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Prevalence and Molecular Characterization of *P. aeruginosa* Isolates in the West Bank-Palestine for ESBLs, MBLs and Integrons

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Authors' contributions

This work was carried out in collaboration between all authors. Authors GA, AS and KA designed the study, wrote the protocol, and wrote the first draft of the manuscript. Authors GA, AS and SO managed the literature searches and analyses of the study performed. Authors GA, AS and KA managed the experimental process and identified the species of microorganisms. Authors GA and SAS edited and reviewed the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study aimed to determine the prevalence and molecular characterization of extended spectrum beta-lactamases (ESBLs), metallo-beta-lactamases (MBLs) and integrons among clinical isolates of *Pseudomonas aeruginosa*.
Place and Duration of the Study: Department of Biology and Biotechnology, An-Najah National University-Nablus, Palestine, during October 2015-April 2016.

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Methodology: Fifty-one clinical isolates of *Pseudomonas aeruginosa* were obtained from different health centers in the West Bank-Palestine. Genes of ESBLs, MBLs and integrons were tested for in the isolates by conventional and/or molecular methods.
Results: This study showed that 21.6% of *P. aeruginosa* isolates were ESBL producers using conventional methods. The prevalence of MBLs by conventional and molecular methods was 60.8% and 29.4% respectively. The most dominant MBL gene among MBL-producing *P. aeruginosa* isolates was bla_{VIM} gene (60%), while the prevalence of bla_{IMP} and bla_{SPM} + bla_{VIM} among the MBL-producing *P. aeruginosa* isolates was 33.3%, 6.7% respectively. Neither bla_{GIM} nor bla_{SIM} was detected. Result of the current research showed that 23.5% of *P. aeruginosa* isolates carried class I integrons. In these clinical isolates neither class 2 nor class 3 integrons were detected. Analysis of ERIC-PCR profiles of 15 *P. aeruginosa* isolates harboring MBL genes showed 4 identical clones circulating among the hospitals from which isolates were collected.
Conclusions: The present study showed high prevalence of MBLs and ESBLs among clinical isolates of *P. aeruginosa* in the West Bank-Palestine. Based on results of this study, effective measures should be taken to control the spread of ESBL- and MBL-producing pathogens including *P. aeruginosa*.

Keywords: Pseudomonas aeruginosa; metallo-beta-lactamases; extended spectrum beta-lactamases; integrons; Palestine.

1. INTRODUCTION

Pseudomonas aeruginosa (P. aeruginosa) is a ubiquitous organism, rarely a member of the normal microbial flora in humans. It commonly affects patients with cystic fibrosis, severe burns, neutropenia, and the mechanically ventilated and is associated with high case fatality rates [1]. Rates of colonization for specific sites in humans are 0 to 2% for skin, 0 to 3.3% for the nasal mucosa, 0 to 6.6% for the throat, and 2.6 to 24% for fecal samples [2]. However, during hospitalization the colonization rates may exceed 50% [3].

The dissemination of this pathogen in healthcare settings is often difficult to control, due to the development of different antibiotic resistance mechanisms such as intrinsic resistance mechanisms and acquired mechanisms, through mutations in different chromosomal loci or horizontal acquisition of resistant genes carried on plasmids, transposons or integrons [4]. Beta-lactams such as ticarcillin, piperacillin, ceftazidime, cefepime, aztreonam, and the carbapenems have an extremely important therapeutic value against *Pseudomonas* infections. Molecular classification based on the amino acid sequence divides β-lactamases into class A, C, and D enzymes, which utilize serine for β-lactam hydrolysis and class B metalloenzymes, which require divalent zinc cations for substrate hydrolysis [5]. Class B β-lactamases, termed metallo-β-lactamases, which are the most clinically important carbapenemases because they are capable of hydrolyzing all β-lactam antimicrobial agents, except the monobactam subgroup, and are not susceptible to β-lactamases inhibitors [6]. Currently, the carbapenem hydrolyzing β-lactamases (metallo β-lactamases) belong to the Bush and Jacoby group 3 of β-lactamases [5]. *Pseudomonas aeruginosa* producing MBLs was first reported from Japan in 1991, then a wide distribution of these resistance determinants and have been reported from various parts of the world including Asia, Europe, Australia, South America and North America [7]. The emergence of acquired MBLs among *P. aeruginosa* represents an epidemiological problem, because these enzymes, virtually confer resistance to all β-lactams and are frequently associated with resistance to aminoglycosides; and genes that are encoding MBL enzymes most commonly are carried on mobile genetic elements that able to spread horizontally [6,8].

