Effect of Combination of Antibiotics on *Enterobacter cloacae* isolated from Different Clinical and Environmental Sources in Diyala Province

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Abstract

This study included collection of 300 clinical and environmental samples (vaginal swabs, wounds, hands of the workers, patients' bed, surgical tools, and floors) from the hospitals of Diyala Province. The results showed that 40 (21.6%) of the isolates were belonging to Enterobacteriaceae and 10 (25%) were *Enterobacter cloacae*, by using diagnostic phenotypic, biochemical tests and the identification was confirmed by using regular api20E and VITEK 2 system. The results of the investigation of some virulence factors showed that *Enterobacter cloacae* was not able to produce haemolysin. The formation of biofilm was detected by micrometer tube method, and *Enterobacter cloacae* was able to formation biofilm at a percentage of 90%, while all isolates of *Enterobacter cloacae* were not able to produce urease. All the isolates (100%) have Siderophores, 70% of the isolates were able to produce bacteriocin, while 60% of the isolates were able to produce β-lactamase. In regards to the resistance to antibiotics, the results showed that 90% of the isolates were resistant to cefixime and tobramycin. imipenem was the most effective antibiotic and showed 100% activity. The minimum inhibitory concentration (MIC) for 5 antibiotics (amoxicillin, cefotaxime, ciprofloxacin, streptomycin, and nalidixic acid) ranged between 64-1024, 32-1024, 2-1024, 128-1024, 8-1024 µg\ml for the five antibiotics, respectively. The results showed that the combination of ciprofloxacin, streptomycin, and cefotaxime at a ratio of 1:3 was the best among other ratios, while the combination of cefotaxime and ciprofloxacin showed 80% synergistic effects against four isolates. In addition, combination of streptomycin and cefotaxime showed 80% synergistic effect against four isolates. Outcome of the plasmid profile for the isolates *Enterobacter cloacae*1 indicated that isolate contains one band of mega

plasmid. Curing was conducted by using acridin orange and plasmids were lost at a concentration of 1024 µg / ml for *Enterobacter cloacae*1. Results indicate that all isolates showed resistance to antibiotics ciprofloxacin and Co-trimoxazole, while lost resistance to amikacin, augmentin, ampicillin, gentamycin and cefotaxime.

Keywords: *Enterobacter cloacae*, Virulence factors, Antibiotics, Plasmid curing.

التأثير الخلطي لمضادات الحياة على *Enterobacter cloacae* المعزولة من مصادر سريرية وبيئية مختلفة في محافظ ديالى

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الخلاصة

تضمنت الدراسة جمع 300عينة سريرية وبيئية مختلفة (مسحات مهبل ، الجروح ، ايدي العاملين ، أسرة المرضى، ادوات الجراحة والارضيات) من مستشفيات محافظة ديالى. أظهرت النتائج بان 40 (21 %) من العزلات تعود الى العائلة المعوية و 10(25 %) كانت *Enterobacter cloacae* ، وباستخدام التشخيص المجهري والكيموحيوي ، وتم تأكيد التشخيص باستخدم نظام api20E ونظام VITEK 2 . نتائج التحري عن عوامل الضراوة بينت ان *Enterobacter cloacae* غير قادرة على انتاج الهيمولايسين وان تكوين الغشاء الحيوي كانت بنسبة 90 % بينما كانت جميع العزلات غير قادرة على انتاج انزيم اليوريز .جميع العزلات (100 %) كانت تمتلك حوامل الحديد و 70 % من العزلات قادر على انتاج البكتريوسين، بينما 60 % قادر على انتاج أنزيمات البيتالاكتاميز. أوضحت النتائج ان 90 % من العزلات مقاومة cefixime وtobramycin ، وكات العزلات حساسة بنسبة 100% للامبينيم . التركيز المثبط الادنى (MIC) لخمسة مضادات حياة amoxicillin) ،cefotaxime ،ciprofloxacin ،nalidixic acid و streptomycin) كانت 64 - 1024 ، 32 -1024 ، 2- 1024 ، 128 – 1024، 8- 1024 مكغم / مل على التوالي . أشارت نتائج خلط مضادات ciprofloxacin و streptomycin وcefotaxime ان افضل نسبة خلط هي 1 : 3 وان خليط cefotaxime و ciprofloxacin اعطى تأثير تأزري بنسبة 80 % ضد اربع عزلات ، وكذلك خليط streptomycin وcefotaxime اعطى تأثير تأزري بنسبة 80 % ضد اربع عزلات . نتائج النسق البلازميدي للعزلة 1 *Enterobacter cloacae* بينت احتواء العزلة على حزمة بلازميدية واحدة كبيرة الحجم. اجريت عملية تحييد للبلازميدات باستخدام صبغة الاكردين البرتقالي بتركيز 1024 مكغم / مل ، واشارت النتائج الى ان العزلة احتفظت بصفة مقاومة مضادات الحياة ciprofloxacin وCo-trimoxazole وفقدت صفة مقاومة مضادات الحياة amikacin ، augmentin ، gentamycin ، ampicillin و cefotaxime

