Efficacy of Combination of Meropenem with Ciprofloxacin, and Nitrofurantoin Against Resistant E. coli Isolated from Patients with Urinary Tract Infections: In vitro Study

Maysaa ali abdul khaleq[1], Abdulkareem H. Abd[2], Ihsan Salah M.R. Rabeea[3], and Thuraya J. Mohamed[4].

[1] Department of pharmacotheraputic College of Pharmacy, Al-Mustansiryh University, E. mail maysaa_ali82a
[2] Department of pharmacology College of Medicine, Al- Nahrain University.
[3] Department of pharmacology & Toxicology College of Pharmacy, Kufa University.
[4] Department of Teaching Laboratories Al-Kadhemia Teaching Hospital.

الخلاصة

جمعت خمسة وسبعون عزلة من الاشيريشيا القولونية من ادرار مرضى المجاري البولية الذين راجعوا مستشفى الكاظمية واليرموك التعليمي في بغداد للفتره من ٢٠٠٩/١١/٢٢ الى ٢٠١٠/١٥/٣ ومنهم تم اختيار خمسة وعشرين عزلة اعتماداً على ما أبدته من مقاومة عالية و متعددة للمضادات الجرثومية ثم حددت التراكيز المثبطة الدنيا (MIC) للمضادات (الميروبنيم النايتروفيورانتوين ، و السبروفلوكساسين) وقد أظهرت النتائج بان مضاد الميروبنيم هو الا ٌكثر فعالية و ذلك بتثبيطه نمو البكتريا بأقل تركيز مقارنة بالمضادات الأخرى.

تضمنت هذه الدراسة استقصاء تأثير اتحاد المضادات الحيوية ضد (25) عزلة من *E. coli* (*in vitro*) وقد أظهرت النتائج أن مزج الميروبنيم مع بقية المضادات الحيوية (السبروفلوكساسين ، النايتروفيورانتوين) يشير الى تاثير تازري عالي عند استعمال ربع التركيز المثبط الادنى (MIC) لكل مضاد حيوي . بينما اتحاد النايتروفيورانتوين مع السبروفلوكساسين في بعض العزلات يشير الى تأثير اضافي فقط عند استعمال نصف التركيز المثبط الادنى (MIC) لكل مضاد حيوي.

شملت ا ٌلدراسة ايضاً دراسة ا ٌلنمط البلازميدي ل (25) عزلة من ال *E. coli* بأستخدام عدة لعزل البلازميد بواسطة نظام ال Miniprep وقد أظهرت النتائج بأن العزلات (٣٢،٣٧،٥٧،٦) حاملة لبلازميد مقاومة ألمضادات الحيوية.

Abstract

Seventy five E. coli isolates were collected from urine of patients with urinary tract infections in AL-Kadhumia and AL-Yarmook teaching hospitals in Baghdad for a period between 22/11/2009 to 15/3/2010, from these samples twenty five isolates were selected according to their pattern of the highest resistance as these showing multi-drug resistances and tested to specify their minimum inhibitory concentration for (meropenem, nitrofurantoin, and ciprofloxacin), meropenem was found having the lowest MIC comparing with others.This study also includes in vitro effects of various combinations of three types of antimicrobials (meropenem, nitrofurantoin, and ciprofloxacin) against twenty five E. coli isolates. Among combinations the combination of meropenem with the other types of antimicrobials showed high synergistic effect when 1/4+1/4 MIC for each antimicrobial were used. While combinations of nitrfurantoin with ciprofloxacin in some isolates showed additive effect when 1/2+1/2 MIC for each antimicrobial were used. The plasmid profile for the (25) E. coli isolates were studied using Pure Yeild ™ plasmid Miniprep system- Cat.# A1220 – Promega- USA. In order to determined the presence of plasmid for antimicrobials resistance.

Introduction

Urinary tract infections (UTIs) are one of the most common bacterial infections in humans both in the community and hospital setting (1). Escherichia coli have been documented to be the most important pathogen associated with symptomatic urinary tract infections (2).plasmid DNA molecule is separate from, and can replicate independently of, the chromosomal DNA.(3)

In this study we use combination of meropenem (which is a broad spectrum antimicrobial agent with more activity against gram-negative bacilli and less activity against gram-positive cocci than is imipenem) (4), with nitrofurantoin (which is only drug clinically proven for use against E.coli or Staph saprophyticus), and ciprofloxacin (which is abroad spectrum antimicrobial agent that is active against gram positive and gram negative bacteria) (5).

