19], in which he explained that using a temperature of more than 37°C led to the curing of antibiotic resistance in several cases of the family Enterobacteriaceae, such as *Proteus mirabilis* and *Serratia marcescens*, at a temperature of 45°C. The occurrence of curing at a temperature of 45°C may be due to the multiplication of R plasmids carrying resistance genes antibiotic substance is sensitive to heat, and heat sensitivity may be due to there were some possible mechanisms, such as the loss of some interactions, including Protein-Protein or DNA-Protein or loss of various receptor bindings proteins during this temperature range, which leads to the inhibition of gene transfer or its instability [20].

Curing of Plasmids by Urea:

The ability to cure plasmids in the *Citrobacter* strains was studied using urea at different concentrations (100, 200, 400) µmol, which were indicated in Tables (4, 5, 6). The results showed that the use of urea at a concentration of (100 µmol) did not have any curing effect on the bacterial plasmids, while the neutralizing effectiveness of urea appeared at the concentration (200,400 µmol) as all bacterial isolates showed sensitivity to the antibiotics under study: (Tetracycline10µg, Oxytetracycline30µg, Doxycycline10µg).

The results of this study agreed with the results of the researcher [21] when using urea as a curing substance for some species of the Enterobacteriaceae family at different concentrations. Increasing the urea concentration in the medium to (200 µmol) or more led to an increase in the curing rate in *Citrobacter* bacteria.

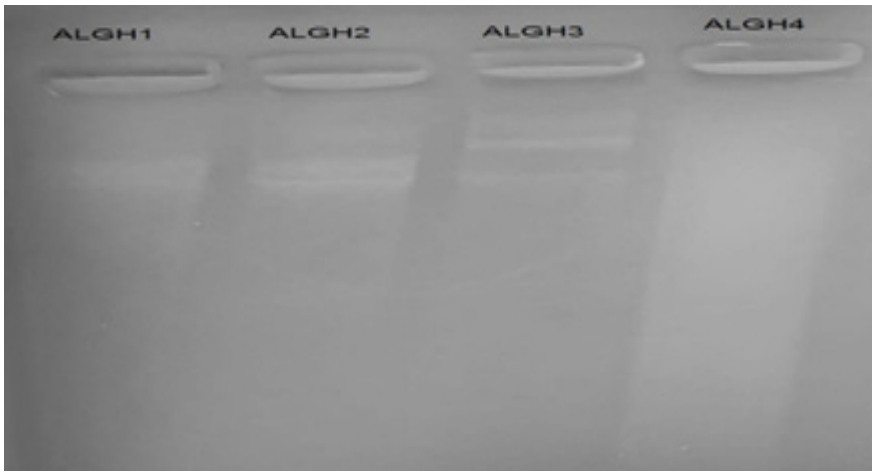

Fig.1. Electrophoresis 2%, Vol 50 for 90 min for Plasmid content of *Citrobacter* strains,

Urea is one of the curing substances for plasmids, and the mechanism of curing mediated by urea may be due to a change in the effectiveness of the enzymes responsible for DNA replication, thus stopping the plasmids from replicating, or it affects the centers of plasmid replication initiation, and this mechanism may depend on the fact that urea is a substance that denatures the protein, or perhaps it depends on the occurrence of mutation through its entry between a pair of nucleotides, leading to the formation of an inactive protein (denaturation). Thus, resistant bacterial cells are transformed into sensitive cells after treating them with urea [13].

Urea therapy is a technique that is not widely used, but preliminary exploratory studies are promising and there is increasing evidence suggesting that urea therapy may be a suitable treatment option in the acute emergency phase due to the short time required for treatment, the fact that it is a simple process, and uses readily available materials. actually. Urea consists of ammonia and carbonate, and the ability of ammonia to inhibit pathogens has been proven in reports, for many types of microorganisms such as bacteria, viruses, and parasites [22].

Curing of Plasmids by using natural agents:

Onions and Garlic have been used as natural plasmid curing agents, since they are plants used daily by humans' diet in order to break lines of bacterial resistance of antibiotics. The effectiveness of each of them was studied on bacterial isolates of the *Citrobacter* strains at different concentrations (1%, 5% and 10%) for both onions and garlic alcoholic extract [23].