Worldwide, the IMP and VIM types are the most commonly detected MBLs in *P. aeruginosa* [9]. VIM-type MBLs are predominant in the Mediterranean region [6,10]. However, IMP-type appears to be the dominant MBL in *P. aeruginosa* isolates from Korea [11,12]. Outbreaks of bla_{VIM}-producing *P. aeruginosa* have been also reported in Greece [13], Italy [14] and Kenya [15]. Outbreaks of bla_{IMP}-producing *P. aeruginosa* have been also reported in Korea [11].

In Tunisia, the prevalence of imipenem resistant *P. aeruginosa* varies between studies and has ranged from 16% to 37.6% [4]. Since November 2002, bla_{VIM-2} producing *P. aeruginosa* has been isolated at Charles Nicolle hospital of Tunisia,

mainly in surgery and intensive care unit [8]. In Saudi Arabia, it was shown that, *bla*$_{VIM-2}$ is the dominant MBL gene in MBL-producing isolates, and the prevalence of MBL was 20.57% of *P. aeruginosa* isolates, and 38.57% of isolates were found resistant to imipenem [16]. In Korea, *P. aeruginosa* isolates harbouring *bla*$_{IMP-6}$ also exhibited a higher level of resistance to meropenem than imipenem [12]. In some countries, such as Korea and Brazil, MBL-producing *P. aeruginosa* constitutes nearly 10% [17,18]. On the other hand, the prevalence of MBLs was high in many countries. In India, 28.57%-54.5% of *P. aeruginosa* produce MBLs [19,20].

Rare information is available about the prevalence and molecular characterization of β-lactamases in the West Bank-Palestine. This study aimed to determine the prevalence and molecular characterization of ESBL- and MBL-producing *P. aeruginosa* using phenotypic tests and multiplex PCR technique among clinical isolates recovered from the West Bank-Palestine, and to determine the prevalence of class 1, 2 and 3 integrons in these isolates.

2. MATERIALS AND METHODS

2.1 Bacterial Strains Collection and Identification

A total of 51 isolates of *P. aeruginosa* were collected from different clinical samples in 2015-2016. The clinical samples included 12 urine, 18 wound swabs, 6 sputum, 1 sputum trap, 4 ear swabs, 1 nasal swab, 3 rectal swabs, 4 skin swabs, 1 blood, 1 abscess. Medical centers and number of isolates from each included in this study were An-Najah National University Hospital-Nablus (n=12), Rafidia Hospital-Nablus (n=8), Medicare Lab-Nablus (n=1), Patient's Friends Society-Nablus (n=2), Palestinian Medical Complex-Ramallah (n=19), AL-Amal Hospital-Jenin (n=1), Al-Shamal lab-Jenin (n=1), Ghannam Lab-Jenin (n=1), Al-Zakah Hospital-Tulkarem (n=1), Thabet Hospital-Tulkarem (n=2), Al-Hussein Hospital-Beit Jala (n=2), Martyr Yasser Arafat Hospital-Salfeet (n=1). These isolates were identified by API 20 E system.

2.2 Antibacterial Susceptibility Test

Antimicrobial sensitivity testing was carried out according to instructions by the Clinical and Laboratory Standard Institute (CLSI) using the disk diffusion method [21]. The isolates were tested for resistance against ciprofloxacin (CIP) 5 µg, nalidixic acid (NA) 30 µg, cefotaxime (CTX) 30 µg, meropenem (MEM) 10 µg, norfloxacin (NOR) 10 µg, ceftazidime (CAZ) 30 µg, tetracycline (TE) 30 µg, imipenem (IPM) 10 µg, and trimethoprim/sulfamethoxazole (1.25 / 23.75 µg). Mueller Hinton agar (MHA) plates were swabbed with a 6-8 h old culture of the bacterial strains; antibiotic disks were placed on the MHA plates containing the inoculum. Then, the plates were incubated at 37℃ for 24 h. The zones of inhibition were measured and the isolates were classified as resistant or susceptible according to the criteria recommended by CLSI guidelines [21].

