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# Distribution of Aminoglycoside Resistance Mediated by 16S rRNA Methylation among IraqI Isolates of Escherichia coli and Pseudomonas aeruginosa

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**Abstract**
One hundred clinical isolates of Escherichia coli and Pseudomonas aeruginosa (58 , 42 isolates respectively ) were obtained from patients suffering from different infections at Baghdad \Iraq teaching hospital . These isolates were diagnosed using api 20E .Results of primary screening test for aminoglycoside resistance using determination the minimum inhibitory concentration revealed that all the isolates conferring multidrug resistance and the highest resistance was against kanamycin, while the lowest was against amikacin .Phenotypic detection of Extended spectrum β–lactamase ( ESβLs) was preformed and the results showed that 84% of the isolates gave positive results. Highly resistant isolates (20 for each ) were selected for the genetic study using polymerase chain reaction technique (PCR) to determine aminoglycoside resistance mediated by methylation 16S rRNA beside detection blaCTX –M gene responsible for ESBLs production .Seven 16S rRNA methylase genes were amplified ,the ArmA (846 bp), RmtA(635bp), RmtB(584bp), RmtC(711bp), RmtD (500 bp), RmtF(453bp) and npmA (641bp) beside amplifying blaCTX –M gene ( 550bp) .Out of 20 E.coli isolates ,16(80%)gave positive results for ArmA gene, while non of P.aeruginosa harboured this gene. Only one isolates out of 20(5%) harboured RmtB methylation gene in E.coli isolates, while 3 isolates out of 20(15%) contains RmtC gene and 1 isolates(5%) harboured RmtD gene in E.coli isolates while in P.aeruginosa showed 3 isolates out of 20 (15%) positive results in this gene. The sixth methylation gene was npmA was detected in only one isolate (5%) out of 20. For blaCTX –M gene , it was detected in all E.coli isolates (100%) while it was detected in 17(85%) of P.aeruginosa. This is the first report in Iraq for the emergence of 16S rRNA methylases among E.coli and P.aeruginosa in correlation with ESβLs production .
**Key words:** Aminoglycoside resistance, 16S rRNA methylation genes, and ESβLs blaCTX –M genes.

## 1.Introduction:

Aminoglycoside antibiotics are currently used for the treatment of a broad range of infections caused by both Gram-positive and Gram-negative bacteria (1). They inhibit bacterial protein synthesis by irreversibly binding to 30S subunit of bacterial ribosome leading to cell death (2). Resistance to these antibiotics in pathogenic bacteria are either to production of aminoglycoside-modifying enzymes , impaired uptake of the antibiotics or decreasing intracellular antibiotic accumulation beside mutation that may occurs in the target ribosomal site (3) . In 2005, a new type of mechanism had been emerged represented by ribosomal protection by methylation 16S rRNA A site (3,4) . Usually this mechanism involved methylation step occur via a methylase enzyme at specific nucleotides in 16S rRNA A sit causing limitation in binding between antibiotics to its target due to loss affinity of binding thus causing high-level resistance to aminoglycosides (5, 6). Methylation of 16S rRNA A sit conferred by a single gene( armA) had been described in uropathogenic E. coli (7, 8) . Now most of methylation is mediated by a set of genes designated as ArmA, RmtA, RmtB, RmtC, RmtD, RmtF and NpmA (9,10,11,12).Usually armA gene is the most predominant between Enterobacteriaceae and Acinetobacter spp. while rmtD type had been identified in Enterobacteriaceae and P.auroginosa especially in Asia and Europe (11, 12,13) . The six methylase enzymes which expressed from the six genes have been described to be carried on mega plasmid and the pathogenic bacteria producing such enzymes have the ability to resist all aminoglycoside group(10, 11( . Associations between 16S rRNA methylase and extended-spectrum β-lactamase (ESBLs) production specially blaCTX –M group have been reported (10, 11,14) . The aims of this study were to evaluate the prevalence of seven 16S rRNA methylase genes(ArmA, RmtA, RmtB, RmtC, RmtD, RmtF and NpmA) among locally isolated E.coli and P.auroginosa (in Iraq) using polymerase chain reaction (PCR) and studying the correlation between the presence of these genes with blaCTX –M gene responsible for ESBLs production.

## 2.Materials and methods:

### 2.1 Collection and diagnosis of Bacterial isolates

One-Hundred clinical isolates of *E.coli*, *P.auroginosa* were isolated from patients admitted to several teaching hospitals in Baghdad during a period between July 2011 and December 2011. They were obtained

from the midstream urine from patients with urinary tract infections (68 isolates), Sputum from patients suffering respiratory tract infection (8 isolates), wounds infections (6 isolates) and from bacteraemia (18) isolates . Bacterial diagnosis including morphological and biochemical tests were done according to Atlas *et al.* (15) followed by the complementary API 20E test.

**2.3 Antibiotic susceptibility tests:**

Resistance of the Isolates towards six type of aminoglycoside antibiotics including amikacin, kanamycin, gentamicin, neomycin, Paromomycin, Streptomycin was performed by determination the Minimum Inhibitory Concentrations according to the CLSI (16). *Escherichia coli* ATCC 35218 was used as a negative control .