We noticed the onion is used as a curing factor that could not happen curing of resistance genes to antibiotics (Tetracycline, Oxytetracycline, Doxycycline) at the concentrations used (1%, 5% and 10%) as mentioned in material and methods. The reason for this may be attributed to the genes responsible for resistance to the antibiotics were applied to another type of plasmid or to the chromosomes, and the bacterial species of the *Citrobacter* genus under study are not affected by the plant extracts of onions, as the transferred bacterial colonies remained as they were and no area was shown to inhibit bacterial growth, and the reason may be due to the absence of areas of inhibition on the bacterial growth medium means that the bacterial plasmid is not affected by the plant extract or that the onion plant does not possess an active substance that inhibits bacterial growth.

The results of the study differed from the results reached by [24], who noted happening of curing of plasmid and loss of resistance to more than one antibiotic for family strains Enterobacteriaceae, such as *Serratia, E. coli*, and *Klebsiella*, which have been studied and isolated from hospital infections.

Concerns regarding the lack of effect of plant sources on pathogens may arise because plants continue to be the fundamental and vital source for combating etiological agents, particularly in the wake of the antibiotic resistance outbreak and the emergence of multidrug-resistant and extensively drug-resistant strains that require the use of natural plants and herbs in place of antibiotics. Consequently, they advised using plants from different hosts until compounds that are affected to bacteria, researching active substances work on their own or combining them with other substances like nanoparticles in order to be used as a promising treatment in a variety of infections caused by bacteria and prevent developing pathological issues [25].

The results of curing plasmids in order to remove resistance to antibiotics in bacterial isolates of the *Citrobacter* genus using garlic extract were shown in Table (7) , the use of garlic as a curing agent at a concentration of (1%) did not cure the resistance genes antibiotics(Tetracycline, Oxytetracycline, Doxycycline), and all clinical isolates of the *Citrobacter* genus showed high resistance to these antibiotics, while table (8) demonstrates that using garlic extract (5%) did not cure the genes of both (Tetracycline, Doxycycline). For all ALGH1,ALGH2&ALGH3 strains of the *Citrobacter* , while sensitivity appeared all bacterial isolate presented against (Oxytetracycline) number of growing colonies ranged between 24-27 colonies, while the results in Table (9) indicates that using garlic as a curing agent at a concentration of (10%) resulted in the curing of antibiotic resistance genes (Tetracycline, Oxytetracycline, Doxycycline). All(ALGH1,ALGH2&ALGH3) of the *Citrobacter* genus showed high and moderate sensitivity to these antibiotics, and the number of growing colonies ranged between 24-29 colonies.

The use of the curing factors of the plasmid

may create a good way to reduce the spread of the multiple resistance to antibiotics by plasmids and the fact that most well-known plasma curing such as Acridine orange and Ethidium bromide and SDS are not suitable for medical uses or treatment applications due to their toxic nature, and that each worker of this is factors are limited to a limited number of plasmids [25]. After the curing process, the plasmids that contain resistance genes are removed from the cells, making them sensitive to antimicrobials in the absence of these genes on the chromosome. This allows us to know whether the resistance is encoded by plasmid or by chromosome. Therefore, we performed a sensitive test (after curing) to antimicrobials, that isolations showed their resistance before curing. The results showed a variation in the sensitive before and after the curing, which demonstrates the important role of the plasmid in resistance.

The curing that appeared in some bacterial isolates may be due to the number of factors, including those related to the bacterial cell itself and the permeability of the cell membrane in it contain plant extracts and chemical agents, and these substances can stop the multiplication of plasma carrying the characteristic of resistance to antibiotics.

Some bacterial genera may possess two or more plasmids originally, as indicated by studies such as: *E. coli* and *Citrobacter*, while other genera may have only one plasmid, such as *Klebsiella*. Therefore, curing experiments using plant extracts, chemical compounds, or physical agents may remove only one plasmid, two, or all of them, or they may not remove or affect their plasmid content. Therefore, the resistance pattern of pathogenic bacteria may or may not change [26].

