Genetic study of relationship between resistance of Enterobacter aerugenes to some antibacterial agents and plasmid containinig

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دراسة وراثية للعلاقة بين مقاومة بكتريا *Enterobacter aerugenes* لبعض المضادات البكتيرية ومحتواها البلازميدي

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الخلاصة :

هدفت الدراسة الى تحديد العلاقة بين مقاومة بكتريا *Enterobacter aerugenes* الوراثية لبعض المضادات الحياتية ومحتواها البلازميدي. اذ تم جمع (70) عينة ادرار من اشخاص مصابين بالتهاب المجاري البولية حسب التشخيص السريري وتم الحصول على (15) عزلة تعود لبكتريا *Enterobacter aerugenes*. تم اجراء فحص المقاومة لمضادات البكتيريا بأستخدام (11) مضاداً بكتيرياً وكانت نسبة المقاومة لمضاد النايتروفيورانتين (100%) ، الترايميثوبريم (90%) ، اميكاسين (35%) ، نورفلوكساسين (45%) ، سيفوتاكزيم (50%) ، سفترياكزون(38%) ، كلورامفنيكول (64%) ، دوكسوسايكلين (80%) ، جنتامايسين (58%) ، سبروفلوكساسين (53%) ، اميبينيم (10%) . اشارت البيانات الى ازدياد نسبة اصابة المجاري البولية بهذه البكتريا . اظهرت البكتريا مقاومة كاملة لمضاد النايتروفيورانتين ومقاومة شبه كاملة لمضاد الترايميثوبريم مما يؤدي الى عدم استخدامها مستقبلاً . ارتبط مقاومة هذه البكتريا المختلفة لمضادات الحياة وراثياً بزيادة الحزم البلازميدية ، حيث كلما كان عدد الحزم البلازميدية أكثر كانت البكتريا أكثر مقاومة لمضادات الحياة المستخدمة في الدراسة .

Abstract

The present study aimed to determine the relationship between the genetic resistance of Enterobacter aerugenes to some antibacterial agents, and its plasmid containing. For this purpose (70) samples of urine were collected from patients with clinically diagnosed urinary tract infection, there were (15) isolate Enterobacter aerugenes. The Antibiotic susceptibility test was used through using (11) antibiotics and resistance bacteria Enterobacter aerugenes was nitrofurantion (100%) , trimethoprim (90 %) , amikacin (35 %) , norfloxacin (45%) , cefotaxime (50 %) , ceftriaxon (38 %) , chloramphenecol (64 %) , imipenem (10 %) , doxocyclin (80 %) , gentamycin (58 %) and ciprofloxacin (53 %) . The data indicated the increase infection of urinary tract infection that caused by Enterobacter aerugenes . There are two antibiotics nitrofurantion and trimethoprim must not use to treatment against Enterobacter aerugenes in the future because the bacteria gave high resistance these antibiotics. The resistance of Enterobacter aerugenes to antibiotics used in this study linked with increasing bounds of plasmid.

1. Introduction

Urinary tract infection is among the most common nosocomial and community acquired infections. information on prevailing levels of antimicrobial Resistance among common pathogens associated with urinary tract infection is useful in making and appropriate choice of empiric therapy [1] . Resistance to antibiotic treatment in patients with urinary tract infection is a representative example of the increasing proplem of antimicrobial resistance[2]. The bacteria *Enterobacter aerugenes* is apportunistic pathogen and its one types very important cause urinary tract infection and nosocomial infection in addition to wound and blood stream infections because it has multidrug resistance to antibiotics [3,4,5,6,7] . *Enterobacter aerugenes* is an important pathogen in hospital acquired infection. It generally exhibits resistance to avariety of broad-spectrum antimicrobial agents, including beta-lactamase[8]. The existence of aprevalent resistant clone of *Enterobacter aerugenes* has been reported[9] . *Enterobacter aerugenes*; anosocomial pathogen, is frequently exhibiting multidrug resistance mechanisms associated with achange in membrane permeability[10]
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2-Materials and methods

2 – 1 : Specimens

The study includes collecting (70) urine samples from some hospitals in Baghdad Governorate. Then the isolates were cultured on macConkeys and blood agar plates as well as used analytic profile index twenty enterobacteriacea (API20E) to identificate the level bacteria species .

2 – 2 : Antibacterial susceptibility testing

Antibiograms were tested according to (Bauer et al.(1966) [11] as the following:

1.Preparation of bacterial supernatant by use normal saline and compare turbidity bacterial supernatant with standard turbidity (MacCferland) that refer to about(1.5×10^8) cell/ml.

2.The cotton swabs were used to spreading part of bacterial supernatant on the plates Muller-Hinton agar. The antibacterial discs put on the isolate cultured in Muller-Hinton agar (five discs in one plate) by using sterile forces.

3.The plates Muller-Hinton agar were incubated in the incubater at (37c°) for (24) houre.The inhibition zones were measured Th 1 s were expressed as susceptible or resistant according to the (NCCLS,2007) [12].

2 – 3 : DNA plasmid extraction Used boiling method to obtained DNA plasmid according to (Holmes and Ouigly.1981) [13] as the following:

1.Transport (1.5 ml) from bacterial supernatant to abendrof tube (all one isolate alone) and separared by using microcentrefuge in speed (5000 rbm/minute) for (5) minutes.

2.Add (350µl) from solution Sucorose Tris-Hcl EDTA (STET) and (25µl) from solution lysozyme (10mg/ml) to the deposit and mixed solution by using vortex for (3 seconds).

