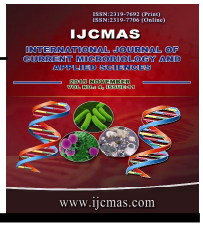
Int.J.Curr.Microbiol.App.Sci (2015) 4(11): 167-176

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 11 (2015) pp. 167-176**
http://www.ijcmas.com



Original Research Article

Effect of Carbapenem Resistant Metallo-Beta-Lactamase Positive *Pseudomonas aeruginosa* on Mortality and Morbidity of Intensive Care Unit Nosocomial Infections

Wesam Hatem Amer*

Lecturer of Medical Microbiology and Immunology, Faculty of Medicine, Tanta University, Egypt
**Corresponding author*

A B S T R A C T

Carbapenem resistant metallo-beta–lactamase positive *Pseudomonas aeruginosa* (CR-MBLP-PA) is a threat in intensive care units (ICU) of our hospitals. Influence of CR-MBLP-PA on mortality and morbidity of ICU in comparison with CR-MBLN (negative)-PA was studied. 48 PA isolated from ICU nosocomial infections. CR isolates were tested for MBL by imipenem and meropenem + ethylene-diamine-tetra-acetic acid (EDTA) combined disc test. The effect of MBL on ICU mortality and morbidity was studied. MBL was the most common mechanism of CR in PA (80%, 32/40). Higher incidence of mortality in CR-MBLP-PA than CR-MBLN-PA (43.8% vs. 25%, p=0.2), 50% of mortalities in both types of isolates due to ventilator associated pneumonia (VAP) (7/14, 1/2) respectively. Higher morbidity of CR-MBLP-PA proved by shorter duration of stay in ICU till death, longer duration of stay till improvement, higher Charlson comorbidity score in comparison with CR-MBLN-PA. Increased incidence of multidrug resistant (MDR) and pan drug resistant strains (PDR) in CR-MBLP-PA in comparison with CR-MBLN-PA (p=0.05). So CR is the main influence on mortality than the presence of MBL. However there was increased virulence and therefore morbidity of CR-MBLP-PA than CR-MBLN-PA.

Keywords

Pseudomonas aeruginosa, Metallo-beta–lactamase, Mortality, Morbidity, ICU

Introduction

PA is a common cause of nosocomial infections with high increase of mortality and morbidity due to emergence of MDR and PDR strains (Yogeesha *et al.,* 2011). Nowadays, carbapenems are considered as the last solution for treatment of resistant PA (Hammami *et al.,* 2011). However resistance to carbapenems is increased due to several mechanisms: efflux pumps, mutations that alter the expression function of porins and penicillin binding proteins (PBPs) and production of carbapenemase (Nordmann and Poirel, 2002). Metallo-beta–lactamases (MBLs) which are classified as (class β beta-lactamases) are common in PA, they are characterized by their resistance to all penicillins, cephalosporins, beta-lactamase inhibitors and carbapenems but poor

hydrolysis of aztreonam and can be inhibited by metal ion chelator (EDTA) (Tängdén and Giske, 2014).

There are many studies about the incidence of MBLs in nosocomial PA with different localities (Hirakata *et al.,* 2003; Hemalatha *et al.,* 2005; Gupta *et al.,* 2006; Asghar *et al.,* 2012; Toval *et al.,* 2015). But there is limited knowledge about the influence of CR-MBLP-PA on morbidity and mortality. Due to the widespread incidence of CR-MBLP-PA in our hospitals, it was necessary to study their effect on morbidity and mortality.

Materials and Methods

This study was performed on PA nosocomial infections isolated from Emergency and Pediatric Intensive Care Units (EICU and PICU) of Tanta University Hospitals for a period of 6 months with prior approval from Institutional Ethical Committee to determine the influence of CR-MBLP -PA and CR-MBLN-PA on mortality and morbidity. Infections not fulfilling Centre for Disease Control criteria for nosocomial infections were excluded (Garner *et al.,* 1988).

