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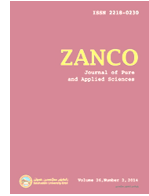

Phenotypic and Molecular Detection of Metallo-β –Lactamase Producing *Pseudomonas aeruginosa* Isolates From Different Clinical Infections in Erbil

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ABSTRACT

Metallo-β-lactamase (MBL) producing *Pseudomonas aeruginosa* has been documented to be a critical nosocomial infection. Itwas continuous intrinsic and acquired resistance to a various group of antimicrobial agents and its resistance ability to develop multidrug resistance lead to a severe therapeutic problem. The study aimed to identify the molecular characterisation of clinical isolates of Metallo-β -lactamase *P. aeruginosa*in Erbil hospitals. This study was carried out during the period from October 2017 to March 2018. A total of 300 clinical specimens were collected from patients (urine 124, wound 80, burns 40, bronchial wash 30, and sputum 26) aged 15-65 years attending Rizgary, West emergency, Erbil teaching hospitals. Out of 300 specimens, 50 isolates of *P. aeruginosa*were recovered and accounted for 1٦% of hospitalised infection isolates, the diagnosis of *P. aeruginosa*isolates was confirmed phenotypically and genotypically via the amplification of *16SrRNA*geneby using PCR technique. All isolates were tested toward the different class of antimicrobials by using agar diffusion method and VITEK 2 system. Levofloxacin, Norfloxacin, and Imipenem was the most effective antimicrobial, and most of the isolates were high resistance to (P, L,V, PI, R, CHL, E, B, A, N, TE, G, MEM, CEF, CTX, ATM).The lowest resistance was to IMP, LEV and NOR. Out of 50 of isolates,14 (28%) were found to produce MBL.*16SrRNA*were used to confirm *P. aeruginosa* and *bla*VIM, *bla*IMP used to detect the MBL. All isolates were positive for *16SrRNA,* while 12 (85%) and 8 (57%) were positive for *bla*VIM and *bla*IMP genes. In conclusion, the present study proved thatMetallo-β-lactamase is producing *P. aeruginosa*isolated had phenotypic characterisation which strongly correlated with according to genotypic characterisation. To our knowledge, this is the first attempt in Erbil city.

1. INTRODUCTION

Pseudomonas aeruginosa is a Gram's negative opportunistic pathogen has emerged as one of the most problematic of the nosocomial pathogens; considered multi-resistant infections in both community and hospital settings. It causes infections in cancer, burn, urinary tract, surgical wound, eye, blood, ear infection, sepsis cystic fibrosis, and intensive care unit (Arciola *et al.,* 2001).

Pseudomonas aeruginosa producing Metallo β-lactamase (MBL) was first ducomented in Jaan in 1991 and since then has been reported from different countries of the world including Asis, Europe, Australia, South America, and North America (Arciola *et al.,* 2001). Ambler classification[1] that divides β-lactamases into four classes (A, B, C and D) based upon their amino acid sequences. Ambler originally specified two classes: class A, the active-site serine β-lactamases; and class B, the metallo-β-lactamases that require a bivalent metal ion usually Zn^{2+} for activity.
Metallo β-lactamases belong to Ambler class B and have the ability to hydrolyse a wide group of β-lactam agents, such as Penicillins, Cdephalosporins, and Carbapenems and it consist of four enzyme groups known as VIM, IMP, SME, and NDM. These enzymes are required Zinc for their catalytic activity and is inhibited by metal chelators, like EDTA and thiol-based compounds (Asmaa *et al.,* 2001). Due to its an extremely persistence organism and usually found in soil, plants, water, humans, animals, and in hospital settings, *P. aeruginosa* is a common pathogen in hospitals generally in intesive care unit (ICU) although has the ability to colonize helthy sunjects, in addition to, exposure to variuos calss of antimicrobials may potentially increase in resistance and subsequence lead to certain mutation and may alter the bacterial genes which encode the drug targets (Azhar, 2017).