Material and Methods

The E. coli identification depended on morphological, biochemical testes in addition to API 20E system. Susceptibility of isolates to seventeenth antimicrobials was tested using disk diffusion assay according to modified Kirby–Bauer method (6). Meropenem, nitrfurantoin , amikacin and imepenem were to be the most effective antimicrobials, while the other antimicrobials were less effective. Minimum inhibitory concentration (MIC) was determined using tubes dilution method (7). The combination of antimicrobials weather it's synergistic, additives, antagonistic, or indifference depending on the fractional inhibitory concentration (FIC) was determine as follow: (≤0.5) synergism, (0.5–<1) additive, (1–<4) indifference,(≥4) antagonism, and calculated using the following equation (8).

$$FIC = \frac{\text{MIC for antibiotic in combination}}{\text{MIC for antibiotic alone}}$$

Plasmid DNA isolated using Pure Yeild ™ plasmid Miniprep system, according to the manufacture manual. Then the extracted plasmid DNA was loaded in 0.8% agarose gel stained with ethidium bromide and electrophoresis for 60 minutes at 2V/Cm using 1X TBE buffer. Then agarose gel was visualized using UV-transluminator.

Result and discussion

Colonies of E. coli had marked as a flat smooth and pink in color as a result of lactose fermentation in the media on MacConky agar, while on blood agar it gave small pink convex colonies surrounded by zone of β- haemolysis. In Microscopic Examination it showed as small single bacilli non spore forming with red color (gram –negative bacteria), it occurred separately and singly, but often they are accumulated in groups. The result of biochemical tests for most of E. coli showed its ability to catalase production and lactose fermentation while it gave a negative result in Oxidase, Urease and Simmon Citrate tests. Further identification of the isolates was done by using Api 20E system, as in Figure (1).

Antimicrobial Sensitivity Test

1-Qualitative Method (Disc Diffusion Test)

In this study we found that antimicrobials sensitivity among E. coli isolates varied according to the nature of antimicrobials. The percentage of resistant isolates to each antimicrobial is shown in Figure (2).

Standard disc diffusion assay was used to detect the sensitivity of pathogenic bacteria and results obtained were compared with those of Clinical and laboratory standard institute [9].The results of the current study (Figure 2) revealed that most of E. coli isolates resist the β- lactam antimicrobials (like ampicillin and amoxicillin) [10].noted the high resistance rates of gram positive and gram negative species to penicillins and some of cephalosporins. Increasing of bacterial resistance rates to this group of antimicrobials may be a result of either production of β-lactamase enzyme that had the ability to destroy the β- lactam ring in these antimicrobials [11, 12]. Also it may be due to minimizing the interaction of antimicrobials with target site (Penicillin Binding Proteins) [13].Augamentin (amoxicillin + clavulanic acid) had more activity than other penicillin due to its presence of clavulanic acid, which inhibit β- lactamase enzyme, and increase the spectrum of amoxicillin against gram- positive and gram- negative bacteria [14].

Many research illustrated the higher activity of imipenem and meropenem (related to carbapenems group) against gram- positive and gram- negative bacteria [15] .

Regarding aminoglycoside group, amikacin was more active than gentamicin on the current E. coli isolates, many researches showed that the increasing resistance against aminoglycoside group was due to production of the modified enzymes and losing outer membrane pores, which are responsible of permeability of surface cell layer to antimicrobials [16]. The current results (Figure 2) was in agreement with that of Shevelev et al. (2002) [17] who found in a study that the resistance percentage of the isolates to amikacin was (0%) , while the resistant rate to gentamicin was (48.6%). The results also was in agreement with Bashir et al.(2008) [18] who found in a study in Pakistan that the resistance percentage of the isolates to gentamicin was (49%) . Resistant to tobramycin was (40.7%) and this result was near that found by Pape et al. (2004) [19] who found that the resistant percentage of E. coli to tobramycin was (30%).

Many studies were illustrated the activity of naldixic acid, and most of quinolones antimicrobials against wide range of bacteria that were in a good agreement with the currently result. For example the resistant rate to ciprofloxacin was (40.7%) this result was comparable to the result of Shamm et al.(2001) [20] found in a study that the resistant percentage of E. coli to ciprofloxacin was (39%).

Resistance to pipracillin was (85.5%), this result was in agreement with that of Bujdakova et al.(1998) [21] who found that (86%) of E. coli isolates resistant to pipracillin , and this may be due to the ability of E. coli to develop resistance to these antimicrobials through the production of β-lactamase enzyme which break the β-lactam ring of pipracillin.

Resistance to nitrofurantoin was (2.6%), this result was in agreement with Akyar (2008) [22] who found that the resistant rate of E. coli against nitrofurantoin was (3%).

Resistance to trimethoprim/ sulfamethoxazole (SXT) was (43.4%), this result may be attributed to the wide use of (SXT) as empirical therapy for urinary tract infection, however this result was in agreement with Gupta; Hooton and Stamm (2001) [23] who found that the resistance to (SXT) among E. coli isolates from patient with UTIs has increased, with a prevalence of resistance which is reported 30 to 50 percent.