Different specimens after consent from every patient (19 endotracheal aspirates, 11 wound swabs, 12 urine and 6 blood samples) were collected and processed according to standard laboratory procedures (Govan, 2006). Data regarding prognostic factors and pre-disposing risk factors were collected from medical records and in consultation with treating doctors and nurses.

Susceptibility to amikacin, gentamicin, netilmicin, ciprofloxacin, piperacillin, piperacillin-tazobactam, ceftazidime, cefepime, imipenem, meropenem, aztreonam, polymyxin-B and colistin was determined by Kirby-Bauer's disc diffusion method (Oxoid, Thermofisher Scientific, UK) according to Clinical and Laboratory Standards Institute guidelines (CLASI, 2014).

PA isolates resistant to imipenem or meropenem or both were subjected to the screening test for MBL production by imipenem and meropenem + EDTA combined disc test as described previously by Yong *et al.* (2002). Isolates with enhancement of zone size of more than or equal to 7 mm between imipenem or meropenem + EDTA disc compared with imipenem disc or meropenem alone were considered as CR-MBLP-PA others were considered as CR-MBLN-PA as shown in figure 1.

Severity of patient's condition was assessed by Charlson's comorbidity score, mean duration of stay in ICU until improvement, until death and pre-disposing risk factors (Veerappa *et al.,* 2014).

Data were analyzed using statistical package of social science (SPSS) version 21. Qualitative data were described using number and percent and was compared using Chi square or Monte Carlo test. Normally quantitative data was expressed as Mean ± SD and compared using student t-test. Statistical significance is at $p \leq 0.05$.

Results and Discussion

In the present study there were 48 non duplicate isolates of PA from EICU and PICU patients. There was no difference between both ICUs as regard the number of PA isolates, sensitivity to carbapenems, presence of MBLs and incidence of mortality. The incidence of CR-PA was 83.3% (40/48), CR-MBLP-PA was 80% (32/40) and mortality was 33.3% (16/48) (Table 1).

The overall mortalities were among CR-PA patients with increased crude mortality among CR-MBLP-PA as compared with CR-MBLN-PA [43.8% (14/32) vs. 25% (2/8), p = 0.2] but without significance. There was increase of morbidity in CR-MBLP-PA that is explained by shorter duration of stay in ICU till death (4.62 ± 1.12 vs. 15.0 ± 1.41, p<0.001), longer duration of stay till improvement (20.58 ± 2.93 vs. 6.33 ± 1.37, p<0.001), higher Charlson comorbidity score (5.03 ± 0.82 vs. 3.0 ± 0.76, p<0.001) in comparison with CR-MBLN-PA. There was improvement of 75% of cases with CR-MBLN-PA (6/8) vs. 56.3% of cases with CR-MBLN-PA (18/32) but without significance. The previous treatment with imipenem was considered as a risk factor for acquisition of MBLs 68.8% of CR-MBLP-PA were treated with Imipenem previously in comparison with 12.5% in CR-MBLN-PA (p=0.006) (Table 2).

There was no significant difference between CR-MBLP-PA and CR-MBLN-PA in antibiotic susceptibility pattern. No reported resistance to colistin and polymexin B in all CR-PA. Sensitivity to netilmycin was equal in both CR-MBLP-PA and CR-MBLN-PA (12.5%, 4/32, 1/8 respectively). There was intermediate sensitivity to aztreonam in CR-MBLP-PA only (28%, 9/32). Also intermediate sensitivity was reported to piperacillin in CR-MBLP-PA, CR-MBLN-PA [46.9% (15/32), 37.5% (3/8) respectively] and to piperacillin-tazobactam [40.6% (13/32), 37.5% (3/8), respectively] (Table 3).