It has been understood that the resistance development in *P. aeruginosa* is multifactorial with mutations in genes encoding porins, efflux pump, penicillin-binding proteins, and chromosomal β-lactamases, all contributing to resistance to β-lactamases, carbapenems, aminoglycoside, and quinolones (Azhar, 2017). *P. aeruginosa* is an important pathogen in hospitalised patients usually their morbidity and mortality are due to its multiple resistance mechanisms. Therefore, as the therapeutic option becomes restricted, the search for a new agent is the priority (Ryan *et al.,* 2011).

P. aeruginosa is responsible for about 10% - 20% of nosocomial infections as bacteremia and sepsis in ICU, cystic fibrosis, pneumonia, urinary tract infection and wound infection. Multidrug resistance (MDR), P. aeruginosa phenotype, is defined as resistance to one antibiotic in three or more anti-pseudomonas, antimicrobial classes (carbapenems, aminoglycoside, fluoroquinolones, and cephalosporin) (Mohammed, 2015). The phenotypic method such as biotyping, serotyping, and molecular methods such as plasmid profile analysis and PCR were used for an epidemiological purpose (Shukriyah, 2013).

Genes responsible for the MBL productions in *P. aeruginosa* are typically part of an integron structure and are carried on transferable plasmids or transposons, but also might be a part of chromosomes. Accordingly, due to its integron-associated gene cassettes, *P. aeruginosa* isolates producing MBL are capable for resistant to the different broad variety groups of antimicrobial agents which further can be transferred to various types of bacteria (Alla *et al.,* 2014). MBL-producing organisms infections is linked with greater rates of mortality, morbidity, and healthcare costs. The international epidemiology of MBL-producing *P. aeruginosa* is still unknown in most countries (Sunite *et al.,* 2016), which at least due to partly lack of proper screening and

recommendations. In some countries, such as Egypt, Brazil, and Korea the proportion of MBL-producers among imipenem-resistant *P. aeruginosa* has been estimated to 27%, 20%, and 11.4, respectively (Clare *et al.,* 2006).

Nosocomial infection involving multi-resistant Metallo-β-lactamase producing *Pseudomonas aeruginosa* is a growing problem worldwide (David *et al.,* 1990). Accordingly, this study focused was designed according to the proposed project of the Ministry of Higher Education and Scientific Research for trying to solve the problem in hospital and health care centres. On the causes of the continuous antibiotic resistance and MBL producing by *P. aeruginosa* that prevalent in hospitals especially that caused the hospitalised infections based on the identify the gene responsible between the isolates collected from different clinical samples by using the Polymerase chain reaction (PCR) and genotypic characterisation for MBL. The current study aimed to detect the presence of MBL-producing P. aeruginosa isolates from the different clinical infections and genotypic method.

2. MATERIALS AND METHODS

2.1. Patients and sample collection

A total of 300 clinical samples from both gender (140 males and 160 females) with different age, whom are suffering from different clinical infections (Urine 124, Wound swab 80, Burn swab 40, lower respiratory tract 30, and sputum 26) from Erbil hospitals during the period from October 2017 to March 2018 were enrolled in this study. Depending on clinical criteria that diagnosed by physician and laboratory criteria of infections based on diagnosis microbiology (Hussam, 2016). All specimens were collected by clean sterilised cotton swabs or containers under the supervision of clinical consultant physicians. Each collected swab was collected and placed in a sterile tube with transport media till reaching the laboratory to be inoculated on culture media then inoculated into blood agar, MacConkey agar, and cetrimide agar by streaking method and incubated at 37 °C for 24 hours (Asmaa *et al.,* 2015).

2.2. Phenotypic identification of bacteria

Phenotypical identification of *P. aeruginosa* isolates was made by Gram staining. Colony morphologies on MacConkey's agar, Cetrimide agar for pigment production, growth at 42∘C. The biochemical tests included by using API 20 NE identification system for non-enteric Gram-negative rods and VITEK2 automatic system (BioMerieux) was used with the ID-GNB card for identification of Gram-negative bacilli for phenotypical confirmation by using Gram-negative card (Yoshichika *et al.,* 2000).