2- Quantitative Method (Minimum Inhibitory Concentration) (MIC)

Table 1 showed that MIC of meropenem ranged from (0.003-12.5µg/ml) this result was in agreement with Marie et al. [24] who found in his study that E. coli was moderately susceptible to meropenem at MIC (8µg/ml) .

The results of this study also showed that the MIC of nitrofurantoin ranged from (3.125 to 25 µg/ml), Garau (2008) conclude that microorganisms considered susceptible to nitrofurantoin if their minimum inhibitory concentration (MIC) was (32 µg/ml) or less [25] . Resistance to nitrofurantoin may be chromosomal or plasmid mediated and involves inhibition of nitrofuran reductase. Acquired resistance to nitrofurantoin in E. coli continues to be rare [26]. But MIC of ciprofloxacin ranged from (25-800 µg/ml), this result was compatible with Muhammad Asif who found in his study that the MIC of Ciprofloxacin in E. coli was rang from (1-256 µg/ml) [27].

3- Antimicrobials Combination:

The result in Table (2) shows that the synergistic effect noticed from combination of meropenem with ciprofloxacin on isolate No. (1, 2, 3, 4, 7, 10, 13, 24, 28, 37, 41,42,43, 44,45,47, 51, 55, 57, 58, 67) of E. coli , this result was in agreement with Sueke (2010) and Neu (2005), whose found that the synergistic effect obtain from combination of meropenem with ciprofloxacin against E. coli isolates [28][29]. While isolate No. (6) show additive effect of meropenem with ciprofloxacin combination .

The synergistic effect also noticed from combination of meropenem with nitrofurantoin (Table 3) when its effect tested on isolates No. (2, 3, 4, 7, 10, 13, 24, 28, 32, 37, 41,42 ,43, 44,45,47, 51, 55, 57, 58, 67) while isolate No. (1) show additive effect but isolates No. (6, 11 and 35) showed no effect when use 1/2+1/2 MIC for each antimicrobials .This result may be attributed to compatibility of both strains to resist both antimicrobials.

Extraction of Plasmid DNA

The result of Figure (3) indicate that each of the isolates (A6 , A37)containing two bands of plasmid DNA with approximate molecular weight (2000 and 1900) bp comparing with molecular weight marker. Also, isolates No.(A32, A57) containing one plasmid DNA with approximate molecular weight (2000) bp when comparing with molecular weight marker.

There are many studies referred to the isolation of antimicrobial resistance plasmid from E. coli. Joseph et al. (2001) [30] found in their study that E. coli isolates contain plasmid coding for resistance of aminoglycoside antimicrobials, including gentamicin and tobramycin. Also, March Galimand et al. (2003) [31] found in their study that E. coli isolated from patient suffering from urinary tract infection contain plasmid coding high level of resistance to aminoglycoside.

Piddock (1999) [32] found in his study that E. coli contain plasmid coding for resistance of flouroquinolone .Sisson et al. (2002) [33] found in their study that resistance to nitrofurantoin may be chromosomal or plasmid mediated. Minch chau phuc Nguyen et al. [34] found in their study that the plasmid gene that confers resistance to azithromycin had recently emerged in non multidrug resistant E. coli; Philippon; Arlet and Jacoby (2002) [35] found in their study that E. coli contains plasmid coding for resistance of ampicillin. In the other hand, other E. coli isolates that show no plasmid may be due to carrying plasmids with low copy number.