2.3 Detection of ESBL Production by Phenotypic Test

All *P. aeruginosa* isolates showed resistance to 3rd generation cephalosporins were tested for the presence of ESBLs by combination double disk test (CDDT). Normal saline suspensions of all *P. aeruginosa* isolates were adjusted to McFarland's 0.5 standard and heavily subcultured on MHA plates. Four discs namely ceftazidime (CAZ) 30 µg, and ceftazidime plus clavulanic acid (30/10 µg), cefotaxime (CTX) 30 µg, and cefotaxime plus clavulanic acid (30/10 µg) were placed at a distance of 20 mm on inoculated MHA plates. The plates were incubated for 24 hrs at 37℃. The presence of an ESBL-producing *P. aeruginosa* was confirmed if an increase in the zone diameter equal or greater than to 5 mm for both ceftazidime and cefotaxime tested in combination with clavulanic acid *vs.* its zone diameter when tested alone [22].

2.4 Detection of MBL Production

2.4.1 Combined disc diffusion test (CDDT)

Normal saline suspensions of all *P. aeruginosa* isolates were adjusted to the McFarland 0.5 standard and used to inoculate MHA plates with zinc sulfate at a final concentration 70 µg/ml. Two discs of imipenem (10 µg), meropenem (10 µg) and ceftazidime (30 µg) were placed on the inoculated MHA plate. Seven hundred and fifty µg of EDTA solution was added to one disc of imipenem (10 µg), meropenem (10 µg) and ceftazidime (30 µg). Then, the plates were incubated at 37℃ for 24 h, the MBL-positive isolates were distinguished from the MBL-negative isolates based on the criterion of more than 7 mm increase in the inhibition zone for imipenem and/or meropenem or ≥ 4 mm increase in the inhibition zone for ceftazidime with the discs to which EDTA was added [23,24].

Table 1. Target genes for PCR amplification, amplicon size and primer sequences that were used in this study

Group	Targets	Primer sequence 5′→3′		Expected amplicon size (bp)	Primer mix	References
Metallo-β-lactamases (Class B)	*bla*$_{Imp}$	Imp-F 5-GGAATAGAGTGGCTTAAYTCTC-3 Imp-R 5-CCAAACYACTASGTTATCT-3	52ºC	188bp	1	[26]
	bla$_{Vim}$	Vim-F 5-GATGGTGTTTGGTCGCATA-3 Vim-R 5-CGAATGCGCAGCACCAG-3	52ºC	390bp	1	[26]
	bla$_{Gim}$	Gim-F 5-TCGACACACCTTGGTCTGAA-3 Gim-R 5-AACTTCCAACTTTGCCATGC-3	52ºC	477bp	1	[26]
	bla$_{Spm}$	Spm-F 5-AAAATCTGGGTACGCAAACG-3 Spm-R 5-ACATTATCCGCTGGAACAGG-3	52ºC	271bp	1	[26]
	bla$_{Sim}$	Sim-F 5-TACAAGGGATTCGGCATCG-3 Sim-R 5-TAATGGCCTGTTCCCATGTG-3	52ºC	570bp	1	[26]
Integrases	*intI1*	intI1 F 5-GCATCCTCGGTTTTCTGG-3 intI1 R 5-GGTGTGGCGGGCTTCGTG-3	58ºC	457bp	2	[27]
	intI2	intI2 F 5-CACGGATATGCGACAAAAAGG T-3 intI2 R 5-GTAGCAAACGAGTGACGAAATG-3	58ºC	789bp	2	[27]
	intI3	intI3 F 5-AT TGCCAAACCTGACTG-3 intI3 R 5-CGAATGCCCCAACAACTC-3	58ºC	922bp	2	[27]

2.4.2 Double disc synergy test (DDST)

Normal saline suspensions of all *P. aeruginosa* isolates were adjusted to the McFarland 0.5 standard and used to inoculate MHA plates. ceftazidime disc (30 µg) was placed on MHA medium inoculated with test *P. aeruginosa* isolate and 20 mm apart, a blank filter study disc impregnated with 10 µl of 750 µg EDTA solution was added, another ceftazidime disc was placed on the far side of the medium. Then, the plates were incubated at 37℃ for 24 h. Enhancement of the zone of inhibition in the area between ceftazidime disc and the EDTA disc in comparison with the zone of inhibition on the far side was interpreted as a positive result. The same was conducted with imipenem (10 µg) and meropenem (10 µg) discs [18].