2.3. Antimicrobial sensitivity test

The susceptibility of the isolates to the following antibacterial agents was tested by the disc diffusion method, also known as the Kirby-Bauer disc diffusion method was carried out by using disks (Bioanalyse/Turkey) on Mueller Hinton agar interpreted according to the clinical laboratory standard institute guidelines CLSI guidelines (CLSI, 2017): Penicillin (P,10ug), Amoxicillin/ Clavulanic acid (AMC, 20/10 ug), Amikacin (AK, 10 ug), Neomycin (N, 10 ug), Lincomycin (L, 10 ug), Levofloxacin (LEV, 5ug), Vancomycin (V, 30 ug), Bacitracin (B, 10 ug), Norfloxacin (NOR, 10 ug), Erythromycin (E, 10 ug), Piperacillin (PI, 10 ug), Tetracycline (TE, 30 ug), Imipenem (IMP, 10 ug), Meropenem (MEM, 10 ug), Cefotaxime (CTX, 30 ug), Ceftazidime (CEF, 30 ug), Aztreonam (ATM, 10 ug), Chloramphenicol (CHL, 30 ug), Rifampicin (R, 5 ug). Also, the isolates were to awarded to the different class of antimicrobials by VITEK2 automatic system for confirmatory susceptibility tests of isolates (CLSI, 2017).

2.4. Phenotypic detection of MBL

The Imipenem-EDTA combined disc test was performed, test organisms were inoculated on to plates of (Firdous *et al.,* 2011). Two (10 μg/ml) Imipenem discs were placed on the plate in distance 30mm and appropriate amounts of 4 μl of EDTA solution were added to one of them to obtain the desired concentration of (750 μg/ml). The inhibition zone of the Imipenem and Imipenem-EDTA discs were compared after 16-18 hours of incubation at 37 ˚C. The result of combined disc test, IMP-EDTA disc, increase in inhibition zone by ≥7 mm than the IMP disc alone was considered as MBL positive (Mariappan *et al.,* 2014). A Solution of a 0.5 m (Molarity) EDTA was prepared by dissolving 16.8 gm of EDTA in 100 of distilled water and its pH was adjusted to 8.0 by using NaOH and sterilized by autoclaving (Agnieszka *et al.,* 2017).

2.5. DNA extraction and Purification Kit

DNA templates were prepared according to the previously described method (Smet *et al.,* 2009). The extraction kit was provided by (GeNet Bio, Korea) as follow steps: The bacterial cells harvested from overnight culture cell 100-200 µl by centrifuge at 13000 rpm for 30 seconds then discard the supernatant followed by adding 200µl of resuspension buffer for resuspend the cell pellet. Then adding of 20 µl of Proteinase K solution for lyses the bacterial cells then mixed by vertexing and incubated at 56˚C until the cell is completely lyses followed by spin down briefly the tube for removing the reaming drops. Adding of 200 µl of binding buffer to the sample then mix well by pulse-vertexing for 15 sec then incubated at 56˚C for 10 minutes, then adding 200 µl of Ethanol and mixed well by vertexing for 15 sec after that spin down to get remaining drops from clinging under the lid. Transfer the lysate into spin tube then centrifuge 13000 rpm for 1 min followed by transferring the lysate to the new collection tube for filtration then adding of washing buffers and discarding of flow through two times then centrifuge once more to completely removing of remain ethanol. Then the spin column transfer to collection tube then adding 200 µl of elution buffer then stored at -40 ˚C.

2.6. Estimation of DNA yield and purity

The extracted genomic DNA was checked by using Nanodrop spectrophotometer to estimate the concentration and purity of extracted DNA through reading the absorbance at (260 /280 nm) (Mai *et al.,* 2014).

