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Molecular detection of antibiotic resistance genes and biofilm production among uropathogenic bacteria

Rawa Abdul Redha Aziz[1] and Elaf Sameer[2]

[1]Doctor Lecturer; Al-Karkh University of Science, College of Science, Department of Microbiology, Iraq; dr.rawaaziz@kus.edu.iq

[2] Lecturer Asisstant; Al-Karkh University of Science, College of Science, Department of Microbiology, Iraq; elaf_sameer@kus.edu.iq

Abstract

Objective(s): UTI is showed to be the most popular infection among Iraqi community and nosocomial UTI and has high number of samples in clinical units and catheterized UTI patients. Aim of study is to detect the antibiotic resistant genes and biofilm production among Iraqi uropathogenic bacteria. **Material and Methods:** Fifty isolates of each *Escherichia coli* and *Pseudomonas aeroginosa* obtained from catheterized UTI patients admitted to Al- Najaf city hospitals. Vitek 2 automated system was used for identifying isolates. Antibiotic sensitivity was performed using disk diffusion method. PCR studies were done for detecting antibiotic resistance genes such as *bla $_{AMP}$, bla$_{VIM}$, bla $_{OXA58}$, rmtC, rmtF, rmt D*, and *parC*. Microtiter plate assay was used to detect biofilm production ability. **Results:** Antibiotic sensitivity test revealed 60% of *E. coli* was MDR, 30% was XDR, 10% was PDR. *P. aeroginosa* showed 2% was pan drug resistant isolate, while there were 48% isolates considered as MDR and 50% were XDR. Antibiotic resistant genes were distributed among *E. coli* and *P. aeroginosa* isolates as follows: [50% and 68% for *bla $_{AMP}$*], [90% and 55% for *bla$_{VIM}$*], [80% and 50% for *bla$_{OXA58}$*], [60% and 48% for *rmtC*], [58% and 40% for *rmtF*], [49% and 90% for *rmtD*], [80% and 78% for *ParC*], respectively. Microtiter dish assay demonstrated that 86% of *E. coli* and 90% of *P. aeroginosa* isolates were biofilm producers. **Conclusion:** Uropathogenic bacteria are being more antibiotic resistant by many means, causing serious problems for UTI catheterized patients in Al-Najaf city, Iraq.

Key Words: Uropathogens, Biofilm, XDR, PCR, and microtiter assay

1. Introduction

Many pathogens have the ability to invade urinary tract tissues, colonize and initiate infections that could be sometimes severe, especially when they are antibiotic resistant and biofilm producers. UTI is considered as a most infection causing high morbidity across the world, and the majority of clinical samples are related to it (1). The most common uropathogenic bacteria infecting urinary tract and kidneys are *E. coli, Proteus sp., P. aeruginosa, Acinetobacter sp., Klebsiella sp., Enterobacter sp., and Citrobacter sp., S. saprophyticus, Enterococcus sp., and Coagulase-negative Staphylococcus* (2). It is studied that these bacteria are more aggressive where they are able to make different biofilm stages and easily invade and initiate infection (2). *E. coli* bacteria is found to be the most common cause of UTI since this bacterium has many virulence factors (3) and it is followed by *P. aeruginosa* which is also very common uropathogenic that can cause complicated UTI and has high morbidity and mortality incidence rates (3). UTI incidence across the world is found to be 150-250 million in 2016, especially among women (4). It is found that 50% of females have had infected by uropathogenic bacteria at least once in lifetime and some of them with chronic pyelonephritis (5). Despite many antibiotics are prescribed for treating UTI patient's worldwide, this infection is getting hard to treat and might be fatal (6). High antibiotic resistant patterns have been found among uropathogenic bacteria for a wide range of antibiotic classes such as trimethoprim-sulfamethoxazole, fluoroquinolones, ciprofloxacin, and others, leading to ineffective treatment (7); therefore, many factors should be taken into account when treating UTI, for example antibiotic resistant levels of pathogens [MDR, XDR, and PDR] (8 and 9). It is shown that multidrug resistant extended-spectrum beta-lactamase (ESBL) producing bacteria uropathogen can cause severe urinary tract infections (2). Consequently, aim of study is to detect the antibiotic resistant genes and biofilm production ability among Iraqi uropathogenic bacteria.

2. Materials and Methods

2.1 Specimens: Fifty isolates of each *E. coli* and *P. aeroginosa* obtained from UTI catheterized patients admitted to Al- Najaf city hospitals. Vitek 2 automated system was used for identifying isolates and found that they were 99.9% probability being *E. coli* and *P. aeroginosa.*

2.2 Susceptibility test: It was done using disk diffusion method according to (10) towards piperacillin (100µg), ampicillin (10 µg), tetracycline (30µg), meropenem (10µg), cefepime (30µg), aztreonam (30µg), amikacin (10µg), ticarcillin-clavulanic acid (75/10µg), trimethoprim (5µg), levofloxacin (5µg), imipenem (10µg), gentamycin (10µg), cephalothin (30 µg), ceftazidime (30µg), tigecycline (15µg), colistin sulphate (25µg), and disk prepared in laboratory for polymyxin B (32µg). Negative control used was *E. coli* (HB101). Isolates were put into antibiotic resistant levels (MDR, XDR, and PDR) according to method done by Aziz and Al Jubori (9).

2.3 Genotyping assay: DNA was extracted for all isolates manually using Wizards kit to be used in PCR process. Different genes related to antibiotic resistant were used (Table 1).

Primer	Sequences (5'_3') F	Sequences (5'_3') R	Size product	Tm
bla$_{AmpC}$	F-ATGCAACAACGACAATCCATC	R-GTTGGGGTAGTTGCGATTGG	1150	**58**
bla$_{AVIM}$	F-GTTTGGTCGCATATCGCAAC	R-AATGCGCAGCACCAGGATAG	382	**57**
rmtC	F-CGA AGA AGT AAC AGC CAA AG	R-ATC CCA ACA TCT CTC CCA CT	711	**53**
rmtD	F-TCAAAAAGGAAAAGGACGTG	R-CGATGCGACGATCCATTC	500	**52**
rmtF	F-GCGATACAGAAAACCGAAGG	R-GGCAGGAGCTTCATCAGAA	453	**52**
OXA58	F 5'-AAGTATTGGGGCTTGTGCTG-3	R 5'-CCCCTTGCGCTCTACATAC-3	599	**52**
par C2	F-GTTACCGTATGCGAGCGGTA	R-TGATTTCACCTGAGGACGGC	314	**57.1**

Table (1): Antibiotic resistant genes were used in this study (9).