الكلمات االمفتاحية : *Enterobacter cloacae* ، عوامل الفوعة، مضادات الحياة، تحييد البلازميدات

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Introduction

Enterobacteriaceae genera are ubiquitous Gram negative bacteria that are found in soil, food, and water while making up a significant portion of the bacterial normal flora of human and animal guts [1]. Members of *Enterobacteriaceae* are involved in various infectious diseases such as urinary tract, intra-abdominal and pelvic infections, bacteremia, wound and tissue infections, and endocarditis [2]. *Enterobacter cloacae* is part of the normal flora of the gastrointestinal tract of 40 to 80% of people and is widely distributed in the environment [3,4]. Like most members of the family *Enterobacteriaceae*, this bacterium is capable of causing opportunistic infections in hospitalized or debilitated patients [5,6]. *Enterobacter cloacae* has an intrinsic resistance to ampicillin and narrow-spectrum cephalosporins and exhibits a high frequency of mutation to resistance to expanded-spectrum and broad-spectrum cephalosporins [7]. These characteristics, associated with the frequent endogenous carriage of *E. cloacae*, may result in abnormally high levels of this organism in the bowels of hospitalized patients, especially those who have received cephalosporins [8,9]. Plasmid-mediated quinolone resistance is increasingly reported worldwide for *Enterobacteriaceae* (5). This resistance is related to Qnr-like proteins belonging to the pentapeptide repeat family that protect DNA from quinolone binding to type II topoisomerase [10,11]. Various multidrug resistance (MDR) phenotypes that confer active protection against environmental toxic compounds by efflux mechanisms have been described in *Enterobacteriaceae* [12-14]. One of these drug ejection systems, the efflux detected in resistant gram-negative bacteria, depends on membrane energy and efficiently expels structurally unrelated antibiotic molecules across the bacterial envelope via a tripartite complex comprising an inner membrane pump, a periplasmic fusion protein, and an outer membrane channel [15,16].

Enterobacteriaceae involved in extra intestinal infections, (mainly *Escherichia coli*) are known to possess virulence-associated characteristics that distinguish them from random fecal isolates. A number of studies have elucidated the epidemiology and significance of these virulence-associated properties, including somatic antigens, adhesions, serum resistance, and production of enterotoxins, colicins, siderophores, and Hemolysin [13].

The aim of this research is to find out bacteriological characteristics and the effect of antibiotics on the growth of *Enterobacter cloacae*.

Materials and Methods

Samples Collection, Isolation and Identification

A total of three hundred samples from clinical and environmental sources (vaginal swabs, wounds, hands of the workers, patient's bed, surgical tools, and floor) were collected from hospitals of Diyala Province during the period from 27/08/2012 to 1/12/2012. Initial identification of strains of *Enterobacter* spp was done on the MacConkey agar and blood agar (Biolife, Italiana). Biochemical identification of isolates of *Enterobacter* spp was carried out by different biochemical tests and the identification was confirmed by using regular api20E and VITEK 2 system.