2.5 DNA Isolation and PCR Amplification

2.5.1 DNA isolation

Pseudomonas aeruginosa genome was prepared for PCR according to the method described previously [25]. Briefly, cells were scraped off an overnight MHA plate, washed with 800 µl of 1X Tris-EDTA buffer (10 mM Tris-HCl, 1 mM EDTA [pH 8]), then the pellet was resuspended in 400 µl of sterile double distilled H_2O, and boiled for 10-15 min. Then, the cells were incubated on ice for 10 min. The deposit was pelleted by centrifugation at 11,500 X *g* for 5 min. DNA concentration was determined using a spectrophotometer and the samples were stored at -20ºC.

2.5.2 Detection of MBL genes by multiplex PCR assay

Polymerase Chain Reaction (PCR) analysis was performed for bla_{VIM}, bla_{IMP}, bla_{SPM-1}, bla_{GIM-1} and bla_{SIM-1} genes according to method described previously [26]. Sequence of primers and size of amplicons are described in Table 1. The PCR reaction mix with a final volume of 25 µl, was performed with 12.5 µl of PCR premix (ReadyMix^{TM} Taq PCR Reaction Mix with $MgCl_2$, Sigma), 0.4 µM of each primer and 3 µl of DNA template. The cycling conditions were: initial denaturation at 94ºC for 5 min, followed by 36 cycles of denaturation at 94ºC for 40 s, annealing at 50ºC for 50 s and extension at 72ºC for 1 min, were followed by a final extension step at 72ºC for 5 min. The PCR products were then detected by electrophoresis through 1.5% agarose gels to determine the size of amplified fragment after staining with a final concentration 0.5 µg/ml of ethidium bromide dye.

2.5.3 Detection of class 1, 2 and 3 integrons

All *P. aeruginosa* isolates were tested for the presence of integrons *intI1*, *intI2* and *intI3* using primers described previously [27]. Primer sequences and size of amplicons are presented in Table 1. The PCR reaction mix with a final volume of 25 µl, was performed with 12.5 µl of PCR premix (ReadyMix^{TM} Taq PCR Reaction Mix with $MgCl_2$, Sigma), 0.4 µM of each primer and 150-300 ng of DNA template. The amplification process was carried out using the thermal cycler (Mastercycler personal, Eppendorf, Germany) according to the following thermal conditions: initial denaturation at 94ºC for 2 min followed by 30 cycles of denaturation at 94ºC for 40 s, annealing at 56ºC for 40 s, and extension at 72ºC for 40 s, were followed by a final extension step at 72ºC for 5 min. The amplified PCR products were detected by electrophoresis through 1.5% agarose gels to determine the size of amplified fragments after staining with a final concentration 0.5 µg/ml of ethidium bromide dye.

2.5.4 ERIC-PCR

ERIC- (Enterobacterial repetitive intergenic consensus) PCR was performed using Primer ERIC1: 5`-ATG TAA GCT CCT GGG GAT TCA C-3` and Primer ERIC2: 5`-AAG TAA GTG ACT GGG GTG AGC G-3`. Each PCR reaction mix (25 µl) composed of 10 mM PCR buffer pH 8.3; 3 mM MgCl2; 0.4 mM of each dNTP; 0.8 µM of each primer; 1.5U of Taq DNA polymerase and 3 µl (150-300 ng) of DNA template. DNA amplification was carried out using the thermal cycler (Mastercycler personal, Eppendorf, Germany) according to the following thermal conditions: initial denaturation for 3 min at 94ºC was followed by 40 cycles of denaturation at 94ºC for 50 s, annealing at 50ºC for 60 s and extension at 72ºC for 2 min, were followed by a final extension step at 72ºC for 5 min. The PCR products were analyzed by electrophoresis through 1.5% agarose gel. The gel image was scored using binary scoring system that recorded the absence and presence of bands as 0 and 1, respectively. A binary matrix was analyzed by the unweighted pair group method for arithmetic averages (UPGMA), using SPSS statistical software version 20 (IBM). The number of different bands in each fingerprint was considered for comparison of bacterial strains as previously described [28], based on the following

criteria: identical clones (no different band), "closely related clones" (have 1 different band), "possibility different clones" (have two different bands), "different clones" (have three or more different bands).