2.7. Preparing the Primers

Lyophilised forward and reverse primers of 16SrRNA gene (F-GGG GGA TCT TCG GAC CTC A, R- TCC TTA GAG TGC CCA CCC G) expected product size 900bp, MBL genes *bla*$_{VIM}$ (F-GTT TGG TCG CAT ATC GCA AC, R- AAT GCG CAG CAC CAG GAT AG) expected product size 380bp, *bla*$_{IMP}$ (F- GAA GGC GTT TAT GTT CAT AC, R- GTA TGT TTC AAG AGT GAT GC) expected product size 600bp were prepared depending on manufacturer's instruction by dissolving the lyophilised sample with nuclease-free water to give a final concentration of (100 pM/µl) (as a stock solution) then rotating down briefly. To prepare 10µM of working primer (working aliquot) re-suspended 10 pM/µl of stock primer in 90µl of deionised water to reach a final concentration 10µM.These primers synthesized by GeNet Bio company (Korea).

2.8. Amplification

PCR was done according to manufacturer protocol for amplification of target genes in a final volume 20µl. The amplification was performed

using hot start Master Mix 2x (GeNet Bio), specific primers for target genes (1µl forward and 1µl reverse), 8µl of *P. aeruginosa* DNA extract as a template. Sterile distilled water was used instead of DNA template to ensure the absence of contaminants in the reaction preparations as a negative control. The PCR conditions started with thermocycler program according to showed in the (Table 2).

Table 1- The PCR thermocycler program for *P. aeruginosa* target genes

Steps	Temperature	Time	Cycle
Initial Denaturation	**95ºC**	**5 min**	**1**
Denaturation Annealing Elongation	**95ºC 50-60ºC 72 ºC**	**30 secs 30 secs 30-60 secs**	**30-45**
Final extension	**72 ºC**	**5 min**	**1**

3. RESULTS AND DISCUSSION

A total of (300) three hundred were collected from different clinical specimens (urine 124, wound swab 80, burn swabs 40, bronchial wash 30, and sputum 26) from patients attending Hawler teaching hospital, Rizgary hospital, and West Emergency hospital in Erbil city during the period from October 2017 to March.

3.1. Collection and identification of *P. aeruginosa* isolates

The obtained *P. aeruginosa* yields and their frequencies from total studied isolates were 5(4%), 14(17.5%), 16(40%), 8 (26.6%) and 7(26.9%) for each of 124 urine, 80 wound, 40 burn, 30 bronchial, and 26 sputum specimens respectively. The highest *P. aeruginosa* frequency occurred in the burn followed by wound infections, and the lowest frequency was found among urine specimen as shown in the (Table 3) and (Fig. 1 &2).

In Brazil (Milena *et al*,.2012) found that the percent of *P. aeruginosa* was15% from the burn which is lower than our results. (Nasih *et al*,.2014) maintained 17% *P. aeruginosa* from the patients. Alla *et al,* (2014) obtained 18% from the burn. (Hussam, 2016) Who collected 30% isolates from 142 clinical swabs from patients in Baghdad hospitals which is by our results. Also, our results agreed with (Nasih *et al*,.2014) who collected 30% from 985 burn samples in Sulaymaniyah hospitalized patients. Asmaa *et al.,* (2015) mentioned that 38% *P. aeruginosa* isolates obtained among 100 samples of burn-in Baghdad hospitals. Sunite (2016) reported that the percent of *P. aeruginosa* was 39% from burn patient. *Pseudomonas aeruginosa*, are among the most common causes of nosocomial as well as community-acquired infection. Burn wounds are a suitable site for multiplication of bacteria and are a more persistent more abundant source of infection than other types of wounds, mainly due to the larger area are included, and longer time of patient stay in the hospital (Delissable and Aibile, 2004). Ali (2016) found that among 60 isolates 81% of isolates can produce pigments among patients in Baghdad hospitals. Another study by (Shukriyah, 2013) reported that among 100 isolates 96% of isolates could produce pigments in Arbil city which is an excellent agreement with our results.