Detection of some virulence factors:

Ability *of Enterobacter cloacae* to produce some virulence factors was investigated and these factors were as follows:

Haemolysin production

The bacterial isolate was streaked on three types of blood culture plates, human blood agar, sheep blood agar and rabbit blood agar. Then the plates were incubated at 37ºC for 24 hours and the haemolytic activity was observed and the kinds of haemolysin (alpha, beta, and delta) were detected according to the type of blood that has been used [17].

Urease production

The bacterial isolate was inoculated heavily over the slope surface of urea agar medium, and incubated at 37^0C. The tube was examined after 4 hours and then after overnight incubation. No tube being reported negative until after 4 days of incubation. Urease-positive cultures changed the colour of the medium to purple-pink [18].

Siderophores

Minimum M9 *inoculated* with *E. cloacae.* The plates were incubation at 37 ºC for 48 hour. The bacterial growth was evidenced by the ability of bacteria to produce siderophores [19].

Bacteriocin production

The brain heart infusion used to activated the all isolates and grown TSA medium then incubated at 37°C for 24 hours. After incubation worked tablets by piercing cork in the middle, and placed on the surface of nutrient agar , that inoculums with amount (0.1 ml) of the bacterial isolates test, mentioned after comparing it with McFarland standard then incubated dishes at 37°C for 24 hr, results positive inhibition zones around the piece agar containing strain producer [20].

Detection of the bacterial ability to produce slime layer and biofilm formation

The ability of *Enterobacter cloacae* to produce slime layer and biofilm formation was tested by three methods; Tube method, Congo red agar method, and microliter tube method [21,22].

Antimicrobial susceptibility test

Fourteen antibiotics disks including β-Lactam group, quinolones group and aminoglycoside group have been used to test the sensitivity of *E. cloacae* by using the Mueller Hinton agar plates. The incubation was done at 37ºC. The interpretation of inhibition zones around the discs was according to the guidelines of the clinical and laboratory standards institute [23]. The inhibition zones were compared with the reference *Escherichia coli* ATCC 25922.

Effect of combination of antibiotics on the growth of *Enterobacter cloacae*

This method included a combination of three antibiotics (ciprofloxacin, streptomycin and cefotaxim), in three different concentrations to detect their effect on the growth of *E. cloaca.* The checkerboard titration method as described previously [24] for the determination of synergistic effect between ciprofloxacin, streptomycin and Cefotaxime for *E. cloacae.*

Detection of β-lactamase Production

The acidometric method for the detection of β-lactamase was described by Sykes and Matthew [25].

Detection of ESBL Production:

Disk approximation method described by Coudron *et al*. [26] was followed for detection of ESBLs in isolates. Disks containing 30 µg cefotaxime, ceftazidime, ceftriaxone, and aztreonam were placed 15 mm (edge to edge) from a disk of augmentin (20 µg amoxicillin

plus 10 µg clavulanate) and then incubated for 16-20 hr at 35°C. Any enhancement of the zone of inhibition between a β-Lactam disks and Augmentin disk gave an indication that the test isolate contains ESβL whose activity is inhibited by clavulanic acid. All bacterial isolates that were positive to β-lactamase production were tested for their ability to produce ESβL enzymes.

Detection of Metalloβ-Lactamase Production

A method used by Bhalerao *et al*. [27] was adopted in this study using two imipenem (10 mg) to be the distance between them 3cm, and then the 10ug EDTA solution to one of the drives of imipenem. Incubated at a temperature 37C° for (18-24) hr, after note areas of inhibition zone, increase of inhibition the zone above 7 mm on the disk Imipenem with EDTA compared with the Imipenem disk alone , the result is positive and bacteria is productive Metallo **β**- lactamase (MBL**).**

Determination of MICs of ESBL-Producing Isolates

The two- fold agar dilution susceptibility method was used for the determination of MICs of a number of different antibiotics, the ranges of appropriate dilutions of antibiotics for MIC determination were used as 0.5 -1024 µg/ml [23]. The MIC values were compared with the break points recommended by CLSI [23].