The researcher [27] indicated that despite the removal of the plasmid from the Gram-negative bacteria, but the bacteria maintained their resistance to some antibiotics, and this can be explained by the presence of genes resistant to these substances on the chromosome. The inability of the drug to reach its target due to decreased cell membrane permeability mostly in Gram-negative bacteria due to the presence of an outer membrane, or that the resistance was not linked to genes in the first place, but rather a function of other mechanisms, for example the presence of efflux pumps, or the fact that the target site changed in composition, or the action of the antigen was inactivated, so the phenomenon of curing of plasmids is not sometimes sufficient to get rid of resistance, but it nevertheless works to reduce the problem of resistance bacteria to antibiotics or bacterial virulence [28]. Our advice to complete these experiments by detection of active ingredient in the garlic by some methods like HPLC or GC-mass.

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TABLE 1. Limits of sensitivity and resistance of *Citrobacter spp* to the antibiotics.

Antibiotics	Antibiotic symbol	Antibiotic concentration (µg/disc)	Antibiotic family	S	I	R
Tetracycline	TE	µg 10	Tetracyclines	19≤	16-20	14≥
Doxycycline	D0	µg 10		≥16	14-16	≤12
Oxytetracycline	T	µg 30		≥19	15-18	£ 14
Ciprofloxacin	CIP	µg 10	FlouroQuinolone	21≤	16-20	≤15
Levofloxacin	LVX	µg 5	Quinolone	≥17	14-16	≤13
Naldixic acid	NA	30 µg		19≤	14-18	13≥
Norfloxacin	NOR	µg 10		≥17	13-16	≤12
Trimethoprim-sulfamethoxazole	SXT	µg 10	Sulfonamide	19≤	16-18	≤15
Amikacin	AK	µg 10	Aminoglycosides	≥17	15-16	≤14
Gentamicin	GM	10 µg		³ 15	13-14	£ 12
Ceftazidime	CAZ	30 µg	Cephalosporins	≥21	18-20	£ 17
Ceftriaxone	CRO	10 µg		³ 19	14-18	£ 13
Ceftriaxone	CRO	10 µg		³ 27	25-26	£ 24

TABLE 2. The resistance of four Citrobacter spp. Against common antibiotic

Antibiotics	Antibiotic symbol	ALGH1	ALGH 2	ALGH 3	ALGH4
Tetracycline	TE (10µg)	R	R	R	R
Doxycycline	DO (10µg)		R R	R	R
OXYtetracycline	T (30 µg)	R	R	R	R
Ciprofloxacin	CIP (10µg)	S	S	S	S
Levofloxacine	LEV (5µg)	S	S	S	S
Norfloxaine	(NOR (10µg	S	S	S	S
Trimethoprim	TMP (10µg)	S	S	S	S
Amikacin	AK (10µg)	S	S	S	S
Gentamycin	CN (10µg)	S	S	S S	I
Ceftazidime	CAZ (30µg)	R	I	I	S
Nalidixic acid	NA (10µg)	S	S	S	S
Ceftriaxone	CRO (10µg)	R	I	S	I

R: resistance, S: sensitive, I: Intermediate

TABLE 3. The effect of high temperature (45°C) on the curing of *Citrobacter* plasmids

Name of the *Citrobacter* isolate	Number of grown colonies		
	Tetracycline 10 µg	Oxytetracyline30 µg	Doxycycline 10 µg
ALGH1	23	8	19
ALGH2	25	10	20
ALGH3	25	13	18

TABLE 4. The effect of Urea at a concentration (100 µmol) on curing Citrobacter plasmids

Name of the *Citrobacter* isolate	Number of grown colonies		
	10µg Tetracycline	**30µg** Oxytetracycline	**Doxycycline 10µg**
ALGH1	50	50	50
ALGH2	50	50	50
ALGH3	50	50	50

TABLE 5. The effect of Urea at a concentration (200 µmol) on curing Citrobacter plasmids

Name of the *Citrobacter* isolate	Number of grown colonies		
	Tetracycline 10µg	Oxy Tetracycline 30µg	10 µg Doxycycline
ALGH1	24	14	18
ALGH2	21	17	21
ALGH3	22	15	21

TABLE 6. The effect of Urea at a concentration (400 µmol) on curing *Citrobacter* plasmids