3. Results

3.1 *E. coli* isolates: antimicrobial sensitivity studies revealed high resistant rates among isolates towards antibiotics used in the study. Isolates were classified into 60% MDR, 30% XDR, and 10% PDR (Figure 1).

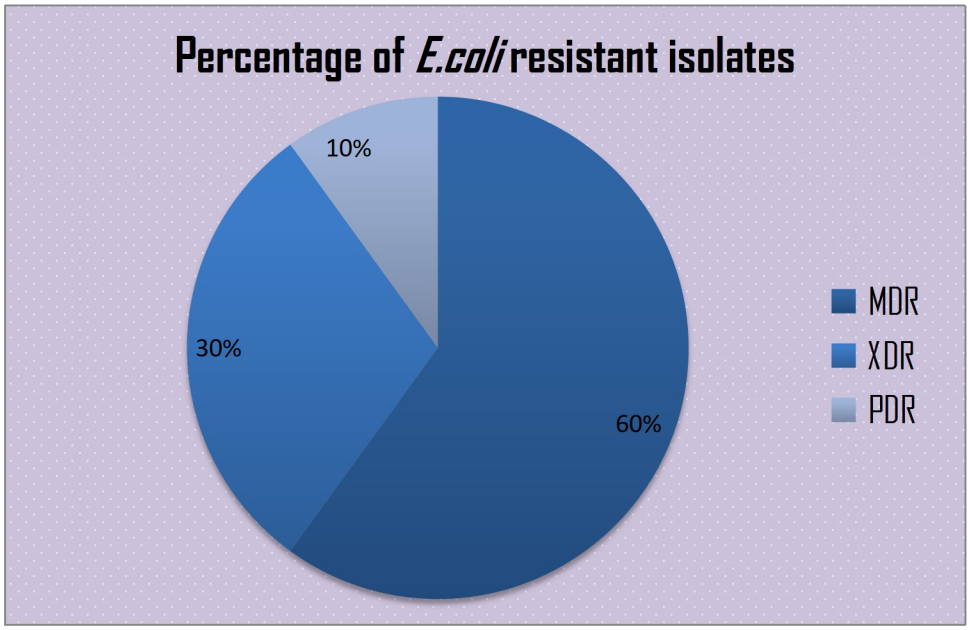

Figure 1: Antibiotic resistant levels of *E. coli* isolates.

PCR studies reported that 50% of isolates were positive for *bla* $_{AMP}$ (1150bp) (Figure 2). There was also 90% of isolates had bla_{VIM} gene (382bp) (Figure 3). In addition, bla_{OXA58} (599bp) was screened and found among 80% of the isolates (Figure 4). Gene *rmtC* (711bp) was found in 60% of isolates (Figure 5). There also was 58% of isolates had *rmtF* (453bp) gene (Figure 6), and 49% of isolates had *rmtD* (500bp) gene (Figure 7). *ParC* (314bp) was found among 80% of the isolates (Figure 8).

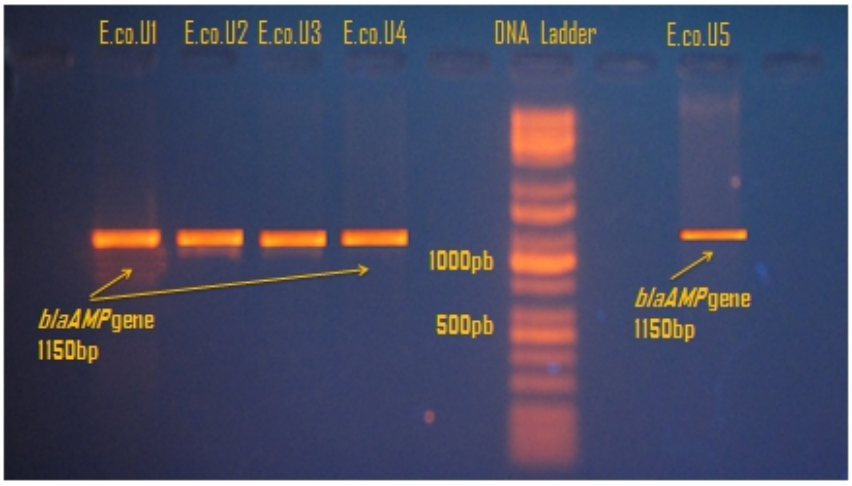

Figure 2: 50% of *E. coli* isolates were positive to *bla* $_{AMP}$ (1150pb) tested by PCR and gel electrophoresis (70W/cm for 90min; 1% agarose) compared with DNA ladder.

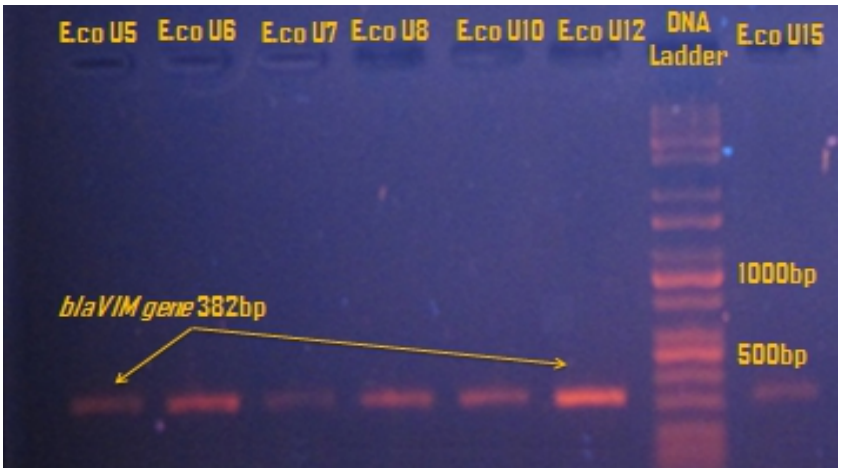

Figure 2: 90% of *E. coli* isolates were positive to *bla* $_{VIM}$ (382pb) tested by PCR and gel electrophoresis (70W/cm for 90min; 1% agarose) compared with DNA ladder.