**2.4 Detection of β-lactamases production:**

Primary screening test to detect extended-spectrum β-lactamases production was performed by culturing bacterial isolates on Muller Hinton medium then a cefotaxime 30µg disc was submitted on the surface of the plat . The detection of β-lactamases production was performed using the Rapid ESβL Detection kit (MAST Group, UK). This kit includes four tests: preliminary β-lactamase screening kit, Metallo-β-lactamases, ESβLs confirmation and Amp C detection test .The test was performed according to the procedure suggested by the manufacturing company in which one drop of test substrate (approximately 20µl) was dispensed onto the filter pad of the strip immediately before testing. Using a loop, one colony was picked up and were spread on the filter pad and any change in color observed around the streaked line was considered a positive result. The test strip was observed after 2 to 15 minutes at room temperature, and the result was read after 15 minutes.

**2.5 Plasmid DNA extraction :**

Plasmid DNA was extracted according to alkaline lysis method from overnight bacterial growth following the procedure of Crosa *et al.*(17) . The extract was used as a DNA template for the PCR technique.

**2.6 Detection of methylase and ESBLs genes using PCR.**

The *armA* , *rmtA, rmtB, armC, rmtD*, *rmtF, npmA* and $bla_{CTX-M}$ genes were detected by PCR using specific primers listed in table(1). The reaction mixture was prepared according to the procedure that suggested by the manufacture company (KAPA, south Afriqa) . PCR products were electrophoresed in 1.% agarose gels and visualized under UV light according to Sambrook and Russell (18).The following primers were used in the current study.

**Table 1: Primers used for detection specific genes.**

| Primers name | primers sequence 5′----------→3′ | Product size (bp) | Primer annealing | Reference from |
|---|---|---|---|---|
| *rmtA-F* | CTA GCG TCC ATC CTT TCC TC | 635 | 58 | Haldorsen( 26) |
| *rmtA-R* | TTG CTT CCA TGC CCT TGC C | | | |
| *rmtB-F* | CCC AAA CAG ACC GTA GAG GC | 584 | 59 | Lee *et al.*(10) |
| *rmtB-R* | CTC AAA CTC GGC GGG CAA GC | | | |
| *rmtC-F* | CGA AGA AGT AAC AGC CAA AG | 711 | 53 | Doi *et al.* (29) |
| *rmtC-R* | ATC CCA ACA TCT CTC CCA CT | | | |
| *rmtD-F* | TCAAAAAGGAAAAGGACGTG | 500 | 52 | Tijet *et al* ., ( 20) |
| *rmtD-R* | CGATGCGACGATCCATTC | | | |
| *rmtF –F* | GCGATACAGAAAACCGAAGG | 453 | 51 | According to this study |
| *rmtF –R* | GGCAGGAGCTTCATCAGAA | | | |
| *armA-F* | CCGAAATGACAGTTCCTATC | 846 | 53 | Wassef *et al.*,( 19) |
| *armA-R* | GAAAATGAGTGCCTTGGAGG | | | |
| *npmA-F* | CTCAAAGGAACAAAGACGG | 641 | 53 | Tijet *et al*., (20) |
| *npmA-R* | GAAACATGGCCAGAAACTC | | | |
| $bla_{CTX-M}$ F | CGCTTTGCGATGTGCAG | 550 | 55 | Nasehi *et al*., (21) |
| $bla_{CTX-M}$R | ACCGCGATATCGTTGGT | | | |

**3. Results and discussion :**

Aminoglycoside antibiotics are widely used in clinical settings, especially for treating life-threatening infections caused by Gram-negative bacteria. They bind to the highly conserved A-site of the 16S rRNA of the prokaryotic 30S ribosomal subunits, interfering with the protein synthesis with subsequent bacterial death (7,20). The most frequently encountered mechanism of resistance to aminoglycosides is their structural modification by specific enzymes produced by resistant organisms. The three classes of such enzymes are aminoglycoside acetyltransferases (AAC), aminoglycoside nucleotidyltransferases (ANT or AAD), and aminoglycoside

phosphotransferases (APH) (20). The ribosomal protection by methylation of 16S rRNA in aminoglycoside-producing actinomycetes gives high level resistance to intrinsic aminoglycosides(7), Since 2003, methylation of 16S rRNA has emerged as a serious threat to the class through the action of plasmid mediated methyltransferase enzymes(5).

Results of MICs for six aminoglycoside antibiotics (kanamycin, amikacin, gentamicin, neomycin, paromomycin and streptomycin), were determine using the two fold agar dilution method. An isolate was characterized as resistant if the MIC value was greater than the breakpoint as defined by CLSI (16), while it will be susceptible if it is less than the breakpoint. *E.coli* and *P.aeruginosa* showed variable degrees of resistance after comparing the results with that in CLSI (16) .The results are illustrated in figure (1). Out of (58) *E.coli* isolates, all (100%) showed their resistant to gentamicin , neomycin (MIC ranged between 32–1024 µg /ml), and kanamycin (MIC ranged from128–1024 µg /ml), 57(98.2%) isolates were resistant to streptomycin (MIC ranged from 16–256 µg /ml), 56(96.5%) for the new used antibiotic paromomycin (MIC ranged from 16–1024µg /ml) and only 20(34.4%) isolates were resistant to amikacin when (MIC ranged from 64–128 µg /ml), thus amikacin had the highest activity as disc diffusion test followed by the activity paromomycin.

