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RESEARCH ARTICLE

# EFFICACY OF COMBINATION OF NITROFURANTOIN WITH GENTAMICIN, AND CIPROFLOXACIN AGAINST RESISTANT *E. COLI* ISOLATED FROM PATIENTS WITH URINARY TRACT INFECTIONS: *IN VITRO STUDY*

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**ABSTRACT**

The aim of this study to determined in vitro effects of various combinations of three antimicrobials (nitrofurantoin, gentamicin and ciprofloxacin) against twenty five *E. coli* isolates and the role of plasmidDNA in antimicrobial resistance.

Method: Seventy five *E. coli* isolates were collected from urine of patients with urinary tract infections in AL-Kadhimia and AL-Yarmook teaching hospitals in Baghdad for a period between 22/11/2012 to 15/3/2013, 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 (nitrofurantoin, gentamicin and ciprofloxacin. The plasmid profile for the twenty five *E. coli* isolates were studied using Pure Yield ™ plasmid Miniprep system- Cat.# A1220 – Promega- USA. In order to determined the presence of plasmid for antimicrobials resistance.

Result :Among combinations the combination of nitrofurantoin with gentamicin showed high synergistic effect when 1/4+1/4 MIC for each antimicrobial were used. While combinations of nitrofurantoin with gentamicin and ciprofloxacin in some isolates showed additive effect when 1/2+1/2 MIC for each antimicrobial were used. ), nitrofurantoin was found having the lowest MIC comparing with others.Extraction of plasmidDNA indicates the presence of antimicrobial resistance plasmid in (A6, A37, A32, and A57) isolates.

Conclusion: Nitrofurantoin had more effect on E.coli and with high synergistic effect in combination with gentamicin against resistant Ecoli.that 18 isolates show synergistic effect, only2 isolate show additive effect, while combination of nitrofurantoin and quinolon better to be avoided that in vitro show antagonist effect.

**Keyword:** Urinary tract infection, E.coli, minimum inhibitory concentration, plasmid DNA.

## 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 Nitrofurantoin which is a synthetic nitrofuran that is used to prevent and treat urinary tract infections [4].with Ciprofloxacin which is a synthetic chemotherapeutic antimicrobial of the fuoroquinolone drug class [5], and aminoglycosides which are polar compound with more activity against aerobic gram-negative bacilli and little activity against an aerobic bacteria and use with other antimicrobial agent against gram positive bacteria [6].

## 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 [7]. 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 [8]. 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 [9].

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

Plasmid DNA isolated using Pure Yield ™ 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

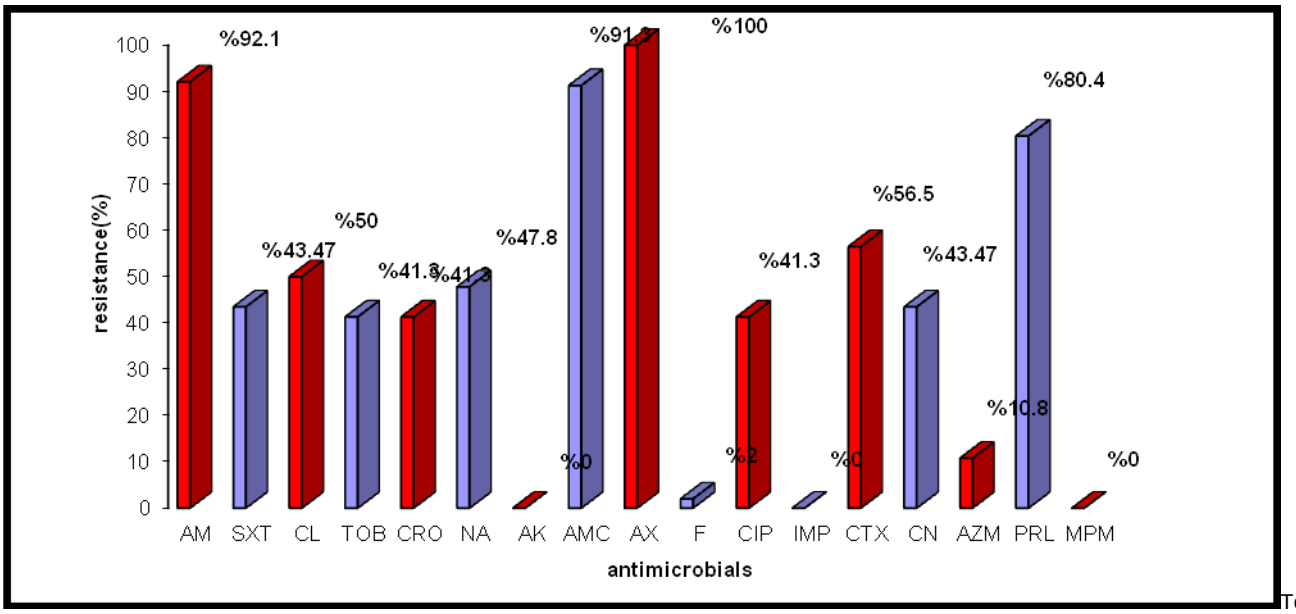
Colonies of *E. coli* had marked as a flat smooth and pink in color as a result of lactose fermentation in the media on MacConky agar, while on blood agar it gave small pink convex colonies surrounded by zone of β- haemolysis. In Microscopic Examination it showed as small single bacilli non spore forming 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}.

