A Study on Relationship of Small Plasmid with Multidrug-Resistance Pattern in Pathogenic *E.coli* Local Isolates.

Zainab M. Naji, Haider Y. Ahmed And Hayder J. Mohamed
Department of Biology-College of Science-Al-Mustansiriyah University

Submitted : 29/03/2011 Accepted : 29/05/2011

الخلاصة

عزلت ثمانون عزلة سريرية لبكتريا اشرشيا القولون من مرضى مصابين بالتهاب المسالك البولية من مستشفى مدينة الطب في بغداد , حيث تمت دراستها للتحقق من مقاومتها ضد تسعة مضادات مايكروبية وذلك باستخدام طريقة الانتشار بالاقراص. غالبية العزلات المدروسة أظهرت مقاومة مختلفة للمضادات الحيوية المختبرة, فمقاومة الكلوكساسيلين و الترايمثبريم كانت 100٪ والأموكسيسيلين90 ٪. اما الجنتامايسين والسبروفلوكسين فكانت 60 ٪.

تم اختيار ثلاث عزلات هي المرقمه 2, 4, 7 لعزل البلازميدات باستخدام طريقة محورة عن طريقة العزل بالقاعدة دون استعمال انزيم الايسوزايم اوالفينول كلوروفورم. و قد وجد ان العزلات تحمل بلازميدات صغيرة يترواح وزنهاالجزيئي حوالي (2–4) كيلو قاعدي. تحيد البلازميدات في العزلات الثلاثة باستخدام صبغة الاكردين البرتقالي اظهر ان العزلات الطافرة خسرت محتواها البلازميدي بالاضافة الى مقاومتها للمضادات الحيوية بنسبة 80% الى كل من مضادات الجنتامايسين , أموكسيسيلين، كلوكساسيلين، و سيفتاكسيم في عزلة رقم 2 ونسبة 50 ٪ لمضاد الاموكسيسيلين والجنتامايسين في العزلتين رقم 4 و7.

ABSTRACT

Eighty clinical isolates of *Escherichia coli* isolated from UTI patient at Medical City Hospital were studied to investigate antimicrobial resistance to nine antimicrobial agents. The majority of multiple drug resistance isolates showed different antibiotics resistance by using disc diffusion method. The current isolates were resistance to Cloxacillin and Trimethprim 100%, Amoxicillin 90% while the Gentamycine and Ciprofloxine 60%. Afterwards, three isolates no.2, no.4, no.7, were subjected to plasmid isolation using modifying alkaline methods without Lysozyme and phenol chlorophorm. Isolates were carrying small plasmid estimated about (2-4) kb cured with Acridin orange , the mutated isolates loss their plasmid and antibiotic resistance about 80% to Amoxicillin Gentamycine, Cloxacillin,

and Cephtaxim in isolate no.2 and about 50% to Gentamycine and amoxicillin in isolates no.4 and no.7.

INTRODUCTION

Escherichia coli has been established as etiological agents of human gastroenteritis, urinary tract infection (UTI) and diarrhea disease of human [1]) this pathogenic bacteria resistant as a result of genetic mutations or acquisition of pre- existing genes that confer resistance [2] which occur either in the deoxyribonucleic acid (DNA) of bacteria chromosome or in the extra chromosomal transferable DNA called plasmid [3] .Phages and transposons are also able to transfer antibiotic resistance genes [4].

Intergenic and intragenic conjugal transfer of multiple antibiotic resistance genes was determined among bacteria [5] as well as transformation up take of extra cellular DNA [6].

The antibiotic resistant mutation arise spontaneously are generally resistant to only one antibiotic, however *E. coli* shown resistance to a number of older antimicrobial agents (Ampicillin, tetracycline,trimethoprim-sulfamethoxazole, chloramphenicol, and streptomycin) , kanamycin, gentamycin and nalidixic acid was also observed [7] as well as dramatic increase in resistance to the B-lactum with specific reference to the more recent cephalosporins to aminoglycosides [8],[9]

In this study we report the multi drug resistance (MRD) phenotype for the local resistance strain isolates from medical city hospital and plasmid related their resistancy by using alkaline procedure with out Lysozyme for extraction of plasmid.

MATERIAL AND METHOD

Bacterial strains: - All 80 isolates used in the study were isolated from 80 UTI patient of medical city hospital in Baghdad.