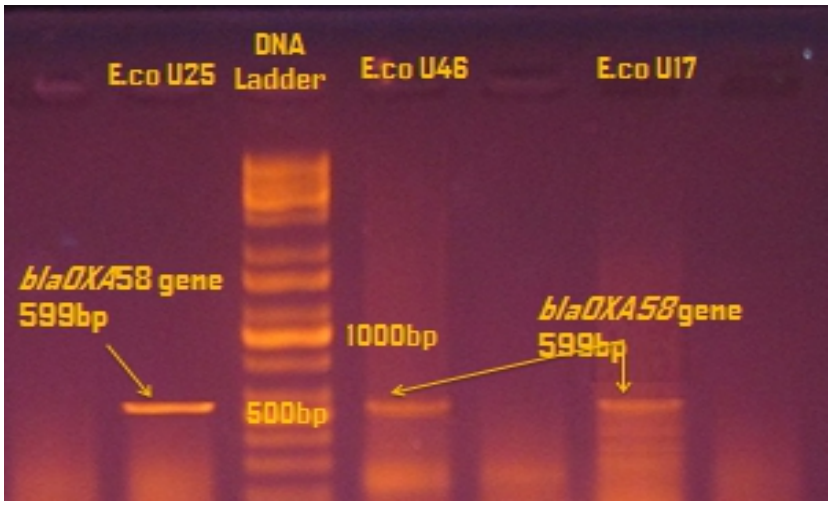

Figure 4: 80% of *E. coli* isolates were positive to *bla*$_{OXA58}$ (599pb) tested by PCR and gel electrophoresis (70W/cm for 90min; 1% agarose) compared with DNA ladder.

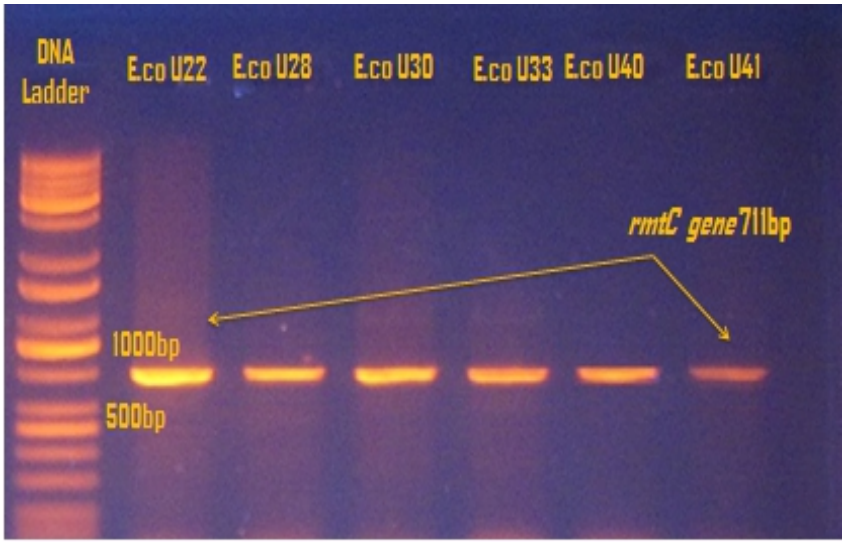

Figure 5: 60% of *E. coli* isolates were positive to *rmtC* (711pb) tested by PCR and gel electrophoresis (70W/cm for 90min; 1% agarose) compared with DNA ladder.

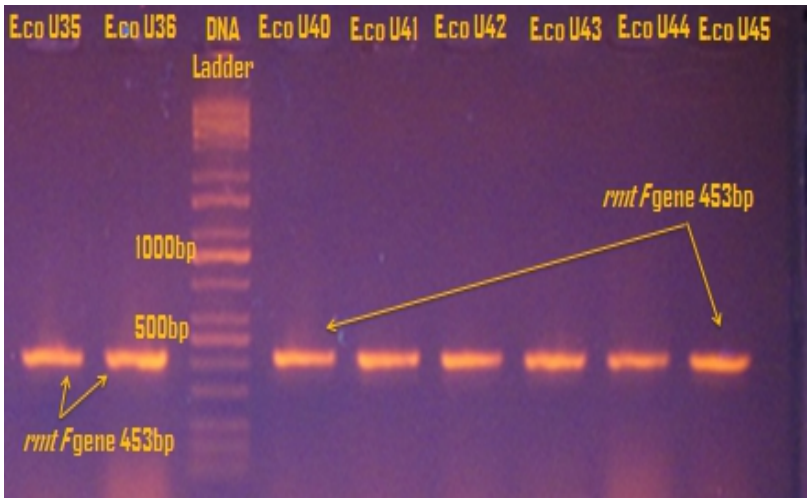

Figure 6: 58% of *E. coli* isolates were positive to *rmtF* (453pb) tested by PCR and gel electrophoresis (70W/cm for 90min; 1% agarose) compared with DNA ladder.