Table 2- Distribution of clinical samples and *P. aeruginosa* isolates

Source of sample	No. of the collected sample	No. of positive culture	Percen. %
Urine	124	5	10%
Wound	80	14	28%

Burn	40	16	32%
Bronchial wash	30	8	16%
Sputum	26	7	14%
Total	300	50	16.66%

3.2. Antibacterial sensitivity of *P. aeruginosa* isolates

Fifty *P. aeruginosa* isolates were screened for their resistance to nineteen different types of antibacterial agents which include (Penicillin, Amikacin, Neomycin, Lincomycin, Gentamycin, Vancomycin, Levofloxacin, Norfloxacin, Bacitracin, Erythromycin, Piperacillin, Tetracycline, Ceftazidime, Aztreonam, Cefotaxime, Chloramphenicol, Rifampicin, Imipenem, and Meropenem). Table (4) illustrates that the isolates were vary in their response to the use of antimicrobial agents and the highest resistance percentage was100% to Penicillin, Lincomycin, Vancomycin, Piperacillin, Rifampicin, and Chloramphenicol, while for Erythromycin was 96%, Bacitracin 82%, Amikacin 78%, Neomycin 78%, Tetracycline 24%, Gentamycin 20%, Meropenem 20% Ceftazidime 16%, Cefotaxime 16%, Aztreonam 16%, and lowest resistance percentage was for Imipenem 4%, Levofloxacin, and Norfloxacin 2%. The isolated bacteria were grouped according to their resistance to the used antimicrobial agents as shown in the (Table 4).

P. aeruginosa isolates across countries are increasingly resistant to a higher number of antimicrobial agents. Ali (2016) described that among 60 isolates of *P. aeruginosa*, 30% resist to 14 different antibacterial agents but all isolates were resistant to G, L, CEF, ATM, PI, and CTX. On the other hand, Produção *et al.,* (2006) demonstrated that the pattern of antibacterial resistance of pathogenic bacterium of *P. aeruginosa*, usually variable from one clinical location to another. Robert (1998) reported that the *P. aeruginosa* is inherent resistance to many antibacterial agents owing to impermeability multidrug efflux pump and a chromosomal AmpC *B. lactamase* and useful activity is seen among Aminopenicillin, 1st to the 4th generation of Cephalosporin monobactam, Carbapenem, amino Glycosides, and Fluoroquinolones. Johann *et al.,* (2005) reported that 20% of *P. aeruginosa* isolates from clinical samples obtained from the surgical units of Ahmadu Bello university teaching hospital in Nigeria were sensitive to imipenem which is in a good agreement with our results that among 50 isolates 28% were resistant to Imipenem and Meropenem. Amina (2013) reported that the *P. aeruginosa* have multidrug resistance gene against various antibacterial agents which was located on the chromosomal DNA. Also, our results showed that all the isolates were highly resistant to Chloramphenicol and Vancomycin. These results agreed with those reported by (Mai *et al.,* 2014) who found that the resistant to Chloramphenicol and Vancomycin were 100% and 100% respectively. Asma *et al.,* (2015) pointed that more than 50 isolates of *P. aeruginosa* among different clinical specimen showed 96% resistance to Amikacin, 96% to Lincomycin, 92% to Erythromycin, 86% to Chloramphenicol, 75% to Amikacin, Neomycin, and 70% to Bacitracin respectively. Hussam (2016) reported that *P. aeruginosa* isolates from burn cases were entirely resist (100%) for each of the following antibacterial agents Penicillin, Lincomycin, Vancomycin, and Piperacillin while exhibited moderate resistance rate (80%) to Amikacin, Rifampicin, Chloramphenicol, and Erythromycin, and were

