Jundishapur J Microbiol. 2016 November; 9(11):e30257. doi: 10.5812/jjm.30257.
Published online 2016 October 2. **Research Article**

Carbapenem Susceptibility and Multidrug-Resistance in *Pseudomonas aeruginosa* Isolates in Egypt

Hany Hashem,[1,*] Amro Hanora,[1] Salah Abdalla,[2] Alaa Shawky,[3] and Alaa Saad[4]

[1]Departement of Microbiology and Immunology, Faculty of Pharmacy, Suez Canal University, Egypt
[2]Departement of Microbiology and Immunology, Faculty of Pharmacy, Qassim University, KSA
[3]Departement of Microbiology and Immunology, Faculty of Pharmacy, Cairo University, Egypt
[4]Departement of Clinical Pathology, Faculty of Medicine, Suez Canal University, Egypt

[*]*Corresponding author*: Hany Hashem, Departement of Microbiology and Immunology, Faculty of Pharmacy, Suez Canal University, Egypt. Tel: +2-0111488-8862, Fax: +2-06433230741, E-mail: hany2515@yahoo.com

Received 2015 June 22; **Revised** 2015 October 17; **Accepted** 2016 September 24.

Abstract

Background: Resistant *Pseudomonas aeruginosa* is a serious concern for antimicrobial therapy, as the common isolates exhibit variable grades of resistance, involving beta-lactamase enzymes, beside native defense mechanisms.
Objectives: The present study was designed to determine the occurrence of Metallo-β- Lactamases (MBL) and Amp C harboring *P. aeruginosa* isolates from Suez Canal university hospital in Ismailia, Egypt.
Methods: A total of 147 *P. aeruginosa* isolates, recovered from 311 patients during a 10-month period, were collected between May 2013 and February 2014; the isolates were collected from urine, wound and sputum. Minimum inhibitory concentration (MIC) determined by agar dilution methods was $\geq$2 μg/mL for meropenem and imipenem. Identification of *P. aeruginosa* was confirmed using API 20NE. Metallo-β- Lactamases and Amp C were detected based on different phenotypic methods.
Results: Overall, 26.5% of *P. aeruginosa* isolates (39/147) were carbapenem resistant isolates. Furthermore, 64.1% (25/39) were MBL producers, these isolates were screened by the combined disc and disc diffusion methods to determine the ability of MBL production. Both MBL and Amp C harbored *P. aeruginosa* isolates were 28% (7/25). Sixty-four percent of *P. aeruginosa* isolates were multidrug resistant (MDR) (16/25). The sensitivity toward polymyxin, imipenem, norfloxacin, piperacillin-tazobactam and gentamicin was 99%, 91%, 88%, 82% and 78%, respectively. The resistance rate towards cefotaxime, ceftazidime, cefepime, aztreonam and meropenem was 98.6%, 86%, 71.4%, 34% and 30%, respectively.
Conclusions: Multidrug resistance was significantly associated with MBL production in *P. aeruginosa*. Early detection of MBL-producing *P. aeruginosa* and hospital antibiotic policy prescription helps proper antimicrobial therapy and avoidance of dissemination of these multidrug resistance isolates.

Keywords: *Pseudomonas aeruginosa*, MBLs, Amp C, MDR

1. Background

Almost 10% of hospital-acquired infections are mainly caused by *Pseudomonas aeruginosa* (1). Acquired resistance is due to the production of plasmid-mediated Amp C β-lactamase, Metallo B-Lactamase enzymes (MBL) and Extended Spectrum B-Lactamase (ESBL) (2). Carbapenems are the elective drugs for treatment of multi-drug resistant (MDR) strains; recently, the increase of carbapenem-resistant *P. aeruginosa* has become a serious challenge worldwide (3). Furthermore, MBL are able to hydrolyze this category of antibiotics and their catalytic activities are not inhibited by inhibitors like sulbactam, clavulanic acid and tazobactam (4). However they are sensitive to metal chelates like EDTA, which are used to detect MBL activities of organisms (5). Since the late 1970s, Amp C β-lactamases have gained extended significance as one of the mechanisms of resistance in gram negative bacteria (6). Amp C enzymes are partially capable of hydrolyzing all β-lactams, poorly inhibited by clavulanic acid, and distinguished from ESBLs by their ability of cephamycins hydrolysis (7).