Plasmid profile (Plasmid DNA analysis)

Plasmid DNA of the one isolate has been extracted by using the Pure Yield™ Plasmid Miniprep Kit (Promega U.S.A). Plasmid DNA was analyzed by electrophoresis on 0.7% agarose gel containing 0.5µg/ml of ethidium bromide [28] and pass the electricity (7 volt ⁄ cm2) for 1-1.5 hr until the pigment arrives to other side of the agarose gel. The agarose has been tested by using ultra violate transilluminator in a wavelength of 302nm.

Curing of plasmid DNA

To determine if resistant gene is encoded by plasmid, acridin orange was used to eliminate the plasmid from the strain. The strains were grown with acridin orange (16, 32, 64, 128, 256, 512, and 1024 µg/ml) and then spread on Muller Hinton containing different antibiotics and the others was without. Replica plates for both media were incubated at 35ºC. Plasmid was

considered to be eliminated from those colons that to grow on antibiotics-free medium only [28,29].

Results and Discussions

Isolation and Identification of *E. cloacae*

The results showed that 115 samples (38.3 %) were negative for bacteriological test, while 185 samples (61.7 %) were positive. Out of 185 clinical and environmental samples, forty samples (21.6%) were belonging to Enterobacteriaceae. Diagnostic tests showed *E .cloacae* lactose fermented the smaller size of the colonies and semi-viscous and confer the identification by api-20E. The results showed that 10 isolates (25 %) were identifies as *E. cloacae.* Four (40 %) isolates of *E. cloacae* were isolated from vaginal swab samples, while one species (10 %) was isolated from wounds. Two isolates (40%) of *E. cloacae* were isolated from the patient's beds, while the samples from the floors did not show any pollution. In addition, two (40%) isolates from hands of the workers, and one (10%) isolate from surgical instruments sources. These results were comparable to the results of the study conducted by Shareef and Noore [30] who found 11.13 % of bacterial isolates collected from different surgical halls were identified as *E. cloacae.*

Detection of some virulence factors

Haemolysin and Siderophores Production

The results indicated that *E. cloacae* is a non-Haemolycine producer (Table 1), because it possesses special regimes to withdraw iron , digested and representation in tissue which is called the aerobactin system as it is producing Haemolysin alternative route in the absence of the aerobactin genes [31]. The rate of siderophores production by *E. cloacae* was 100% (Table 1) and these results agreed with those of Mokracka *et al*. [32] who found that the siderophores production by *E. cloacae* was 99%. The species of the family Enterobacteriaceae are divided into two groups, depending on their ability to produce aerobactin; the first group includes the genera *Proteus, Serratia* and *Salmonella* which have low ability to produce aerobactin, while the second group includes species with high rate to produce aerobactin like

E. coli. The isolates were able to configure siderophores system; they are capable of producing siderophores on blood agar. This result indicates the presence of a relationship between the production of haemolysin and composition of siderophores.

Biofilm production

Three methods were used to detect biofilm production, Congo red (or CRA), tube method and microliter tube method. Results showed that microliter tube method is the best method for the detection of biofilm formation. The results showed that *E. cloacae* was able to formation biofilm with percentages of 90%, 80%, and 90% when detected by Cong –red method , tube method and microliter tube method respectively (Table 1). This result agree well with the results obtained by Bunyan [33] as she reported that *K. pneumonia*e produce biofilm with 100% by using microliter tube method, but does not agree with the results of Al-Salihi [34] who reported a ratio of 22%. There are several possible environmental factors that affect the production of biofilm including, oxygen, temperature, and other factors [16].

Production of Urease and Bacteriocin

The involvement of *E. cloacae* in the production of urease means the lysis of urea and liberation of ammonia that works to raise the pH and in turn changes the color of phenol reagent from red to pink and this can be used as an evidence for the ability of bacteria to produce tis enzyme [35]. The results showed that *E. cloacae* did not produce enzyme (Table 1). Results have shown that 70 % *E. cloacae* produced bacteriocin. These results are very similar to those of Al - Dhumaina [36] who reported that *E. cloacae* produce Bacteriocin with 67% percentage. Production of bacteriocin coded by genes located on plasmid; bacteriocin gene, specific antibody HIV immunity gene and lysis gene [37].