3. RESULTS

3.1 Antibiotic Susceptibility

Results of this study showed that all *P. aeruginosa* isolates were resistant to trimethoprim/sulfamethoxazole, most of isolates were resistant to nalidixic acid, ceftriaxone and cefotaxim. The most effective tested antibiotics against these clinical *P. aeruginosa* isolates were norfloxacin, ciprofloxacin and ceftazidime. Results of antibiotic resistance against the clinical *P. aeruginosa* isolates are presented in Table 2. In addition, all of the isolates were multi-drug resistant.

3.2 Detection of β-lactamase Producing *P. aeruginosa* and Integrons

Results of the current study showed that 21.6% of the tested clinical *P. aeruginosa* isolates were ESBL-producers using conventional techniques. The prevalence of MBL genes in these isolates were 60.8% and 29.4% using conventional techniques and multiplex PCR technique, respectively. The prevalence of detected genes was 17.6% for bla_{Vim},, 9.8% for bla_{Imp} and 2% for bla_{Spm} and bla_{Vim} togather. Neither bla_{GIM} nor bla_{SIM} was detected in these isolates. In this study it was shown that bla_{Vim} gene was the most common (60%) among *P. aeruginosa* isolates that carried MBL genes. There was 22 isolates tested positive for MBL using conventional tests only, but negative with multiplex PCR assay. Also in this study, there were 6 isolates of *P. aeruginosa* carried MBL genes using PCR assay only, but considered as non MBL producers using conventional tests. In addition, ESBL and MBL enzymes were coexisted in 9 isolates of *P. aeruginosa* in this study. Results of this research regarding the prevalence of MBLs are presented in Table 3 and Fig. 1.

Table 2. Antibiotic resistance of 51 *P. aeruginosa* isolates collected from different clinical samples

Antibiotic	Resistant strains	
	No.	%
Trimethoprim/ Sulfamethoxazole	51	100
Tetracycline	38	74.5
Nalidixic acid	50	98
Ceftriaxone	49	96.1
Meropenem	23	45.1
Imipenim	25	49
Cefotaxim	49	96.1
Ciprofloxacin	10	21.6
Norfloxacin	11	19.6
Ceftazidime	13	25.5

A total of 12 of clinical isolates of *P. aeruginosa* (23.5%) were carried class I integrons. Nine of class I integrons positive isolates were detected in either MBL producers and/or ESBL producers *P. aeruginosa* isolates. Results of integrons are presented in Fig. 2. Classes II and III integrons were not detected in these isolates.

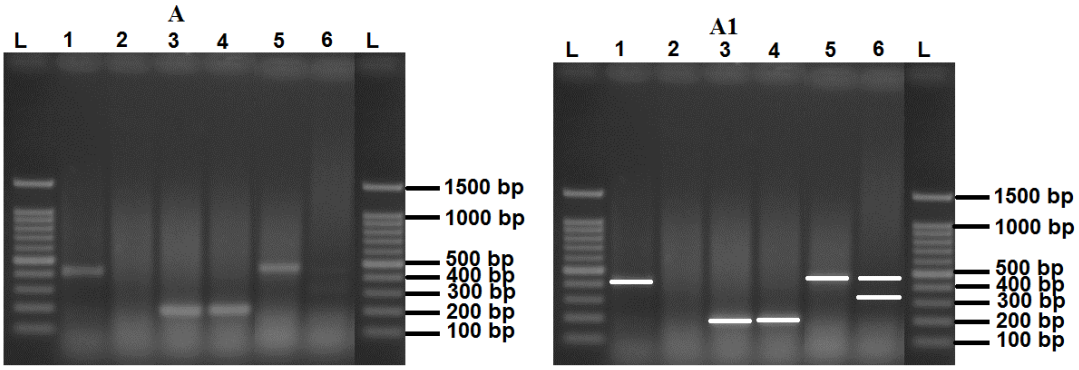

Fig. 1. A: Multiplex PCR profile specific for metallo-β-lactamase genes detected in the clinical isolates of *P. aeruginosa* by multiplex PCR. Lanes L donate for DNA ladder; lanes 1 and 5 donate for bla_{VIM} gene; lane 2 represents a negative sample; lanes 3 and 4 donate for bla_{IMP} gene and lane 6 donates for both bla_{VIM} and bla_{SPM} genes
A1: It is the same as A but bands are demarcated to be obvious