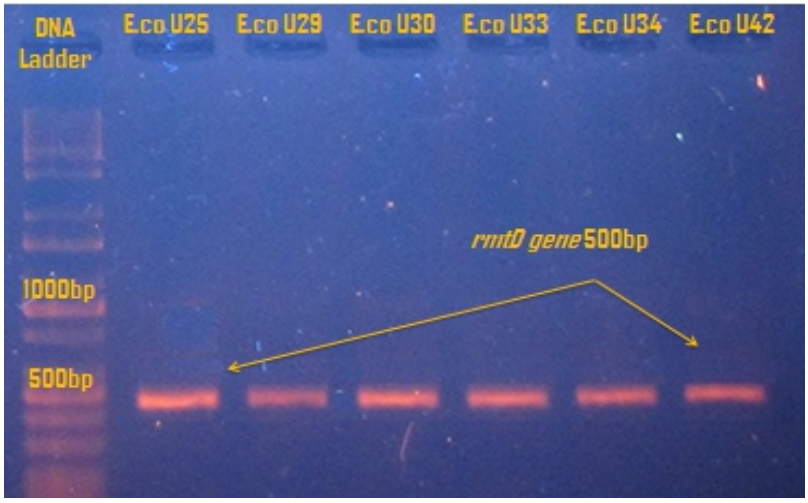

Figure 7: 49% of *E. coli* isolates were positive to *rmtD* (500pb) tested by PCR and gel electrophoresis (70W/cm for 90min; 1% agarose) compared with DNA ladder.

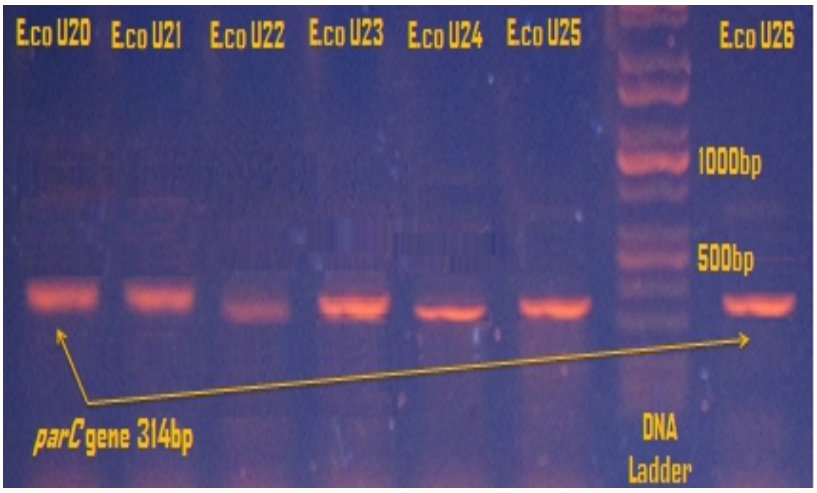

Figure 8: 80% of *E. coli* isolates were positive to *parC* (314pb) tested by PCR and gel electrophoresis (70W/cm for 90min; 1% agarose) compared with DNA ladder.

Microtiter plate assay showed that 86% of *E.coli* isolates were biofilm producers with a range of 0.5-0.9 OD550 nm, while the rest of isolates showed no biofilm production ability (Figure 9).

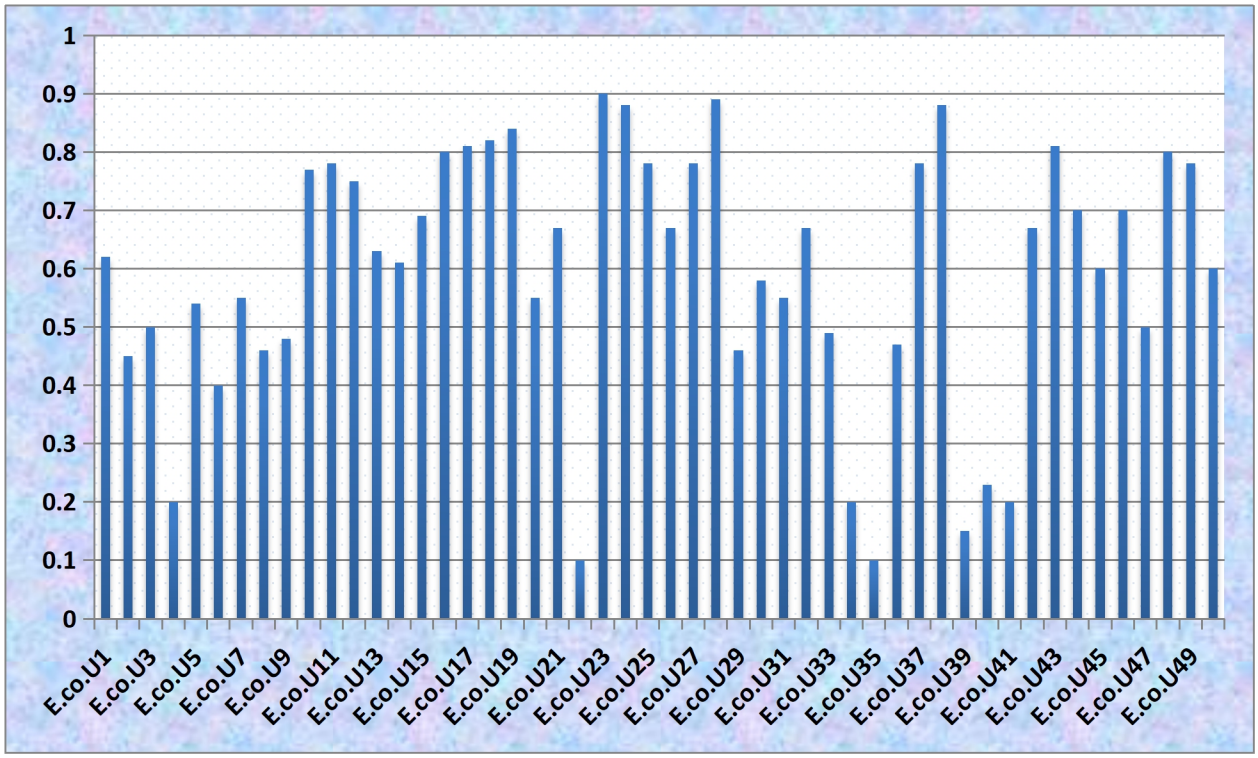

Figure 9: Average of three measures of *E. coli* isolates with a range of 0.5-0.9 OD550 nm using microtiter plate assay

3.2 *Pseudomonas aeroginosa.* antimicrobial sensitivity test showed 5% of isolates was pan drug resistant, while there were 45% isolates considered as multidrug resistant (MDR) and 50% were extensively drug resistant (XDR) (Figure 10).