There was more frequency of predisposing risk factors in CR-MBLP-PA than CR-MBLN-PA but without significance (Table 4). As MBLs catalyze all classes of β-lactams, there increased incidence nowadays will be a clinical problem (Walsh *et al.,* 2005). So there early detection is mandatory. Several phenotypic methods were approved for their detection (Pitout *et al.,* 2005; Behera *et al.,* 2008). However (Yong *et al.,* 2002) found 100% sensitivity and specificity by imipenem-EDTA disks for detection of MBLs in *Pseudomonas*. This test is cheap, easy and sensitive so it can be done in small laboratories, although polymerase chain reaction is highly sensitive and specific but it is costly and not available in all hospitals. By this test the prevalence of MBLs in the present study was 80%, that prove the abuse of antibiotics especially carbapenems in our institute. Also Irfan *et al.* (2008) and Kali *et al.* (2013) reported 100% prevalence of MBLs in ICU *Pseudomonas* by imipenem-EDTA disks. Lower ratios were proved by De *et al.* (2010) and Abd El-Baky *et al.* (2013) in ICUS of different localities 33.33%, 53.4%, respectively.

There were several studies in the prevalence, epidemiological data and risk factors of MBLs in *Pseudomonas* (Kumar *et al.,* 2012; Ranjan *et al.,* 2014). But there is lack of studies on the relation between MBLs, mortality and morbidity in our ICUS. So the present study is the first one to determine the influence of CR-MBLP-PA on mortality and morbidity in ICU patients of our hospitals. There was no difference between PICU, EICU in the prevalence of CR, MBLs and even mortality. However there was significance in mortality between CR- PA and CS(sensitive)-PA infections as all mortalities were from CR- PA (16/48, 33.3%, p=0.02), however this significance not found between CR-MBLP-PA and CR-MBLN-PA infections (43.8% vs. 25%, p=0.2), this was in agreement with (Veerappa *et al.,* 2014). So CR is the main influence on mortality (due to limited choices of antibiotics) irrespective to the mechanism of resistance. However Laupland *et al.* (2005) and Zavascki *et al.* (2006a,b) found significance between CR-

MBLP-PA and CR-MBLN-PA in case – fatality rates (25% vs. 13%; p=0.05, 51.2% versus 32.1% respectively), due to large number of cases included in these studies (228, 298 patients with IR- PA respectively).

Several studies all over the world reported the mortality ratios in CR-MBLP-PA in comparison with CR-MBLN-PA (De *et al.,* 2010, Laupland *et al.,* 2005 and Zavascki *et al.,* 2006a). But there is lack of resources on its relation with morbidity. In this study there was higher morbidity of CR-MBLP-PA that explained by short duration till death, long stay in ICU till improvement and high Charlson score significant in comparison with CR-MBLN-PA, this in agreement with (Veerappa *et al.,* 2014). Also (Lucena *et al.,* 2014) explained the severity of MBL-PA by faster progression of patients to death and faster onset of infection. So CR-MBLP-PA can be more virulent than CR-MBLN-PA and these patients will have bad outcome and increased burden on our hospitals. However, there is need for more studies on the virulence factors of MBLP-PA.

There was significance between CR-MBLP-PA and CR-MBLN-PA as regard the previous treatment with imipenem (68.8% vs. 12.5%, p=0.006), so previous intake of imipenem is a risk factor of MBLs in PA. This may be due to the overuse of imipenem as an empirical antibiotic in our ICUs, which will increase the mortality, morbidity and cost of these patients due to failure of this empirical treatment. Also Leppe *et al.* (2002) identified that consumption of imipenem is the major cause of CR and MBLs in PA. Several studies documented risk factors of MBLs, previous treatment with antibiotic is one of these risk factors that lead to acquisition of MBLs in both *Acinetobacter* species and PA (De *et al.,* 2010; Kumar *et al.,* 2012).