For the (42) isolates of *P.aeruginosa*, all (100%) showed their resistant to gentamicin , neomycin (MIC ranged from 16–1024 µg /ml), and paromomycin (MIC ranged from 32–1024µg /ml), while 39(92.8%) isolates shown their resistance to kanamycin (MIC ranged from 64–1024 µg /ml) and streptomycin (MIC ranged from16–1024 µg /ml), and only 20(47.6%) isolates were resistant to amikacin (MIC ranged from 64–128 µg /ml). From noticing the results of *E.coli* isolates ,it's clear that the high-level of resistance was toward neomycin, gentamicin and kanamycin (100%), streptomycin (98.2%) and Paromomycin (86.2%) when the MIC of some isolates reached 1024 µg /ml (neomycin, gentamicin, kanamycin and paromomycin) while the break point for these antibiotic was only 16 µg /ml except for kanamycin which is 64 µg /ml. It could be said that the best in it activity was to amikacin when the highest titer of MIC was 128 µg /ml as a maximum rate for some isolates while the break point for this antibiotic is (64 µg /ml).

Risberg (22) illustrated that the values of MIC values for gentamicin against *E.coli* isolates (0.5-256) µg/ml. These values are much lower than the result of this study, while Yamane *et al*. (4) reported that the MIC values reached to 1024 µg/ml, thus it agreed with this study. For paromomycin MIC was between (32-1024) µg/ml, Al-Agamy (23) reported that the rang of MIC for streptomycin reached (16-1024) µg/ml which is a full agreement with the current study. The result of this study coincide with the results of Fritsche *et al*. (24) in Brazil who reported that MIC(16-128) µg/ml for amikacin, while Gonza′les-Zorn *et al.* (25) reported higher values of MIC towards this antibiotic when the average was (32-256) µg/ml.

In respect to the results of *P.aeruginosa*, Gad *et al*.(26) found that the rang of MIC values for kanamycin and gentamicin were between (32-1024) µg/ml and (16-1024) µg/ml respectively which is coincide with the current study. Yamane *et al*. (4) reported that the rang of MIC for neomycin was between(16-1024) µg/ml which convenient with this study, while Sekiguchi *et al*.(27) found that the rang of MIC for streptomycin was more than 64µg/ml, and in the study of Gad *et al*.(26) and Lim *et al*. (28) they reported that the rang of MIC to amikacin was between (64-128) µg/ml which is convenient with the current study

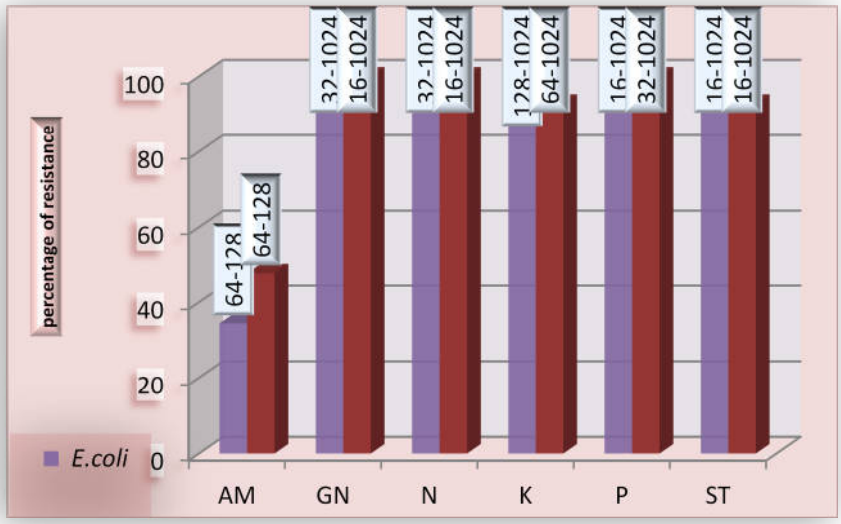



**Figure (1) : The percentage and MIC values of some aminoglycoside against *E.coli* and *P.aeruginosa* isolates. AK:**amikacin**, GN:**gentamicin**, N:**neomycin**, K:**kanamycin**, P:** paromomycin**, ST:**streptomycin

. The high resistance of our clinical isolates may contributed to plasmid- or chromosome encoded modifying enzymes or due to defects in uptake of antibiotic which result from impermeability resistance beside changing in the target side for the antibiotic action or the newly discovered methylation mechanism Giedraitienė *et al*.,(30).

Table (2) shows the results of β-Lactemase screening test in which , 84(84%) isolates gave positive results in the preliminary screening of β-lactamases distributed as 50 out of 58(86.2%) for *E.coli* and 34 out of 42(80.9%) for *P.aeruginosa*, 83% were ESβLs producers distributed as 48/58(82.7% ) of *E.coli* and 35/42(83.3%) isolates for *P.aeruginosa* . In MβL, the rate was reduced to 75/100(75%) and it was distributed as 45/58(77.5%) for *E.coli* and 30/42(71.4% ) for *P.aeruginosa*, finally 70% of the isolates were AmpC producer and they distributed as 33/58(56.8%) and 37/42(88.09%) for *E.coli* and *P.aeruginosa* respectively.. According to Alvarez-Ortega *et al.*,(31) they reported that the β-lactamase enzymes are mostly encoded by transferable plasmids, and these enzymes had been found predominately in *P.aeruginosa* and Enterobacteriaceae especially in *E.coli*. ESβL-producing isolates sometimes demonstrated resistance to an even broader range of β-lactams . However, co-resistance with non-β-lactams are also frequently seen in ESβL-producing gram negative bacteria particularly to aminoglycosides, beside that genes encoding ESβLs are typically carried on the same self-transferable plasmids that often carry other determinants of antibiotic resistance Kaleem *et al.* ,(32). Acquired MβL genes are located on integron that reside on mobile genetic elements such as plasmids or transposons, thus, enabling widespread dissemination Enwuru *et al.*,( 33). Carattoli (34) found AmpC β-lactamases family includes most chromosomal enzymes in both of *E. coli* and *P. aeruginosa*.