sensitive to Imipenem, Meropenem, and tazobactam in a percentage rate (10%) for each antibacterial. Resistance mediated by *P. aeruginosa* can be attributed both to an inducible, chromosomally mediated beta-lactamases that can render broad-spectrum cephalosporin indicative and to a plasmid-mediated beta-lactamase that can lead to resistance to several Penicillin and Cephalosporin (Hussen, 2010). The mechanisms of bacterial resistance to aminoglycoside antibacterial in clinical isolates are usually controlled by enzymatic inactivation of the antibacterial, since nine different enzymes that catalyse the phosphorylation, acetylation, re-adenylation of aminoglycosides have now been identified in bacteria (Eigner *et al.,* 2005). The evolution of multi-resistant *P. aeruginosa* and its mechanisms of antibiotic resistance mechanisms include reduced cell permeability, efflux pump, changes in the target enzymes and inactivation of the antibiotics (Drieux *et al.,* 2008). *P. aeruginosa* is a versatile opportunistic pathogen. It is the predominant cause of wound infection in pre-antibiotic and persists as critical pathogen, strongly studies as a significant cause of nosocomial infections. In recent years, a marked increase in the number of hospitals acquired infections due to multidrug resistance has been reported from many countries (Ekrem and Rokan, 2014).

Table 3- Resistance of *P. aeruginosa* to understudy to different antimicrobial agents

Antimicrobial agent	No. of Isolates	% of Resistant
Penicillin	50	100%
Amikacin	37	74%
Neomycin	37	74%
Lincomycin	50	100%
Gentamycin	10	20%
Vancomycin	50	100%
Levofloxacin	2	4%
Norfloxacin	2	4%
Bacitracin	40	80%
Erythromycin	48	96%
Piperacillin	50	100%
Tetracycline	12	24%
Ceftazidime	8	16%
Aztreonam	8	16%
Cefotaxime	8	16%
Chloramphenicol	50	100%
Rifampicin	50	100%
Imipenem	2	4%
Meropenem	10	20%

3.4. Metallo β-lactamase production in *P. aeruginosa*

Combined Imipenem-EDTA disc method performed for detection of the ability of *P. aeruginosa* isolates to produce MBL. That is responsible for their resistance to beta-lactam antimicrobial agents like Imipenem and Meropenem. Among 50 isolates of *P. aeruginosa* 14 (28%), of the isolates were MBL producers, the inhibition zone with Imipenem-EDTA is more than 7 mm than the IMP disc alone. The remaining *P. aeruginosa* isolates 36 (72%) were non-MBL producers (Fig. 1&2).

In the present study, 28% of isolates have Metallo β-lactamase, and this result agreed with (Ami *et al.,* 2008) who found that 21% of *P. aeruginosa* isolates from ICU patients have MBL enzyme, also agreed with (Tanzinah *et*

al., 2010) who reported that 25% of the isolates have MBL enzyme. In contrast, these results do not agree with (Johann *et al.,* 2005) who documented that 66.66% of the isolates have MBL enzyme, and also didn't agree with (Shukriyah, 2013) who demonstrate 69% of isolates have MBL enzyme. Our result also compatible with (Hallem *et al.,* 2011) who detected that 28% of *P. aeruginosa* isolates in Tehran, Iran was MBL producers. Johann *et al.,* (2005) who detected that 30% of *P. aeruginosa* isolates in largely centralised laboratory Canada was MBL producers by using combined Imipenem-EDTA disc method. The present study indicates the high prevalence of MBL of diverse mechanisms. To confilct these problems, epidemiological studies should be done in hospital settings to determine the source of infection. Early detection of these MBL producing isolate in a routine laboratory could help to avoid treatment failure, as often the isolates producing this enzyme show a susceptible phenotype in routine susceptibility testing. Furthermore, strict antibiotic policies and measure to limit the indiscriminative use of Imipenem and Meropenem in the hospital sittings should be undertaken to minimize the emergence of this great multiple MBL producing pathogen, and to prevent spreads would leave no other option to treat Gram-negative nosocomial infections (Al-Haidary, 2010).