Name of the *Citrobacter* isolate	Number of grown colonies		
	Tetracycline 10µg	Oxy-Tetracycline 30µg	Doxycycline 10µg
ALGH1	22	14	22
ALGH2	24	15	18
ALGH3	19	17	21

TABLE 7. The effect of Garlic extract at a concentration of 1% on the curing of Citrobacter plasmids

Name of the *Citrobacter* isolate	Number of grown colonies		
	Tetracycline 10µg	Oxy Tetracycline 30µg	10 µg Doxycycline
ALGH1	50	50	50
ALGH2	50	50	50
ALGH3	50	50	50

TABLE 7. The effect of Garlic extract at a concentration of 1% on the curing of Citrobacter plasmids

Name of the *Citrobacter* isolate	Number of grown colonies		
	Tetracycline 10µg	Oxy Tetracycline 30µg	10 µg Doxycycline
ALGH1	50	50	50
ALGH2	50	50	50
ALGH3	50	50	50

TABLE 8. The effect of garlic extract at a concentration of 5% on the curing of Citrobacter plasmids

Name of the *Citrobacter* isolate	Number of grown colonies		
	Tetracycline 10µg	Oxy Tetracycline 30µg	10 µg Doxycycline
ALGH1	50	24	50
ALGH2	50	26	50
ALGH3	50	27	50

TABLE 9. The effect of garlic extract at a concentration of 10% on the curing of *Citrobacter* plasmids

Name of the *Citrobacter* isolate	Number of grown colonies		
	Tetracycline 10µg	OxyTetracycline 30µg	10 µg Doxycycline
ALGH1	26	18	24
ALGH2	28	16	25
ALGH3	29	18	24

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التحري عن بعض عوامل ضراوة جراثيم Citrobacter spp المعزولة من إصابات الحروق ومحاولة تثبيطها

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أجريت الدراسة الحالية على أربع عزلات من بكتريا *Citrobacter freundii* تم تحديدها جزيئيا سابقا ومسجلة في بنك الجينات العالمي تحت الأسماء ALGH1 (رقم الانضمام OQ703592)، ALGH2 (رقم الانضمام OQ703593) ALGH3 (رقم الانضمام OQ703594)، وALGH4 (رقم الانضمام OQ703595). تم تحليل خصائص المضادات الحيوية ضد 12 مضاد حيوي، أظهرت جميع العزلات مقاومة 100٪ لـ (tetracycline، Doxycycline، oxytetracycline)، في حين أظهرت الدراسة أن الأميكاسين، تريميثوبريم، حامض الناليديكسيك، نورفلوكسين، ليفوفلوكساسين و سيبروفلوكساسين هي الأفضل في التأثير كمضاد للجراثيم ضد بكتريا *Citrobacter spp*. تم إجراء تجارب التحييد لهذه البلازميدات بالطرائق الفيزيائية (درجة الحرارة المرتفعة) والكيميائية (اليوريا بتراكيز مختلفة) والطرائق الطبيعية باستخدام نبات البصل والثوم بثلاثة تراكيز (1٪، 5٪، 10٪)، وكان استخدام اليوريا مؤثرا وفعالاً في تحييد البلازميدات بتركيزات 200 و 400 ميكروجرام/مل. درجات حرارة عالية تصل إلى 45 درجة مئوية تحييد البلازميدات المقاومة لجنس *Citrobacter*. وعلى حد علمنا تعتبر هذه الدراسة الأولى محليا التي سجلت استخدام نبات البصل والثوم في التحييد للبلازميد. وكانت النتائج فشل البصل في جميع التراكيز ونجاح الثوم في كل من التركيزين 5٪ و10٪ في التحييد باستخدام مستخلص الثوم (5٪) لم يحييد جينات كل من (تتراسيكلين، دوكسيسايكلين). بالنسبة لجميع سلالات ALGH1 وALGH2 وALGH3 من البكتيريا *Citrobacter*، في حين ظهرت حساسية جميع العزلات البكتيرية المقدمة ضد (Oxytetracycline) وتراوح عدد المستعمرات النامية بين 24-27 مستعمرة، أن استخدام الثوم كعامل محيد بتركيز (10٪) أدى إلى تحييد الجينات المقاومة للمضادات الحيوية (التتراسيكلين، أوكسي تتراسيكلين، دوكسيسيكلين).