**Table (2): The percentage of β-lactamase types produced by *E.coli* and *P. aeruginosa* isolates.**

| Test / Bacteria | Preliminary screening test | ESβLs | MβL | AmpC |
|---|---|---|---|---|
| *E.coli* out of 58 | 50(86.2%) | 48(82.7%) | 45(77.5%) | 33(56.8%) |
| *P. aeruginosa* out of 42 | 34(80.9%) | 35(83.3%) | 30(71.4%) | 37(88.09%) |
| Total (100) | 84(84%) | 83(83%) | 75(75%) | 70(70%) |

### 3.1 Detection of 16S rRNA methylase, ESβLs genes

The presence of *ArmA, RmtA, RmtB, RmtC, RmtD, RmtF* and *NpmA* and $bla_{CTX-M}$ were investigated by PCR amplification technique using primer sets as described previously. Twenty isolates from each species were selected depending on their ability to resist amikacin and gentamicin beside their ability to produce ESBLs. Among these 40 isolates, *armA* gene was detected in 16 *E.coli* isolates (Figure 3) which shows agarose gel

electrophoresis of *armA* PCR products for *E.coli* isolates. As it clear the positive results in lanes 1,2,3,4,6,7 and the amplified segment was 846bp while lane 5 shows negative result. In contrast to *E.coli* none of *P. aeruginosa* isolates gave positive result f this gor ene. This result is in line with the results of Zhou *et al.*(35) who showed the prevalence rate of *ArmA* in *E.coli* was 67.2% and this result is relatively closed with the current result.

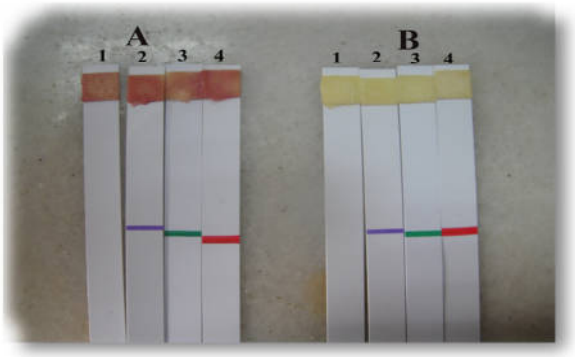
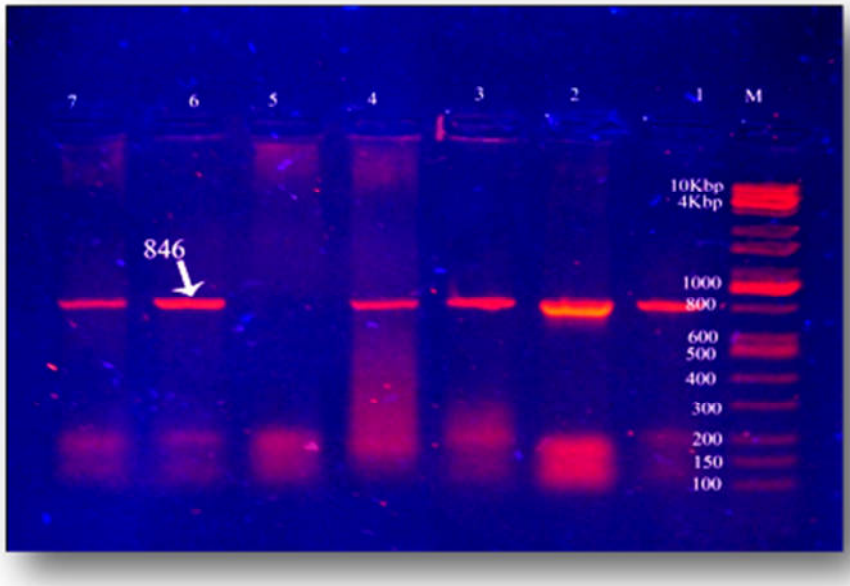



**Figure (2):Detection of β-lactemase types using easy strips test**
**A-1:positive result for primary screening test of *E.coli* no.37.**
**A-2: positive result for ESβLs of *E.coli* no.37.**
**A-3: positive result for MβL of *E.coli* no.37.**
**A-4: positive result for AmpC of *E.coli* no.37.**
**B 1-4: negative result for all above tests of the standard strain (*E.coli* ATCC 35218).**




**Figur (3): garose gel electrophoresis (1% agarose, 7 V/cm for 90min) for *armA* gene of *E.coli* isolates using plasmid DNA as a template, lane M : 100bp DNA ladder,lanes (1,2,3,4,6,7) are positive results with 846bp amplicon, lane 5 shows a negative result.**

For detection the second methylation gene *RmtA*, the results showed that it was not found in any of locally isolated bacteria in spite of trying a different optimizing conditions for PCR experiments and Doi and Arakawa (36) in Japan found 0.9% (1 out of 111) of *E.coli* harbored this gene

For the detection of third methylation gene with amplified size (584bp), the screening test for *RmtB* gene showed that only one *E.coli* isolate (no.37) out of 20 ( 5%) harbored this gene, and such data is similar with Wassef *et al.*(19) and Haldorsen (26) who reported that 6.1% and 4% respectively of the *E.coli* gave a positive results for this gene .Figure (4) shows the results for amplification *RmtB* gene in which only one *E.coli* isolate gave positive shine band of 584bp as compared with 100bp DNA ladder. As with *RmtA* gene, non of *P.aeruginosa* isolates gave a positive PCR product for this gene, which is so far so good as it not being detected either in the world Haldorsen, (26).