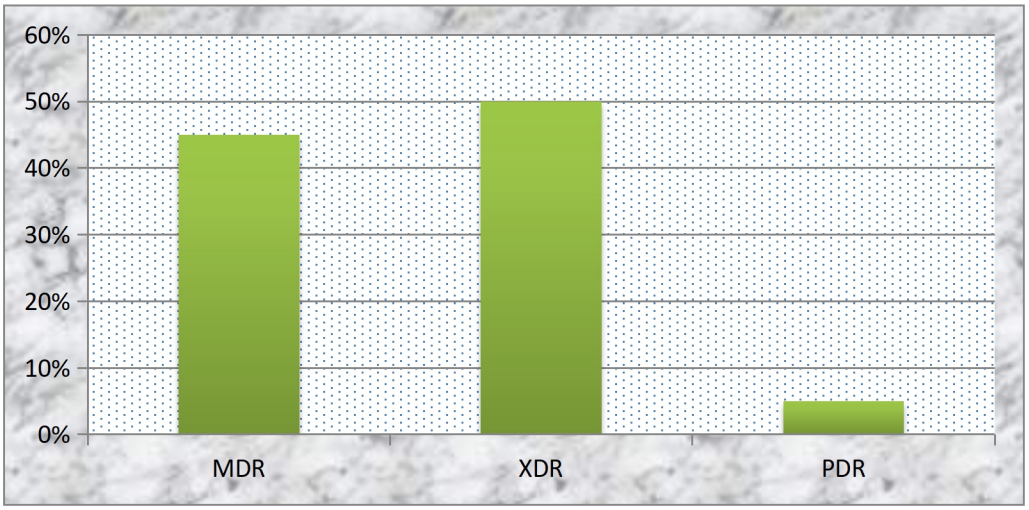

Figure 10: Antibiotic resistant levels of *Pseudomonas aeroginosa* isolates.

Genetic screening for antibiotic resistant genes showed 68% of isolates had *bla* $_{AMP}$ gene (1150bp) (Figure 11). The gene *bla*$_{VIM}$ (382bp) was found among 55% of isolates (Figure 12), and there was 50% were *bla*$_{OXA58}$ (599bp) positive (Figure 13). The gene *rmtC* (711bp) was found among 48% of isolates (Figure 14), and *rmtF* (453bp) was harbored by 40% of isolates (Figure 15); however, 90 % of isolates had *rmtD* (500bp) gene (Figure 16). Last gene tested, *ParC* (314bp), was found among 78% isolates (Figure 17).

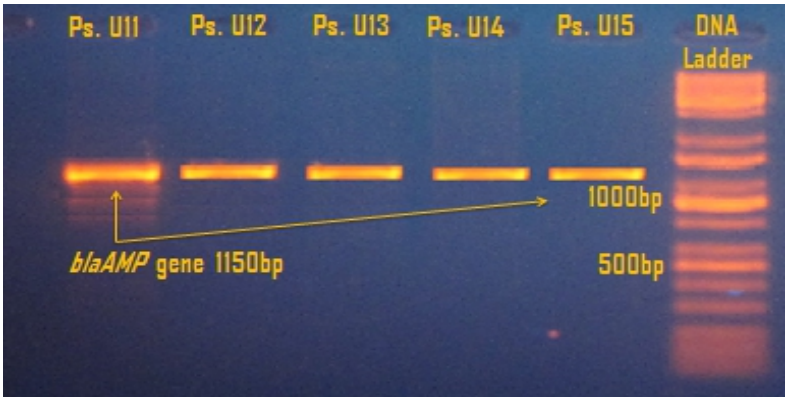

Figure 11: 68% of *P. aeroginosa* isolates were positive to *bla* $_{AMP}$ (1150pb) tested by PCR and gel electrophoresis (70W/cm for 90min; 1% agarose) compared with DNA ladder.

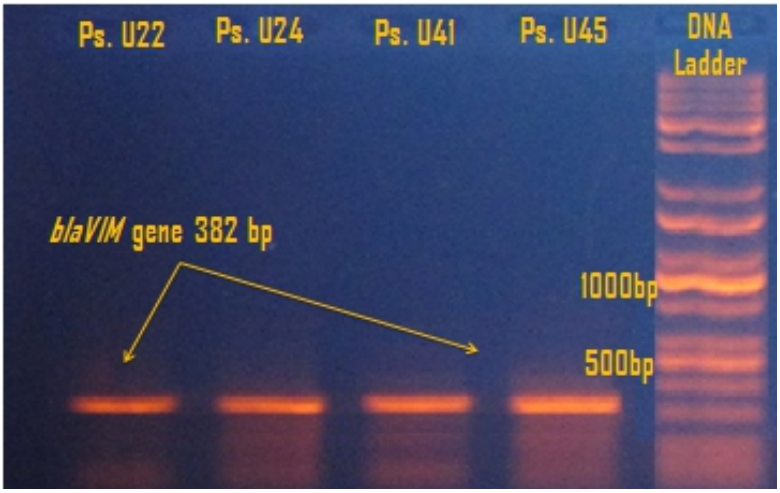

Figure 12: 55% of *P. aeroginosa* isolates were positive to *bla* $_{VIM}$ (382pb) tested by PCR and gel electrophoresis (70W/cm for 90min; 1% agarose) compared with DNA ladder.