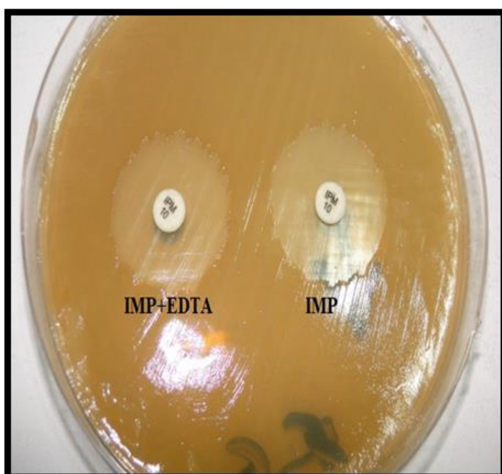

Figure (1): Combined disc diffusion test MBL negative

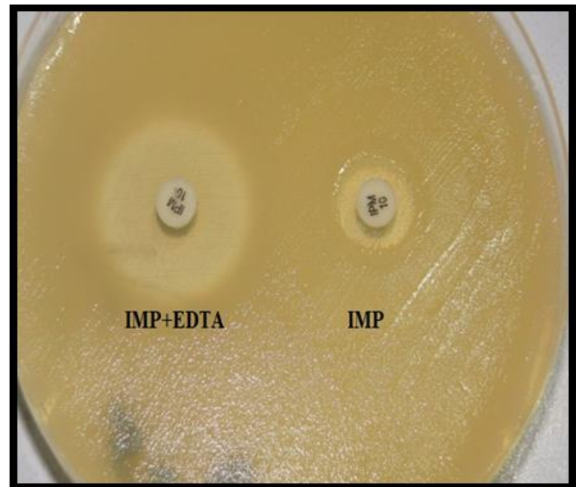

Figure (2): Combined disc diffusion test MBL positive

3.5. Molecular aspect of *16SrRNA* and MBL genes *bla*$_{VIM}$, *bla*$_{IMP}$ of *P. aeruginosa* isolates

The outcome of PCR amplification among 50 isolates, 14 (28%) was found to be MBL producers by Combined Imipenem-EDTA disc method (phenotypic method) and was genotypically confirmed by PCR amplification of the *16SrRNA* (Fig. 3). The amplification of the *16SrRNA* gene showed that among fourteen MBL isolates all of them carried this gene.

Among 14 phenotypic Metallo β-lactamase isolates the results achieved by using PCR revealed that 12 (85%) isolates have *bla*$_{VIM}$ genes (Fig. 4), while 8 (57%) isolates carried *bla*$_{IMP}$ genes (Fig. 5).

MBLs genes were reported by (Mai *et al.,* 2014) which among 122 clinical samples in Cairo Egypt 85% were carried *bla*$_{VIM}$ genes and 54% were positive for *bla*$_{IMP}$ genes which strongly agreed with our findings. However, our finding agreed with (Alla *et al.,* 2014) among 75 isolates in Baghdad hospitals 70% was carried *bla*$_{VIM}$ genes, 40% was carried *bla*$_{IMP}$ genes. This finding was supported by results of previous studies demonstrating

MBLs genes. Amina (2013) mentioned that 100 *P. aeruginosa* isolates 60 (60%) carried *bla*$_{VIM}$ genes and 40% carried *bla*$_{IMP}$ genes. In worldwide nosocomial outbreaks, the VIM is the most dominant MBL gene associated with outbreaks due to MBL producing *P. aeruginosa*. In our study, 71% of MBL isolates carried MBL genes this is agreed with (Ali, 2016) who found that 68% of isolates carried MBL genes. Most of the phenotypic methods were used to detect MBL in *P. aeruginosa* and are very useful for laboratory detection and research purposes, but the confirmation of the results nowadays become a strong point in medicine and to ensure the quality of research and feature of antimicrobials uses, however, the results sometimes maybe differ due to sensitivity and specify of certain genes.