As regard antibiotic susceptibility, several studies reported more significant resistance in MBLP-PA in comparison with MBLN-PA to all antibiotics (Hemalatha *et al.,* 2005; Varaiya *et al.,* 2008; Ranjan *et al.,* 2014). While in the present study there was no statistical significance in the susceptibility pattern between CR-MBLP-PA and CR-MBLN-PA, this was similar to results of (Veerappa *et al.,* 2014). These differences between studies may be due to small sample size in the present study and different antibiotic practices in each locality. In this study, all CR-PA isolates were sensitive to both colistin and polymyxin B. They are the last therapy after resistance to azetronam (Zavascki *et al.,* 2006b). However their use is still limited due to their unavailability in our hospitals, their nephrotoxicity and neurotoxicity (Falagas and Kasiakou, 2006). While there was 100 % resistance of all CR-PA isolates to all cephalosporins, aminoglycosides except netilmycin (87.5%) and 97.5% for ciprofloxacin. Intermediate sensitivity to piperacillin–tazobactam, piperacillin and aztreonam (40.6%, 46.9%, 9% respectively) was detected in MBL-PA isolates. This interesting finding was detected in other studies (Veerappa *et al.,* 2014; Gibb *et al.,* 2002; Crespo *et al.,* 2004). So these drugs can be used in combinations for treatment of MBLP-PA.

According to (Pinheiro *et al.,* 2008) MDR isolates are resistant to at least three or more of anti-pseudomonal drugs and PDR isolates are resistant to all antibiotics except colistin or polymyxin B. Higher mortality and morbidity in CR-MBLP-PA due to more frequent MDR (21 vs. 3) and PDR strains (11 vs. 5) in comparison with CR-MBLN-PA with significance (p=0.05), this was in agreement with other studies that reported the association of MDR and PDR strains with MBL isolates were statistically significant in comparison with MBLN-PA (Laupland *et al.,* 2005; Ranjan *et al.,* 2014).

The presence of MDR and PDR strains leave limited choices of antibiotic and thus will increase the mortality ratios in ICU.

It was relevant in this study, that the underlying disease is a very important impact factor on mortality as there were 8 mortalities in VAP patients, representing 50 % of mortalities in CR- MBLP-PA and CR-MBLN-PA (7/14 & 1/2 respectively), however there is no deaths in urinary tract infection patients. So serious diseases like VAP, regardless to its cause, will increase the mortality and morbidity, thus increase patient cost and worsen the prognosis in ICUs (Melsen *et al.*, 2011; Kollef *et al.*, 2012).

Table.1 Incidence of CR, MBL and mortality in EICU, PICU

	Department			
	EICU (24)	**PICU (24)**	**Total (48)**	**p**
CR				
Resistant	20(83.3%)	20(83.3%)	40(83.3%)	1.000
Sensitive	4(16.7%)	4(16.7%)	8(16.7%)	
MBL				
Positive	17(85%)	15(75%)	32(80%)	0.695
Negative	3(15%)	5(25%)	8(20%)	
Mortality				
Died	7(29.2%)	9(37.5%)	16(33.3%)	0.540
Not died	17(70.8%)	15(62.5%)	32(66.7%)	

R: Carbapenem resistance, MBL: metallo-beta-lactamase, EICU: Emergency intensive care unit, PICU: Pediatric intensive care unit, $p \leq 0.05$ significant.

Table.2 Distribution of prognostic factors in CR-MBLP-PA and CR-MBLN-PA patients

	CR-MBLP-PA (32)	**CR-MBLN-PA (8)**	**p**
Mortality	14 (43.8%)	2(25%)	0.2
Mean duration of stay till death	4.62 ± 1.12	15.0 ± 1.41	<0.001*
Mean duration of stay till improvement	20.58 ± 2.93	6.33 ± 1.37	<0.001*
Charlson comorbidity score	5.03 ± 0.82	3.0 ± 0.76	<0.001*
Previous treatment with imipenem	22 (68.8%)	1 (12.5%)	0.006*

CR-MBLP-PA: Carbapenem resistant MBL positive *Pseudomonas aeruginosa*, CR-MBLN-PA**:** Carbapenem resistant MBL negative *Pseudomonas aeruginosa*

Table.3 Relation between MBL and resistance rates of isolates to different antibiotics