Reference

1-David, S.; and Howes ,MD.Urinary tract infection in female;2009;27.
2- Musa-Aisien, AS.; Ibadin ,OM.; and Ukoh ,G. Prevalence and antimicrobial sensitivity pattern in urinary tract infection in febrile under-55 at a childrenś emergency unit in Nigeria.Annual of tropical pediatric; 2003;23:39-45.
3- Lipps G. Plasmids: Current Research and Future Trends; Caister Academic Press;2008.
4- Henry, F.; and Chambers, MD. Basic and clinical pharmacology .10th ed. In: Bertram G. and Katzung M.D. Lang medical book /Mc Graw Hill;2007; PP. 726 -770.
5- Laurence, L.; Brunton ,Phd.; Keith, L.; Paker, MD.,Ph: In Goodman and Gilmanś .Manual of pharmacology and therapeutics. 11th ed. Mc Graw- Hill companies;2008; PP.707.
6-Vandepitte, J. ;and Verhaegen, J.; Engbaek, K.; Rohner, P.; Piot ,P.; Heuck ,C.C. Basic laboratory procedures in clinical bacteriology. 2nd ed. World health organization;2003; PP.30 :109-115.
7- Michael, J.; Leboffe and Bruton, E.; Pierce. A photographic atlas for the 3ed ed. Microbiology laboratory /Douglas, N. Morton, Inc.2005.
8- American society for microbiology. Instructions to Authors.S.I.: Antimicrob. Agents chemother;2002; 46:i-xix.
9- Clinical and laboratory standard institute (CLSI) .Performance standard for antimicrobial susceptibility testing. 17th informational supplement; Document M100-S17; Clinical and laboratory standards institute;2007;Vol.27.No.1.
10- Akther, J.; Qutub, M.; and Qadri, S. Antimicrobial susceptibility testing and patterns of resistance at tertiary care center in Saudi . Med. J.; 2001;22(7):569- 576.
11- Gupta, K.; Scholes, D.; and Stamm, W. Increasing prevalenceof antimicrobial resistance among pathogens causing acute uncomplicated cystitis in women. JAMJ;1999; 281:736-738.
12- Murray, P.R.; Baron, E.J.; Pefaller, M.A.; Tenover, F.C.; and Yolken, R.H. Manual of clinical microbiology. 7th ed. ASM press. USA.;1999.
13- Clark,W.G.; Brater, D.C.; and Johnson, A.R. Medic pharmacology Gothś in to chemotherapy mechanisms of antimicrobial. International press;1992.
14- Normann,P.; Ronse, E.; Nass ,T.; Duport, C.; and Labia, R. Characterization of a novel extended-spectrum β- lactamase from Pseudomonas areuginosa. Antimicrob.Chemother;1993; 37(5): 962-969.

15- Gales, AC.; Sader, HS.; And Jones, RN. Urinary tract infection trends in latin American hospitals: report from the sentry antimicrobial surveillance program (1997-2000). Diagnostic microbiology and infect Dis.;2002;44:289-299.

16- Vatopoulos, A.; Tsakris, A.; Tzouvelekis, L.; Legakis, N.; Pitt,T.; and Komninou, Z. Diversity of aminoglycosides resistance in Enterobacter Cloacaein Greece .J. Clin.Microbiol. Infect.Dis.;1992 ; 11(2):131-138.

17- Shevelev, A.; Reshedko, G.; Edelstein, I.; Kozlova, L.; Korovina, N.;Zorkin, S.; Katosova, L.; Papajan, A.; Marusina, N.; Alumetova, L.;Vjalkova, A.; Agapova, E.;and Fokina, B.Mechanisms of resistance to aminoglycosides (amg) E. coli isolates from children with community –aquired urinary tract infections, 4th European congress of chemotherapy and infection. Paris; France; 2002.

18- Bashir, MF.; Qazi, JI.; Ahmad, N.; and Riaz, S. Diversity of urinary tract pathogens and drug resistant isolates of Esherichia coli in different age and gender groups of Pakistanis. Tropical journal of pharmaceutical research; 2008;7(3): 1025-1031.

19- Pape, L; Gunzer, F; Ziesing, S; Pape, A; Offiner, G; and Ehrich, JH.Bacterial pathogens, resistance patterns and treatment options in community acquired pediatric urinary tract infection. Klin padiatr; 2004;216(2): 83-86.

20- Sahm, D.F.,C.; Thornsberry, C.; Mayfield, D.C.; Jones, M.E.; and Karlowsky,J.A. Multi- drug resistant urinary tract isolates of Esherichia coli : Prevalence and patient demographics in united states in 2000.Antimicrob.Agents; Chemother;2001;45:1402-1406.

21- Bujdakova, H.; Lausova, A.; Jankovicova, S.; Prodinger, W.M.; Kallova, J.;Milosovic, P.; and Kettner, M. Study of β-lactam resistance in ceftazidime resistant clinical isolates of enterobacteriaceae. J.Antimicrob. Agents ;1998;10: 136-414.

22- Akyar, I. Antibiotic resistance rates of extended spectrum beta lactamas producing Escherichia coli and Klebsiella spp. Strains isolated from urinary tract infection in a private hospital. Microbiyol Bul. National center of biotechnology information,U.S. National library of medicine; 2008.

23- Gupta, K.; Hooton, TM.; and Stamm, WE. Increasing antimicrobial resistance and the management of un complicated community acquired urinary tract infections. Ann.Internal medicine;2001;135: 41-50.

24- Mari- Frederique; Laurent poirel; Claire poyart; Helene reglier- poupet; and Patrice nordmann. Ertapenem resistance of Escherichia coli.;2007;Vol.13.Number 2.

25- Garau: " Other antimicrobials of interest in the era of extended spectrum beta-lactamases: Fosfomycin, Nitrofurantoin and Tigecyclin". Clin. Microbiol. Infect;2008; PP. 1:198-202. suppl. 14.