Table 1: The virulence factors produced by *Enterobacter cloacae*

Virulence factors	Percentage (%)
Haemolysin Production	0
Siderophores Production	100
Urease production	0
Biofilm production	90, 80 ,90
Bacteriocin production	70

Detection of Production of β-Lactamase, Extended Spectrum β-Lactamase and of Metallo β-Lactamase

The results showed that 60 % of *E. cloacae* could produce β-Lactamase, while the rate of production of ESBs and Metallo β-Lactamase were 10%, 10%, respectively (Table 2). Sarojamma and Ramakrishna [38] reported that the production of extended spectrum β-Lactamase enzyme by bacterial cells in any hospital depends on various factors such as the use of antibiotics, through put of species producing among persons employed in hospitals, and type of sterilization units used in hospital, especially in intensive care units. The study carried out by Castanheira and others [39] on 39 isolates of Enterobacteriaceae showed it was resisted carpinem by *E. coli, E. cloacae* and *K. pneumonia*, isolated from India , 15 isolates carry genes $bla_{\text{NDM-1}}$ and 10 isolates carry $bla_{\text{OXA-181}}$ which coded for resistance of carpenim, and also possesses $bla_{\text{VIM-5}}$. (Table 2). The availability of some isolates which gave a negative results with β-Lactamase, Indicates the availability of other resistance mechanisms such as non-production β-Lactamase like target site, or having a barrier permeability and efflux pump [40,41].

Table 2: Percentage of β-Lactamase enzymes production by *Enterobacter cloacae*

β-Lactamase enzyme	Percentage of production (%)
β-Lactamase	60%
Extended Spectrum β-Lactamase	10%
Metallo β-Lactamase	10%

Antimicrobial susceptibility test

Sensitivity test of the isolates towards 14 antibiotics including β-lactams group, aminoglycoside, quninolos, sulfa group, nitrofurans and augmentin was done by measuring the diameter of clear zone and compare it with the information CLSI 2012 [23]. Results demonstrated that *E. cloacae* was resistant to cefixime, ceftazidime, aztreonam, cefotaxime, piperacillin and ampicillin by 90%, 80%, 70%, 80%, 80%and 80%, respectively. In addition, *E. cloacae* showed resistance to amikacin, gentamycin and tobramycin by 30%, 10%, and 10 %, respectively. Furthermore, the rate of resistance to ciprofloxacin group was 10 %, while the rate of the resistance to Co-trimoxazol and Augmentin was 50 %. Nitrofurans and imipenem have high effectiveness against *E. cloacae* (90 % and 100 %, respectively (Table

3). The relatively high percentages of antibiotic resistance among these ß-lactamase producing isolates indicate that these isolates possess an enzymatic mechanism of resistance. The major mechanism of antibiotic resistance in gram- negative bacteria that causing clinically significant infections was the expression of β-lactamase enzymes, of which there are several classes including plasmid-encoded and chromosomally encoded enzymes [42,43]. This may explain the bacterial resistance to aminoglycoside by three mechanisms which are: modulating molecule mediated enzymes modified adenylating, phosphorylating and acetylating, or mutation chromosomal such as a mutation in the gene encoded for the target protein in subunit (30S), causing the loss of anti-familiarity to link to a protein target and reduce permeability of the bacterial cell [44]. The cause of the resistance of the isolates to quninolos could be due to a change in the target site to link to counter the enzyme, as happens in the change *gyr* A which is one of the building blocks of an enzyme (DNA gyrase) [45].