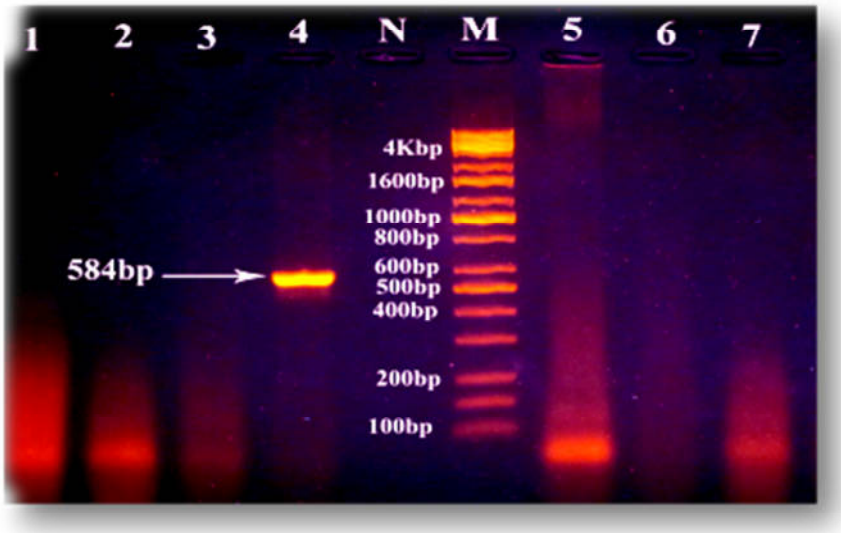



**Figure 4 :Agarose gel electrophoresis (1% agarose, 7 V/cm in 90min) for *RmtB* gene in *E.coli* isolates using template prepared by boiling method, lane M: 100bp DNA ladder, lanes 4: *RmtB* PCR products with 584bp amplified size : lanes (1,2,3,5,6,7 ) are negative result, lane N shows negative control without template .**

For the detection of fourth methylation gene which was *RmtC*, the results showed that 3 *E.coli* isolates(no.37,52,56) out of 20(15%) gave positive results, and this result agreed with Castanheira *et al.*(37) who found that only 1 isolate out of 6 (16.6%) harbored this gene, while Fritsche *et al.*(24), Bercot *et al.*(2010), and Haldorsen ( 26 ) failed to detect a positive result for this gene. Figure (5) shows the results of the amplified *RmtC* gene in *E.coli* isolates with amplified bands (711bp) as compared with 100bp DNA ladder. Also this gene was not detected in the *P.aeruginosa* isolates of this study .

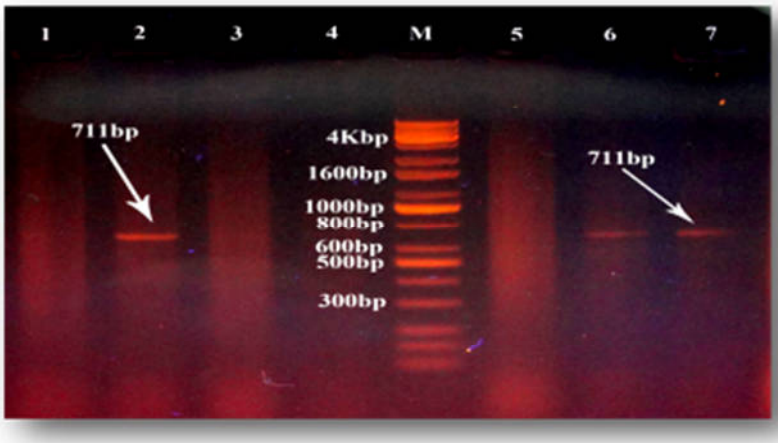



**Figure 5 :Agarose gel electrophoresis (1% agarose, 7 V/cm in 90min) for *RmtC* gene in *E.coli* isolates, lane M : 100bp DNA ladder, lanes (2,6,7) PCR products for *RmtC* gene (711bp), lane (1,3,4,5) the negative results.**

The results of detection the fifth gene which was *RmtD* revealed that only one isolates (5% ) of *E.coli* (no.37) harboured this gene, and this result relatively agreed with the result of Tijet *et al.*(20) who found that the ratio of prevalence of *RmtD* reached in *E.coli* 0.7% (7 out of 1064) in a study carried in Argentina. From the other hand the prevalence of *RmtD* gene in locally isolated *P.aeruginosa* reached 3 isolates (no.14,28,37) out of 20 (15% ) , and this result similar with the results of Castanheira *et al.*(37) in Brazil who found that 4(15.4%) isolates of *P.aeruginosa* out of 26 gave positive results, while the rate reached 6.2% (1 out of 16) for *P.aeruginosa* isolates in Haldorsen (26) study. Figure (6) shows the results of amplifying *RmtD* gene from two isolates of *E.coli* and *P.aeruginosa* which gave bands of (500bp) as compared with 100bp DNA ladder.