3.3 ERIC-PCR Analysis

ERIC-PCR typing of 15 clinical *P. aeruginosa* isolates carried different MBL genes revealed a 4 ERIC PCR profiles (clusters) at a 75% similarity level. Results of ERIC-PCR typing showed that at least there are 4 identical clones circulating among these hospitals. These clones are C1CL1 (isolates 9 and 14); C2CL1 (isolates 1 and 3); C3CL1 (isolates 5 and 12) and C4CL1 (isolates 4, 7 and 11). Results of ERIC-PCR analyses are presented in Figs. 3 and 4.

4. DISCUSSION

Pseudomonas aeruginosa is one of the most common life-threatening bacteria that cause several complications to the hospitalized patients. The great clinical relevance of this Gram-negative pathogenic microorganism is due to its innate resistance or its ability to develop resistance to many classes of antibiotics. One of these mechanisms is producing MBL enzymes, which could hydrolyze β-lactam ring in β-lactam antibiotics. Nowadays, one of the great

Table 3. Prevalence of MBL genes among 51 isolates of *P. aeruginosa* detected by multiplex PCR and conventional technique

Technique	Metallo-β-lactamase (MBL) genes no. (%)				
	bla_{VIM}	bla_{IMP}	bla_{SPM}+bla_{VIM}	bla_{GIM}	bla_{SIM}
PCR technique	9 (17.6)	5 (9.8)	1 (2)	0 (0)	0 (0)
	Total 15 (29.4)				
Conventional technique	31 (60.8)				
Distribution according to methods	Positive by both techniques: (n=9) Positive by PCR only: (n=6) Positive by conventional only: (n=22)				

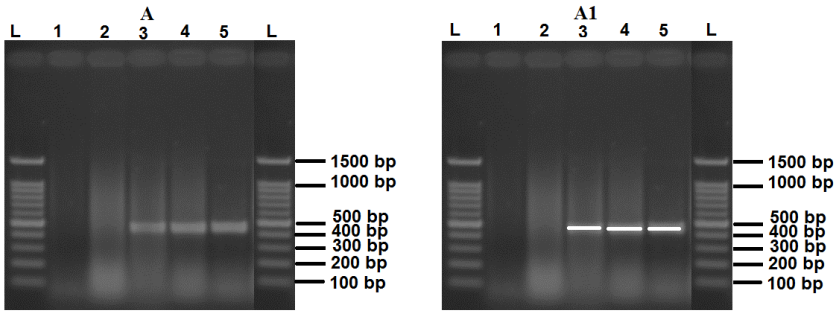

Fig. 2. A: PCR profile specific for integrase genes detected in clinical isolates of *P. aeruginosa*. Lanes L donate for DNA ladder, Lanes 1 and 2 represent negative control and negative integrase sample, respectively, while Lanes 3, 4 and 5 represent positive for integrase 1
A1: It is the same as A but bands are demarcated to be obvious