Susceptibility testing:- Antimicrobial susceptibility testing bacteria isolates was done using the disc diffusion method as described by the National Committee for clinical Laboratory Standards [10] the antimicrobial agents used were Cloxacillin **Cx** (30µg) , Nalidix acid **Nal** (30 µg), Cefatoxime **Ce** (30µg), Ceftriaxone **Cro** (30 µg) , Trimethprin **Tr** (5 µg) Gentamycine **G** (10 µg) Ciprofloxacine **Cp** (5 µg), Imipenem **IMP** (10 µg) and Amoxicillin **Amp** . (10 µg) .

Resistance was evaluated according to the reference zone diameter interpretation standers of NCCLS [11]

Plasmid curing:-Acridine orange for plasmid curing was used in this experiment, by incubating *E.coli* isolates overnight at 37C in loury broth (LB) with different concentration of acridine orange ranging from 100 to 500 µg \ ml [12] discreet single colonies were marked and sub cultured for antibiotics resistance the colonies that failed to show it resistances were selected as cured strain and screened for plasmid [13] .

Plasmid isolation: the alkaline plasmid method without Lysozyme was used for plasmid DNA isolation [13] and modified alkaline lysis procedure of plasmid without using phenol or chlorophorm [14] .

Electrophoresis of plasmid DNA: - 1% agarose in TBE buffer was used as described Maniatis [15]

RESULT AND DISCCUSION

Increase in drag resistance trend has been reported earlier [16] [17]. *E.coli* clinical isolates have often been used as an indicator of the dissemination of acquired resistance genes [1].

In this study all 80 UTI isolates were screened for their resistance profiles show different class of resistance of multiple drug resistance (MRD) mutants against 9 antimicrobial agents. (Cloxacillin, Nalidix acid, Cephtaxime, Ceftriaxone, Trimethprin, Gentamycine, Ciproflaxin, Amoxicillin and Imipenem).

The 9 antimicrobial agent tested showed various degrees of resistance against Cloxacillin and Trimethprin 100%. Amoxicillin 90%, while Gentamycine and ciprofloxine was 60%

All isolates were sensitive to Imipenem 10 µg .

In order to study the relationship of multi druge-resistant in *E.coli* isolates and their indigenous plasmid, three isolates were chosen No (2, 4, 7) for plasmid profile and curing experiment, depend on their MDR to antimicrobial agents

(Cx, Nal ,Amp ,Cro. Tr, Ce, G and Cp)

Plasmid profile results were obtained using the modified alkaline lysis procedure of plasmid without using phenol or chlorophorm [15].

Plasmid molecular weight in this experiment ranged from (2-3Kb) for the isolates no.(4 and 7) which harbor one plasmid fig. No. 1 E,F and (2-4.5) kb this agree with previous studies reported that a bacterial isolates carrying law molecular weight plasmids Ranging in size from 2.3- 30Kb, [18] ,[19] , [1] thus the inability of these isolates to harbor plasmid with

large molecular weight does not affect its ability to confer resistance to the various antibiotics [1] the result also show that isolate no. 2 harbor two plasmid [1] fig.No.1 A

Plasmid curing experiment with acridine orange in liquid culture showed elimination of plasmid corroborated by lost of resistancy for antimicrobial agents, 80% of the treated cells isolate (no.2) lost its resistancy to Gentamycine, Cloxacillin, Cephtaxim, and Amoxicillin. 50% of the curing Isolates (4 and 7) lost its resistance to Gentamycine and amoxicillin. Plasmid extraction for curd cells showed lost of plasmid for the three selected isolates (2, 4, and 7) fig. No.1, B, C, D The result show the successes of liquid method in plasmid curing [20]

The results in our study of *E.coli* UTI isolates may led to the suggestion that there may be the presence of multiple plasmid in the mutants or a plasmid carrying multiple resistance determinants [21] and these kinds of plasmid may be related to R- plasmid or R factor
which exhibited widely within the Enterobacteriacea *E.coli, Kelbsiella, Proteus, Pseudomonas and Salmonella* [2],[19],[20] [23] .

The evolution of multi-resistance antibiotic among Gram – negative bacteria has been widely reported as antimicrobial resistance change over time and some pathogens that were once considered routine to treat are developing resistance to almost all antibacterial agents. The continued mismanaged selective antimicrobials agents contributed towards the emergence of multiple drug resistant bacteria, and that has been regarded as an inevitable genetic response to antimicrobial therapy [24], [25]

Our suggestion that we need for periodic antibiotic resistance survey to help local physicians on the best treatment strategies to the current antimicrobial resistance patterns of UTI pathogens in our communities.