2. Objectives

Multidrug-resistant *P. aeruginosa* are the most prevalent bacterial isolates amongst burned and respiratory infected patients. Our study objective was to define the antibiotic susceptibility profiles of *P. aeruginosa*, as well as MBL and Amp C β-lactamases detection.

3. Methods

3.1. Bacterial Analysis

The study included one hundred and forty-seven (147) clinical specimens of isolates of *P. aeruginosa* collected between May 2013 and February 2014, from Suez Canal university hospital in Ismailia, Egypt, with different sources of infections. All *P. aeruginosa* samples were isolated by standard microbiological procedures, identified using API 20NE (BioMerieux, France), and stockpiled in Luria-Bertani broth medium (Merck, Germany) having 30% glycerol at -80°C.

3.2. Drug Susceptibility Testing

Drug susceptibility testing and interpretation were performed according to clinical laboratory standards institute guidelines (8), using disk diffusion method for antimicrobial agents, including Piperacillin (PRL), Ceftazidime (CAZ), Cefotaxime (CTX), Ceftriaxone (CRO), Cefepime (FEP), Gentamicin (CN), Amikacin (AK), Tobramycin (TOP), Polymyxin (PB), Norfloxacin (NOR), Aztreonam (ATM), Imipenem (IPM), Meropenem (MEM) and Piperacillin-Tazobactam (TZP) [Oxoid, England]. Multi-Drug Resistant *P. aeruginosa* isolates were resistant to at least three classes of the following compounds β-lactams, fluoroquinolones, and aminoglycosides. *Pseudomonas aeruginosa* ATCC 27853 was run simultaneously with the tested organisms for quality control of the susceptibility testing.

3.3. Phenotypic Detection of Metallo B-Lactamase Production

Imipenem and meropenem resistant strains were screened for carbapenemase activity by Modified Hodge Test (MHT) (9). Positive *P. aeruginosa* strains were tested for MBLs production by Imipenem/EDTA double disk synergy test (10) and disk potentiation test (11).

3.3.1. Modified Hodge Test (MHT)

Suspension of overnight culture of *E. coli* ATCC 25922 was adjusted to 0.5 McFarland standard, using a sterile cotton swab on the surface of a Mueller-Hinton agar (Oxoid, England). After drying, 10 μg of imipenem disk was placed in the middle of the plate and the test organism was heavily streaked from center to periphery of the plate in four different directions, and it was allowed to stand for 15 minutes at room temperature. The plate was incubated overnight at 37°C. The presence of distorted zone of inhibition, a 'cloverleaf shaped' due to carbapenemase production by the test strain, was considered as positive results.

3.3.2. Imipenem-EDTA Double Disk Synergy Test (DDST)

The IMP-EDTA double disk synergy test was performed for detection of Metallo-β-lactamases. Liquid overnight culture of the tested isolate was adjusted to a turbidity of 0.5 McFarland standards, and spread on the surface of a MHA plate. After drying, a 10-μg imipenem disk, and a blank sterile filter paper disk (6 mm in diameter) were placed 10 mm apart from edge to edge. Ten microliters of 50 mM zinc sulfate solution was added to the 10-μg imipenem disk (MBLs requires divalent cations at the active site for their activation, usually zinc). Ten microliters of 0.5-M EDTA (Sigma, USA) solution was added to the blank filter paper disk. After overnight incubation at 37°C, the presence of a stretched growth inhibition zone between the two disks was interpreted as positive for MBL.

3.3.3. Disk Potentiation Test

Turbidity was adjusted to 0.5 McFarland standard of the tested strains and inoculated on Mueller Hinton agar plate. Two imipenem disks (10 μg) were placed on the plate wide apart, and 10 μL of 0.5-M EDTA solution was added to one imipenem disk. The inhibition zones of the imipenem and imipenem-EDTA disks were compared after 24 hours of incubation at 37°C. The increase in inhibition zone with the imipenem and EDTA disk was $\geq$ 7 mm when compared to the imipenem disk alone; it was deliberated as MBL-positive isolates.