	CR-MBLP-PA	CR-MBLN-PA	p
Amikacin			
Resistant	32(100.0%)	8(100.0%)	-
Intermediate	0(0.0%)	0(0.0%)	
Sensitive	0(0.0%)	0(0.0%)	
Netilmycin			1.000
Resistant	28 (87.5 %)	7(87.5%)	
Intermediate	0 (0.0 %)	0 (0.0 %)	
Sensitive	4(12.5%)	1 (12.5 %)	
Gentamicin			
Resistant	32(100.0%)	8(100.0%)	-
Intermediate	0(0.0%)	0(0.0%)	
Sensitive	0(0.0%)	0(0.0%)	
Ceftazidime			
Resistant	32(100.0%)	8(100.0%)	-
Intermediate	0(0.0%)	0(0.0%)	
Sensitive	0(0.0%)	0(0.0%)	
Cefepime			
Resistant	32(100.0%)	8(100.0%)	
Intermediate	0(0.0%)	0(0.0%)	-
Sensitive	0(0.0%)	0(0.0%)	
Ciprofloxacin			1.000
Resistant	31(96.9%)	8(100.0%)	
Intermediate	0(0.0%)	0(0.0%)	
Sensitive	1(3.1%)	0(0.0%)	
Aztreonam			
Resistant	23(71.9%)	8(100.0%)	0.162
Intermediate	9(28.1%)	0(0.0%)	
Sensitive	0(0.0%)	0(0.0%)	
Piperacillin-tazobactam			
Resistant	19(59.4 %)	5 (62.50%)	1.000
Intermediate	13 (40.6 %)	3 (37.5 %)	
Sensitive	0(0.0%)	0(0.0%)	
Piperacillin			
Resistant	17 (53.1 %)	5(62.50%)	0.709
Intermediate	15(46.9 %)	3(37.5%)	
Sensitive	0(0.0%)	0(0.0%)	
Colistin			
Resistant	0(0.0%)	0(0.0%)	-
Intermediate	0(0.0%)	0(0.0%)	
Sensitive	32(100.0%)	8(100.0%)	
Polymyxin B			
Resistant	0(0.0%)	0(0.0%)	-
Intermediate	0(0.0%)	0(0.0%)	
Sensitive	32(100.0%)	8(100.0%)	

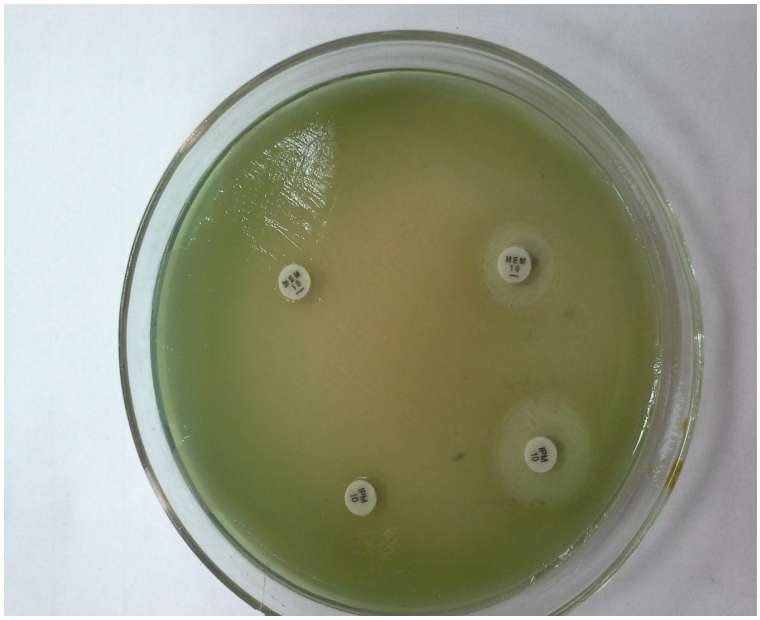
$p \leq 0.05$ is significant

Table.4 Relation between MBL and underlying infections

	CR-MBLP-PA (n = 32)	CR-MBLN-PA (n = 8)	Chi-square	
			X2	P-value
Postoperative wound infection	9(28.1%)	2 (25.0 %)	0.034	0.859
Urinary Catheter infection	4 (12.5%)	1 (12.5%)	0.001	0.999
VAP	15(46.9%)	4(50.0%)	0.031	0.874
Central line infection	4 (12.5%)	1 (12.5%)	0.001	0.999