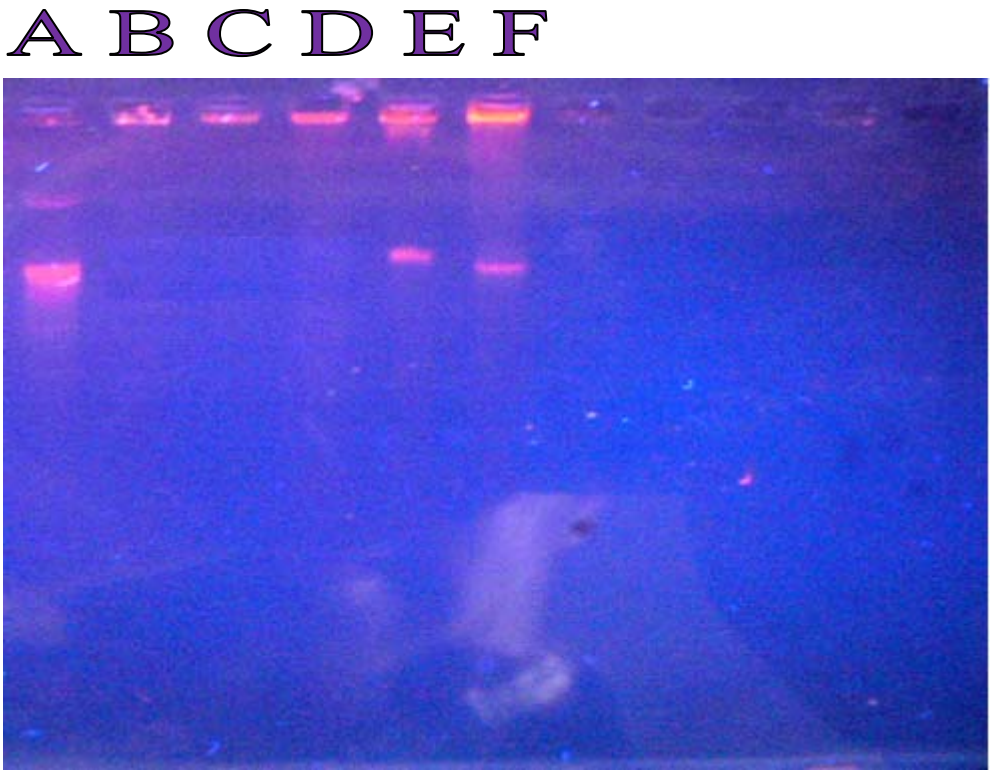

Fig.1 Agarose gel electrophoresis 1% of purified plasmid DNA for for original and curd *E.coli* MDR local isolates,
(A) – isolates no.2, (E) – isolates no.4 and (F) – isolates no.7.
(B), (C) and (D) represent the cured isolates for no.2, 4, and 7

REFERNCES

[1]-Uchechi N .Ekwenye and Erinmakaz." Investigating of plasmid DNA and antibiotic resistance in some pathogenic organisms". Afri J. of Biotech. vol.6 (7), 877-880. (2007).

[2]-Brown JC, Shanahon PMA, Jesudason MV, Thomas CJ and Amyes.SG," Mutation responsible for reducing susceptibility to 4-quinolones in clinical isolate of multi-resistant *Salmonella Typhi* in India". J. Anti chemo. 37: 891-900. (1996).

[3]-Summers .DK. "The biology of plasmids". Cambridge: Mass Blackwell Science Ltd. (1996).

[4]-Kokjohn TA "Transduction mechanism and potential for gene transfer in the environment".73-97. (1988).

[5]-Hasan M. Zahid , Zinat Mahal and Mamun R.Chowdhury." Intergenic and intragenic conjugal transfer of multiple antibiotic resistance determinants among bacteria in the aquatic environment of Bangladesh" .Afri J of Biotech. vol.8(2): 155-160 . (2009).

[6]- Stewart, G. J. The mechanism of natural transformation, 139–164. In S. B. Levy and R. V. Miller (ed.), Gene transfer in the environment. McGraw-Hill Publishing, New York, N.Y (1989).

[7]- Alessandro B., Lucia P., Marta B., Connie F., Yolanda V., Elisa G., Ana Liz V., Antonia M., Chiara L., Filippo B., Marianne S., Angela B., Herlan G., Hugo R., Torkel F., Göran K., Eduardo G., Franco P. and Gian Maria R."Multi- resistant commensal *Escherichia coli* in children, Peru and Bolivia" .Emerging infec.dis. Vol.12(6): 907 – 913. (2006)

[8]-Jacoby,G.A."Genetics of extended-spectrum beta-lactamase". Eur.J.Clin.Microbiol.Infect.Dis.,23(suppl.1):2-11. (1994)

[9]- Stratchounski L., Abrarova E., Edelshtein I., Suvorov M., Reshedko G., Krechikova O.,Iljina V., Gugutsidze E., Furletova N. and Petrochenkova N. , " Antimicrobial resistance pattern of gram negative urinary tract pathogens isolated from outpatients in Russia" In: proceedings of the 9th European Congress .Berlin. March 21-34, (Poster#PO344) (www.antibiotic.ru). (1999)

[10]- National Committee for Clinical Laboratory Standards,."Methods for dilution antimicrobial Susceptibility Test for Bacteria that grow Aerobically" .4th Edn, Approved standard M7-A4. National committee for Clinical Laboratory Standards, Wayne,Pa(1997).