3.4. Detection of AmpC β-lactamase

Metallo β-lactamase producing isolates were screened for Amp C β-lactamase; cefoxitin (Oxoid, England) inhibition zone diameter < 18 mm were considered as positive for Amp C β-lactamase production (12).

3.4.1. Amp C Test

Test principle was established on use of Tris-EDTA to permeabilize a bacterial cell and release β-lactamases into the outside environment. Amp C (13) disks (disk of filter paper 6-mm in diameter containing Tris-EDTA) were prepared by applying 20 μL of a 1:1 mixture of saline and 100 μL Tris-EDTA to sterile filter paper disks, permitting the disks to dry, and storing them at 8°C (14).

An adjusted 0.5-McFarland suspension standard of overnight culture of cefoxitin-susceptible *E. coli* ATCC 25922 was made and a lawn of culture was inoculated on the surface of a Mueller-Hinton agar plate (8). Amp C disks were rehydrated with 20 μL of saline, and several colonies of *P. aeruginosa* were applied to a disk. The cefoxitin disk (30 μg) was placed on the inoculated surface of the MHA. The inoculated Amp C disk was nearly touching the cefoxitin antibiotic disk. The plate was incubated overnight at 37°C.

3.4.2. Disk Antagonism Test

Inducible Amp C β-lactamases was detected as, 0.5 McFarland of test (15) isolate was swabbed on MHA plate, ceftazidime (30 μg), and cefoxitin (30 μg) disks were placed 20 mm apart from center to center. Presence of inhibition zone blunting in the ceftazidime disk was considered inducible Amp C β-lactamase.

3.4.3. Amp C Inhibitor Method (12)

A disk containing 30 μg of cefoxitin and another containing cefoxitin with 3-Aminophenylboronic Acid (APB) (16), were placed on the agar. Inoculated plates were incubated overnight at 35°C. Comparison of zone size of cefoxitin - APB disk and cefoxitin only disk was more $\geq$ 5 mm recorded as Amp C β-lactamase producer.

3.5. Minimum Inhibitory Concentrations Determination of Carbapenem

Carbapenems MICs, determined for MBLs producers by the agar dilution method, were graded serially to obtain drug concentrations ranging from 1024 to 0.125 μg/mL of the respective commercial preparation of imipenem [500 mg powder, Manufacturers: Glaxo Smithklein, Cairo, Egypt] and meropenem [500 mg powder, Astra Zeneca pharma, Cairo, Egypt], and were taken for the study of antibiotics, according to the Clinical and Laboratory Standards Institute (CLSI) (8).

4. Results

One hundred and forty-seven (147) non-duplicate *P. aeruginosa* clinical isolates were collected from Suez Canal university hospital. The clinical specimens were collected from clinically diagnosed patients and separated into six groups, according to the source of infection as shown in Table 1.

4.1. Metallo-β- Lactamase-producing Pseudomonas aeruginosa Isolates

Thirty-nine (39, 26.5%) out of 147 were carbapenem (IMP & MEM) resistant *P. aeruginosa* isolates. Metallo-β- Lactamases producers were 25 isolates (17%), and in relation to clinical specimens shown in Figure 1, which were confirmed by imipenem-EDTA double disk synergy test and disk potentiation test.

4.2. Carbapenem Minimum Inhibitory Concentrations

Minimum Inhibitory Concentration determination for imipenem and meropenem was done by the agar dilution technique; (39) Carbapenemase-producing *P. aeruginosa* isolates are summarized in Table 3.

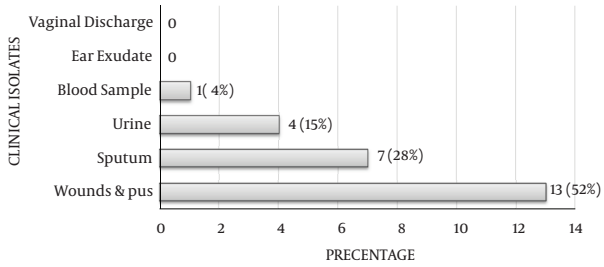

Figure 1. Metallo-β- Lactamases Producer Numbers and Percentage in Relation to Source of *P. aeruginosa* Clinical Isolates

4.3. Metallo-β-Lactamases Produced by Pseudomonas aeruginosa in Relation to Age

Patients infected with (MBL) *P. aeruginosa* (68%, 17/25 patients) mainly belonged to the 51 to 70 year-old age group, as detailed in patients age distribution curve of Figure 2. In addition, MBLs prevalence in males was 60% (15/25) and in females was 40% (10/25).