**Fig. 1: Identification of *E. coli* by Api20E stem.**

### Antimicrobial Sensitivity Test

### 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.




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**

**Fig. 2: Percentage of resistant *E. coli* isolates to antimicrobials.**

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 [10].The results of the current study (Figure 2) revealed that most of *E. coli* isolates resist the β- lactam antimicrobials (like ampicillin and amoxicillin) [11].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 [12, 13]. Also it may be due to minimizing the interaction of antimicrobials with target site (Penicillin Binding Proteins) [14].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 [15].

Many research illustrated the higher activity of imipenem and meropenem (related to carbapenems group) against gram-positive and gram- negative bacteria [16].

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 [17]. The current results (Figure 2) were in agreement with that of Shevelev *et al*. (2002) [18] 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 were in agreement with Bashir *et al.* (2008) [19] 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) [20] 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) [21] 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) [22] 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) [23] 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) [24] 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.

**Quantitative Method (Minimum Inhibitory Concentration) (MIC)**

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

| ***E. coli* isolates** | **Gentamicin µg/ml** | | **Ciprofoxacin µg/ml** | | **Nitrofurantoin µg/ml** | |
|---|---|---|---|---|---|---|
| | **MIC** | **MBC** | **MIC** | **MBC** | **MIC** | **MBC** |
| A1 | 300 | 300 | 200 | 300 | 12.5 | 25 |
| A2 | 200 | 300 | 300 | 400 | 12.5 | 25 |
| A3 | 300 | 300 | 300 | 300 | 25 | 50 |
| A4 | 300 | 480 | 300 | 400 | 25 | 50 |
| A6 | 480 | 480 | 800 | 800 | 25 | 50 |
| A7 | 300 | 300 | 300 | 300 | 12.5 | 25 |
| A10 | 480 | 480 | 50 | 100 | 12.5 | 25 |

| | | | | | | |
|---|---|---|---|---|---|---|
| A11 | 12.5 | 12.5 | 25 | 25 | 3.12 | 6.25 |
| A13 | 300 | 300 | 200 | 300 | 12.5 | 25 |
| A24 | 300 | 300 | 300 | 300 | 25 | 50 |
| A28 | 200 | 200 | 200 | 200 | 12.5 | 25 |
| A32 | 480 | 480 | 50 | 100 | 12.5 | 25 |
| A35 | 200 | 300 | 200 | 300 | 25 | 50 |
| A37 | 480 | 480 | 800 | 800 | 25 | 50 |
| A41 | 100 | 200 | 200 | 300 | 6.25 | 12.5 |
| A42 | 100 | 200 | 200 | 300 | 6.25 | 12.5 |
| A43 | 200 | 300 | 200 | 300 | 25 | 50 |
| A44 | 200 | 300 | 50 | 50 | 1.6 | 3.125 |
| A45 | 200 | 300 | 300 | 400 | 12.5 | 25 |
| A47 | 480 | 480 | 800 | 800 | 25 | 50 |
| A51 | 100 | 200 | 200 | 300 | 6.25 | 12.5 |
| A55 | 200 | 300 | 50 | 50 | 1.6 | 3.125 |
| A57 | 480 | 480 | 300 | 300 | 25 | 50 |
| A58 | 300 | 300 | 200 | 300 | 12.5 | 25 |
| A67 | 300 | 300 | 200 | 300 | 12.5 | 25 |
| LSD value | 137.95 * | 118.38 * | 219.05 * | 210.11 * | 8.397* | 16.80* |

*** (P<0.05), LSD: Least significant difference, MBC: minimum bactericidal concentration**

Table 1 showed that 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]. While MIC of gentamicin ranged from (12.5 to 480 µg/ml), this result was in agreement with Jakobsem *et al*. [25] who found in his study that the MIC of gentamicin distributed from (8-› 512 µg/ml).