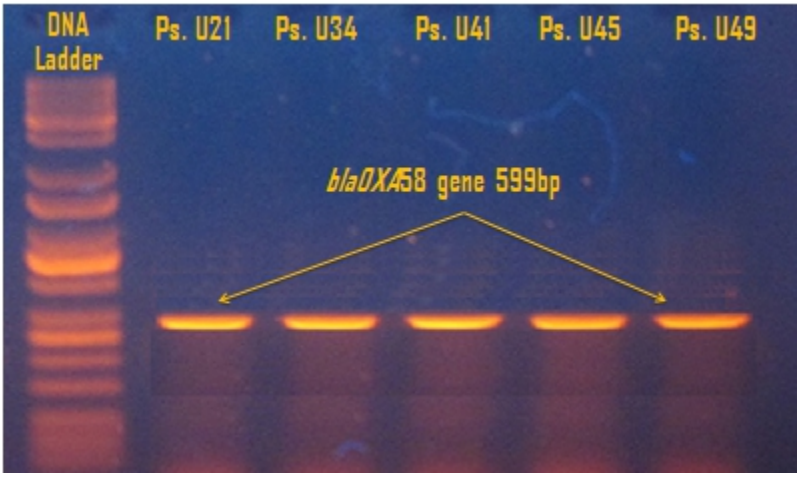

Figure 13: 50% of *P. aeroginosa* isolates were positive to *bla* $_{OXA58}$ (599pb) tested by PCR and gel electrophoresis (70W/cm for 90min; 1% agarose) compared with DNA ladder.

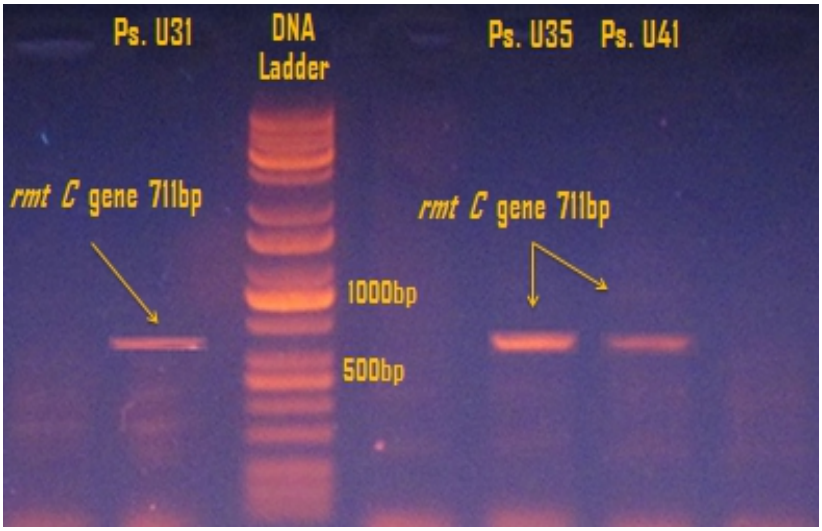

Figure 14: 48% of *Pseudomonas aeroginosa* isolates were positive to *rmtC* (711pb) tested by PCR and gel electrophoresis (70W/cm for 90min; 1% agarose) compared with DNA ladder.

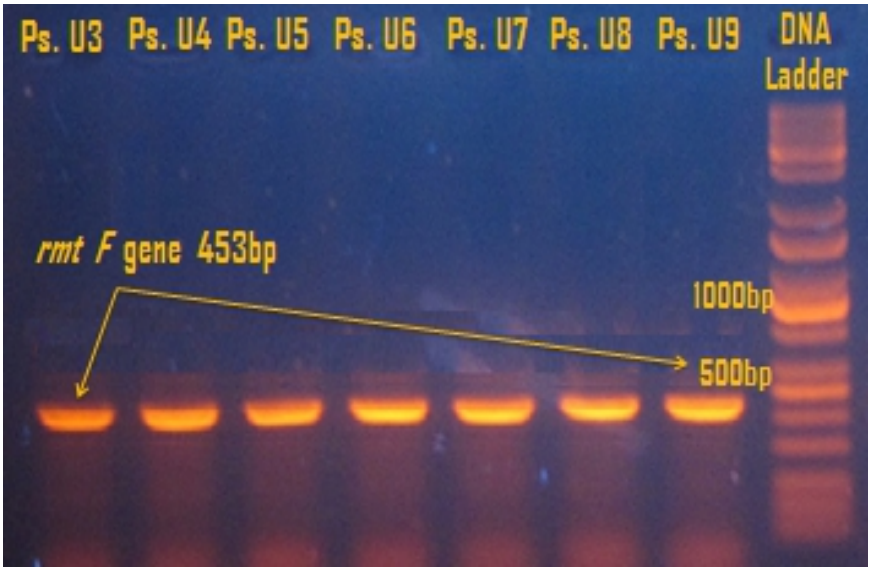

Figure 15: 40% of *P. aeroginosa* isolates were positive to *rmtF* (453pb) tested by PCR and gel electrophoresis (70W/cm for 90min; 1% agarose) compared with DNA ladder.

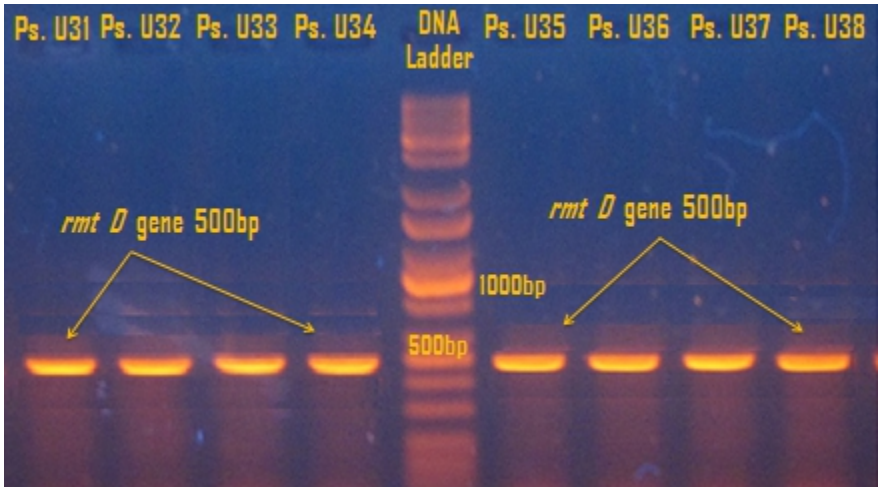

Figure 16: 90% of *P. aeroginosa* isolates were positive to *rmtD* (500pb) tested by PCR and gel electrophoresis (70W/cm for 90min; 1% agarose) compared with DNA ladder.