Table 3: The percentages of antimicrobial susceptibility by *Enterobacter cloacae.*

Antibiotic	Percentages of susceptibility		
	S	I	R
Ampicillin	10%	10%	80%
Amikacin	90%	0%	10%
Augmentin	20%	30%	50%
Aztreonam	20%	0%	80%
Cefixime	10%	0%	90%
Cefotaxime	10%	10%	70%
Ceftazidime	20%	0%	80%
Ciprofloxacin	60%	30%	10%
Co-trimoxazole	50%	0%	50%
Gentamycin	70%	0%	30%
Imipenem	100%	0%	100%
Nitrofurans	90%	10%	0%
Piperacillin	20%	0%	80%
Tobramycin	10%	0%	90%

S= Sensitive I= Intermediate R=Resistant

Multiple Antibiotic Resistances

The spread of genes that carry the resistance to antibiotics is a problem that threatens the world's health. The plasmids contribute to the spread of this resistance are mediated by pathogenic bacteria [46]. The results showed that 3.3% of *E. cloacae* had the capacity of

multiple antibiotics resistance to 11 out of 14 antibiotics. Pieboji *et al.* [47] documented a significant difference in the spread of the pattern of multiple resistances to antibiotics among gram negative, bacteria isolated from inpatients and those isolated from outpatients. The resistance to antibiotics may be due to the random use of antibiotics, which increases bacterial resistance to various antibiotics [48].

Determination of Minimum Inhibition Concentration of a number of antibiotics

The results presented in (Table 4) indicate that the MIC values for amoxicillin ranged between 1024- < 1024 ug/ml. The high proportion of resistance to Amoxicillin may be related to one of these mechanisms; destroying antibiotic mediated β-lactamase or failure of antibiotics penetration and access to target location (penicillin binding protein) (PBPs) [49]. MICs value of cefotaxime ranged between 32 and < 1024**.** The high MIC values -may be related to the presence of several factor of resistance, especially in production of some enzymes such as β-lactamase (OXA- 4, OXA-5, OXA -6 OXA-7, OXA-10 [50,51]. The results showed that values of MIC of streptomycin ranged from 128 to 1024 ug/ml for *E. cloacae*. This resistance may be caused by the production of enzymes by the streptomycin-resistant bacteria that modify the antibiotic and thus loses its effectiveness or come as a result of the loss of some bacterial outer membrane proteins. Ciprofloxacin values of MIC recorded (2-64 µg /ml, while the values of MIC of nalidixic acid between (8- < 1024). Ribera and others [52] Pointed that the resistance of quninolos group as a result of mutations in the genes *par*C and *gyr*A responsible for the synthesis of enzymes (DNA gyrase), or through the mechanism of efflux pump.

Table 4: The values of the minimum inhibitory concentrations (MIC) of some antibiotics for *Enterobacter cloacae.*

Antibiotic	MIC Value / µg\ml	Break point µg /ml
Amoxicillin	1024- < 1024	≥16
Cefotaxime	32- < 1024	≥64
Streptomycin	128 -1024	≥32
Ciprofloxacin	2-64	≥4
Nalidixic acid	8- < 1024	≥32

Effect of Combination of antibiotics in *E. cloacae*

Combination of antibiotics by using the chess method described by Mandal *et al*. [53], calculated the coefficient of factors inhibition concentration (FIC) of the five isolates of *E. cloacae* that showed resistance to antibiotics. In this study, we combined ciprofloxacin, streptomycin and cefotaxime. Combination of antibiotics by 1:0, 5, 1:1, 1:2, and 1:3 and the ratios of Cefotaxime are fixed. The results showed that the 1:3 combination ratio of cefotaxime /streptomycin, cefotaxime /ciprofloxacin was the best, as evidenced by the impact resulting from the combination ratio of the isolates under study. (Tables 5 and 6) show a significant decrease in MIC values to antibiotics after combination of ciprofloxacin and streptomycin with cefotaxime when compared with the use of antibiotic alone. The results of combination of ciprofloxacin with cefotaxime showed a synergistic effect against four isolates (80%), while antagonistic effect against one isolate, as shown in (Table 5). The combination of streptomycin with cefotaxime was accompanied with a synergistic effect against four isolates (80%) and addition effect against one isolate (Tables 5 and 6). These results agreed with those of Farjadian and others [54] and those of Fish and others [55] who demonstrated the importance of the combination of ciprofloxacin and ceftazidime, which gave high efficiency in treating various injuries by *Pseudomonas aeruginosa*. The use of β-Lactam group with aminoglycosides group or fluorinated quninolos group gave higher effectiveness against Gram negative and positive bacteria than giving them individually. The ability of cefotaxime to destroy cell wall may reinforced the streptomycin which act as an inhibitor of manufacturing proteins in Gram negative bacteria, which produces synergistic impact [55].