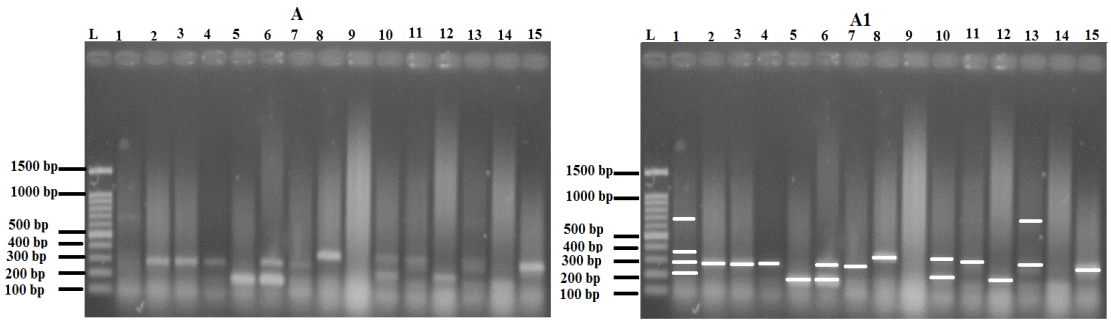

Fig. 3. A: ERIC-PCR profile of 15 clinical isolates of MBL-producers *P. aeruginosa* recovered on 1.5% agarose gel. Lanes L represent DNA ladder, while other lanes represent ERIC-PCR products
A1: It is the same as A but bands are demarcated to be obvious

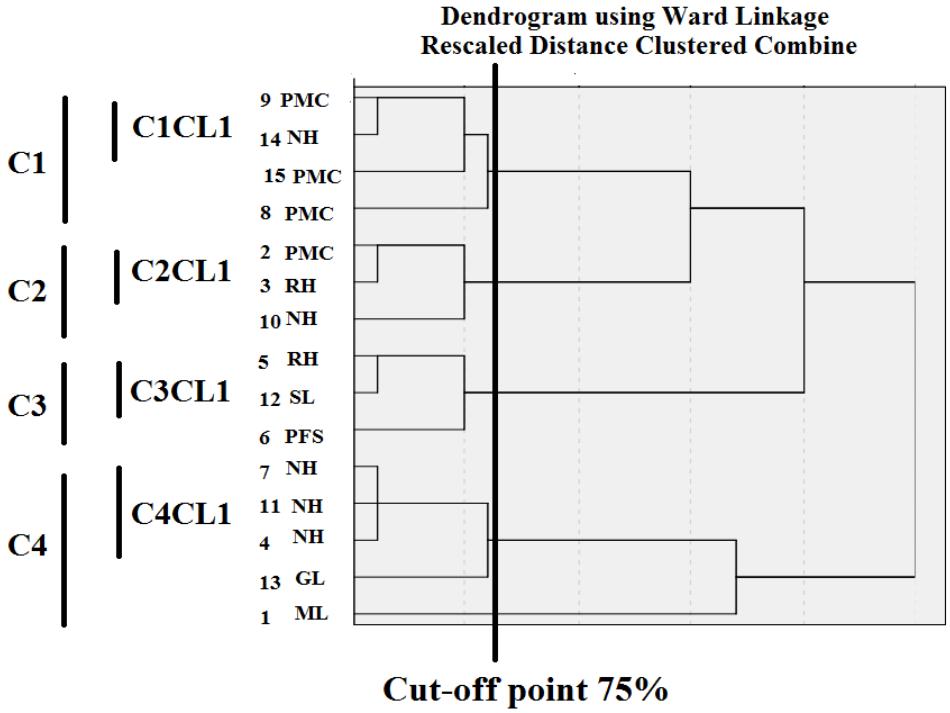

Fig. 4. Dendrogram of 15 clinical *P. aeruginosa* isolates had MBL-genes based on UPGMA method derived from analysis ERIC-PCR-profiles at a 75% similarity level
C: Cluster, CL: Clone, PMC: Palestinian medical complex, RH: Rafidia hospital, NH: Al-Najah National University Hospital, SL: Al-Shamal lab, PFS: Patient's friends society, GL: Ghnnam lab, ML: Medicare labs

challenges is developing of resistance against carbapenem derivatives, which are considered the drugs of choice for the treatment of infections caused by multi-drug resistant Gram negative rods [16]. This is due to the stability of these agents against majority of β-lactamases and their high rate of permeation through bacterial outer membranes [29].

Results of the current study showed that the prevalence of ESBL among *P. aeruginosa* isolates in Palestine is high and it was 21.6% using phenotypic test. In different countries of the Middle East, the prevalence of ESBL producing *P. aeruginosa* ranged from 7.4%-39.2% [30-32].