**Antimicrobials Combination**

**Table 2: the Result of combination of nitrofurantoin with gentamicin (1/4+1/4 MIC).**

| ***E. Coli* isolates** | **MIC of nitrofurantoin before combination (ug/ml)** | **MIC of nitrofurantoin after combination (ug/ml)** | **MIC of gentamicin before combination (ug/ml)** | **MIC of gentamicin after combination (ug/ml)** | **FIC** | **Result** |
|---|---|---|---|---|---|---|
| A1 | 12.5 | 3.12 | 300 | 75 | 0.5 | Syn |
| A3 | 25 | 6.25 | 300 | 75 | 0.5 | Syn |
| A4 | 25 | 6.25 | 300 | 75 | 0.5 | Syn |
| A6 | 25 | 6.25 | 480 | 120 | 0.5 | Syn |
| A7 | 12.5 | 3.12 | 300 | 75 | 0.5 | Syn |
| A10 | 12.5 | 3.12 | 480 | 120 | 0.5 | Syn |
| A13 | 12.5 | 3.12 | 300 | 75 | 0.5 | Syn |
| A24 | 25 | 6.25 | 300 | 75 | 0.5 | Syn |
| A37 | 25 | 6.25 | 480 | 120 | 0.5 | Syn |
| A42 | 6.25 | 1.56 | 100 | 25 | 0.5 | Syn |
| A43 | 25 | 6.25 | 200 | 50 | 0.5 | Syn |
| A45 | 12.5 | 3.12 | 200 | 50 | 0.5 | Syn |
| A47 | 25 | 6.25 | 480 | 120 | 0.5 | Syn |
| A51 | 6.25 | 1.56 | 100 | 25 | 0.5 | Syn |
| A55 | 1.6 | 0.4 | 200 | 50 | 0.5 | Syn |
| A57 | 25 | 6.25 | 480 | 120 | 0.5 | Syn |
| A58 | 12.5 | 3.12 | 300 | 75 | 0.5 | Syn |
| A67 | 12.5 | 3.12 | 300 | 75 | 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**

The result in Table (2) shows that the synergistic effect noticed from combination of nitrofurantoin with gentamicin when tested on isolates No. (1, 3, 4, 6, 7, 10, 13, 24, 37, 42, 43, 45, 47, 51, 55, 57, 58, 67,) while isolate No. (2) Show additive effect but other isolate show no effect.

**Table 3: 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** |
|---|---|---|---|---|---|---|---|
| A2 | F+CN | 12. 5 | 6. 25 | 200 | 100 | 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 CIP: ciprofloxacin; CN: gentamicin; F: nitrofurantoin.**

On the other hand there is no synergistic effect noticed from combination of nitrofurantoin with ciprofloxacin Table (3), only there is additive effect noticed when tested on isolates No. (4 and 32).This result was in agreement with Call *et al.*(1978) whose found that nitrofurantoin and quinolon antimicrobials are mutually antagonistic in vitro. It is not known whether this is of clinical significance, but the combination should be avoided.