26- Calla, Mc. DB.; Kaiser, C.; and Green, MHL : Genetic of nitrofurazone resistance in Escherichia coli. J Bacteriol ;1978; pp.133:10-16

27- Muhammad Asif: studies on prevalence, characterization and development of resistance in clinically significant gram negative bacilli against commonly used antibiotics. Biological sciences;2003; PP.238.

28- Sueke, HM.: An in vitro investigation of synergy or antagonism between antimicrobial combination against E. coli isolates;2010; pp.190-222.

29- Neu, H.C.: Synergy and antagonism of combination with quinolones; European journal of clinical microbiology and infectious diseases; 2005; PP.255- 261; Vol.10.

30- Joseph, W. Chow; Vivek, kAk.; IIyou;Susan, J. Kao; Joanne Petrin; Don,B. Clewell ; Stephen, A. Lerner; George, H. Miller; and Karen, J. Shaw Aminoglycoside resistance genes aph (2)-Ib and aac-Im detected together in strains of both Escherichia coli and Enterococcus faecium. Antimicrobial agents and chemotherapy; 2001; Vol.45.PP. 2691-2694.

31- MarcGalimand;PatriceCourvalin;andThierryLambert.Plasmid-mediated high – level resistance toaminoglycosides in Enterobacteriaceae due to 16S rRNA methylation, antimicrobial agents and chemotherapy; 2003;Vol. 47.PP.2565-2571.

32- Piddoch, LJ. Mechanisms of flouroquinolon resistance. Drugs;1999;58 (suppl2)11-18.

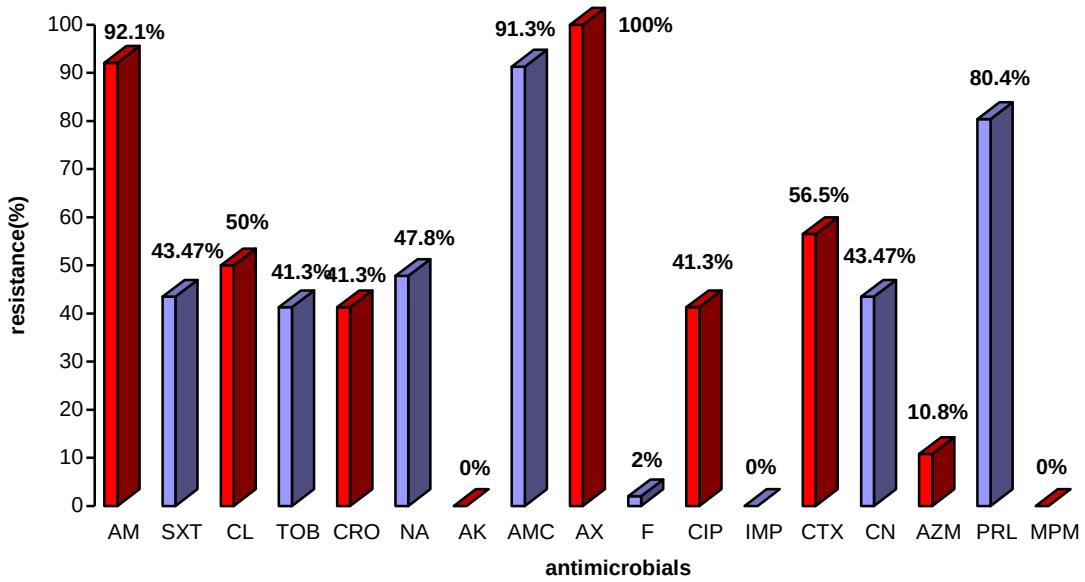
33- Sisson, G.; Goodwin, A.; Raudonikiene, A.; Hushes, N.J.; Mukhopadhyay ,A.K.; Berg, D.E.; and Hoffiman ,P.S.Structural and mechanistic studies of Escherichia coli nitroreductase with the antibiotic nitrofurazone. Antimicrobial agents and chemotherapy;2002; 46, 2116-212.

34- Minhchan Plac Ngugen; Paul Louis Woerther; Mathilde Bouvet; Antoine Andremon; Roland Leclercq; and Annuie Canu .Escherichia coli as reservoir for macrolide resistance genes.Emerging infectious disease;2009;Vol. 15. No.10.

35- Philippon, A.; Arlet, B.; Jacoby, GA. Plasmid –determined AmpC- type β-lactamases. Antimicrobial agent chemotherapy; 2002; 46:1-11.