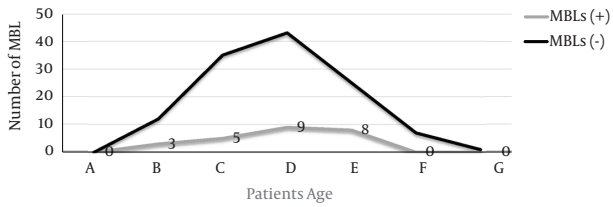

Figure 2. Age Distribution of Patients Infected With Metallo-β-Lactamases (+) and MBL (-) bacteria. Age in years; A: (0-20); B: (21-40); C: (41-50); D: (51-60); E: (61-70); F: (71-80); G: (81-100)

4.4. AmpC β -lactamase Detection

Metallo β-lactamase positive isolates (17) were screened for co-existence of Amp C. The potential Amp C β-lactamase producers, detected by the cefoxitin-screening test, were seven (28%) positive isolates. Among the seven screening positive isolates, one (4%) *P. aeruginosa* isolate revealed the presence of inducible Amp C β-lactamases by disk antagonism test, and plasmid mediated Amp C was detected in five (20%) *P. aeruginosa* isolates.

5. Discussion

Pseudomonas aeruginosa infection is a major cause of serious complications in hospitalized patients of developing countries (18, 19). Metallo-β-Lactamases have been identified from clinical isolates worldwide. Senda et al. reported an increasing frequency over the earlier few years,

Table 1. Distribution of *Pseudomonas aeruginosa* in Clinical Samples

Isolated Group	Sources of *P. aeruginosa* Isolates	Number of Isolates (n = 147)	Percentage
Group I	Wounds & pus swabs	63	43%
Group II	Sputum	34	23%
Group III	Urine	29	20%
Group IV	Blood sample	11	7%
Group V	Ear exudate	7	5%
Group VI	Vaginal discharge	3	2%

Table 2. Antimicrobial Susceptibility Profiles of (147) *Pseudomonas aeruginosa* Isolates

Antimicrobial Agent(s)	Concentration (μg)	Resistant, No. (%)	Intermediate, No. (%)	Sensitive, No. (%)
PRL	100	83 (56)	-	64 (43.5)
CAZ	30	111 (75.5)	16 (11)	20 (14)
CTX	30	141 (96)	4 (3)	2 (1.3)
CRO	30	121 (82)	11 (7)	15 (10)
FEP	30	97 (66)	8 (5)	42 (28.5)
CN	10	27 (18)	5 (3.4)	115 (78)
AK	30	28 (19)	6 (4)	113 (77)
TOP	10	29 (20)	8 (5)	110 (75)
PB	300 IU	2 (1.3)	-	145 (99)
NOR	10	16 (11)	2 (1.3)	129 (88)
ATM	30	23 (16)	27 (18)	97 (66)
IMP	10	11 (7)	2 (1.3)	134 (91)
MEM	10	35 (24)	9 (6)	103 (70)
TZP	100/10	26 (18)	-	121 (82)

Abbreviations: PRL, Piperacillin; CAZ, Ceftazidime; CTX, Cefotaxime; CRO, Ceftriaxone; FEP, Cefepime; CN, Gentamicin; AK, Amikacin; TOP, Tobramycin; PB, Polymyxin; NOR, Norfloxacin; ATM, Aztreonam; IPM, Imipenem; MEM, Meropenem; TZP, Piperacillin Tazobactam.

Table 3. Minimum Inhibitory Concentration for (39) Carbapenem Resistant *Pseudomonas aeruginosa* Isolates

Antibiotics	Minimum Inhibitory Concentration in μg/mL						
	$\leq$ 2	4	8	16	32	64	$\geq$ 128
Imipenem	9	4	7	7	3	6	3
Meropenem	2	8	7	8	8	2	4

and bacteria producing these enzymes have been responsible for persistent nosocomial outbreaks that were accompanied by severe infections (20). In our study, the commonest specimen was wound, while pus swabs had a prevalence of 43% (63/147 isolates) and sputum swab 23% (34/147 isolates), followed by other specimens. These findings are consistent with other studies where *P. aeruginosa* was found frequently to cause suppurated skin and respiratory infections (21, 22).