3.The solution put in water bath(100 c°) for (40 second) and separated solution by useing microcentrefuge in speed (13000 rbm/minute) for (10) minutes.

4.Remove the viscouse pellet and add (40µl) from solution botassium acetate and (420µl) from isopropyl alchohol , this material mixed and save in (-20 c°) for (1-2) hour.

5.separate solution by using microcentrefuge in speed (13000 rbm / minute) for (15) minute and add (50µl) Tris-Hcl EDTA and became ready for the electrophoresis.

2 – 4 : DNA plasmid electrophoresis in gel agarose

The gel electrophoresis used in detection of plasmid DNA according to (Maniatis *et al* 1982) [14] as the following:

1.Preparation gel agarose in concentration(0.7%) by using Tris-Hcl boric acid EDTA (TBE).The gel agarose heated to the boiling degree and cooling in (45-50 c°) and add (10µl) ethidium bromide in concentration(0.5µg / ml).

2.The comb fixation in the slab to creat wells that contianinig the sample and add gel agarose carefully and abandon for (30 minute) to solding.

3.Remove comb from gel agarose carefully and fixation the slab in electrophoresis instrument and add Tris-Hcl boric acid EDTA to cover surface of the gel agarose.

4.Put (10µl) from the sample that will be tested in abendrof tube and add (5µl) loading buffer and mixed carefully.

5.The samples were put in wells and pass the electricity (5 volt / cm^2) for (1-2) hour until the pigment arrive to other side to the gel agarose.

6.The agarose test by using ultraviola 2 ısilluminator in wave length (360) nanometer.

3- Results and Discussion

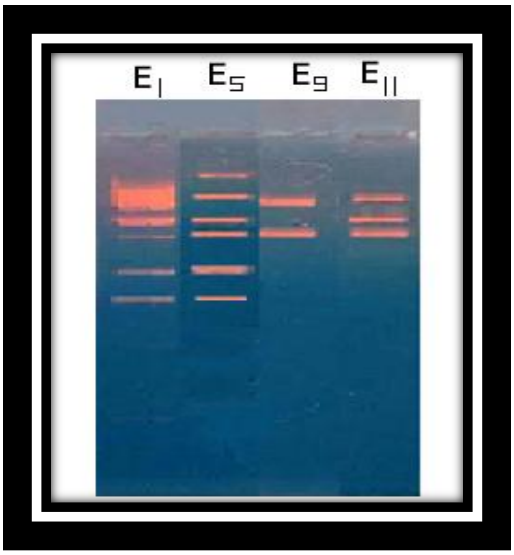
We received and examined (70) urine speciemens during the study period cultured the speciemens on the blood and macConkeys agar plates . Then after incubation period late lactose fermented will be taken , after that identification the isolates by analytic profile index enterobacteriacea (API20E) . The results were (15) isolates of *Enterobacter aerugenes* . Then antibiotics susceptibility test was used by using (11) antibiotics disks. The results clarved that the resistance nitrofurantion (100%) , trimethoprim (90 %), amikacin (35 %) , norfloxacin (45%) , cefotaxime (50 %) , ceftriaxon (38 %) , chloramphenecol (64 %) , imipenem (10 %) , doxocyclin (80 %) , gentamycin (58 %) and ciprofloxacin (53 %) , after that choice (4) isolates resistance to antibiotics in differential levels E_5, E_1, E_{11} and E_9 who appeared 10,8,6 and 4 respectively of antibiotic resistance. Extraction and gel electrophoresis of plasmid DNA were used for these four isolates and the results show that these isolates contained number of DNA plasmid bound differed from isolate to another (shape 1) . The isolate become more resistant when contain more bounds .

Table (1) Resistance the *Enerobacter aerugenes* to the Antibiotics

Bacteria	Antibiotics	%
Enerobacter aerugenes	nitrofurantion	(100%)
	trimethoprim	(90 %)
	amikacin	(35 %)
	norfloxacin	(45%)
	cefotaxime	(50 %
	ceftriaxon	(38 %)

	chloramphenecol	(64 %)
	doxocyclin	(80 %)
	gentamycin	(58 %)
	ciprofloxacin	(53 %)
	imipenem	(10 %)

containinig to the Plasmid :Shape (1)



isolates (E_1 , E_5 , E_9 , E_{11})

Urinary tract infections constitute one type of problems of the most frequently encountred conditions in clinical medical practice requiring antimicrobial therapeutic intervention . The research was done to known relationship between the presence of extended spectrum beta lactamase encoded plasmid (ESBL) and the drug resistance of *Enterobacter aerugenes* . This bacteria can show resistance to Gentamycin , Amikacin and Ciprofloxacine as well as a resistance to betalactam drug [15] . *Enterobacter aerugenes* have other mechanisims to resistance antibiotics like modification of outer memberane and change metabolic pathway may be resistant chloramphenicol , Trimethoprim and betalactam antibiotics [16,17] . Other mechanisim efflux pump may appear multidrug resistance to antibiotics [18,19] . The isolates *Enterobacter*

aerugenes in this study appear differential resistance to antibiotics used in this study and increase resistance with increasing number of bounds plasmid to this bacteria .

5- Conclusion

This study indicated the spread infection of urinary tract infection that was caused by Enterobacter aerugenes . This bacteria has 4 rential levels to resistance common antibiotics that used in treatment . more resistance wa itrofurantion (100%) and trimethoprim (90%) , these antibiotics can not be used in treatment of UTI caused by Enterobacter aerugenes in the future . The increase resistance Enterobacter aerugenes to antibiotics in this study linked with increasing plasmid bounds .

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