**Extraction of Plasmid DNA**

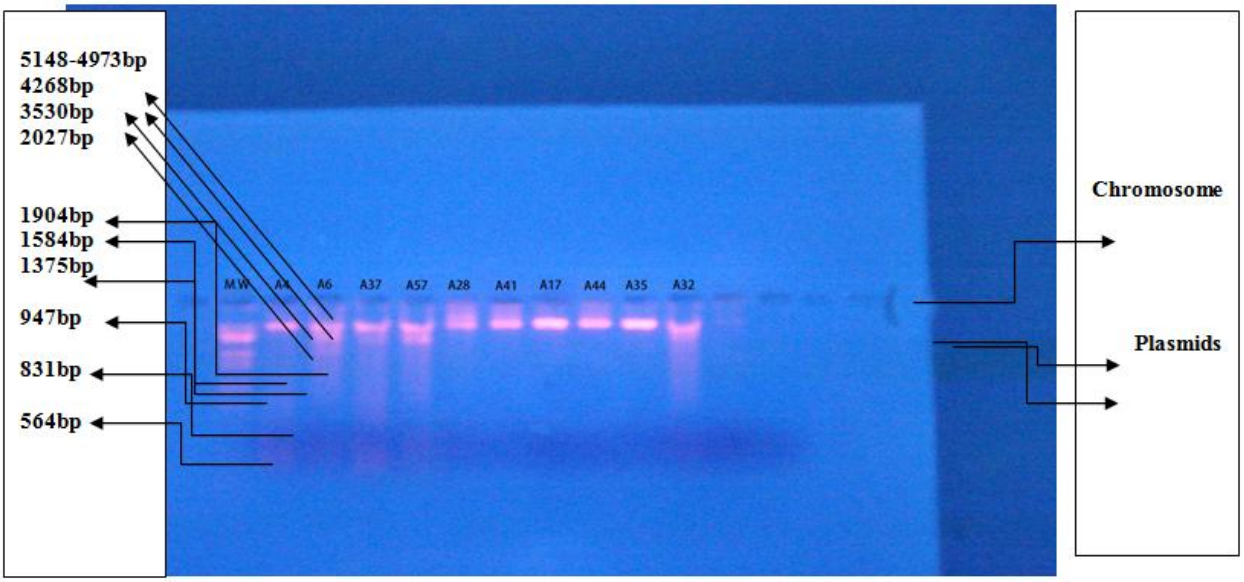



**Figure 3: plasmid profile of *E. coli* strains Lane (A6, A37, A57, A32): Plasmid DNA extracted from *E. coli* strains; M.W: Molecular weight marker of lambda DNA digested with EcoRI+HindIII . Electrophoresis was carried in 0.8% agarose gel at (2V/Cm) for 30 min.**

The result of Figure (3)and (4) 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) [28] 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) [29] found in their study that *E. coli* isolated from patient suffering from urinary tract infection contain plasmid coding high level of resistance to aminoglycoside.

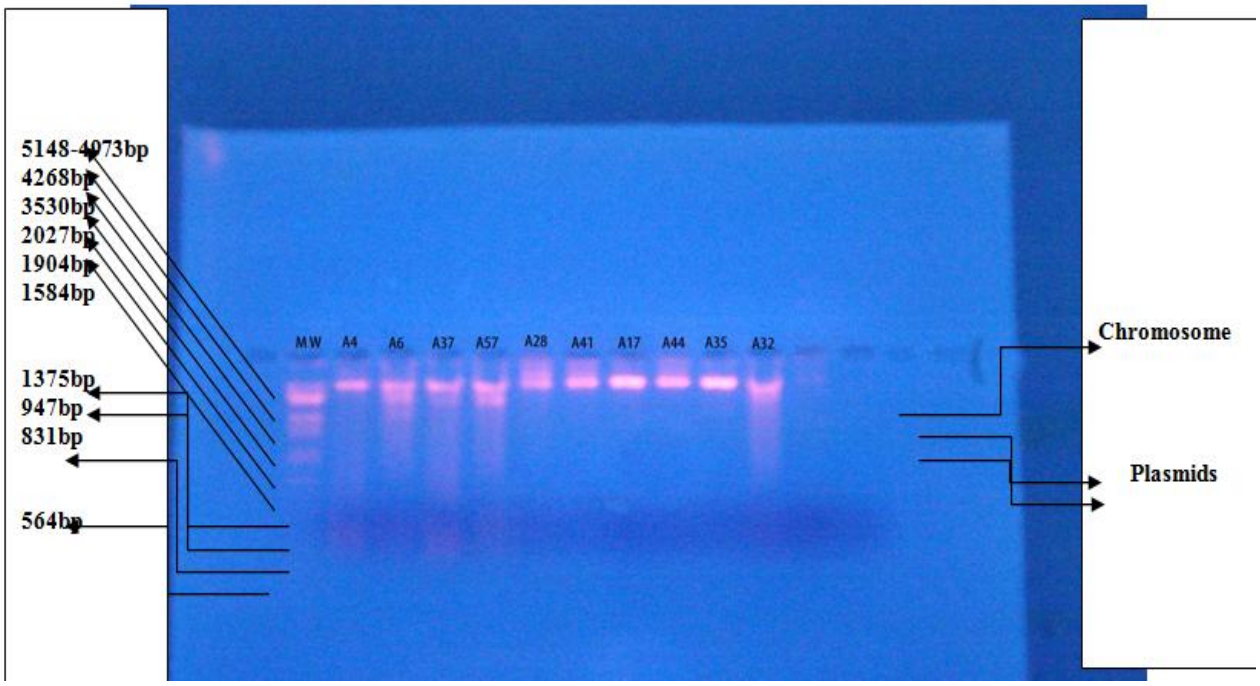



**Figure 4: plasmid profile of *E. coli* strains isolated from UTIs patients Lane (A6, A37, A57, A32): Plasmid DNA extracted from *E. coli* strains; M.W: Molecular weight marker of lambda DNA digested with EcoRI+HindIII . Electrophoresis was carried in 0.8% agarose gel at (2V/Cm) for 60 min.**

Piddock (1999) [30] found in his study that *E. coli* contain plasmid coding for resistance of flouroquinolone .Sisson *et al.* (2002) [31] found in their study that resistance to nitrofurantoin may be chromosomal or plasmid mediated. Minch chau phuc Nguyen *et al.* [32] 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) [33] 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.

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