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Rate of induction of resistance in Fecal *E-coli* against cefquinome as compare to ceftriaxone after continuous passage in *vivo*

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Abstract:

The study was conducted to evaluate the development of resistance for fecal *E. coli* against third and fourth generations of cephalosporins . This bacteria was chosen because they represent the pool of resistance elements that are available for transfer to other bacterial species including pathogens.Three groups of 5 (mice) were used. 89.25µg/Kg) of ceftriaxon and(12.5µg/Kg) of cefquinome, were given a sub therapeutic dose orally for the first and second groups respectively. While the third group were given distilled water only as a control. Then isolation, purification and identification of fecal *E-coli* from GIT was done. Morphological and biochemical tests had been used to make sure that isolated bacteria was *E.coli*. It has been observed that the mean value minimum inhibitory concentration (MIC) of the isolated bacteria from both of first and second groups was compared with the control group.MIC of both antibacterials in comparison with the control group which were 1.18 , 1.37µg/ml for ceftriaxone ,cefquinome respectively. While the MIC values for ceftriaxone and cefquinome were 16.00 and 4.6 µg/ml respectively .this means that the significancy ($P<0.05$) was 13.55 folds and 3.35 folds in the third and fourth generation generations respectively in comparison with the control of each antibacterial .We concluded that antibiotic resistance may not be only a consequence in pathogenic bacteria but also in normal flora which could contribute this resistance to other microorganism.

Key word :MIC :Minimum Inhibition Concentration , PBPs: penicillin-binding proteins

معدل استحداث المقاومة في الاشيريشيا القولونية البرازيه ضد السفكوينوم مقارنة مع السفترياكزون بعد تكرار تمريرها داخل الجسم الحي

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الخلاصة:

أجريت هذه الدراسة لتَقييم تطويرِ المقاومةِ في الاشيريشيا القولونية البرازيه ، في جسم الكائن الحي ضد الجيل الثالث والرابع من السيفالوسبورين الشائعة الاستعمال في الإنسان والحيوان ، وقد اختيرت هذه البكتيريا لكونها تمثل محيط من

عناصر المقاومة والتي تكون متاحة إلى أنواع أخرى من البكتيريا وبضمنها المرضية. أخذت في البداية ثلاث مجاميع (فئران) كل مجموعة تحتوي على خمسه أفراد . أعطيت (89.25 مكغم/مل) من السفترياكزون و(12.5 مكغم/مل) من سفكوينوم كربع الجرعه العلاجيه مره واحده يوميا ولمده أسبوع ، للمجموعة اللاولى والثانيه على التوالي ، وتم اخذ المجموعة الثالثة كمجموعة سيطرة وإعطائها ماء مقطر فقط ولنفس المدة المذكورة أعلاه ، وبعد انتهاء الفترة المحددة تم عزل الاشيرشيا القولونية البرازيه وبعدها تم إعادة تأكيد هوية الجرثومة وبعدها تم تحديد قيمه التركيز المثبط الأدنى MIC للبكتيريا لكل مجموعة (الأولى والثانية) ومقارنتها مع مجموعة السيطرة . وكانت القيم MIC للمضادات الحيوية هي لمجموعة السيطرة هي 1.18 مكغم/مل للسفترياكزون و 1.37 مكغم/مل للسفكوينوم وبنفس الطريقة تم تحديد قيم MIC للمجموعتين الاولى والثانية بعد استحداث المقاومة لهما وكانت قيم MIC 16.00، 4.6 مكغم/مل للسفترياكزون والسيفكوينوم على التوالي وهذا يعني بأن الاهميه ضمن المستوى ($P<0.05$) كانت 13.55 ضعفا و3.35 ضعفا للجيل الثالث والرابع على التوالي بالمقارنة مع مجموعة السيطرة لكل مضاد حيوي . ولقد استنتج من هذه الدراسة أن مقاومة المضادات الحيوية قد لا تكون فقط للبكتيريا المسببة للأمراض ولكن أيضا تحصل للفلورا الطبيعية التي يمكن أن تسهم في نقل هذه المقاومة إلى الكائنات الحية الدقيقة الأخرى.

Introduction:

The increase in the number of resistant and multiresistant strains of bacteria is a major concern of health officials worldwide, particularly with the decline in the number of new antibiotics available for treatment. While much effort has been directed toward management and monitoring of antibiotic use and the prevalence of bacterial within communities (1,2). Furthermore, antimicrobial-resistant is spreading between different bacterial strains in different habitats has been demonstrated (3).