Our results report that 26.5% (39/147) of *P. aeruginosa* strains were resistant to carbapenem antibiotics (imipenem & meropenem) of which, 64% (25/39) were detected as MBL-producers, which is much higher than studies conducted by Navneeth et al., (23), and Hodiwala et al., (24), who revealed 12% and 21% MBL-mediated imipenem resistance in *P. aeruginosa*. In our study the resistance

rates of cefotaxime, ceftazidime, cefepime, piperacillin, aztreonam and meropenem were 98.6%, 86%, 71.4%, 56%, 34% and 30%, respectively. Behera et al. reported 70% resistance to ceftazidime, 75% to piperacillin, 59% to piperacillin/tazobactam, 74% to amikacin, 81% to cefepime, and 69% to aztreonam (25).

The sensitivity testing toward polymyxin, imipenem, norfloxacin, piperacillin-tazobactam, and gentamicin were 99%, 91%, 88%, 82%, and 78%, respectively. In a previous study by Dardi and Wankhede, higher sensitivity rate was reported towards amikacin (83.3%), meropenem (81.7%), tobramycin (80%) and cefepime (66.7%) (26). Multi-Drug Resistance in our study was 64% (16/25), nearly similar to the study of Anvarinejad et al., which reported MDR of 63.5% (17). In the present study, the most common age group affected by MBLs was > 51 year-olds with a prevalence of 68% (17/25), and males with prevalence of 60% (15/25) were more frequently affected than females with prevalence of 40% (10/25), with, male: female ratio being 3: 2. Niranjan et al., showed that MBLs were more prevalent in the age group of 10 to 11 year-olds, with prevalence of 29% (10/34) (27).

Males were 64.7% (22/34) while females were 35.3% (12/34) with male: female ratio being 1.8: 1. Deba et al. in their study on MBLs detection reported that male: female ratio was 1.2: 1 and the most common age group was > 60 year-olds (46.6%) (28). Prevalence of Amp C β-lactamases among MBLs-producing *P. aeruginosa* isolates was 28% (7/25), which was lower than the study conducted by Noyal et al., that reported 46.9% (15/32) were Amp C β-lactamase and MBLs producers (29). Therefore, Amp C β-lactamase could be a significant causative factor for carbapenemase resistance between the isolates in our hospital similar to other studies (30, 31).

Footnotes

Authors' Contribution: Hany Hashem and Amro Hanora participated in designing the experiments; Hany Hashem, Amro Hanora and Salah Abdalla participated in drafting of the manuscript; Hany Hashem carried out all experiments including molecular biology experiments; All authors read and approved the final manuscript.

Financial Disclosure: The authors declared that they had no financial and non-financial competing interests.

Funding/Support: The study was financially supported by Hany Hashem, department of microbiology and immunology, faculty of pharmacy, Suez Canal university in Ismailia, Egypt.