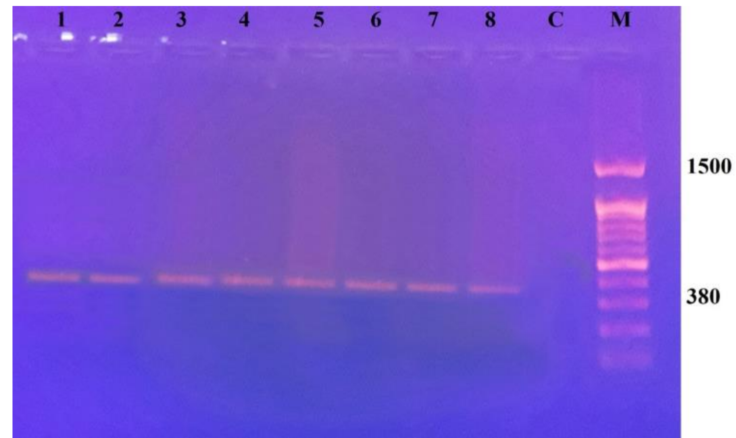

Figure 4: PCR products of a *bla*$_{VIM}$ gene run on 1% agarose gel (30 min /70 vol), lane 1-8 *P. aeruginosa* isolates; lane C negative control; lane M: 100bp size marker.

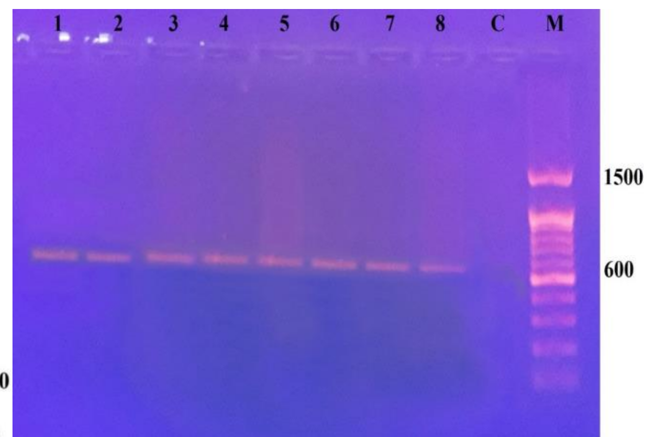

Figure 5: PCR products of a *bla*$_{IMP}$ gene run on 1% agarose gel (30 min /70 vol), lane 1-8 *P. aeruginosa* isolates; lane C negative control; lane M: 100bp size marker.

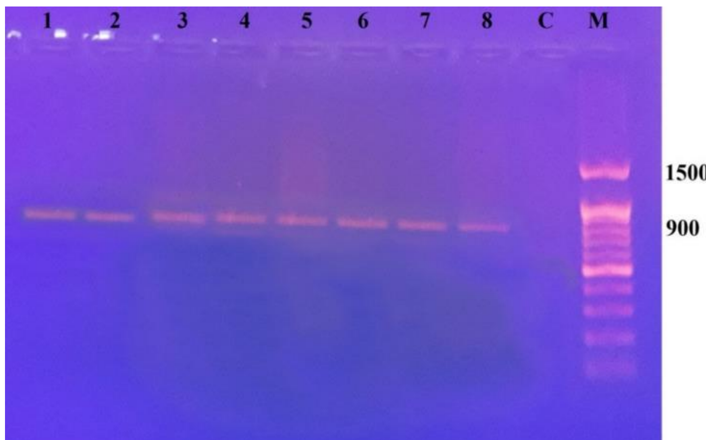

Figure (3): PCR products of *16SrRNA* gene run on 1% agarose gel (30 min /70 vol), lane 1-8 *P. aeruginosa* isolates; lane C negative control; lane M: 100bp size marker.

4. CONCLUSIONS

We can conclude that threats MBL *P. aeruginosa*infection becomes a significant concern often life-threatening to patients and its increasingly growing resistance to various antibacterials are the massive problem in such infection. Majority of studies isolates were multi-resistant to antimicrobial agents which may indicate that they were subject to frequent modifications in their genetic material. Levofloxacin, Norfloxacin, and Imipenem are the most effective antibacterial against *P. aeruginosa*while the most isolates seen to be highly resistant to Penicillin, Lincomycin, Vancomycin, Piperacillin, Rifampicin, and Chloramphenicol. A novel rate isolate is MBL producers. Molecular detection of *16SrRNA* gene is beneficial for *pseudomonas* diagnosis confirmation. Polymerase chain reaction for genotypic characterisation showed that the

majority of MBL isolates are carrying bla_{VIM}, bla_{IMP} genes.

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