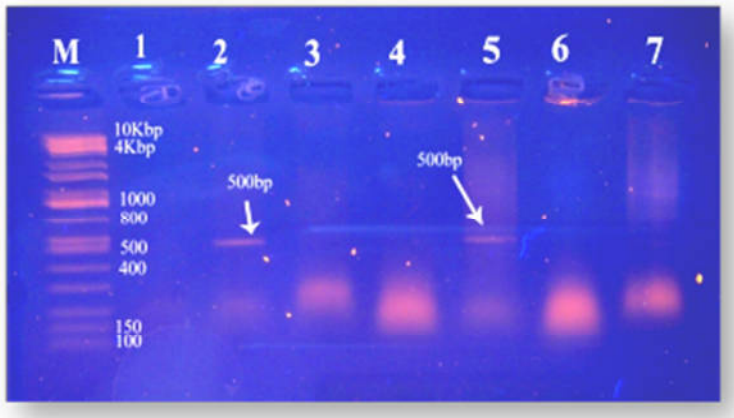



**Figure 6: Agarose gel electrophoresis (1% agarose, 7 V/cm and 90min) for *rmtD* gene in *E.coli* and *P.aeruginosa* isolates using plasmid DNA as a template, (amplified size 500bp) : lane 1 negative control without template, lanes 2,5 clear positive results in *E.coli* and *P.aeruginosa* respectively, while lanes 3,4,6,7 show negative results.**

The sixth amplified gene for a new 16S rRNA methylation named *rmtF* revealed that only one isolates (5% ) of *E.coli* (no.37) harbored this gene, this result didn't agree with Hidalgo,(38) in India who found that 11 isolates of *E.coli* out of 11 carry a new 16S methylase gene, Figure (7) shows the result of the *rmtF* for *E.coli* which gave clear sharp band of 453bp size as compared with 100bp DNA ladder.

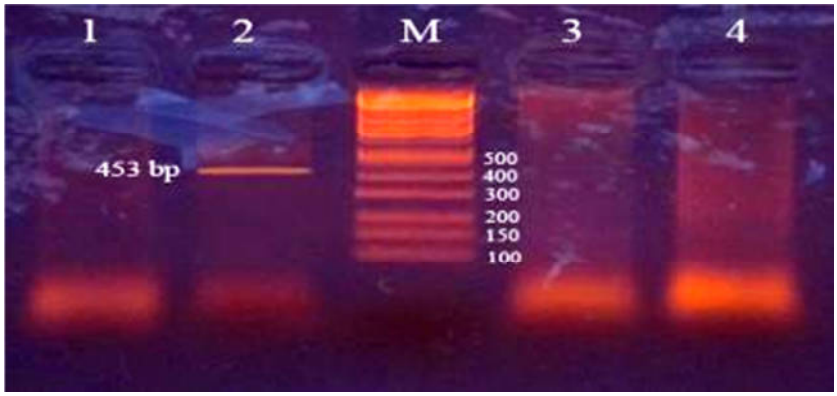



**Figure 7 :Agarose gel electrophoresis (1% agarose, 7 V/cm for 90min) for *rmtF* gene in *E.coli* isolates using Boiling method, line M (DNA ladder) 100bp molecular marker, line 2 positive result with product size 453bp amplicon, line (1, 3,4) illustrate negative result.**

The last amplified gene for 16S rRNA methylation was *npmA* and also only *E.coli* isolate ( no.37) out of 20 (5%) gave a positive result thus our result didn't agree with Wachino *et al.* (39) who found that 34% of *E.coli* isolates verified a positive results, but Fritsche *et al.*(24) and Haldorsen (26) didn't find such positive PCR products neither in *E.coli* nor in *P.aeruginosa* and this result agreed with the current study since none of the locally isolated *P.aeruginosa* gave a positive results. Figure (8) shows the result of the *npmA* for *E.coli* which gave clear sharp band (641bp )

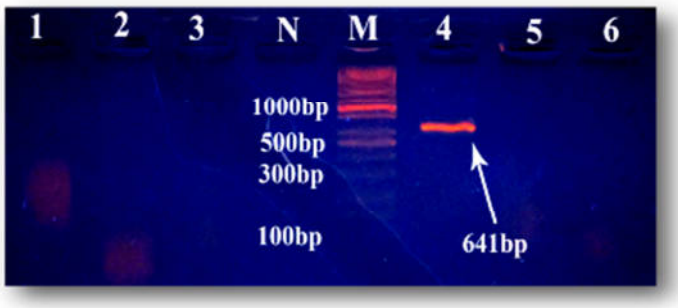



**Figure8:Agarose gel electrophoresis (1% agarose, 7 V/cm for 90min) for *npmA* gene in *E.coli* isolates using plasmid DNA as a template , lane M (DNA ladder) 100bp molecular marker, lines N negative control without template, lane 4 positive result with product size 641bp amplicon, lanes (1,2,3,5,6) illustrate negative result.**

In *E.coli*, the current results showed that the prevalence of $bla_{CTX-M}$ gene (amplified size 550bp) which was present in all the twenty isolates 100% and this result very close to Bell *et al*. (40) in Australia when they found that the percentage of this gene in *E.coli* reached 95%. Figure (9) shows the amplified $bla_{CTX-M}$ segments in *E.coli* isolates which gave bands of (550bp) as compared with 100 bp DNA ladder. Here, the used template was plasmid DNA since most of $bla_{CTX-M}$ gene responsible for ESβLs is under plasmid control. From the other hand, the results showed that the prevalence of $bla_{CTX-M}$ gene in *P.aeruginosa* reached (17/20)85%, and this result goes in line with Al-Grawi (41) in Baghdad who found that 80% of *P.aeruginosa* isolates harbor $bla_{CTX-M}$ gene. Figure (10) shows the result of the amplified $bla_{CTX-M}$ gene in *P.aeruginosa* isolates which also gave amplified bands with (550bp)