Table 5: The effect of combination of Ciprofloxacin with Cefotaxime by (1: 3) in the values of MIC of *Enterobacter cloacae*.

strain	MIC Value of concentration 1:3				
	CTX µg\ml	CIP µg\ml	Combination µg\ml	FIC Index	Type of effect
E1	1034	64	16	0.26	Synergism
E6	256	8	4	0.26	Synergism
E10	32	2	4	2012	antagonism
E29	32	2	0.5	0.02	Synergism
E30	512	16	0.5	0.03	Synergism

* CTM = cefotaxime *CIP =ciprofloxacin * E = *E. cloacae*

Table 6: the effect of combination of Streptomycin with Cefotaxime by (1: 3) in the values of MIC of *Enterobacter cloacae*.

Isolates	MIC Value of concentration 1:3				
	CTX µg\ml	S µg\ml	Combination µg\ml	FIC Index	Type of effect
E1	1034	512	16	0.04	Synergism
E6	256	256	8	0.06	Synergism
E10	32	256	16	0.56	addition
E29	32	256	16	0.31	Synergism
E30	512	512	32	0.125	Synergism

* CTM = cefotaxime *S= streptomycin * E = *E. cloacae*

Plasmid profile and Plasmids curing

The content of plasmid was studied and the results showed that *E. cloacae1 c*ontain one mage band plasmid. The relationship between the content of plasmid and antibiotic resistance was studied by using plasmid curing and the results indicated that *E. cloacae* had lost band of plasmids when the isolates were treated with a grading orange at a concentration of 1024 μg /mL. In addition, the results indicated that all isolates showed resistance to ciprofloxacin and Co-trimoxazol, while they did not show resistance to other antibiotics such as Augmentin, amikacin, gentamycin, cefotaxime, and ampicillin. It is clear from these results that the genes responsible for the resistance to antibiotics Ciprofloxacin, and Co-trimoxazol may be located on the chromosome, while the genes responsible for the resistance to other antibiotics may be located on plasmids. The results regarding gentamycin and ampicillin are in agreement with the findings of Rasool *et al*. [56] who showed that the isolates lost their resistance to these antibiotics after using acridine orange.

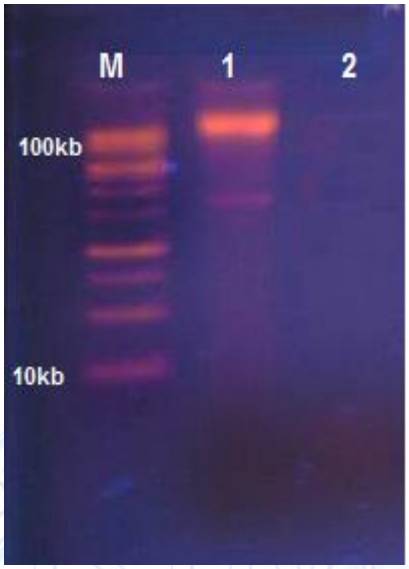

Figure 1: Agarose gel electrophoresis of plasmids from *E. cloacae*; Lane 1. Ladder DNA; 2 Plasmid content of *E. cloacae*. 3 Losing of plasmid band from *E. cloacae*, Lane

Conclusion

This study indicates that the spread of urinary tract infection (UTI) is caused by *E. cloacae.* This bacterium has differential levels of resistance to common antibiotics that used in treatment, and the highest resistance was to nitrofurans (100%) and Co-trimoxazol (90%) and consequently these antibiotics cannot be used in the treatment of UTI caused by *E. cloacae* in the future. The increased resistance of *E. cloacae* to antibiotics shown in this study can be linked with increasing plasmid bands.

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