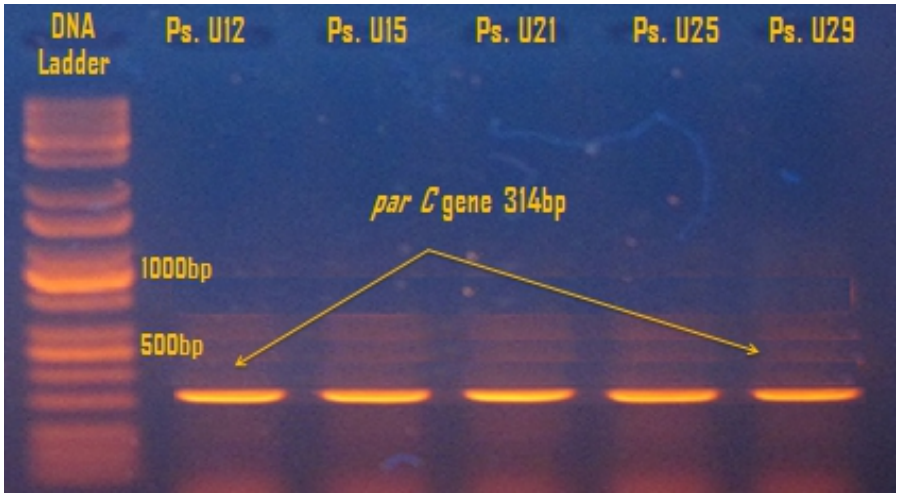

Figure 17: 78% of *P. aeroginosa* isolates were positive to *parC* (314pb) tested by PCR and gel electrophoresis (70W/cm for 90min; 1% agarose) compared with DNA ladder.

Microtiter dish assay revealed that 90% of *P. aeroginosa* isolates were biofilm producers with a range of 0.4-0.7 OD550 nm (Figure 18).

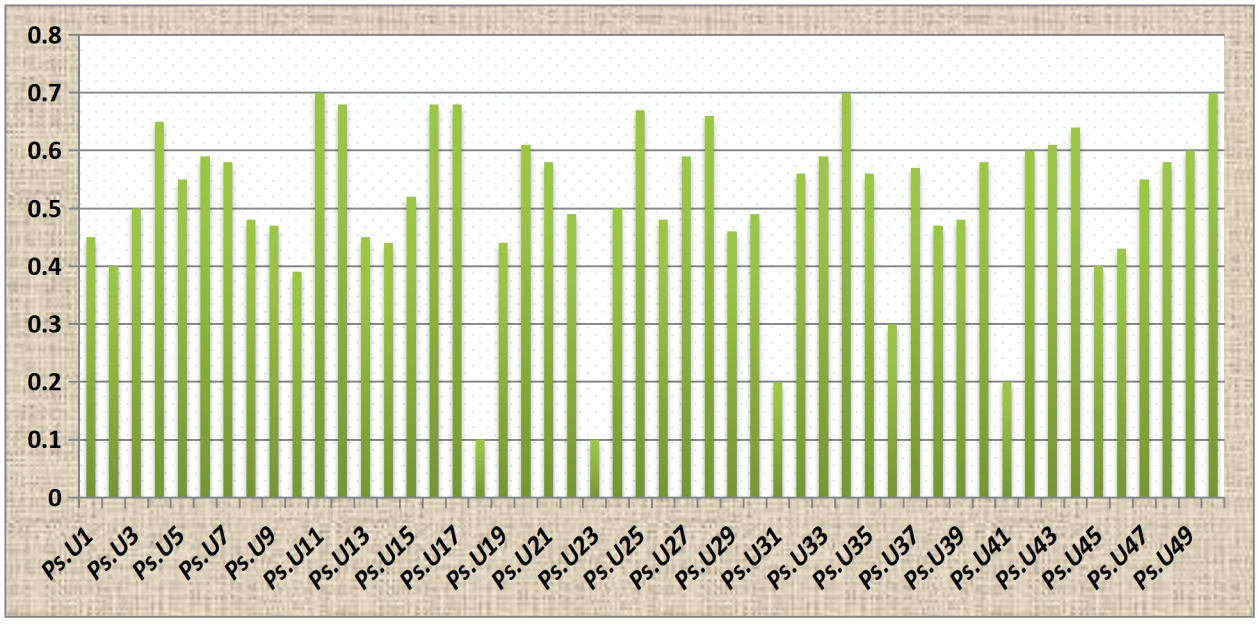

Figure 18: Average of three measures of *P. aeroginosa* isolates at OD550 using microtiter plate assay.

4. Discussion

Antibiotic resistant pathogens could cause serious infections that are hard to treat, especially when they are at high resistant levels MDR, XDR, and PDR (9 and 11). Antibiotic sensitivity test conducted in this study revealed that E. coli isolates had different antibiotic resistant levels. They showed to be 60% MDR, 30% XDR, and even 10% were PDR. *P. aeroginosa* also showed different patterns of resistant, however, they were 45% MDR, 50% XDR, and only 5% were pan drug resistant. Similar results showed by Aljanaby and Aljanaby (11), Mishra *et al.* (12), Aljanaby and Gafil (13), Falagas *et al.* (14), and Ensor *et al.* (15). Mishra *et al.* (12) found that different uropathogenic bacteria like *E. faecalis, S. aureus, C. freundii, E. aerogenes, E. coli, K. oxytoca, K. pneumoniae, P.vulgaris* and *P. aeruginosa* isolated from UTI patients in Saudi Arabia were multidrug resistant towards different antibiotics such as aminoglycosides, β-lactams, fluoroquinolones, cotrimoxazole and nitrofurantoin (12). It was also reported that 126 uropathogenic bacteria include *E. coli*, *Pseudomonas aeroginosa,* and many others could be highly resistant to amoxicillin, third generation cephalosporins and imipenem. They concluded that 69.3% of *E. coli* and 88.8 % of *P. aeroginosa* isolates were MDR (16).