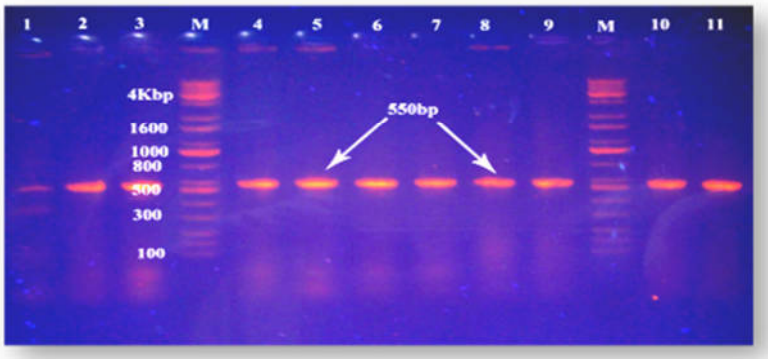



**Figure 9: Gel electrophoresis (1% agarose, 7 V/cm for 90 min)for $bla_{CTX-M}$ gene for some *E.coli* isolates using plasmid DNA as a template. lane M 100bp DNA ladder, lanes (1,2,3,4,5,6,7,8,9,10,11) positive results with 550bp amplicon.**

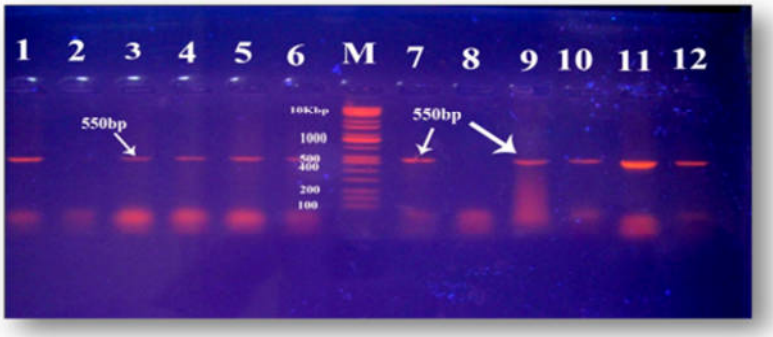



**Figure 10: Gel electrophoresis (1% agarose, 7 V/cm for 90min) of $bla_{CTX-M}$ gene in *P.aeruginosa* isolates using plasmid DNA as a template. lane M :100bp DNA ladder , lanes (1,3,4,5,6,7,9,10,11,12) positive results for $bla_{CTX-M}$ gene with 550bp amplicon, while lanes 2 and 8 show negative results**

In conclusion, *ArmA* gene was the most prevalent among the other genes of methylation, and all kind of methylation genes were detected in the locally isolates *E.coli* except *RmtA*. One of the interesting results in the current study is that one isolates of *E.coli* (no. 37) table (3) harbored the six methylation genes beside $bla_{CTX-M}$ gene. In *P.aeruginosa* only one methylase gene (*RmtD)* gene was reported in three isolates(no.14, 28,37) in addition to $bla_{CTX-M}$ gene, according to this result, there evidence to the emergence of methylation genes in locally isolates *E.coli* more than *P.aeruginosa* despite of the high rate of resistance toward aminoglycoside antibiotic .In addition to resistance by methylation 16S rRNA mechanisms , mutation in 23S rRNA, efflux mechanisms, aminoglycoside modifying enzymes (AMEs) and impermeability of the cell wall may cause reduced susceptibility to aminoglycosides and the most prevalent was efflux mechanisms according to Al-Grawi (41). In this study it seem to be an association between 16S rRNA methylases and ESβL-coding genes is taken a place as clear in table (3) which shows the correlation between $bla_{CTX-M}$ gene and 16S rRNA methylase genes, since these genes are sometimes located on the same conjugative plasmid Haldorsen, (26). Other finding which was noticed in the current study, that two or more 16S rRNA methylase genes were presented in the same isolates beside $bla_{CTX-M}$ gene (*E.coli* no.37,52 and 56), especially *E.coli* (no.37) that contained six types 16S rRNA methylase genes and $bla_{CTX-M}$ gene. For *P.aeruginosa,* the three isolates which harboured methylase gene *RmtD* (no. 14, 28 and 37) were also harboured $bla_{CTX-M}$ gene . The responsible genes are mostly located on transposons Tn*1548* within transferable plasmids, which provides them with the potential to spread horizontally and may in part explain the already worldwide distribution of this novel resistance mechanism . Some of these isolates have been found to co-produce ESβL or MβL, contributing to their multidrug-resistant phenotypes Doi and Arakawa, (29). This study underlines the co-association of 16S rRNA methylase and ESβL-encoding genes linked together resulting in the multidrug-resistant for both of *E.coli* and *P.aeruginosa,* which considered the first report in Iraq and may become a clinical problem. Worryingly, an increase in the prevalence of ESβL, led to the prevalence of aminoglycoside resistance is likely to increase in the coming years.