VAP: ventilator associated pneumonia

Fig.1 Imipenem and meropenem + EDTA combined disc test for metallo-beta- lactamase detection (MBL), the left side: is the Imipenem and Meropenem (10µg) alone show resistance, the right side: Imipenem and Meropenem + 750 µg EDTA combined disc show positive for MBL as both discs zones increased ≥7mm



To conclude CR–PA is widely spread in ICUs with MBLs production is the most prevalent mechanism of resistance. So determination of MBLs in laboratories by simple and easy test like imipenem-EDTA combined disk test is very important to limit MBL gene spread in Enterobacteriaceae by plasmid and to decrease its incidence in ICUs by infection control measures. CR-MBLP-PA was associated with increased mortality rates. In addition CR- MBLP-PA was significantly related to increased morbidity that evidenced by decrease of mean duration till death, increased mean duration till improvement and increased Charlson comorbidity score thus it will lead to decrease prognosis and increase the cost of ICU patients. Frequent use of imipenem in ICUs was related to increased incidence of MBLs. VAP was the most serious underlying disease related to increase to mortality. However there was no significant difference in antibiotic resistance pattern between CR- MBLP-PA and CR-MBLN-PA but still MBLP-PA more serious due to its association with high incidence of MDR and

PDR strains with limited choices of antibiotic. Fortunately this study reported no resistance to colistin and polymexin in all CR-PA either MBL positive or negative reported, but they will be the last resorts after them we will be returned to the era before antibiotic. So, very strict infection control measures are very important to decrease incidence of MBLs. More studies with larger scale are still needed on the virulence factors of CR-MBLN-PA.

Acknowledgments

I would like to profusely thank the staff of Microbiology laboratory of Infection Control Unit, Faculty of Medicine, Tanta University for their cooperation in the collection of isolates used in this study.

Conflict of interest: Nil

Reference

Abd El-Baky, R.M., Abd El-Azeim, N.H., Gad, G.F.M. 2013. Prevalence of extended-spectrum beta-lactamase, AmpC beta-lactamase, and metallo-beta-lactamase among clinical isolates of *Pseudomonas aeruginosa*. *J. Adv. Bio. Bioeng.*, 1: 22–29.

Asghar, A.H. 2012. Antimicrobial susceptibility and metallo-β-lactamase production among *Pseudomonas aeruginosa* isolated from Makkah Hospitals. *Pak. J. Med. Sci.*, 28(5): 781–786.

Behera, B., Mathur, P., Das, A., Kapil, A., Sharma, V. 2008. An evaluation of four different phenotypic techniques for detection of Metallo-β -lactamase producing *Pseudomonas aeruginosa*. *Indian J. Med. Microbiol.*, 26: 233–7.

Clinical and Laboratory Standards Institute, 2014. Performance Standards for Antimicrobial susceptibility testing. 24th informational supplement, CLSI document M100-S24. CLASI, Wayne, PA.

Crespo, M.P., Woodford, N., Sinclair, A., Kaufmann, M.E., Turton, J., Glover, J. *et al.* 2004. Outbreak of carbapenem-resistant *Pseudomonas aeruginosa* producing VIM-8, a novel metallo-b-lactamase, in a tertiary care center in Cali, Colombia. *J. Clin. Microbiol.*, 42: 5094–101.

De, A.S., Kumar, S.H., Baveja, S.M. 2010. Prevalence of metallo-βlactamase producing *Pseudomonas aeruginosa* and *Acinetobacter* species in intensive care areas in a tertiary care hospital. *Indian J. Crit. Care Med.*, 14(4): 217–9.

Falagas, M., Kasiakou, S. 2006. Toxicity of polymyxins: a systematic review of the evidence from old and recent studies. *J. Crit. Care*, 10: R27.

Garner, J.S., Jarvis, W.R., Emori, T.G., Horan, T.C., Hughes, J.M. 1988. CDC definitions for nosocomial infections. *Am. J. Infect. Control*, 16: 128–40.