E. coli (4)

Figure 1: Identification of E. coli by Api20E system



ToB:

Tobramycin; CN: Gentamicin; Sxt: Triomethoprime and sulfamethoxazole; Cip: Ciprofloxacin; Na: Naldixic acid; Ctx: Cefotaxime; Ipm: Imipenim;Am: Ampicillin; CL: Cephalexin; CRO:Ceftriaxone;AMC:Amoxicillin and Clavulonicacid; F:Nitrofurantoin; AZM:Azithromycin;PRL:Pipracillin;MPM: Meropenem; AX:Amoxicillin AK:Amikacin

Figure2: Percentage of resistant E. coli isolates to antimicrobials

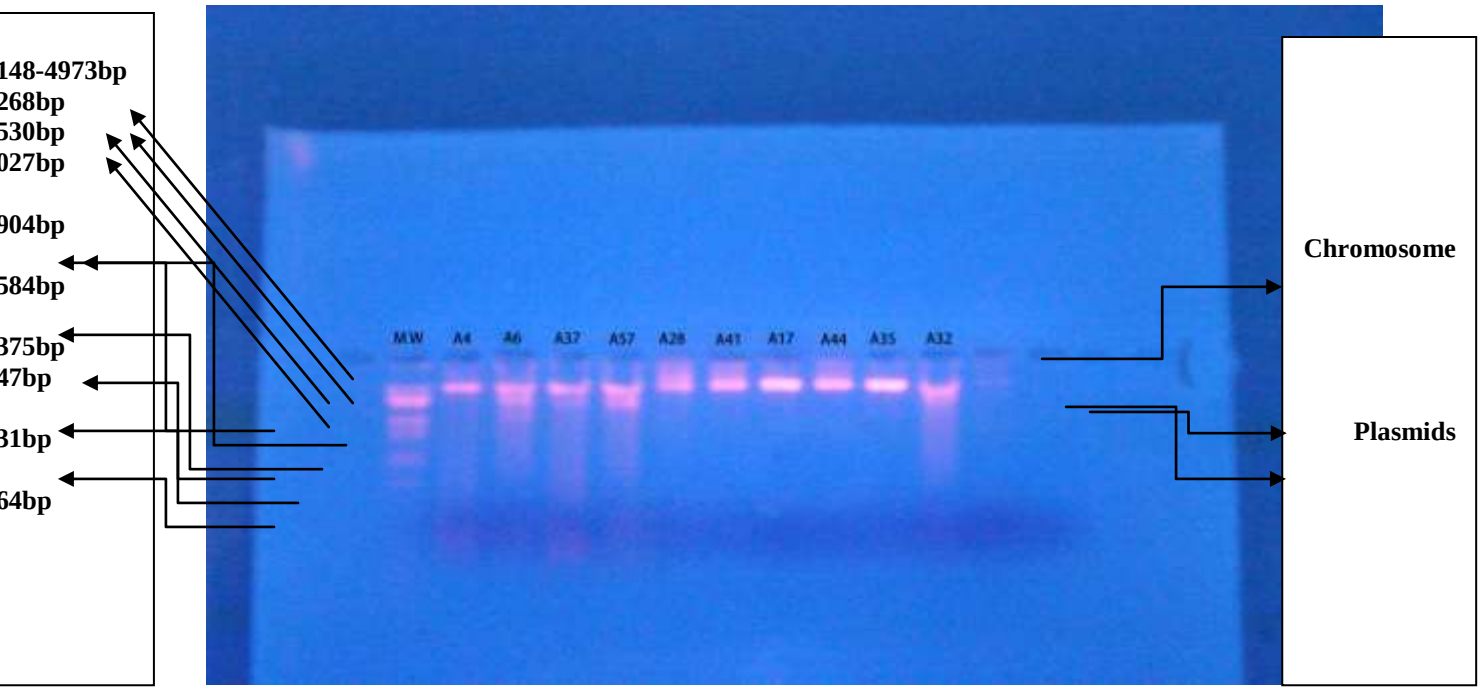


Figure 3: plasmid profile of E. coli strains Lane (A6, A37, A57, A32): Plasmid DNA extracted from E. coli strains; M.W: Molecular weight marker of lambda DNA digested with EcoRI+HindIII . Electrophoresis was carried in 0.8% agarose gel at (2V/Cm) for 30 min.