[11]- National Committee for Clinical Laboratory Standards," Performance standards for Antimicrobial susceptibility Testing Ninth Information Supplements" NCCLS Document M100-S9. National committee for Clinical Laboratory Standards, Wayne,Pa. (1999).

[12]- Fujii T, Takeo M, Maeda Y., "Plasmid- encoded genes specifying aniline oxidation from Acinetobacter sp.starin ". Microbiology 143: 93-99. (1997).

[13]-Biarmbaim HC,Doly J " A rapid alkaline extraction procedure for screening recombinant DNA" .Nucleic Acid Res.7:1513-1523 .(1979).

[14]-He,M.,Wield,A.and Kaderbhai,M.A". A simple single – step procedure for small scale preparation of *Escherichia Coli* plasmid". Nucleic Acids Res.18, 1660. (1990).

[15]- Maniates, T., E.F.Fritsch and J.Samborck,"Molecular cloning, A laboratory manual.", Cold Spring Harbor ,New York. (1982).

[16]- -Rasool , SA., Ahmed B. and Iqbal. A. " Multiple drug resistance among indigenous clinical *Staphylococci* In vivo manipulation and characterization of the R-plasmids." Proc.Pak.Congr.Zool., 13:439-447.(1993).

[17]-Ghazala,S.S." Plasmids of indigenous *Pseudomonads* : Characterization and gene manipulation." Ph D thesis University of Karachi .pp1-190. (2000).

[18]- Ogunledun,A., Daini O.A. and Sule- Odu A.O, Amballi A.A. ,Fakoya A.O.,Iwalokun b.A."Antibiotic resistance and R- Plasmids of *K.Pneumoniae* in asymptomatic bacteriuria." Afr.J.Med.Pharm.Sci. 1:27-34. (2000).

[19] Franiczek R., DolnaL,Kryzanowska B.,Szufnarowski K.,Kowalska-Krochmal B. and Zielinska M.."Conjugation transfer frequency of resistance gene from ESBL- produces Enterobacteriacea strains isolated from patients hospitalized in pediatric words."(in Polish). Medycyna doswiadczalna i Microbiologai.58 (1): 41-51. (2006).

[20]-Abdel–Salalm,M.S., Abdel–Salam,A.Z., Ibrahim,S.A., Abosereh,N.A and Sa'eb,A.T.M." Resistance plasmid of indigenous *Pseudomonas* in Egypt." .J. Applied Science Research, 3(9):873-878. (2007).

[21]- Martínez-Martínez, L., Hernández-Allés, S., Albertí, S., Tomás, J. M., Benedí, V. J. and Jacoby, G. A. ". In vivo selection of porin – deficient mutant of *Klebsiella pneumonia* with increased resistance to cefoxitin and expanded spectrum cephalosporins." *Antimicrob Agents Chemother* ., 40:342-348.(1996).

[22]-Okeke IN, Lamikanra A. and Edelman R.Socio- "economic and behavioral factors leading to acquired bacterial resistance to antimicrobial agents in developing countries." Emerg. Infect Dis., 5:18-27. (1999).

[23]- Deniyi B.A.A, Amajoyi C.C and.Smith S.I ."Plasmid profile of multidrug resistant local uropathogenic *Escherichia coli*, *Klebsiella* spp., *Proteus* spp. and *Pseudomonas* spp.Isolates .J Bio.Sci. 6(3): 527-531.(2006).

[24]-Sahm, D.F., Thormsberry C., Mayfield D.C., Jones M.E., and Karlowsky J.A.".Multidrug resistant urinary tract isolation of *E.coli* .Prevalence and patients demo- graphics in the united State in 2000" . Antimicrobs. Agent. Chemother., 45:1402-1406. (2001).

[25]-Iroha I.R , Oji A.E. and Esimone C.O." Antimicrobial resistance pattern of plasmid –mediated extended –spectrum B- Lactamase producing strain of *Escherichia coli".* Sen.Res and Assay.,vol 3 (6) : 215-218.(2008).