Gibb, A.P., Tribuddharat, C., Moore, R.A., Louie, T.J., Krulicki, W., Livermore, D.M. *et al.* 2002. Nosocomial outbreak of carbapenem-resistant *Pseudomonas aeruginosa* with a new bla(IMP) allele, bla(IMP-7). *Antimicrob. Agents Chemother.*, 46: 255–8.

Govan, J.R. 2006. *Pseudomonas aeruginosa*. In: Collee, G., Barrie, P.M., Andrew, P.F., Anthony, S. (Eds). Mackie and McCartne practical medical microbiology, 14th edn. Churchill Livingstone, New York. Pp. 413–24.

Gupta, E., Mohanty, S., Sood, S., Dhawan, B., Das, B.K., Kapil, A. 2006.

Emerging resistance to carbapenems in a tertiary care hospital in north India. *Indian J. Med. Res.,* 124: 95–8.

Hammami, S., Boutiba-Ben, B.I., Ghozzi, R., Saidani, M., Amine, S., Ben Redjeb, S. 2011. Nosocomial outbreak of imipenem-resistant *Pseudomonas aeruginosa* producing VIM-2 metallo-beta-lactamase in a kidney transplantation unit. *Diagn. Pathol.,* 6: 106.

Hemalatha, V., Sekar, U., Kamat, V. 2005. Detection of metallo betalactamase producing *Pseudomonas aeruginosa* in hospitalized patients. *Indian J. Med. Res.,* 122: 148–52.

Hirakata, Y., Yamaguchi, T., Nakano, M., Izumikawa, K., Mine, M., Aoki, S. 2003. Clinical and bacteriological characteristics of IMP-type metallobeta-lactamase-producing *Pseudomonas aeruginosa*. *Clin. Infect. Dis.,* 37: 26–32.

Irfan, S., Zafar, A., Guhar, D., Ahsan, T., Hasan, R. 2008. Metallo-β-lactamase-producing clinical isolates of *Acinetobacter* species and *Pseudomonas aeruginosa* from intensive care unit patients of a tertiary care hospital. *Indian J. Med. Microbiol.,* 26(3): 243–245.

Kali, A., Srirangaraj, S., Kumar, S., Divya, H.A., Kalyani, A., Umadevi, S. 2013. Detection of metallo-beta-lactamase producing *Pseudomonas aeruginosa* in intensive care units. *Aust. Med. J.,* 6(12): 686–93.

Kollef, M.H., Hamilton, C.W., Ernst, F.R. 2012. Economic impact of ventilator-associated pneumonia in a large matched cohort. *Infect. Control Hosp. Epidemiol.,* 33: 250–256.

Kumar, S.H., De, A.S., Baveja, S.M., Gore, M.A. 2012. Prevalence and risk factors of metallo β-lactamase producing *Pseudomonas aeruginosa* and *Acinetobacter* species in burns and surgical wards in a tertiary care hospital. *J. Lab. Physicians,* 4(1): 39–42.

Laupland, K.B., Parkins, M.D., Church, D.L., Gregson, D.B., Louie, T.J., Conly, J.M. *et al.* 2005. Population-based epidemiological study of infections caused by carbapenem-resistant *Pseudomonas aeruginosa* in the Calgary Health Region: importance of metallo-b-lactamase (MBL)–producing strains. *J. Infect. Dis.,* 192: 1606–12.

Leppe, P.M., Grusa, E., Reichl, H., Hogel, J., Trautmann, M. 2002. Consumption of imipenem correlates with β-lactam resistance in *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.,* 2920–2925.

Lucena, A., Dalla Costa, L.M., Nogueira, K.S., Matos, A.P., Gales, A.C., Paganini, M.C. *et al.* 2014. Nosocomial infections with metallo-beta-lactamase-producing *Pseudomonas aeruginosa*: molecular epidemiology, risk factors, clinical features and outcomes. *J. Hosp. Infect.,* 87(4): 234–40.

Melsen, W.G., Rovers, M.M., Koeman, M., Bonten, M.J.M. 2011. Estimating the attributable mortality of ventilator-associated pneumonia from randomized prevention studies. *Crit. Care Med.,* 39: 2736–2742.