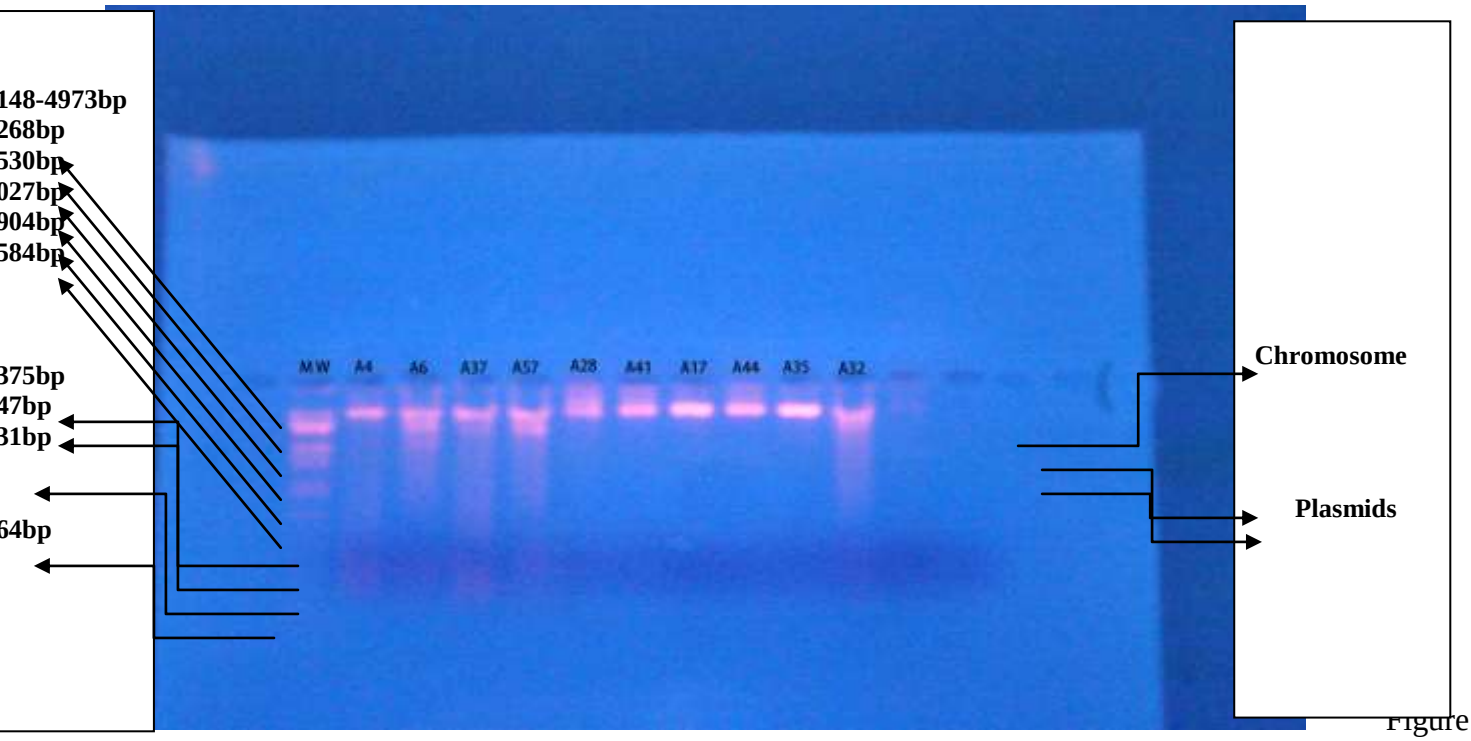


Figure 4: plasmid profile of E. coli strains isolated from UTIs patients Lane (A6, A37, A57, A32): Plasmid DNA extracted from E. coli strains; M.W: Molecular weight marker of lambda DNA digested with EcoRI+HindIII . Electrophoresis was carried in 0.8% agarose gel at (2V/Cm) for 60 min.

Table 1: MIC value for three antimicrobials (µg/ml) tested against E. coli isolates

E. coli isolates	Meropenem µg/ml		Ciprofoxacin µg/ml		Nitrofurantoin µg/ml	
	MIC	MBC	MIC	MBC	MIC	MBC
A1	0.12	0.125	200	300	12.5	25
A2	1.25	12.5	300	400	12.5	25
A3	0.12	1.25	300	300	25	50
A4	1.25	1.25	300	400	25	50
A6	0.12	1.25	800	800	25	50
A7	1.25	1.25	300	300	12.5	25
A10	12.5	12.5	50	100	12.5	25
A11	0.003	0.003	25	25	3.12	6.25
A13	0.12	0.12	200	300	12.5	25
A24	0.12	1.2	300	300	25	50
A28	0.03	0.03	200	200	12.5	25
A32	12.5	12.5	50	100	12.5	25
A35	1.25	1.25	200	300	25	50
A37	0.12	1.25	800	800	25	50
A41	12.5	12.5	200	300	6.25	12.5
A42	12.5	12.5	200	300	6.25	12.5
A43	1.25	1.25	200	300	25	50
A44	0.06	0.06	50	50	1.6	3.125
A45	1.25	12.5	300	400	12.5	25
A47	0.12	1.25	800	800	25	50
A51	12.5	12.5	200	300	6.25	12.5
A55	0.06	0.03	50	50	1.6	3.125
A57	0.12	1.25	300	300	25	50
A58	0.12	0.12	200	300	12.5	25
A67	0.12	0.125	200	300	12.5	25
E. coli isolates	Meropenem µg/ml		Ciprofoxacin µg/ml		Nitrofurantoin µg/ml	
	MIC	MBC	MIC	MBC	MIC	MBC
LSD value	4.945 *	5.418 *	219.05 *	210.11 *	8.397 *	16.80 *