References

1. National Nosocomial Infections Surveillance S. National Nosocomial Infections Surveillance (NNIS) System Report, data summary from January 1992 through June 2004, issued October 2004. *Am J Infect Control.* 2004;**32**(8):470–85. doi: 10.1016/S0196655304005425. [PubMed: 15573054].
2. Manchanda V, Singh NP. Occurrence and detection of AmpC β-lactamases among Gram-negative clinical isolates using a modified three-dimensional test at Guru Tegh Bahadur Hospital, Delhi, India. *J Antimicrob Chemother.* 2003;**51**(2):415–8. [PubMed: 12562713].
3. Rossolini GM, Mantengoli E. Treatment and control of severe infections caused by multiresistant Pseudomonas aeruginosa. *Clin Microbiol Infect.* 2005;**11 Suppl 4**:17–32. doi: 10.1111/j.1469-0691.2005.01161.x. [PubMed: 15953020].
4. Queenan AM, Bush K. Carbapenemases: the versatile beta-lactamases. *Clin Microbiol Rev.* 2007;**20**(3):440–58. doi: 10.1128/CMR.00001-07. [PubMed: 17630334] table of contents.
5. Arakawa Y, Shibata N, Shibayama K, Kurokawa H, Yagi T, Fujiwara H, et al. Convenient test for screening metallo-beta-lactamase-producing gram-negative bacteria by using thiol compounds. *J Clin Microbiol.* 2000;**38**(1):40–3. [PubMed: 10618060].
6. Bush K, Jacoby GA, Medeiros AA. A functional classification scheme for beta-lactamases and its correlation with molecular structure. *Antimicrob Agents Chemother.* 1995;**39**(6):1211–33. [PubMed: 7574506].
7. Hanson ND. AmpC beta-lactamases: what do we need to know for the future?. *J Antimicrob Chemother.* 2003;**52**(1):2–4. doi: 10.1093/jac/dkg284. [PubMed: 12775673].
8. Wayne PA. Clinical and Laboratory Standards Institute (CLSI) performance standards for antimicrobial susceptibility testing; Tewnty-Second informational supplement.. Approv Stand CLSI Doc M100-S22.; 2012. p. 32.
9. Lee K, Kim CK, Yong D, Jeong SH, Yum JH, Seo YH, et al. Improved performance of the modified Hodge test with MacConkey agar for screening carbapenemase-producing Gram-negative bacilli. *J Microbiol Methods.* 2010;**83**(2):149–52. doi: 10.1016/j.mimet.2010.08.010. [PubMed: 20801167].
10. Lee K, Chong Y, Shin HB, Kim YA, Yong D, Yum JH. Modified Hodge and EDTA-disk synergy tests to screen metallo-beta-lactamase-producing strains of Pseudomonas and Acinetobacter species. *Clin Microbiol Infect.* 2001;**7**(2):88–91. [PubMed: 11298149].
11. Yong D, Lee K, Yum JH, Shin HB, Rossolini GM, Chong Y. Imipenem-EDTA disk method for differentiation of metallo-beta-lactamase-producing clinical isolates of Pseudomonas spp. and Acinetobacter spp. *J Clin Microbiol.* 2002;**40**(10):3798–801. [PubMed: 12354884].
12. Hemalatha V, Padma M, Sekar U, Vinodh TM, Arunkumar AS. Detection of Amp C beta lactamases production in Escherichia coli & Klebsiella by an inhibitor based method. *Indian J Med Res.* 2007;**126**(3):220–3. [PubMed: 18037717].
13. Singhal S, Mathur T, Khan S, Upadhyay DJ, Chugh S, Gaind R, et al. Evaluation of methods for AmpC beta-lactamase in gram negative clinical isolates from tertiary care hospitals. *Indian J Med Microbiol.* 2005;**23**(2):120–4. [PubMed: 15928443].
14. Black JA, Moland ES, Thomson KS. AmpC disk test for detection of plasmid-mediated AmpC beta-lactamases in Enterobacteriaceae lacking chromosomal AmpC beta-lactamases. *J Clin Microbiol.* 2005;**43**(7):3110–3. doi: 10.1128/JCM.43.7.3110-3113.2005. [PubMed: 16000421].
15. Upadhyay S, Sen MR, Bhattacharjee A. Presence of different beta-lactamase classes among clinical isolates of Pseudomonas aeruginosa expressing AmpC beta-lactamase enzyme. *J Infect Dev Ctries.* 2010;**4**(04):239–42. doi: 10.3855/jidc.497.
16. Yagi T, Wachino J, Kurokawa H, Suzuki S, Yamane K, Doi Y, et al. Practical methods using boronic acid compounds for identification of class C beta-lactamase-producing Klebsiella pneumoniae and Escherichia coli. *J Clin Microbiol.* 2005;**43**(6):2551–8. doi: 10.1128/JCM.43.6.2551-2558.2005. [PubMed: 15956362].