**Table (3) Presence of 16S rRNA Methylation Genes and MIC values.**

| No. of isolates | Phenotypic resistance (values of MIC) | | | | | | Genotypic detection | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | AK | GN | N | K | P | S | *ArmA* | *RmtA* | *RmtB* | *RmtC* | *RmtD* | *RmtF* | *Npm A* | *bla*CTX-M |
| E 38 | 64 | 512 | 128 | 256 | 256 | 128 | + | – | – | – | – | – | – | + |
| E 39 | 64 | 1024 | 1024 | 256 | 128 | 128 | + | – | – | – | – | – | – | + |
| E 40 | 32 | 1024 | 512 | 256 | 128 | 128 | – | – | – | – | – | – | – | + |
| E 44 | 32 | 256 | 128 | 256 | 128 | 64 | – | – | – | – | – | – | – | + |
| E 50 | 128 | 1024 | 512 | 512 | 512 | 64 | + | – | – | – | – | – | – | + |
| E 52 | 128 | 1024 | 512 | 512 | 256 | 128 | + | – | – | + | – | – | – | + |
| E 56 | 128 | 1024 | 1024 | 1024 | 1024 | 128 | + | – | – | + | – | – | – | + |
| E 57 | 128 | 1024 | 1024 | 1024 | 1024 | 128 | + | – | – | – | – | – | – | + |
| E 58 | 128 | 1024 | 1024 | 1024 | 1024 | 128 | + | – | – | – | – | – | — | + |
| P4 | 64 | 512 | 1024 | 256 | 256 | 1024 | – | – | – | – | – | – | – | + |
| P 5 | 64 | 1024 | 1024 | 128 | 512 | 512 | – | – | – | – | – | – | – | + |

| No. of isolates | Phenotypic resistance (values of MIC) | | | | | | Genotypic detection | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | AK | GN | N | K | P | S | *ArmA* | *RmtA* | *RmtB* | *RmtC* | *RmtD* | *RmtF* | *Npm* | *bla*CTX |
| E 1 | 64 | 1024 | 512 | 256 | 128 | 32 | + | – | – | – | – | – | – | + |
| E 4 | 64 | 512 | 256 | 256 | 16 | 64 | + | – | – | – | – | – | – | + |
| E 8 | 128 | 128 | 64 | 512 | 32 | 32 | + | – | – | – | – | – | – | + |
| E 11 | 32 | 512 | 64 | 256 | 16 | 32 | – | – | – | – | – | – | – | + |
| E 17 | 128 | 1024 | 512 | 256 | 16 | 128 | + | – | – | – | – | – | – | + |
| E 22 | 32 | 256 | 32 | 256 | 64 | 16 | + | – | – | – | – | – | – | + |
| E 30 | 64 | 1024 | 1024 | 512 | 128 | 32 | + | – | – | – | – | – | – | + |
| E 31 | 64 | 1024 | 1024 | 512 | 128 | 32 | + | – | – | – | – | – | – | + |
| E 32 | 32 | 1024 | 1024 | 256 | 16 | 32 | – | – | – | – | – | – | – | + |
| E 33 | 32 | 512 | 256 | 256 | 64 | 32 | + | – | – | – | – | – | – | + |
| E 37 | 128 | 1024 | 1024 | 1024 | 1024 | 256 | + | – | + | + | + | + | + | + |

| No. of isolates | Phenotypic resistance (values of MIC) | | | | | | Genotypic detection | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | AK | GN | K | N | P | S | *ArmA* | *RmtA* | *RmtB* | *RmtC* | *RmtD* | *RmtF* | *Npm A* | *bla*$_{CTX-M}$ |
| P 9 | 64 | 1024 | 512 | 512 | 256 | 128 | – | – | – | – | – | – | – | – |
| P 14 | 128 | 1024 | 1024 | 1024 | 1024 | 512 | – | – | – | – | + | – | – | + |
| P15 | 64 | 1024 | 512 | 1024 | 128 | 16 | – | – | – | – | – | – | – | + |
| P16 | 64 | 128 | 1024 | 1024 | 32 | 16 | – | – | – | – | – | – | – | + |
| P18 | 128 | 1024 | 256 | 512 | 128 | 256 | – | – | – | – | – | – | – | + |
| P21 | 64 | 1024 | 512 | 1024 | 512 | 1024 | – | – | – | – | – | – | – | + |
| P23 | 128 | 128 | 512 | 1024 | 128 | 128 | – | – | – | – | – | – | – | + |
| P25 | 64 | 1024 | 256 | 1024 | 512 | 64 | – | – | – | – | – | – | – | + |
| P28 | 128 | 1024 | 1024 | 1024 | 1024 | 128 | – | – | – | – | + | – | – | + |
| P31 | 64 | 1024 | 512 | 32 | 512 | 1024 | – | – | – | – | – | – | – | + |
| P33 | 64 | 1024 | 512 | 1024 | 256 | 128 | – | – | – | – | – | – | – | + |

| No. of isolates | Phenotypic resistance (values of MIC) | | | | | | Genotypic detection | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | AK | GN | K | N | P | S | *ArmA* | *RmtA* | *RmtB* | *RmtC* | *RmtD* | *RmtF* | *Npm A* | *bla*$_{CTX-M}$ |
| P34 | 64 | 1024 | 256 | 1024 | 512 | 32 | – | – | – | – | – | – | – | + |
| P35 | 128 | 1024 | 512 | 256 | 512 | 128 | – | – | – | – | – | – | – | + |
| P37 | 128 | 1024 | 1024 | 1024 | 1024 | 512 | – | – | – | – | + | – | – | + |
| P39 | 64 | 512 | 512 | 256 | 512 | 128 | – | – | – | – | – | – | – | – |
| P40 | 64 | 512 | 256 | 512 | 512 | 64 | – | – | – | – | – | – | – | – |
| P41 | 128 | 512 | 1024 | 1024 | 256 | 256 | – | – | – | – | – | – | – | + |
| P42 | 64 | 1024 | 512 | 1024 | 256 | 128 | – | – | – | – | – | – | – | + |

(+) positive, (-) negative.

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