Genotyping studies approved that the ability of antibiotic resistance levels among *E. coli* and *P. aeroginosa* isolates in phenotypic detection referred to having different resistance genes like β-lactamases and metallo β-lactamase (MBLs), 16s ribosomal methylation enzymes, and quinolones resistance genes. PCR studies showed that *E. coli* and *P. aeroginosa* isolates had bla_{AMP}, bla_{VIM}, and bla_{OXA58} genes that confirm the ability to resist different β-lactam antibiotic agents tested in this study, and also would confirm the resistance towards carbapenem agents (imipenem and meropenem) which usually mediated by bla_{VIM} and bla_{OXA58}. Similar results showed by Najjuka *et al.* (17), Kamble (18), Fazeli *et al.* (19), and Kateete *et al.* (20). It was reported that *P. aeruginosa* isolates were carbapenem resistant since they had bla_{IMP}*-like*, bla_{VIM}*-like*, bla_{SPM}*-like*; bla_{NDM-1}*-like* (21). It was also found that clinical *P. aeruginosa* isolates could have AmpC β-lactamases (AmpC), extended spectrum β-lactamases (ESBLs) and metallo β-Lactamases (MBLs) (22). Study revealed that uropathogenic *E. coli* isolated from complicated

UTI were antibiotic resistant due to having ESBL genes and Metallo-betalactamase MBL genes (23).

Aminoglycoside antibiotic agents were also not effective against local *E. coli* and *P. aeroginosa* isolates since they showed to have 16s ribosomal methylation enzymes represents in *rmtC, rmtD,* and *rmtF* genes, which conferred resistancy towards useful clinical aminoglycoside. Similar results were showed by Kateete *et al.* (21), Doi and Arakawa (24), and Wachino *et al.* (25). It was revealed that *Enterobacteriaceae* family members could have methylation of 16S rRNA, resulting in high resistant isolates (24). Other study showed that *rmt C*, *rmtB*, and *rmtA* genes presence among gram negative bacteria were considered as a crucial cause of aminoglycoside resistance, leading to health threat (25). It was demonstrated that 25% of uropathogenic bacteria tested have different aminoglycoside resistant genes such as *ant(4), dfrA, addAB* and *QacE* carried on integron, concluding the possibility of other resistance genes presence in such pathogens (21). Especially found in gram negative bacteria, 16s ribosomal methylation enzymes lead to resistance ability by effecting on antibiotic binding sites (26). As revealed in literature, this resistance ability among uropathogens like *E. coli* and *P. aeroginosa* would lead to rapid world wide spread causing chronic and severe infections (27).

Fourth generation quinolones were also not effective against local isolates since most of them had *parC* gene. It was demonstrated that any mutation in *parC* gene (serine 80) related to topoisomerase IV leads to reduce the affinity of quinolone antibiotics attachments (9). It was revealed that clinical *P. aeroginosa* isolated from different infections had quinolone resistance patterns due to pentapeptide repeat protein *qnr* (21). Białek *et al.* (28) showed that any mutation in genes encoding antibiotic binding proteins would lead to reduce or loss the ability to attach with quinolone agents, turning uropathogenic bacteria to be multidrug resistant. It is studied that uropathogenic *E. coli* isolates were resistant to ciprofloxacin, ofloxacin, and norfloxacin and nalidixic acid in high rates due to having mutation in *gyrA* gene related to DNA topoisomerase II structure (29). Similar results were revealed on uropathogenic *E.coli* that resist ciprofloxacin, norfloxacin and nalidixic acid by PCR (30 and 31). There were four different mutations in *parC* gene confirming quinolone resistance pattern when studying 25 uropathogenic *E.coli* isolated from complicated UTI by DNA sequencing methods (32). It was also studied that uropathogenic

P. aeroginosa could resist ciprofloxacin and levofloxacin due to having of mutations in *gyrA* and *parC* genes (33)

Microtiter dish assay demonstrated that local *E. coli* and *P. aeroginosa* isolates were strong biofilm producers that would suggest being hard to eradicate. Similar findings was revealed by 22). It was showed that 93.3% and 83.3% of biofilm producers *E. coli* and *P. aeroginosa*, respectively could resist nalidixic acid, ampicillin, cephotaxime and cotrimoxazole compared to non-biofilm producers which were mostly sensitive (34). In the same study, they revealed that *E. coli* and *P. aeroginosa* were highly biofilm producers comparing to *Enterococci, K. pneumoniae* , *Acinetobacter, Staphylococci* isolated (34). It was also reported that 60% of *E. coli* isolated from UTI patients were strong biofilm producers when tested by three different means (35). Biofilm is related to chronic and severe UTI, especially when produced by antibiotic resistant uropathogens (36). *E.coli* and *P. aeroginosa* with other isolates tested showed to be resistant to different clinical antibiotic classes and highly biofilm producers (36). Gandee *et al*. (37) revealed that *P. aeroginosa* isolates were good biofilm producers and can resist many antibiotic agents except of ciprofloxacin at 2 µg/mL. Deotale *et al.* (38) showed that *E. coli* isolated from UTI were strong biofilm producers, leading to high level of antibiotic resistance compared with other isolates tested in the study. Also, a study showed that mucoid variant colonies in crystal violet tube-adherence method were produced by E. coli isolates that were trimethoprim-sulfamethoxazole, ciprofloxacin, fosfomycin resistant and ESBL producers (39). Besides, it was demonstrated that 94% of uropathogenic E .coli isolated from complicated urinary tract infection were biofilm producers including 81% of them to be very strong formers as compared with *E. coli* ATCC 25922 positive control (40).

5. Conclusion

As UTI has been the most popular infection among Iraqi community and has high number of samples in clinical units and catheterized UTI patients in Al-Najaf city, Iraq. Findings indicate high resistance pattern against useful antibiotics used clinically and biofilm production was in high rates, suggesting hard treatments trails showed be taken into account.

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