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

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لبعض المضادات البكتيرية Enterobacter aerugenes ومحتفها البلازميدي

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%) , doxycyclin (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 problem of antimicrobial resistance [2]. The bacteria Enterobacter aerogenes is an opportunistic 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 aerogenes is an important pathogen in hospital acquired infection. It generally exhibits resistance to a variety of broad-spectrum antimicrobial agents, including beta-lactamase [8]. The existence of prevalent resistant clone of Enterobacter aerogenes has been reported [9]. Enterobacter aerogenes; nosocomial pathogen, is frequently exhibiting multidrug resistance mechanisms associated with a change in membrane permeability [10].

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 enterobacteriaceae (API 20E) to identify 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 incubator at (37°C) for (24) hours. The inhibition zones were measured. The results 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 separated by using microcentrefuge in speed (5000 rbm/minute) for (5) minutes.
2. Add (350μl) from solution Sucrose 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 using microcentrefuge in speed (13000 rbm/minute) for (10) minutes.
4. Remove the viscous 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 containing the sample and add gel agarose carefully and abandon for (30 minute) to soldering.
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 abendoof 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 ultraviolet illuminator in wave length (360) nanometer.

3- Results and Discussion

We received and examined (70) urine specimen 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 clarified that the resistance nitrofurantion (100% ) , trimethoprim (90 %), amikacin (35 %), norfloxacin (45% ), cefotaxime (50 %), ceftriaxon (38 %), chloramphenicol (64 %), imipenem (10 %), doxocyclin (80 %), gentamycin (58 %) and ciprofloxacin (53 %), after that choice (4) isolates resistance to antibiotics in differential levels E5, E11, E11 and E9 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.

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Table (1) Resistance the Enterobacter aerugenes to the Antibiotics

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Antibiotics</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Enterobacter aerugenes</strong></td>
<td>nitrofurantion</td>
<td>(100%)</td>
</tr>
<tr>
<td></td>
<td>trimethoprim</td>
<td>(90%)</td>
</tr>
<tr>
<td></td>
<td>amikacin</td>
<td>(35%)</td>
</tr>
<tr>
<td></td>
<td>norfloxacin</td>
<td>(45%)</td>
</tr>
<tr>
<td></td>
<td>cefotaxime</td>
<td>(50%)</td>
</tr>
<tr>
<td></td>
<td>ceftriaxon</td>
<td>(38%)</td>
</tr>
</tbody>
</table>
Urinary tract infections constitute one type of problems of the most frequently encountered 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 aerogenes. This bacteria can show resistance to Gentamycin, Amikacin and Ciprofloxacin as well as a resistance to betalactam drug. \[15\]. Enterobacter aerogenes have other mechanisms to resistance antibiotics like modification of outer memberane and change metabolic pathway may be resistant chloramphenicol, Trimethoprim and betalactam antibiotics. \[16,17\]. Other mechanism efflux pump may appear multidrug resistance to antibiotics. \[18,19\]. The isolates Enterobacter
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 have different levels to resistance common antibiotics that used in treatment. More resistance was to nitrofurantoin (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.

References