The present study showed that the Prevalence of MBLs in Palestine is very high and it was 29.4 and 60.8% using multiplex PCR amplification and conventional methods, respectively. In different countries in Middle East, the prevalence of MBLs in clinical *P. aeruginosa* isolates ranged from 20-37.3% [16,30-32]. Methodology (conventional or molecular methods) and type of MBL genes detected in these studies may have an effect on the prevalence between these studies. Worldwide, the bla_{IMP} and bla_{Vim} types are the most commonly detected MBLs in *P. aeruginosa* [9]. Based on these findings, bla_{Vim} gene was the most common one among MBL-producing *P. aeruginosa* isolates. This result is in agreement with other published reports which showed that bla_{Vim} type MBLs is predominant in the Mediterranean region and other parts of the world [6,10,31,33]. However, this result was in contrast to other studies, which showed that bla_{IMP} gene or bla_{SPM-1} gene was the most common in MBL producers *P. aeruginosa* isolates [11,12,33]. In this study, there was 22 isolates of clinical *P. aeruginosa* which showed MBL producers by phenotypic tests but were negative by multiplex PCR assay. This proposed that negative amplification in these phenotypic positive *P. aeruginosa* isolates may be due to these bacterial isolates carried other MBL genes, which could not be amplified by the used primers or these isolates may have MBL genes on the chromosomes of these isolates. On other hand, 6 of the *P. aeruginosa* isolates were negative using conventional methods and positive using PCR

technique. It is thought that the use of other substrates in these conventional methods will increase the sensitivity of these tests. Additionally, the ESBL and MBL coexistence was observed in this study. These results were in agreement with other reports [32,34,35]. The coexistence of different classes of β-lactamases in a single *P. aeruginosa* isolate may pose diagnostic and treatment challenges [34].

In this study, only class I of integrons was detected among tested *P. aeruginosa* isolates and none of these isolates carried other tested classes. This result was consistent with other reports previously published, which showed that the class 1 integrons was the only detected class among clinical isolates of *P. aeruginosa* [36,37]. In current research, 23.5% of tested *P. aeruginosa* isolates carried integrons. This result is in contract to other reports previously published, which showed that the prevalence of class 1 integrons in *P. aeruginosa* isolates is too high and ranged from 40.8%-69.3% [36,37]. Presence of class 1 integrons among multi-drug resistant *P. aeruginosa* isolates or other types of pathogens might be responsible for dissemination of antibiotic resistance gene [27].

Frequency of imipenem and meropenem resistance among *P. aeruginosa* isolates in this study was 49% and 45.1% respectively. This indicates the high level of resistance for carpabenems in Palestine and most of this resistance is due to MBL production. Our study showed that 84% of imipenem resistant and about 81% of meropenem resistant isolates were MBL producers. Other mechanisms may be responsible for imipenem and meropenem resistance. Worldwide, the carpabenems resistance is increasing yearly and it was ranged from 16-98% in the Middle East [4,16,31,32]. High prevalence of antibiotic resistance and high prevalence of MBL-producing *P. aeruginosa* in Palestine may be due to selective pressure of antibiotic imposed by the high rate and misuse of antimicrobial agents, particularly cephalosporins in the country could be the only major cause.

ERIC-PCR analysis was done to study the genetic relationships among 15 *P. aeruginosa* isolates harboring MBL genes. ERIC-PCR profiles for these isolates at 75% similarity level showed that there were 4 clusters with 4 identical bacterial clones circulating among the hospitals from where isolates were collected. From dendrogram, it is noticed that strains of the same clone as in clones C1CL1, C2CL1 and C3CL1 were circulating in different hospitals. This possibly is as a result of medical referrals and transportation of patients among hospitals. Strains of C4CL1 clone were circulating only at Al-Najah National university hospital-Nablus. In previous studies, pulsed field gel electrophoresis (PFGE) was the common technique used to study the genetic relationship among *Pseudomonas* isolates [4,12,27]. Three methods were used in genotyping of *P. aeruginosa* isolates including multi-locus sequence typing, PFGE and ERIC-PCR, the least discriminatory methods was observed by ERIC-PCR [38].

5. CONCLUSION

In conclusion, the high prevalence of MBL-producing *P. aeruginosa* in Palestine underline the necessity of effective measures should be taken to control the spread of MBL-producing *P. aeruginosa* and other β-lactamase-producing pathogens. Correct diagnosis of β-lactamase-producing bacteria including MBL-producing *P. aeruginosa* in due time is obligatory for optimal patient management and for immediate institution of appropriate infection control measures to prevent the spread of these organisms.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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