17. Anvarinejad M, Japoni A, Rafaatpour N, Mardaneh J, Abbasi P, Shahidi MA, et al. Burn patients infected with metallo-beta-lactamase-producing Pseudomonas aeruginosa: Multidrug-resistant strains. *Archives of Trauma Res.* 2014;**3**(2):ee18182. doi: 10.5812/atr.18182.
18. Lari AR, Alaghehbandan R. Nosocomial infections in an Iranian burn care center. *Burns.* 2000;**26**(8):737–40. [PubMed: 11024608].
19. Zafer MM, Al-Agamy MH, El-Mahallawy HA, Amin MA, El Din Ashour S. Dissemination of VIM-2 producing Pseudomonas aeruginosa ST233 at tertiary care hospitals in Egypt. *BMC Infect Dis.* 2015;**15**:122. doi: 10.1186/s12879-015-0861-8. [PubMed: 25880997].
20. Senda K, Arakawa Y, Nakashima K, Ito H, Ichiyama S, Shimokata K, et al. Multifocal outbreaks of metallo-beta-lactamase-producing Pseudomonas aeruginosa resistant to broad-spectrum beta-lactams, including carbapenems. *Antimicrob Agents Chemother.* 1996;**40**(2):349–53. [PubMed: 8834878].
21. Zavascki AP, Barth AL, Gaspareto PB, Gonçalves ALS, Moro ALD, Fernandes JF, et al. Risk factors for nosocomial infections due to Pseudomonas aeruginosa producing metallo-β-lactamase in two tertiary-care teaching hospitals. *J Antimicrob Chemother.* 2006;**58**(4):882–5. doi: 10.1093/jac/dkl327.
22. Elumalai K, Krishnappa K, Anandan A, Govindarajan M, Mathivanan T. Larvicidal and ovicidal activity of seven essential oil against lepidopteran pest S. litura (Lepidoptera: Noctuidae). *Inter J Recent Sci Res.* 2010;**1**:008–14.
23. Navaneeth BV, Sridaran D, Sahay D, Belwadi MR. A preliminary study on metallo-beta-lactamase producing Pseudomonas aeruginosa in hospitalized patients. *Indian J Med Res.* 2002;**116**:264–7. [PubMed: 12807154].
24. Hodiwala A, Dhoke R, Urhekar AD. Incidence of metallo-beta-lactamase producing pseudomonas, acinetobacter & enterobacterial isolates in hospitalised patients. *Int J Pharamcy Biol Sci.* 2013;**3**:79–83.
25. Behera B, Das A, Mathur P, Kapil A. High prevalence of carbapenem resistant Pseudomonas aeruginosa at a tertiary care centre of north India. Are we under-reporting?. *Indian J Med Res.* 2008;**128**(3):324–5. [PubMed: 19052347].
26. Dardi Charan Kaur D, Wankhede SV. A study of Biofilm formation & Metallo-β-Lactamases in Pseudomonas aeruginosa in a tertiary care rural hospital. *Blood.* ;**13**(3):7–23.
27. Niranjan HP, Priyanka BV, Basavarajappa KGJV. Comparison of screening tests for metallo-beta lactamase producing gram negative bacteria. *J Evol Medi Den Sci.* 2012;**1**(2):45–9.
28. Bashir D, Thokar MA, Fomda BA, Bashir G, Zahoor D, Ahmad S. Detection of metallo-beta-lactamase (MBL) producing Pseudomonas aeruginosa at a tertiary care hospital in Kashmir. *African J Microbiol Res.* 2011;**5**(2):164–72.
29. Noyal MJ, Menezes GA, Harish BN, Sujatha S, Parija SC. Simple screening tests for detection of carbapenemases in clinical isolates of nonfermentative Gram-negative bacteria. *Indian J Med Res.* 2009;**129**(6):707–12. [PubMed: 19692754].
30. Perez F, Hujer AM, Hujer KM, Decker BK, Rather PN, Bonomo RA. Global challenge of multidrug-resistant Acinetobacter baumannii. *Antimicrob Agents Chemother.* 2007;**51**(10):3471–84. doi: 10.1128/AAC.01464-06. [PubMed: 17646423].
31. Maragakis LL, Perl TM. Acinetobacter baumannii: epidemiology, antimicrobial resistance, and treatment options. *Clin Infect Dis.* 2008;**46**(8):1254–63. doi: 10.1086/529198. [PubMed: 18444865].