* (P<0.05), LSD: Least significant difference

Table2: Results of combination of meropenem with ciprofloxacin (1/4+1/4MIC**)**

E. Coli isolates	MIC of meropenem before combination (ug/ml)	MIC of meropenem after combination (ug/ml)	MIC of ciprofloxacin before combination (ug/ml)	MIC of ciprofloxacin after combination (ug/ml)	FIC	Result
A1	0.12	0.03	300	75	0.5	Syn
A2	1.25	0.31	300	75	0.5	Syn
A3	0.12	0.03	300	75	0.5	Syn
A4	1.25	0.31	300	75	0.5	Syn
A7	1.25	0.31	300	75	0.5	Syn
A10	12.5	3.12	50	12.5	0.5	Syn
A13	0.12	0.031	200	50	0.5	Syn
A24	0.12	0.03	300	75	0.5	Syn
A28	0.03	0.007	200	50	0.5	Syn
A32	1.25	0.31	50	12.5	0.5	Syn
A37	0.12	0.03	800	200	0.5	Syn
A41	12.5	3.12	200	50	0.5	Syn
A42	12.5	3.12	200	50	0.5	Syn
A43	1.25	0.31	200	50	0.5	Syn
A44	0.06	0.01	50	12.5	0.5	Syn
A45	1.25	0.31	300	75	0.5	Syn
A47	0.12	0.031	800	200	0.5	Syn
A51	12.5	3.12	200	50	0.5	Syn
A55	0.06	0.01	50	12.5	0.5	Syn
A57	0.12	0.03	300	75	0.5	Syn
A58	0.12	0.03	200	50	0.5	Syn
A67	0.12	0.03	200	50	0.5	Syn
LSD value	5.030 *	4.234 *	213.56 *	122.23 *	--	--

* (P<0.05); LSD: Least significant difference; Syn: Synergism; FIC: Fractional Inhibitory Concentration

Table 3: Results of combination of meropenem with nitrofurantoin (1/4+1/4 MIC):

E. Coli isolates	MIC of meropenem before combination (ug/ml)	MIC of meropenem after combination (ug/ml)	MIC of nitrofurantoin before combination (ug/ml)	MIC of nitrofurantoin after combination (ug/ml)	FIC	Result
A2	1.25	0.31	12.5	3.12	0.5	Syn
A3	0.12	0.03	25	6.25	0.5	Syn
A4	1.25	0.31	25	6.25	0.5	Syn
A7	1.25	0.31	12.5	3.12	0.5	Syn
A10	12.5	3.12	12.5	3.12	0.5	Syn
A13	0.12	0.03	12.5	3.12	0.5	Syn
A24	0.12	0.03	25	6.25	0.5	Syn
A28	0.03	0.007	12.5	3.12	0.5	Syn
A32	1.25	0.31	12.5	3.12	0.5	Syn
A37	0.12	0.03	25	6.25	0.5	Syn
A41	12.5	3.12	6.25	1.56	0.5	Syn
A42	12.5	3.12	6.25	1.56	0.5	Syn
A43	1.25	0.31	25	6.25	0.5	Syn
A44	0.06	0.015	1.6	0.4	0.5	Syn
A45	1.25	0.31	12.5	3.12	0.5	Syn
A47	0.12	0.031	25	6.25	0.5	Syn
A51	12.5	3.12	6.25	1.56	0.5	Syn
A55	0.06	0.015	1.6	0.4	0.5	Syn
A57	0.12	0.03	25	6.25	0.5	Syn
A58	0.12	0.03	12.5	3.12	0.5	Syn
A67	0.12	0.03	12.5	3.12	0.5	Syn
LSD value	5.030 *	4.234 *	213.56 *	122.23 *	--	--

* (P<0.05); LSD: Least significant difference; Syn: Synergism; FIC: Fractional Inhibitory Concentrations

Table 4: Antimicrobials combination (1/2+1/2 MIC for each antimicrobials)

E. coli isolates	Antimicrobials combination	MIC of first antimicroal alone (µg/ml)	MIC of first antimicrobial in combination (µg/ml)	MIC of second antimicrobial alone (µg/ml)	MIC of second antimicrobial in combination (µg/ml)	FIC	Results
A1	MPM+F	0.125	0. 0625	12. 5	6. 25	1	Add
A6	MPM+CIP	0.12	0.0625	800	400	1	Add
A4	F+CIP	25	12. 5	300	150	1	Add
A32	F+CIP	12. 5	6. 25	50	25	1	Add

Add: Addition; FIC: Fractional Inhibitory Concentration MPM: meropenem; F:nitrufurantoin; Cip: ciprofloxacin.