The normal flora contains antibiotic resistance genes to various degrees, even in individuals with no history of exposure to commercially prepared antibiotics. Several factors seem to increase the number of antibiotic-resistant bacteria in feces. One important factor is the exposure of the intestinal flora to antibacterial drugs. Antibiotics used as feed additives seem to play an important role in the development of antibiotic resistance in normal flora bacteria., (4). The knowledge of prevalence and patterns of antimicrobial resistance in generic *Escherichia coli* might indicate the pool of resistance elements that are available for transfer to other bacterial species including pathogens(5). Generic *E. coli* are used to monitor changes in prevalence and patterns of resistance as these commensal bacteria are regularly found in the gastrointestinal tract of animals and humans (6).

Ceftriaxone a third generation cephalosporins P, N and C, 7-amino cephalosporinic acid, and with the addition of side chain, it became possible to produce semisynthetic compound,Ceftriaxone is injectable cephalosporin agent,in addition .Ceftriaxone are highly resistant to staphylococcal β- lactamases producers and has a good activity against Gram positive and Gram negative bacteria (7) .it is considered to be the drugs of choice for many infections caused by members of the *Enterobacteraciae* (8). Ceftriaxone, a beta-lactam antibiotic is mainly bactericidal. It inhibits the third and final stage of bacterial cell wall synthesis by preferentially binding to specific penicillin-binding proteins (PBPs) that are located inside the bacterial cell wall.

Cefquinome is an aminothiazolyl cephalosporin extended spectrum beta-lactam and a member of the fourth generation cephalosporins, Cefquinome has been used only in veterinary medicine and only for individual treatment, and is safe and well tolerated. Formulations for the US will be available for parenteral injection, either as multiple or as single-dose products.(9). it is a zwitter-ionic compound with improved penetration into the periplasmatic space of Gram-negative bacilli and enhanced binding to penicillin-binding proteins .Aim of our study that is isolation and identification of fecal *E-coli*

from mice and determine the sensitivity by measuring the (MIC) against cefquinome and ceftriaxone and determination possibility of induction of resistance of these microorganisms against the above mentioned drugs in *vivo* by continuous treatment with sub therapeutic doses(1/4) in mice.

Materials and Methods:

To determine the rate of antibiotic resistance of *fecal E. coli* from healthy mice and to infer it is induced by antibiotic use by giving orally sub therapeutic dose , In the present studies white Swiss BALB/C mice were employed as the test animals,15 mice were taken and divided three groups respectively as following:

1- group one, 5 mice were given a sub therapeutic dose orally (89.25µg/Kg) of ceftriaxon , once daily for one week .

2- Group two 5 mice were given sub therapeutic dose orally (12.5µg/Kg)for cefquinome, giving once daily for one week (10)

3- Group three served are control five mice were given distilled water only for the same period . At the end of 7 days period, isolation, purification and identification of fecal *E-coli* from GIT was done by Fresh specimens of faeces (0.5 g) from 6 normal healthy mice were collected in sterile universal containers. They were transported quickly to the laboratory and processed within an hour of collection, cultured first on the brain heart infusion broth , incubated at 37°C, for 24hrs, transfer to macConkey agar secondly, and transfer to E.M.B agar. The diagnosis depends on the color, shape, size of colonies, also the confirmatory tests had been completed to make sure that isolates bacteria was *E.coli* after that determined the mean MIC to bacteria for each group (first and second**)** which compare with control group.

Results and Discussion:

Induction resistance in *vivo* by giving antibiotic orally at sub therapeutic dose(1/4) for one week.

An interesting observation in this study is the induction of resistance to third and fourth generation cephalosporins in *vivo* . In an experiment designed to induce resistance in *vivo* for fecal *E. coli* to cetriaxone and cefquinome , after exposure of susceptible microorganisms to sub therapeutic dose (1/4) of each of the two drugs for seven days .Then isolation, purification and identification of fecal *E-coli* from GIT was done. Morphological and biochemical tests had been used to make sure that isolated bacteria was *E.coli*., The results of MIC of the two antibacterials for control group (after giving distilled water only**)** estimated by tube dilution method are summarized in table 1 **.**These were 1.18, 1.37µg/ml for ceftriaxone , cefquinome respectively. This results showed that no significant difference in value of MIC with each drug because that third and fourth generation have been found to be effective against most major pathogens, including *E. coli* .