Nordmann, P., Poirel, L. 2002. Emerging carbapenemases in Gram-negative aerobes. *Clin. Microbiol. Infect.,* 8: 321–31.

Pinheiro, M.R.S., Lacerda, H.R., Melo, R.G.L., Maciel, M.A. 2008. *Pseudomonas aeruginosa* infections: factors relating to mortality with emphasis on resistance pattern and

antimicrobial treatment. *Braz. J. Infect. Dis.*, 12(6): 509–515.

Pitout, J.D., Gregson, D.B., Poirel, L., McClure, J.A., Le, P., Church, D.L. 2005. Detection of *Pseudomonas aeruginosa* producing Metallo-β-lactamases in a large centralized laboratory. *J. Clin. Microbiol.*, 43: 3129–35.

Ranjan, S., Banashankari, G.S., Babu, P.R.S. 2014. Comparison of epidemiological and antibiotic susceptibility pattern of metallo-beta-lactamase-positive and metallo-beta-lactamase-negative strains of *Pseudomonas aeruginosa*. *J. Lab. Physicians*, 6(2): 109–113.

Tängdén, T., Giske, C.G. 2014. Global dissemination of extensively drug-resistant carbapenemase-producing Enterobacteriaceae: clinical perspectives on detection, treatment and infection control. *J. Intern. Med.*, 277(5): 501–12.

Toval, F., Guzmán-Marte, A., Madriz, V., Somogyi, T., Rodríguez, C., García, F. 2015. Predominance of carbapenem-resistant *Pseudomonas aeruginosa* isolates carrying blaIMP and blaVIM metallo-β-lactamases in a major hospital in Costa Rica. *J. Med. Microbiol.*, 64(1): 37–43.

Varaiya, A., Kulkarni, N., Kulkarni, M., Bhalekar, P., Dogra, J. 2008. Incidence of metallo beta lactamase producing *Pseudomonas aeruginosa* in ICU patients. *Indian J. Med. Res.*, 127: 398–402.

Veerappa, K., Babu, Y., Visweswaraiah, D.S., Kumar, A. 2014. The influence of imipenem resistant metallo-beta-lactamase positive and negative *Pseudomonas aeruginosa* nosocomial infections on mortality and morbidity. *J. Nat. Sci. Biol. Med.*, 5(2): 345–51.

Walsh, T.R., Toleman, M.A., Poirel, L., Nordmann, P. 2005. Metallo-β-lactamases. The quiet before the storm? *Clin. Microbiol. Rev.*, 18: 306–25.

Yogeesha, K.V., Jayasimha, V.L., Basavarajappa, K.G., Arun, K., Raghu, K.K., Niranjan, H.P. 2011. A comparative study of ventilator associated pneumonia and ventilator associated tracheobronchitis: Incidence, outcome and risk factors. *Biosci. Biotech. Res. Asia*, 8: 195–203.

Yong, D., Lee, K., Yum, J.H., Shin, H.B., Rossolini, G.M., Chong, Y. 2002. Imipenem-EDTA disk method for differentiation of metallo-beta-lactamaseproducing clinical isolates of *Pseudomonas* spp. and *Acinetobacter* spp. *J. Clin. Microbiol.*, 40: 3798–801.

Zavascki, A.P., Barth, A.F., Gon calves, A.LS., Moro, A.L.D., Fernandes, J.F., Martins, A.F. *et al.* 2006a. The influence of metallo-b-lactamase production on mortality in nosocomial *Pseudomonas aeruginosa* infections. *J. Antimicrob. Chemother.*, 58: 387–392.

Zavascki, A.P., Barth, A.L., Gaspareto, P.B., Goncalves, A.L., Moro, A.L., Fernandes, J.F. *et al.* 2006b. Risk factors for nosocomial infections due to *Pseudomonas aeruginosa* producing metallo-beta-lactamase in two tertiary-care teaching hospitals. *J. Antimicrob. Chemother.*, 58: 882–5.