The same process after giving drugs orally (sub therapeutic dose) for induction of resistance the MIC values were set out in tables 2,3 and 4 they were 16.00 and 4.6 µg/ml for ceftriaxone and cefquinome respectively , which mean an increase of 13.55 , 3.35 folds respectively . The third generation showed significantly highest increase (13.55) folds , while the fourth generation showed the lowest increase (3.35) folds.These findings are in agreement with (11) found reduction in the proportion of sensitive coliforms excreted in pigs after chlortetracycline fed at concentrations of 10 or 20 grams per ton. (12), found in their study when used clindamycin, in short-term studies, cause disturbances in the composition of the gut microbiota as well as to select for resistance. These studies have mainly been based on data from isolates and have

indicated a normalization of the flora a few weeks following withdrawal of the treatment However, by using molecular approaches focused on the genus Bacteroides, it is found longterm shifts in the composition of the intestinal microbiota of individual subjects after a short-term administration of clindamycin (13). As mentioned before exposure of microorganism to different levels of antibacterial drug may result in increase in degree of resistance as reported before by many workers. (14) reported the use of broad spectrum antibiotics creates a selective pressure on the bacterial flora, thus increasing the emergence of multiresistant bacteria, which results in a vicious circle of treatments and emergence of new antibiotic resistant bacteria. The gastrointestinal tract is a massive reservoir of bacteria with a potential for both receiving and transferring antibiotic resistance genes, in the context that the reason for the development of resistance in *vivo* is due to replacement of sensitive serotypes by other already resistant serotypes.

At the same time, it is possible to monitor the acquisition and persistence of resistance genes in the community. Together this information should help to provide knowledge of the natural dynamics of the normal microbiota and help us to understand the long-term consequences of antimicrobial treatment. This information is of great importance for the implementation of rational administration guidelines for antibiotic therapies.

Table 1: the result value of MIC of two antibacterials after giving distal water only for one week

No of mice	Value of MIC µg/ml for ceftriaxone	Value of MIC µg/ml for cefquinome
Mice 1	1.25	1.25
Mice 2	2.5	2.5
Mice3	1.25	1.25
Mice4	0.31	0.62
Mice5	0.62	1.25
* Mean ± stander error	1.18 ± 0.37	1.37 ± 0.30

***Number represent mean ± stander error P>0.05**

Table 2 . show initial and final values of MIC of the ceftriaxone , after giving orally , contain sub therapeutic dose (1/4 therapeutic dose) for one week.

No of mice	Initial MIC µg/ml (giving D.W only)	final MIC µg/ml (giving sub therapeutic dose)
Mice 1	1.25	20.00
Mice 2	2.5	10.00
Mice3	1.25	20.00
Mice4	0.31	20.00
Mice5	0.62	10.00
* Mean ± stander error	1.18 ± 0.37 b	16.00 ± 2.44 a

***Number represent mean ± stander error**

Different small letters mean significant (P<0.05) result between concentration within group.

Table 3. show initial and final values of MIC of the cefquinome , after giving orally , contain sub therapeutic dose (1/4 therapeutic dose) for one week.

No of mice	Initial MIC µg/ml (giving D.W only)	final MIC µg/ml (giving sub therapeutic dose)
Mice 1	1.25	3.12
Mice 2	2.5	6.25
Mice3	1.25	1.56
Mice4	0.62	6.25
Mice5	1.25	6.25
* Mean ± stander error	1.37 ± 0.30 b	4.6 ± 0.98 a

***Number represent mean ± stander error**
Different small letters mean significant (P<0.05) result between concentration within group.

Table .(4-13) show values of MIC of the control group and two antibiotics in vivo ,after giving sub therapeutic dose for one week .

ANTIBIOTICS	Value of MIC µg/ml of control groups (giving D.W only)	Value of MIC after giving sub therapeutic dose for one week	Folds of elevation
Ceftriaxone	1.18 ± 0.37 b	16.00 ± 2.44 Aa	13.55
Cefquinome	1.37± 0.30 b	4.60 ± 0.98 Bb	3.35

Numer represent mean ± stander error.

Different small letters mean significant (P<0.05) result between within group.
Different capital letters mean significant (P<0.05) result betwee different groups. L.S.D: 2.9.

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